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Pharmacokinetics of oral tenofovir disoproxil fumarate in pregnancy and lactation: a systematic review

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SUMMARY

Background: Tenofovir Disoproxil Fumarate (TDF), the oral prodrug of Tenofovir (TFV), is advocated in pregnancy to for prevention of mother to child transmission (PMCT) with failure of hepatitis B immunoglobulin and vaccination. The pharmacokinetics of TDF monotherapy for PMCT-HBV is important if deployment is to emulate the success of multiple-ARVs for PMCT-HIV in resource constrained settings.

Methods: This systematic review followed a protocol and is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) guidelines. We included studies that enrolled pregnant women who received oral TDF therapy as monotherapy or in combination with other antiretrovirals (ARVs): irrespective of the reason for receiving the drug (e.g. HIV, HBV or pre exposure prophylaxis); and reported pharmacokinetics.

Results: The AUC, Cmax and Clast, of TFV were decreased in the second and third trimester compared to first trimester or post-partum. In none of the manuscripts was the non-pregnant HBV threshold of Cmax of 300 ng/ml reached, but the EC50 of TFV is lower for treatment of HBV compared to HIV. The TFV concentration in breastfed infants was 0.03% of the recommended infant dose.

Conclusions: Most knowledge of pharmacokinetic of TFV in pregnancy results from studies on HIV involving multiple antiretrovirals. Increased TFV clearance occurred in the second and third trimester when optimal TFV concentrations are required to
maximize suppression of HBV in the window before birth. Dose or duration adjustments will be better conceptualized with concurrent analysis of the PK of TFV monotherapy and Hepatitis B pharmacodynamics in pregnancy.

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Running head: Pharmacokinetics of oral Tenofovir Disoproxil Fumarate in pregnancy and lactation

BACKGROUND

Over 3% of the world populations are infected with Hepatitis B virus (HBV), which is the most significant risk factor for hepatocellular carcinoma. Mother to child transmission (MTCT) is the main route of acquisition [1–4] and the risk of infection is linear to maternal viral load at birth [5]. Perinatal infection occurs in 70–90% of babies born to women with HBe Antigen-positive (HBeAg) HBV and 0–30% in those who are HBeAg-negative [1]. Even with optimal preventive strategies, hepatitis B immunoglobulins (HBIG) after birth in HBeAg-positive mothers in addition to HBV vaccination, MTCT occurs in an estimated 8–32% of cases [6,7]. Unfortunately in low-income countries (LIC) where HBV is most prevalent, HBIG is usually not provided because of access (including homebirth), complexity of production and delivery and short shelf life, cost, and the need for a cold chain [8].

Maternal Tenofovir Disoproxil Fumarate (TDF) therapy is one strategy under consideration to reduce MTCT. Pregnant women could be treated with oral TDF during the course of their pregnancy and, if no further availability, it can be stopped 1 month post-partum. TDF is an oral prodrug of tenofovir (TFV), a nucleotide analogue that was developed and has been widely used as an antiretroviral drug against Human Immunodeficiency Virus (HIV) and HBV. TDF has an oral bioavailability (F) estimated at 20-30%, is formulation dependent [9]. Following absorption and distribution, TFV is converted intracellularly to its active anabolite tenofovir diphosphate (TDP). There is no efficacy threshold level for TFV in HIV, but thresholds for HBV that have been used included a Cmax of 300 ng/ml and a t1/2 of 17 hours (tested in non-pregnant males and females) [10]. The elimination of plasma or serum TFV is more rapid (t1/2 12-16 hours) than the bioactive intracellular TDP (t1/2 87 hours in peripheral blood mononuclear cells (PBMCs), and 96 hours in hepatocytes) [11]. This t1/2 of TFV in PBMCs is helpful since TFV is pharmacologically “forgiving” in the context of poor adherence. TFV exhibits long-lasting anti-HBV activity in cell culture, but has an in vitro 50% effective concentration (EC50) that is lower for treatment of HBV compared to HIV (0.03 ± 0.02 µg/ml with continuous exposure) [12]. TDF is widely regarded as safe in pregnancy after extensive use in HIV-positive pregnant women [13].

Both the pharmacokinetics (PK) [14] and pharmacodynamic (PD) properties [15] of drugs may be affected by physiological changes of pregnancy and reduced exposure has been reported for antiretroviral drugs in pregnancy [14,16] and in lactation [17]. TDF suppression of HBV for prevention of MTCT starts in pregnancy which sets a constraint on the time frame available to reduce the viral load before birth. While a systematic review and meta-analysis supports high efficacy of TDF from 28 weeks given with HBIG and the birth dose to reduce MTCT; HBV DNA remains detectable in 2-38% of participants which is important because the threshold of HBV DNA load to prevent MTCT of HBV without HBIG (reality for resource limited settings) is unknown [6,18–20]. Rebound of HBV DNA to pre-treatment levels within 4-8 weeks occurs following short-course TDF before protective infant HBV antibody levels can be acquired [21]. This may be
important in resource-constrained settings where breastfeeding remains the only source of infant nutrition and HBIG and HB birth dose administration are frequently not provided.

The objective of this systematic review was to summarise the PK of oral TDF for PMTCT of HBV and HIV in pregnant and lactating women. The PK of TDF monotherapy for PMCT-HBV may be important before widespread deployment in low resource settings where optimal provision of HBIG and birth dose are problematic.

**METHODS**

This systematic review followed a protocol and is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) guidelines.

Protocol and registration

This review was registered in advance in PROSPERO (International prospective register of systematic reviews). Registration number: CRD42018082352.

Eligibility criteria

We included studies that enrolled pregnant women who received oral TDF therapy as monotherapy or in combination with other antiretrovirals (ARVs), irrespective of the reason for receiving the drug (e.g. HIV, HBV or pre exposure prophylaxis), and details of the reported pharmacokinetics. Both English and non-English-language studies were included.

Information sources

We searched for publications in Medline, Embase and Ovid Cochrane Central Register of Controlled Trials from 1980 to 15 November 2018. Controlled vocabulary supplemented with keywords was used to search for pharmacokinetic studies of TDF in pregnancy. A manual search of bibliographies of the included studies and relevant systematic reviews was conducted. We also contacted people in the field to make sure we did not miss unpublished papers. The last search was performed on 15 November 2018.

The complete search is listed in the supplementary material.

Study selection

Two independent reviewers screened in duplicate titles and abstracts for potential eligibility. Disagreements were reconciled by consensus or by a third reviewer. Articles that were selected were first screened for eligibility using the title and abstracts. Manuscripts were excluded during the process that did not meet our patient, intervention, control, outcome (PICO) objectives.

Data extraction

For each study, data extraction was done in duplicate using a standardized, pretested form. A third reviewer compared data and resolved inconsistencies by referring to the full text of the articles.
Data items
The exposure parameters used were those that were previously reported as being essential for dosing decision support in pregnancy, i.e. area under the concentration-time curve (AUC, \( \text{AUC}_x \)) as total exposure, or within an interval e.g. in the case of daily dosing as \( \text{AUC}_{0-24} \), minimal (\( C_{\text{trough}} \)) and maximal (\( C_{\text{max}} \)) plasma concentrations [14]. Other distribution parameters collected were standard pharmacokinetic measures including: volume of distribution (\( V_d \)), fraction of drug bound and unbound to plasma proteins (\( f_b \) and \( f_{ub} \)); and elimination parameters (half life (\( t_{1/2} \)) and clearance (CL)).

Risk of Bias Assessment
Two reviewers assessed the risk of bias (i.e., systematic error) independently using the Cochrane Risk of Bias assessment [22]. The quality of evidence (i.e., certainty in the estimates) was evaluated using the Grading of Recommendations Assessment, Development, and Evaluation approach (GRADE) [23].

Criteria used to evaluate quality of evidence were risk of bias, indirectness (surrogate outcomes), imprecision (wide confidence intervals), inconsistency (heterogeneity), and publication bias [24].

RESULTS
The initial search resulted in 668 citations of which 62 were duplicates. We eventually included 11 studies that were published between 2008 and 2018 (Table 1). Six studies informed about TDF PK during pregnancy [25–30] and five on drug concentrations of which three were after a single dose of drugs during childbirth [31–35]. The average weighted kappa for study selection was 0.71 (good, supplementary table 1). The study selection process and reasons for exclusions are shown in Figure 1.

The breastmilk search resulted in 107 citations of which 11 were duplicates. We included four studies in our final analyses (Figure 2). These studies were all conducted in Africa. Three studies included HIV infected women [36–38] and one included HIV negative women who received TDF as part of Pre Exposure Prophylaxis (PrEP [17]).

Assay Methodologies
The PK parameters were tested with high performance liquid chromatography (HPLC) with fluorescence detection which has been validated according to the International Conference of Harmonisation Guidelines in terms of accuracy, precision, specificity, robustness, limits of detection and quantitation, and other aspects of analytical validation [39] or Liquid chromatography–mass or tandem-mass spectrometry (LC-MS/MS) methods, validated for the multiplexed quantification of TAF and TFV [40] (Supplementary Table 2). The analyzed PK samples were of maternal plasma, cord blood, infant plasma, amniotic fluid and breastmilk; and presented as concentration ratios.

Risk of bias within studies
The quality assessment of the studies was based on the GRADE scoring system, (Supplementary Table 3). In this system the studies were scored on study limitations (low, medium or high level), directness, consistency, precision and reporting bias. Most of the studies did not report how patients were selected and
did not report the proportion of patients who agreed to participate resulting in medium levels of study limitations. There were no reporting biases detected.

Results of individual studies

Pharmacokinetics during pregnancy

Five publications described steady-state PK parameters [26–30]. A computer simulated modeling study was also included [25] (Table 2). Of the five steady state PK studies, four included HIV-infected or non-infected pregnant females treated with 300mg TDF in steady state, either as PrEP or as treatment, and only Cressey et al. included HBV infected women. The non-compartmental analyses (NCA) of all publications indicated that the AUC/f and Cmax values were significantly lower in pregnancy compared to non-pregnant women.

The study by Colbers et al. showed that 26% of the patients receiving TDF in pregnancy did not meet the threshold of 2,000 ng/ml*h AUC0-24 (defined as the threshold for TFV efficacy for HIV being the 10th percentile in non-pregnant controls) in the third trimester compared with only 4% of the patients in the postpartum period. One in nine patients that had an AUC below the threshold had a detectable HIV viral load at delivery compared to six out of 25 with AUC above the threshold [28].

Colbers et al. showed that the Cmax returned to normal after delivery [28]. Best et al. showed a similar trend, however this was not statically significantly different between trimesters and the postpartum period [27]. Accordingly the Clast reported in the various studies was decreased in pregnant women and normalized postpartum [29]. Best et al. and Colbers et al. showed a decrease of 20% and 23% in AUC/f comparing the third trimester and post-partum, respectively [27,28]. These studies estimated an increased oral clearance (Cl/f) of TFV in the third trimester compared with postpartum of 57-55 l/H versus 46-43 l/H, respectively. Thus the oral clearance (Cl/f) of TFV increased significantly in comparison to post- partum and non-pregnant women and the AUC is inversely correlated with clearance.

The data from these studies [17,18] was used for the computer modeling [25]. The modeling study used population characteristics of a certain patient population and physicochemical parameters of the drug to simulate drug plasma concentrations. Oral clearance (Cl/f) was estimated to increase until an estimated gestational age (EGA) of 28 weeks and then to slowly decrease again. Thus tenofovir Cmax, Clast and AUC all decreased in HIV pregnant women in the second and third trimester. The absorption time (Tmax) did not change during pregnancy. Best et al. [27] estimated a terminal t1/2 of 16.1 h during pregnancy compared with 12.4 h postpartum [27] which suggests that the exposure parameters are affected mainly by an increased volume of distribution, Vd. There were no data on the intracellular phosphorylation kinetics of TFV in pregnancy.

TFV concentrations

The study from Pyra et al. [30] compared the mean concentrations of TFV during the different trimesters in pregnancy and in pre-pregnant and in non-pregnant women when the drug was provided for pre-exposure prophylaxis HIV. Their data suggests that after the first trimester the mean concentration of TFV decreases to below the non-pregnant concentration (Figure 3). During pregnancy the TFV concentrations were 45–58% lower compared with non-pregnant after adjusting for adherence. Cressey et al. [29] assessed TFV exposures in HIV uninfected HBV infected pregnant women (estimated gestational age 28 weeks), and
showed that TFV exposure was 20% lower in pregnancy compared with postpartum. This was similar to the results reported in studies of HIV infected pregnant women.

**Pharmacokinetics during childbirth**

Three publications provide PK information after a single oral dose (600mg or 900mg) of TDF during childbirth [31–33]. The reported $C_{\text{max}}$ after 600mg TDF differed widely among all studies with 234 (range 83-595) ng/ml, 310 (range 70–520) and 448 (range 110-928) ng/ml. The reported AUCs of the women who received 600mg TDF were 2.73 mg/l*hr (range 1.43-3.55) and 4.22 (range 2.77-24.46) mg/l*hr. The $t_{1/2}$ following the 600 mg dosage was 15.9-19.5 (range 3.3-187.6) hours.

**Cord blood concentrations**

Rimawi et al. [34] compared paired maternal (delivery) and cord blood samples of 10 patients who received a TDF containing ARV regimen. They found a maternal plasma TDF concentration of 94.6 ng/ml with a cord plasma of 38.5 ng/ml, or a median cord plasma/maternal plasma ratio of 1:2.5 (95%CI). Yeh et al. [35] measured drug concentrations at delivery in maternal blood plasma, cord blood plasma, and amniotic fluid. The TFV concentration in the maternal plasma was 5.0 ng/mL and in cord plasma was 30 ng/ml. These two studies report very different cord plasma / maternal plasma ratios which could be due to the different assays used in the studies, LC-MS/MS and HPLC.

**Breast milk**

In four studies, maternal plasma and breastmilk samples, taken within 30 minutes of each other, were compared (Table 3), three studies were done in HIV infected women. The timing of the samples in relation to the TDF dose varied from 1-2 hours after the maternal TDF dose to over 12 hours [36–38,41]. The median TDF concentration in breastmilk varied from 3.2 to 14.1 ng/ml with a maternal plasma concentration of 86.7 to 293.0 ng/ml. This resulted in a breastmilk/maternal plasma ratio of 0.03 to 0.07. TFV was excreted in breastmilk in a very low concentration, lower than the concentrations found in cord blood samples, resulting in a median amount of ingested TFV in infants of 0.03% of the recommended infant dose.

**DISCUSSION**

In order to prevent MTCT of HBV and HIV in pregnancy, the viral load needs to be low or preferably undetectable particularly at the time of birth. HBV DNA suppression may be compromised by lower exposure to TDF in later pregnancy. In this review the previously stated threshold for the $C_{\text{max}}$ of 300 ng/ml for HBV suppression was not achieved. This review shows that the $C_{\text{mean}}$ and $C_{\text{max}}$ of TFV decrease during pregnancy. With $T_{\text{max}}$ remaining the same, the absorption of the drug appears to be unaffected. The decrease in $C_{\text{max}}$ could be a result of the increasing $V_d$, due to an physiological increase of total body water and extracellular fluid during pregnancy [14]. In one published manuscript this led to a sufficient $C_{\text{max}}$ in the first trimester, but $C_{\text{max}}$ then decreased as the pregnancy evolved [14–16]. Drug elimination is increased during pregnancy with increased clearance until a gestation of approximately 28 weeks after which it slowly decreases [27,28,42]. Since TDF is excreted mainly by glomerular filtration, its renal clearance is expected to parallel changes in creatinine clearance during pregnancy [15]. As the AUC is dependent on the clearance, an increase in the creatinine clearance results in a decrease in TFV AUC per trimester of the pregnancy.
This review supports conclusions of Benaboud et al., that dosing escalation be considered from 2nd trimester to achieve similar exposure to non-pregnant adults with the caveat that clinical experience of more than 300 mg daily is limited [42]. Dense and sparse PK sampling under TDF treatment correlated with HBV DNA load across gestation and post-partum (or pre-pregnancy) are required to determine if dose adjustment is needed. If this is needed, it needs to be considered that TDF follows a dose-linear PK [9].

Effect of pharmacokinetics on HBV DNA suppression

While this review shows reduce exposure of TDF in pregnancy the implications of this altered PK on HBV DNA suppression are unclear because of unknowns in the PK PD relationships in the initial clearance phase and the treatment duration factor. Previously it has been suggested that the decrease of AUC, C\text{max} and C\text{min} that occurs in pregnancy does not affect the viral load in HIV patients [28]. However, the women enrolled were taking multiple antivirals, making it unclear if it was the TDF suppressing the virus or one of the other antivirals. Moreover, co-formulated TDF increases the AUC, C\text{max} and C\text{min} of TFV [43].

Treatment with TDF can reduce the HIV or HBV viral load with an adequate length of time before delivery. In non-pregnant chronic HBV infected patients treated with TDF, maximal suppression of HBV DNA plateaus at approximately 32 weeks with good compliance and with a 4 week viral load reduction of approximately $3.9-4.0 \log_{10} \text{IU/ml}$ [44–46]. This reduction is higher than reported in pregnancy, where the HBV DNA decreases between 2.75 and $3.52 \log_{10} \text{IU/ml}$ after 4 weeks of TDF therapy with up to 32% of mothers with an HBV DNA $>200,000 \text{IU/ml}$ at delivery [6,47–49]. If started earlier in pregnancy (gestation of 24 weeks), the viral decline can reach $4.08- 5.23 \log_{10} \text{IU/ml}$ until childbirth, however, in LIC women tend to present later in pregnancy [50]. The HBV DNA viral load needed to omit HBIG is unknown but potentially negated by longer dosing prior to childbirth.

In studies on the PK and PD of TFV in HIV infected individuals, dose escalating data from 75, 150, 300 and 600mg showed a dose-proportional increase in viral suppression until 300mg, the 600 mg dose could not produce a steeper viral decay compared to the 300mg dosage [44,51]. The C\text{max} were proportional to the dosage with $375 \text{ng/ml}$ vs $573 \text{ng/ml}$ after 8 hours for 300mg and 600mg respectively. The studied plateau TFV concentrations in the healthy individuals was higher compared to the described PK data in pregnancy. This suggests that in healthy individuals the maximum achievable HIV viral decay is with 300mg but this might be different in pregnancy when the drug concentrations are lower; or different for HBV. Increasing the dosage of TDF to get a C\text{max} as observed in healthy individuals might produce a steeper viral decay and, as toxicity is related to drug concentrations, would not necessarily increase risks.

More data is needed in the PK/PD of TDF in pregnancy, in particular on the impact of variable circulating blood concentrations on the clearance rate of HBV. This information could guide recommendations on dosing and the duration of treatment in the prevention of MTCT particularly when optimal delivery of HBIG and birth dose vaccination are compromised.

TDF in breastfeeding

TFV concentrations in breastfeeding infants are mostly undetectable or lower than considered of clinical significance. These findings corroborate with findings from animal studies [52] and suggests that usage of TDF in breastfeeding women is safe for the infant but of no therapeutic value. Most liver disease societies including American Association for the Study of Liver Diseases and European Association for the Study of
the Liver support allowance of breast feeding when mothers were taking TDF. In a resource-constrained setting the risk of infant infection from lactation (cracked nipples) due to HBV rebound (typically 4-8 weeks) after TDF cessation at one month post-partum and before protection from infant vaccination is established (when HBIG and birth dose are not accessible), is unknown.

Limitations
This systematic review has several limitations including few available studies most of which are on HIV. The number of studies is disproportionately low compared to the number and ethnicities of pregnant women with HIV and HBV infections worldwide. The two studies reporting on the drug concentrations used different assays to measure the PK which makes comparison difficult. The impact of pharmacogenetics is not reviewed, although it is known that genetic variation of HBV might impact the PK. Lastly, the studies reporting on pharmacokinetics in pregnancy are all based in Europe or USA. This makes the results difficult to translate to patients elsewhere, although no difference in PK has been reported for TFV between these populations in non-pregnant adults.

CONCLUSION
This main two highlights of this review are firstly the limited data on the pharmacokinetics of TDF monotherapy treatment for HBV in pregnant women which suggest lower concentrations of TFV in second and third trimester of pregnancy compared to first trimester and non-pregnant status. Secondly, studies to date have occurred in settings where other HBV risk reduction measures (birth dose, HBIG, TDF during lactation) were optimized and as these are not routinely provided in resource-constrained settings consideration for ensuring the most favourable exposure before childbirth is useful.

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CONFLICT OF INTEREST
EJS declares to have received travels grants from Abbvie and Gilead.

The other authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study

REFERENCES


Supplementary material

Search strategy

Supplementary Table 1. Kappa calculation

Supplementary Table 2. Reporting methodology and limit of quantification of tenofovir assay

Supplementary Table 3. Quality assessment of the studies was based on the GRADE scoring system
<table>
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<tr>
<th>Author</th>
<th>Country (period)</th>
<th>Type of study</th>
<th>Study Population PW- pregnant women</th>
<th>Intervention (drug manufacturer), timing and dose</th>
<th>PK model</th>
<th>PK variables</th>
<th>Steady state/single dose</th>
<th>GRADE score</th>
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</thead>
<tbody>
<tr>
<td>Benaboud [26]</td>
<td>France</td>
<td>Observational drug monitoring</td>
<td>186 PW and 46 non-PW with HIV, EGA 28-38 weeks</td>
<td>TDF oral 300mg OD with other ARV</td>
<td>One and two compartment model</td>
<td>Vc, Q, Vp, ka, Cmin, AUC, CL/F</td>
<td>Steady state</td>
<td>Moderate</td>
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<td>Best [27]</td>
<td>USA clinics from the P1026s cohort (Nov 04-Sep 08)</td>
<td>Multicenter, prospective non blinded</td>
<td>37 PW with HIV infection, EGA 20-38 weeks</td>
<td>TDF oral 300mg OD with other ARV</td>
<td>Two compartment model</td>
<td>Vd/F, Cmax, tmax, C0, C24, AUC, t½, CL/F</td>
<td>Steady state</td>
<td>High</td>
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<tr>
<td>Colbers [28]</td>
<td>PANNA network (Nov 08-Jan 12)</td>
<td>Non randomized, open label, prospective, multicenter</td>
<td>34 PW with HIV, EGA 28-38 weeks, median 33 weeks</td>
<td>TDF oral 300mg OD with FTC</td>
<td>Non compartment model</td>
<td>Cmax, C24, tmax, AUC, t½, CL/F</td>
<td>Steady state</td>
<td>High</td>
</tr>
<tr>
<td>Cressey [29]</td>
<td>Thailand (Jan 2013-Dec 2016) and PANNA network</td>
<td>Data were combined from two trials</td>
<td>154 PW with HBV, EGA 28 weeks</td>
<td>TDF oral 300mg OD with and without FTC</td>
<td>Two compartment model</td>
<td>AUC, C24</td>
<td>Steady state</td>
<td>Moderate</td>
</tr>
<tr>
<td>De Sousa Mendes [25]</td>
<td>France (2014)</td>
<td>PK modeling based on physiology</td>
<td>Male volunteers</td>
<td>TFV, FTC, 3TC</td>
<td>Physiologically-based pharmacokinetic two compartment model</td>
<td>Vss, Cmax, tmax, AUC, Cl/F</td>
<td>Simulation</td>
<td>Moderate</td>
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<tr>
<td>Flynn [31]</td>
<td>USA and Puerto Rico</td>
<td>RCT</td>
<td>31 HIV infected PW, EGA ≥34</td>
<td>600mg TDF, 900mg TDF or 900/600 TDF/FTC before delivery with 2 4mg/kg TDF for infant or 4/3 mg/kg TDF/FTC for child</td>
<td>Non compartment model</td>
<td>Cmax, tmax, C24, AUC, t½, concentration at delivery</td>
<td>Single dose</td>
<td>Moderate</td>
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<td>Hirt [32]</td>
<td>ANRS 12109 study TEMAA in Africa/Asia</td>
<td>Modelling study</td>
<td>38 HIV+ PW, EGA 28-38</td>
<td>NVP/TDF 600mg at delivery and TDF 300mg 7 days pp</td>
<td>Two and three compartment models</td>
<td>V/F, ka, Cmin, Cmax, AUC, CL/F</td>
<td>Single dose</td>
<td>Moderate</td>
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<td><strong>Mirochnick [33]</strong></td>
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<td><strong>Rimawi [34]</strong></td>
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<td><strong>Pyra [30]</strong></td>
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<td><strong>Yeh [35]</strong></td>
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**Breastmilk studies**

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<tr>
<th>Author</th>
<th>Country (period)</th>
<th>Type of study</th>
<th>Study Population</th>
<th>Intervention (drug, timing and dose)</th>
<th>PK variables</th>
<th>Steady state/single dose</th>
<th>GRADE score</th>
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<tr>
<td>Benaboud [36]</td>
<td>Cote d'Ivoire</td>
<td>Prospective as part of the TEmAA study</td>
<td>5 exclusively breastfeeding mothers, HIV+</td>
<td>TDF oral 600mg at delivery (in combination with FTC and NVP) and FTC-TDF OD for 7 days</td>
<td>Drug concentrations in breastmilk and maternal plasma. Simulated minimal and maximal milk doses</td>
<td>Steady state</td>
<td>Moderate</td>
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<tr>
<td>Publication</td>
<td>Type</td>
<td>Authors</td>
<td>Location</td>
<td>Study Design</td>
<td>Participants</td>
<td>Drug Regimen</td>
<td>Key Outcomes</td>
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<tr>
<td>Mugwanya [17]</td>
<td>Original article</td>
<td>Kenya and Uganda (Jan-Jun 2015)</td>
<td>Prospective, open label, single arm</td>
<td>50 HIV negative breastfeeding mothers</td>
<td>TDF oral 300mg OD (PrEP)</td>
<td>Drug concentration in infant plasma, maternal plasma and whole breastmilk, M/P, infant plasma drug-to-milk</td>
<td>Steady state, High</td>
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<tr>
<td>Palombi [37]</td>
<td>Original article</td>
<td>Malawi</td>
<td>Prospective as part of the Option B-Plus approach</td>
<td>47 HIV infected breastfeeding mothers</td>
<td>TDF oral 300mg OD n combination with other ARV</td>
<td>Drug concentration in infant plasma, maternal plasma and whole breastmilk, M/P</td>
<td>Steady state, High</td>
</tr>
<tr>
<td>Waitt [38]</td>
<td>Original article</td>
<td>Uganda and Nigeria</td>
<td>Prospective</td>
<td>27 HIV infected and breastfeeding mothers</td>
<td>TDF 300mg OD in combination with NVP, 3TC, EFV and/or FTC</td>
<td>Drug concentration in infant plasma, maternal plasma and whole breastmilk, M/P</td>
<td>Steady state, Moderate</td>
</tr>
</tbody>
</table>

3TC, Lamivudine; AUC, Area under the plasma concentration-time curve; ARV, Antiretrovirals; C_{max}, Maximum (peak) plasma drug concentration; C_{min}, Minimum plasma drug concentration; C_{0}, Initial (fictive) or back-extrapolated plasma drug concentration at time zero following bolus intravenous injection; C_{24}, plasma drug concentration at time 24 following bolus intravenous injection; C_{through}, Trough plasma concentration (measured concentration at the end of a dosing interval at steady state [taken directly before next administration]); CL/F, Apparent total clearance of the drug from plasma after oral administration; COBI, Cobisistat; DTG, Dolutegravir; EGA, Estimated Gestational Age; EVG, Elvitegravir; RFV, Efavirenz; FTC, Emtricitabine; HIV, Human Immunodeficiency Virus; k_{a}, Absorption rate constant (first-order); M/P, milk to plasma ratio; NVP, Nevirapine; OD, Once Daily; PK, Pharmacokinetic; PrEP, Pre Exposure Prophylaxis; PW, pregnant women; Q, intercompartmental clearance; t\(_{1/2}\), Elimination half-life; TDF, Tenofovir Disoproxil Fumarate; t_{max}, Time to reach maximum (peak) plasma concentration following drug administration; Vc, central volume of distribution; Vd/F, apparent peripheral volume of distribution; Vss, Apparent volume of distribution at steady state; Vp, peripheral volume of distribution;
**Table 2. Pharmacokinetic outcome measurements of TDF in pregnancy**

<table>
<thead>
<tr>
<th>Author</th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$C_{24}$ (ng/ml)</th>
<th>AUC (ng/ml.h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Second trimester</td>
<td>Third trimester</td>
<td>Post-partum</td>
</tr>
<tr>
<td>Benaboud [26]</td>
<td>39 (22-92)</td>
<td>1,600 (900-3,300)</td>
<td>1,600 (900-3,300)</td>
</tr>
<tr>
<td>Best [27]</td>
<td>250 (202-355)</td>
<td>245 (207-334)</td>
<td>54 (40-70)</td>
</tr>
<tr>
<td>De Sousa Mendes [25]</td>
<td>270 (120-910)</td>
<td>40 (34-48)</td>
<td>56 (48-68)</td>
</tr>
</tbody>
</table>

AUC, Area under the plasma concentration-time curve; $C_{24}$, plasma drug concentration at time 24 following bolus intravenous injection; $C_{\text{max}}$, Maximum (peak) plasma drug concentration

**Table 3. Drug concentrations of TDF in breastmilk**

<table>
<thead>
<tr>
<th>Author</th>
<th>$C_{\text{min}}$ in breastmilk (ng/ml)</th>
<th>$C_{\text{max}}$ in breastmilk (ng/ml)</th>
<th>Median ingested TFV dose mcg/kg/day</th>
<th>Infant concentration (ng/ml)</th>
<th>Concentration in maternal plasma (ng/ml)</th>
<th>Breastmilk/plasma ratio (M/P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benaboud [36]</td>
<td>6.8 (5.83-8.75)</td>
<td>14.1 (11.60-16.25)</td>
<td>1.4 (1.1-2.4)</td>
<td>undetectable</td>
<td>152.0 (56.9-321.0)</td>
<td>0.03 (0.01-0.05)</td>
</tr>
<tr>
<td>Mugwanya [17]</td>
<td>3.2 (2.3-4.7)</td>
<td>0.47 (0.35-0.71)</td>
<td>94% undetectable</td>
<td>152.0 (56.9-321.0)</td>
<td>0.03 (0.01-0.05)</td>
<td></td>
</tr>
<tr>
<td>Palombi [37]</td>
<td>5.0 (0-6.1)</td>
<td>24 (0-51.6)</td>
<td>86.7 (73.7-102.6)</td>
<td>293 (176-391)</td>
<td>0.07 (0.06-0.08)</td>
<td></td>
</tr>
<tr>
<td>Waitt [38]</td>
<td>5.98 (0-8.05)</td>
<td></td>
<td>293 (176-391)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C$_{\text{max}}$, Maximum (peak) plasma drug concentration; C$_{\text{min}}$, Minimum plasma drug concentration; TFV, Tenofovir
Figure 1. PRISMA Flow Diagram for pharmacokinetic studies on TDF in pregnancy

Records identified through database searching (n = 668)

Records after duplicates removed (n = 606)

Records excluded, reasons (n = 595)
- 14 Case Report/Case series
- 91 Not on oral TDF
- 109 no PK data
- 35 Not about pregnant women
- 343 Reviews/guidelines
- 1 Protocol
- 2 animal studies

Records screened (n = 606)

Full-text articles assessed for eligibility (n = 11)

Studies included in qualitative synthesis (n = 6)

Studies included in quantitative synthesis (meta-analysis) (n = 6)

Full-text articles excluded for PK data in pregnancy but used for other analyses, with reasons (n = 5)
- 3 single dose of TDF during labour
- 2 reported drug concentrations but no PK data

Additional records identified through other sources (n = 0)

Additional records identified through other sources (n = 0)

Records identified through database searching (n = 668)

Additional records identified through other sources (n = 0)
Figure 2. PRISMA Flow Diagram for pharmacokinetic studies on TDF in breastmilk

Identification

Records identified through database searching (n = 107)

Additional records identified through other sources (n = 1)

Records after duplicates removed (n = 97)

Records excluded, reasons (n = 87)
- Review/guideline: 56
- No TDF per oral: 13
- No breastmilk data: 3
- No pharmacokinetic data: 15

Records screened (n = 97)

Full-text articles assessed for eligibility (n = 10)

Studies included in qualitative and quantitative synthesis (n = 4)

Full-text articles excluded, with reasons (n = 6)
- Review/guideline/expert opinion: 6

Screening

Eligibility

Included
Figure 3. TFV$_{\text{mean}}$ concentration in pregnant (n=37) and non-pregnant (n=97) women in tenofovir pre-exposure prophylaxis [30]

Light grey: TFV$_{\text{mean}}$ in women that had sampling done before and during pregnancy, as per manuscript - these are the same women;
Black stripes: TFV$_{\text{mean}}$ in women that did not had sampling done before pregnancy, as per manuscript - these are the same women;
100% medication event monitoring system and unadjusted for dose; TFV-tenofovir
Supplementary material

Search strategy
Medline, Embase and Ovid Cochrane Central Register were searched using the following search terms:

- pharmacokinetics.fs. or exp pharmacokinetics/ or exp area under curve/ or exp absorption/ or half-life/ or (Pharmacokinetic* or pharmacodynamic* or PK or PD or "PK/PD" or PPK or t\text{max} or \text{c}\text{max} or AUC or "area under the curve" or clearance or elimination or "volume of distribution" or "drug level" or absorption or half-life or ((serum or plasma) adj3 concentration)).

- exp TENOFOVIR/ or (tenofovir or TDF or viread).

- exp Pregnancy/ or exp Pregnancy Complications/ or exp Maternal Health Services/ or Perinatal Care/ or Labor pain/ or Analgesia, Obstetric/ or exp Obstetric Surgical Procedures/ or (antenatal* or prenatal* or puerper* or postnatal* or post-natal* or (post adj natal*) or postpartum or post-partum or (post adj partum) or prepregnancy or pre-pregnancy or (pre adj pregnancy) or preconcept* or pre-concept* or (pre adj concept*) or periconcept* or peri-concept* or (peri adj concept*) or eclamp* or preeclamp* or (pre adj eclamp*) or amniocentes*.ti,ab. or (chorion* adj vill*) or caesarean or cesarean or caesarian or cesarian or cesarien or caesarien or pregnan*).

For the breastmilk data an additional search was done replacing pregnancy terms with the following search terms:

- exp milk, human/ or colostrum/ or exp lactation/ or (Colostrum or Whey or ((breast or human or mother*) adj milk) or lactation or lactating or (breast adj feeding) or breastfeeding).ti,ab,kf.

Supplementary Table 1. Kappa calculation

<table>
<thead>
<tr>
<th>Percent agreement</th>
<th>87.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chance agreement</td>
<td>9/16*100%</td>
</tr>
<tr>
<td>Corrected chance</td>
<td>87.5%-56.25%</td>
</tr>
<tr>
<td>Corrected potential agreement</td>
<td>100%-56.25%</td>
</tr>
<tr>
<td>Kappa</td>
<td>31.25%/43.75%</td>
</tr>
</tbody>
</table>
Supplementary Table 2. Reporting methodology and limit of quantification of tenofovir assay

<table>
<thead>
<tr>
<th>Author</th>
<th>Assay method</th>
<th>Assay referenced</th>
<th>Pharmacology Laboratory</th>
<th>Linear range</th>
<th>Lower limit of quantification (LLOQ)</th>
<th>Assay accuracy and precision</th>
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</thead>
<tbody>
<tr>
<td>Best [22]</td>
<td>LC-MS</td>
<td>J Chromatogr B Analyt Technol Biomed Life Sci. 2006 Jan 2;830(1):6-12. Epub 2005 Nov 2. <strong>Sensitive assay for determining plasma tenofovir concentrations by LC/MS/MS.</strong> Delahunty T et al</td>
<td>USA</td>
<td>10-1500 ng/ml</td>
<td>10 ng/ml</td>
<td>Accuracy and precision were within ± 20% at 10 ng/mL and ± 15% at other quality control concentrations</td>
</tr>
<tr>
<td>Colbers [23]</td>
<td>HPLC fluorimetric detection</td>
<td>States ‘validated’ but not referenced</td>
<td>The Netherlands</td>
<td>15–1500 ng/ml</td>
<td>15 ng/ml</td>
<td>Accuracy/precision not reported</td>
</tr>
<tr>
<td>Cressey [24]</td>
<td>LC-MS/MS</td>
<td>States “ The laboratory participates in an external quality control program” which was referenced but the actual method was not referenced*.</td>
<td>Unknown</td>
<td>20–2500 ng/ml</td>
<td>Not stated</td>
<td>Average accuracy 99-102% and precision was &lt;5% of the coefficient-of-variation.</td>
</tr>
<tr>
<td>De Sousa Mendes [20]</td>
<td>Not applicable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flynn [26]</td>
<td>LC-MS</td>
<td>J Acquir Immune Defic Syndr. 2007 Oct 1;46(2):167-73. <strong>Bioequivalence of efavirenz/emtricitabine/tenofovir disoproxil fumarate single-tablet regimen.</strong></td>
<td>Gilead Sciences, USA</td>
<td>Not stated</td>
<td>10 ng/ml</td>
<td>Precision percent coefficient of variation of &lt;13% and an accuracy within 16%</td>
</tr>
<tr>
<td>Authors</td>
<td>Methodology</td>
<td>Reference</td>
<td>LOQ (ng/ml)</td>
<td>Precision and accuracy</td>
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<td></td>
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<td>----------------------------</td>
<td>-------------</td>
<td>------------------------</td>
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<td></td>
</tr>
<tr>
<td>Mirochnick</td>
<td>LC-MS/MS</td>
<td>Not referenced</td>
<td>5-1,000</td>
<td>Precision ≤6.9% and accuracy ≤±9.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,000 ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rimawi [29]</td>
<td>LC-MS/MS and HPLC</td>
<td>Not referenced</td>
<td>Unknown</td>
<td>Accuracy and precision not reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ther. Drug Monit. 28:517–525. 2006. Full validation of an analytical method for the HIV-protease inhibitor atazanavir in combination with 8 other antiretroviral agents and its applicability to therapeutic drug monitoring. Rezk, N. L., R. D. et al</td>
<td>UNC center for AIDS research</td>
<td>intra- and interday precision (2.0 to 14.3%) and accuracy (88 to 113%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations**

HPLC: High performance liquid chromatography

LC-MS: Liquid chromatography–mass spectrometry
LC-MS/MS: Liquid chromatography–tandem mass spectrometry

Supplementary Table 3. Quality assessment of the studies was based on the GRADE scoring system

<table>
<thead>
<tr>
<th>Study</th>
<th>Study limitations</th>
<th>Directness</th>
<th>Consistency</th>
<th>Precision</th>
<th>Reporting bias</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benaboud [22]</td>
<td>Medium level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Imprecise</td>
<td>Undetected</td>
<td>Moderate</td>
</tr>
<tr>
<td>Best [23]</td>
<td>Low level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Precise</td>
<td>Undetected</td>
<td>High</td>
</tr>
<tr>
<td>Colbers [24]</td>
<td>Low level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Precise</td>
<td>Undetected</td>
<td>High</td>
</tr>
<tr>
<td>Cressey [25]</td>
<td>Medium level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Precise</td>
<td>Undetected</td>
<td>Moderate</td>
</tr>
<tr>
<td>De Sousa Mendes [21]</td>
<td>Medium level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Imprecise</td>
<td>Undetected</td>
<td>Moderate</td>
</tr>
<tr>
<td>Flynn [27]</td>
<td>High level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Precise</td>
<td>Undetected</td>
<td>Moderate</td>
</tr>
<tr>
<td>Hirt [28]</td>
<td>Medium level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Imprecise</td>
<td>Undetected</td>
<td>Moderate</td>
</tr>
<tr>
<td>Mirochnick [29]</td>
<td>Medium level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Imprecise</td>
<td>Undetected</td>
<td>Moderate</td>
</tr>
<tr>
<td>Rimawi [30]</td>
<td>Medium level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Precise</td>
<td>Undetected</td>
<td>High</td>
</tr>
<tr>
<td>Pyra [26]</td>
<td>Medium level</td>
<td>Direct</td>
<td>Unknown, single study</td>
<td>Precise</td>
<td>Undetected</td>
<td>High</td>
</tr>
<tr>
<td>Yeh [31]</td>
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<td>Direct</td>
<td>Unknown, single study</td>
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<td>Undetected</td>
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</tr>
<tr>
<td>Benaboud [32]</td>
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<td>Precision</td>
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<td>Quality</td>
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<tr>
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<td>-----------------------</td>
<td>-----------</td>
<td>------------</td>
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</tr>
<tr>
<td>Mugwanya</td>
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<td>direct</td>
<td>unknown, single study</td>
<td>precise</td>
<td>undetected</td>
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<td>precise</td>
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<tr>
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