PROTOCOL

Protocol ID: **MRCZ/A/1968**

Brief Title: **University of Zimbabwe College of Health Sciences Birth Cohort Study**

Acronym: **UZ-CHS-BC**

Official Title: **HIV Exposure, Disease Acquisition and Progression among Children: Role of Maternal Immunogenetics, Viral Genetic Diversity, HAART Exposure, Co-morbidities and Psycho-Social Status: UZ-CHS Birth Cohort.**

Principal Investigator: **Duri Kerina (PhD)**

Sponsor: **University of Zimbabwe**
College of Health Sciences (UZ-CHS)
Department of Immunology

Version 2, 3 December 2015

Co-investigators

1. Professor E. Gomo
Immunogeneticist
Department of Laboratory Sciences, UZ-CHS.

2. Professor FZ Gumbo
Paediatrician
Department of Paediatrics and Child care, UZ-CHS.

3. Professor S. Rusakaniko
Biostatistician
Department of Community Medicine,
University of Zimbabwe College of Health Sciences
(UZ-CHS)

Co-ordinating Centre (Sponsor)

University of Zimbabwe College of Health Sciences Research Support Centre (UZCHS RSC)
Parirenyatwa Hospital Grounds, Harare

Telephone: +263 4 708020

Email: rscsacoreoffice@gmail.com

Mentors/Advisory Committee

1. **Professor M.Z. Chirenje**
Obstetrician
Department Obstetrics and Gynaecology, UZ-CHS
Email: chirenje@uz-ucsf.co.zw

2. **Professor I. Chitsike**
Paediatrician
Department of Paediatrics, UZ-CHS
Email: chitsikei@gmail.com

3. **Professor L.S. Zijenah**
Immunologist
Department of Immunology, UZ-CHS
Email:lzijenah@gmail.com

4. **Professor B. Stray-Pedersen**
University of Oslo, Institute of Clinical Medicine
Department of Women and Children
Oslo University Hospital, Rikshospitalet
0027 Oslo
E-mail: babill.stray-pedersen@medisin.uio.no

Confidentially Statement

This protocol is confidential and may not be given, without permission of the investigators' to any other persons other than the prospective co-investigators, research site staff and relevant ethics committees and regulatory authorities.

Statement of Compliance

This research study will be conducted in compliance with the International Conference on Harmonisation Tripartite Guidelines on Good Clinical Practice protocol, local good Clinical and Laboratory Practice Guidelines in line with the ethical principles of the Declaration of Helsinki and requirements and guidelines of the Human Research Ethics Committee (HREC) that is; Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee and Medical Research Council of Zimbabwe.

Protocol Signature Page

Sponsor Representative

_____	_____	_____
Name	Signature	Date

Investigator(s)

I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol and will only make changes in the protocol after notifying the sponsor.

I understand that I may terminate or suspend enrolment of the study at any time if it becomes necessary to protect the best interests of the study participants.

I agree to personally conduct and supervise this investigation to ensure that all associates, colleagues, and employees assisting in the running of this study are informed about their obligations in meeting these commitments.

I will conduct the study in accordance with Good Clinical Practice, the Declaration of Helsinki, and the moral, ethical and scientific principles that justify medical research. The study will be conducted in accordance with all relevant laws and regulations relating to clinical studies and the protection of study participants.

I will ensure that the requirements relating to HREC review and approval are met.

I agree to maintain adequate and accurate records and to make those records available for audit and inspection in accordance with relevant regulatory requirements.

I agree to promptly report to the HREC any changes in the research activities and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without HREC approval, except where necessary to ensure the safety of study participants.

Principal Investigator

_____	_____	_____
Name	**Signature**	**Date**

Co-Investigator

_____	_____	_____
Name	**Signature**	**Date**

Co-Investigator

_____	_____	_____
Name	**Signature**	**Date**

Protocol Summary

Full Title	HIV Exposure, Disease Acquisition and Progression Among Children: Role of Maternal Immunogenetics, Viral Genetic Diversity, HAART Exposure, Co-morbidities and Psycho-Social Status: UZ-CHS Birth Cohort
Short Title	UZ-CHS Birth Cohort
Study Design	Prospective cohort study
Study Population	Pregnant women from 28 weeks gestation and their infants
Sample size	**1200** (600 HIV positive (+) and 600 HIV negative (-)
Duration of participation	A **total of 27 months** that is, plus or minus 3 months in pregnancy and a follow up period of 24 months after delivery.
Study intervention	An observational study on how antenatal **co-morbidities** impact on pregnancy outcome, infant growth and development in this era of option B+ of prevention of mother to child transmission of HIV (PMTCT).
Background	Whilst the numbers of human immune deficiency virus (HIV) exposed and infected (HEI) infants are dwindling by the day, courtesy of the current effective PMTCT programs, the converse is true with (HIV) exposed but uninfected (HEU) infants. Despite being HIV uninfected HEU infants, have been shown to have higher morbidity and mortality mainly from infectious diseases just like their HEI counterparts. Understanding why and how this ever growing population of HEU infants is at high risk of acquiring infections is critical as their problems will be of major public health importance in the near future. However, before understanding HEU infants themselves, it is necessary to first understand the impact of theintrauterineenvironment which is key in shaping their immune development and their lifelong health in general. To achieve this, a new research paradigm involving an integrated approach is critical. There is need to clearly discern, in a holistic and synergistic approach involving different specialties, the role of antenatal comorbidities including maternal psychosocial well-being not mention the impact of highly active antiretroviral therapy (HAART) prophylaxis regimen on pregnancy outcome, infant growth, immune and/or neuro-development. More so, how similar or different these factors are among HIV+ and HIV- pregnant women remains poorly described. Evaluation of combinations of soluble biomarkers of different comorbidities may prove more powerful tool in predicting disease outcome. Undertaking research in such a manner within the college not only provides the mostly sought after evidence based clinical care for mothers and their children but also build research capacity within the College. In addition investigators also attain higher degrees for self-actualization not to mention institutional and national professional advancements.
Primary Objectives	1. To determine the prevalence and incidence of HIV and co-infections (hepatitis B virus (HBV) hepatitis C virus (HCV), cytomegalovirus(CMV)intestinal parasites) and determine how these impact on pregnancy outcome, infant growth and immune/neuro-development. 2. To determine of maternal nutritional status and the prevalence and incidence of non-communicable diseases (diabetes, hypertensive disorders, cardiovascular diseases) and how these impact on pregnancy outcome and child growth 3. To determine immunological and soluble biomarkers of HIV transmission, diseases progression for different comorbidities in isolation or combinations among mothers and infants. 4. To correlate maternal plasma viral load, soluble biomarkers immune activation profiles and diseases progression and duration of HAART treatment among HIV+ mothers 5. To determine any association of different HAART regimens and pregnancy outcome, infant growth and immune/neuro-development among HIV+ mothers
Primary end points	1. Prevalence, trends and incidence of comorbidities among HIV+ and HIV- mothers 2. Pregnancy outcomes for HIV-/+ mothers with different comorbidities with or without different HAART regimens 3. HIV and co-infections vertical transmission rates in the era of option B+ among mothers with chronic and acute HIV infection 4. Soluble biomarkers for different comorbidities, acute, chronic HIV infection and vertical transmission

Table of Contents

Table of Tables

Table of Figures

List of Abbreviation and Acronyms

µl	microliter
AIDS	Acquired immune deficiency syndrome
APOBEC3G	Apolipoprotein-B mRNA-editing enzyme catalytic polypeptide-like-3G
BCG	Bacille de Calmette et Guérin
BMI	Body mass index
BST-2	Bone stromal tumour protein-2
BV	Bacterial vaginosis
CD	Cluster of differentiation
CMV	Cytomegalovirus
CNS	Central nervous system
DNA	Deoxyribonucleic Acid
DPIC	Depo-Provera injectable contraception
EBV	Epstein Barr virus
Env	Envelope gene
g/dl	grams per decilitre
GDM	Gestational diabetes mellitus
Gp	Glycoprotein
GUD	Genital ulcer disease
HbA1c	Glycosylated haemoglobin
HBV	Hepatitis Bvirus
HCV	Hepatitis Cvirus
HDL	High density lipoprotein
HELLP	Haemolysis, elevated liver enzymes and low platelet count
HIV	Human immunodeficiency virus
HPV	Human Papilloma virus
HSE	Herpes simplex encephalitis
HSV	Herpes simplex virus
IFN-γ	Interferon-gamma

IGF-1	Insulin growth factor -1
IgG	ImmunoglobulinG
IL	Interleukin
KIR	Killer cell immunoglobulin-like receptors
LTBI	Latent TB infection
MCP-1	Monocyte chemoattractant protein-1
Nef	Negative factor
NK cells	Natural killer cells
OIs	Opportunistic infection
PI	Protease inhibitor
PKPD-VD	Pharmacokinetics- pharmacodynamics-viral dynamics
PMTCT	Prevention of mother to child transmission
PNGs	Potential N-glycosylation sites
RFs	Restriction factors
SAMHD-1	Sterile alpha motif histidine-aspartic domain-1
SSA	Sub-Saharan Africa
STIs	Sexually transmitted infections
TB	Tuberculosis
Th-2	T-lymphocyte helper-2
TIBC	Total iron binding capacity
TLRs	Toll like receptors
TRIM5α	Tripartite motif 5-alpha
TST	Tuberculin skin test
TV	*Trichomonas vaginalis*
UZ-CHS	University of Zimbabwe College of Health Sciences
V3	Envelope gene variable region 3
VDR	Vitamin D receptor
Vif	Viral infectivity factor
Vpr	Viral protein R
Vpu	Viral protein U
VZV	Varicella zoster virus

Executive Summary

Viral, host immunogenetics and environmental factors associated with human immunodeficiency virus (HIV) non-acquisition and/or slow HIV disease progression among paediatric patients remain poorly described. The little that is currently known comes from HIV-1 subtype B studies. The role of innate immunity in protection against HIV infection and disease progression may not be over-emphasised among paediatric HIV infected individuals whose adaptive immunity may not have fully developed. Since HIV-infected children progress more rapidly than adults and have fewer background cofactors such as drug use and co-infections, the effects of host genetic factors on HIV-1 disease may be more clearly identified. While the emphasis has been on increasing availability and coverage of efficacious antiretroviral regimens and strengthening health systems within PMTCT initiatives, there is also a need to address other maternal-related factors which are equally essential. Undiagnosed maternal HIV infection and co-morbidities, nutritional status, pyscho-social factors, unplanned pregnancies, delays in accessing antenatal care, non-disclosure of the pregnant woman's HIV status to her partner and social demography are some of the most significant maternal risk factors associated with vertical transmission of HIV and/or infant morbidity including mortality. Equally important in determining infants 'susceptibility to infections are maternal behavioural, biological and immunogenetic factors. How all these factors in isolation or combination modify the infants', growth, neuro- and immune development remains poorly described. In view of varied population specific differences in the frequencies of protective or susceptibility alleles, it is important to strengthen research efforts towards defining unknown genetic propensity in our own population. Mathematical modelling of these potentially interactive genetic variables alongside with their respective immunological profiles may help to identify potential genetic or/and immunological markers with better diagnostic and/or prognostic values. Thus, there is need for a cohort study to clearly discern the causes and consequences of viral immune profiles and genetics in addition to social dynamics of disease acquisition which are impossible to determine with cross sectional studies. A holistic and integrative research approach involving different disciplines to predict diseases outcomes of the ever growing population of HEU may not be over-emphasised. This is only possible through development of sub-studies nested within this cohort, which may have different study designs, addressing other pertinent comorbidities research questions from different perspectives.Such a research approach is not only cost effective and efficient as it quickly yields incredible maternal and infant scientific observations. HEU and non-progressing HEI infants offer hope that natural and effective HIV control is possible at the same time providing an important insight into the development of the next generation of HIV/AIDS vaccines and immune-based therapies with minimal side effects.

Background

The underlying reason behind the high HIV-1 prevalence in sub-Saharan Africa (SSA) remains elusive. Unlike in Europe and the United States of America(USA) where HIV is confined to intravenous drug users and homosexual men [1], the African epidemic is more widely distributed across the general population with heterosexual penile-vaginal as the main transmission mechanism [2]. However, this observation is inconsistent with the low probability of heterosexual HIV transmission per coital act, as differences in sexual behaviour do not always translate to differences in HIV prevalence or incidence [3;4] pointing to the possibility of other precipitating co-factors that may increase per contact transmission rates in the region. Moreover, this heterosexual mode of transmission does not satisfactorily explain the relatively high proportion of HIV discordant couples common in the region who in some instances continue to bear children[5]. Thus, contact with an infected person represents only a necessary but not sufficient condition for HIV transmission as exposure to the virus without being infected is common, pointing to gaps in our current knowledge on HIV transmission.

Contemporary scientific literature demonstrates that human immunogenetics coupled with ecological factors are important determinants of vulnerability to HIV infection and progression to AIDS [6]. Synergistic relationships between HIV disease burden and viral genotypes, malnutrition including co-infections have been observed, implicating them as possible HIV disease acquisition precipitating factors [7;8]. On average 74% Africans are exposed to two or more parasitic, bacterial or viral infections whilst a good 26% grapple with six or more diseases including non-communicable diseases, some of which are side effect(s) attributed to intake of HAART [9-11]. Relative to single pathogen infections, co-infections may alter HIV transmission dynamics [12]. Single and/or dual pathogen infection studies have quite common. Such studies are essential but they could have reported misleading conclusions taking cognisance that in real local life situation such solitary or double infections are rare. Thus, there is a paucity of information on the prevalence of co-morbidities and how these in isolation or/and combination modify or modulate HIV disease acquisition, immune responses progression, pregnancy outcome, neuro-cognitive development and response to therapy more so in children. The foregoing including other potential social-cultural factors may be central in fuelling the HIV-1 epidemic in SSA. Unless a holistic multi-disciplinary research approach is adopted that will at the same time generate critical mass of local professionals with relevant expertise complimented by cross fertilization of regional and international institutional collaborations, the problem of HIV will always remain.

1.0 Introduction

Use of effective prophylactic HAART in pregnancy has reduced vertical transmission rates of HIV down to about 1% from a high of more than 20% [13;14]. Thus the immediate benefit of HAART in pregnancy remains unquestionable, implying that most infants can now escape HIV infection. Consequently, the number of HEU infants is increasing, representing about 30% of all children born in most parts of SSA[15]. Despite these positive and impressive developments, HEU children have been shown to have substantially increased morbidity and mortality predominantly from infectious causes, particularly between 2 and 6 months of age, compared with their HIV unexposed uninfected (HUU) counterparts[16-21]. This observation is of great concern taking cognisance that over and above their ever increasing numbers, HEU alongside the HIV exposed and infected (HEI) infants, are now maturing into adults[22], entailing that any problems specifically associated with these groups of children will be of major public health importance. So far it is not yet clear whether *in utero* exposure to HIV-1 and/or other co-morbidities including exposure to prophylactic HAART during pregnancy and/or infancy may predispose these infants to such infections.

Preterm deliveries have been the commonest adverse pregnancy outcome among Chinese HIV-infected women, with such babies generally suffering from respiratory distress syndrome, dysplasia, intracerebral hemorrhage and necrotizing enterocolitis [23]. Studies have also shown maternal HIV infection to be associated with being underweight and low height for age z scores among HEU infants [24-26]. Contrary to the above findings, a recent Botswana study has found no significant mortality difference by HIV exposure status and non-exposure in a neonatal intensive care setting but has identified low Apgar scores and extreme prematurity as important risk factors for mortality, pointing to the importance of other related factors [27].Maternal plasma and cervical HIV-1 RNA load are important risk factors for vertical transmission of HIV[28]. Little is known about trends of HIV RNA levels in HIV-infected women conceiving on HAART with or without viral load suppression and how this impacts on pregnancy outcome including infant development. Recent findings suggest that exposure to high maternal HIV-1 viremia *in utero,* even in the absence of perinatal transmission, affects the infant's immune system development resulting in health complications later in life[29]. The role of the mother-infant pair host genetics and HIV diversity in susceptibility to infections is equally critical. Thus, a better understanding of the role of all the foregoing factors, not to mention the obvious shortcomings associated with being born in an HIV affected household is essential for better clinical management of this ever growing HEU population with the long-term aim of addressing most of their unmet health needs.

1.1. HEU Infants Increased *Susceptibility* to Infections

In spite of being HIV uninfected, HEU infants, like their HEI counterparts, have been shown to harbour enteropathogenic *Escherichia. Coli* (E. coli) and Cryptosporidium infections in their stool specimens, an observation that may explain the observed increased mortality [30]. A low full blood count (FBC) at birth has been significantly associated with this increased infant morbidity and mortality [31]. Nasopharyngeal bacterial colonisation with *Staphylococcus aureus, Streptococcus pneumoniae, Moraxella catarrhalis* including *Haemophilus influenza* have been shown to be prevalent among Tanzanian HEU infants at about 6 weeks of age [32]. Acute bronchiolitis has been quite common and more severe among Brazilian HEU infants of less than 6 months of age[33].Interestingly, pulmonary and extra-pulmonary tuberculosis (TB) have also been shown to be prevalent among HEU infants[34]. Malawian and South African studies on hospitalized HEU infants showed serious infectious morbidity due to *Pneumocystis jiroveci* pneumonia, *Cytomegalovirus* (CMV) colitis with perforation, *Pseudomonas* sepsis, haemorrhagic varicella, Group a *Streptococcal* meningitis, endocarditis and gastroenteritis [35-37]. Early breastfeeding cessation has been associated with increased risk of serious gastroenteritis among HEU infants when compared with later breastfeeding cessation [38;39].Maternal prolactin hormone levels "rise with infant suckling. Thus, the more feedings, the higher the level of serum prolactin although stress can also elevate levels. (Cox, Owens, & Hartmann, 1996; Tay 1996). Studies have found out that breastfeeding decreased pneumonia incidence among Kenyans HEU infants with morbidity rates picking up after weaning[40;41;42]. Besides issues surrounding early cessation of breastfeeding, studies have indicated that HEU infants have a wide range of phenotypical and functional immunological abnormalities that may contribute to the high morbidity and mortality from infections.[43;44]. Such factors surrounding the abnormal infant immune development are not well described.

1.2. Immunological Abnormalities of HEU Infants

1.2.1. Adaptive Immunity Anomalies

Cell-mediated immunity and T-lymphocyte maturation have been shown to be altered in HEU new-borns and these abnormalities persist over time [45;46] implying that *in utero* exposure to HIV and/or HAART may cause some of these immune abnormalities. The thymus being the organ responsible for the maturation and selection of T lymphocytes is thus pivotal in allowing the development of a functional immune system [47;48]. The role played by thymus size and output in the shaping of foetal immune development in HEU infants remain poorly described. Absolute numbers of lymphocytes are decreased in HEU infants with feotal exposure to maternal HIV-1 priming the infant immune system leading to an enhanced immune activation and altered T lymphocyte homing [49;50]. Immune activation in HEU infants is also believed to modify interaction between the immune system and HIV pathogenesis[51].Findings have demonstrated a significant disturbance in cytokine network among HEU

neonates exposed to HAART during pregnancy [52;53]. The observation that ARV induces not only quantitative but also qualitative alterations in the cytokine profile warrants attention. Cytokine profile, when properly designed could complement the data from viral load and CD4+ T lymphocyte count in the analysis of the disease status, thus aiding in the decision making among possible therapeutic interventions aimed at persistently decreasing immune activation and chronic inflammation [54;55].Thus, soluble markers in body fluids is a reflection of immune status and therefore may be important in assessing HIV transmission, progression even mortality. Decrease in IL-2, IFN-γ and concomitant increases of IL-4 and IL-10 have been associated with a decline in antigen-specific immune responses, resulting in opportunistic infections [56;57]. Elevated inflammatory biomarkers such as CRP and IL-6, have been associated with HIV disease progression and mortality. Chemokines and defensins in breast milk and cervico-vaginal secretions have been associated with HIV-1 susceptibility and transmission. Trends in plasma and breast milk biomarker levels of immune activation and inflammation as interferon-γ-inducible protein-10 (IP-10), monokine induced by interferon-γ (MIG) and soluble CD14 (sCD14) levels, intestinal fatty acid binding protein (I-FABP) can be compared to HIV-1 RNA level and evaluated for associations with comorbidities. Biomarkers of the acute HIV infection, such as IFNγ, serum amyloid A, IP-10, IL-12, , IL-7 and IL-15, may help predict viral set-point variation and disease progression whilst IL-6, soluble (s) CD14, sCD163, and CRP, D-dimer, fibrin and hyaluronic acid can be clinically useful biomarkers in predicting comorbidities in HIV-1-infected patients both on and without HAART. Evaluation of combinations of soluble biomarkers of different comorbidities may prove more powerful tool in predicting disease outcome. Persistent exposure to viremia alters HIV-specific CD8 response through a tenacious immune activation process leading to naive CD8 T-lymphocyte exhaustion and skewed maturation of memory subsets [58]. Increasing differences in lymphocyte subsets become more apparent as early as six weeks of age with total and naive CD4+ T lymphocytes being significantly higher in American HEU than HEI infants whilst CD8+ lymphocyte positive for human leukocyte antigen DR locus positive (HLA-DR+), CD45 RA+ HLA-DR+ and CD28+ HLA-DR+ were elevated in HEI infants [59]. Soluble CD14 (sCD14) is secreted into plasma and serves as a biomarker of monocyte activation. elevated levels of sCD14 have been associated with $CD8^+$ T-cell activation and with mortality in the setting of HIV infection. Altered CD4/CD8 and the memory/naive ratios have been observed at least in Italian HEU adults[60]. Studies have also shown alterations in immunoglobulin levels and higher B-lymphocyte apoptosis among HEU infants [61;62]. The role of pro-inflammatory lipids high density lipoprotein (HDL), in systemic immune activation in HIV infection remains largely unknown. All in all these findings suggest that foetal exposure to a chronically activated maternal immune system may be the reason behind the observed altered adaptive immune responses which are not yet fully developed. The innate immunity is more important at this tender age of development.

1.2.2. Innate Immunity Anomalies

In view of the under-developed adaptive immunity and the limited exposure to pathogens *in utero* infants rely on the innate immune system for protection against infections. Innate immune cellular population include macrophages, dendritic cells, monocyte and natural killer (NK) cells. Findings have shown the dysfunctionality of the innate immunity of HEU infants. Alterations in NK cell subsets, activation, and cytolytic potential including cytokine production may contribute to the reduced ability to fight infections, a phenomenon observed among these infants [63;64]. Identification of invading pathogens is through pathogen associated molecular patterns that are sensed by patterns recognition receptors such as toll like receptors (TLRs) and C type lectins. TLRs recognise both extra and intracellular pathogens. TLR agonist receptor binding culminate in the downstream production of cytokines, chemokines and antimicrobials. Defensins are effector molecules of the innate immunity with a broad antimicrobial spectrum [65]. Alpha-defensin concentrations in breast milk have been associated with a decreased risk of intra-partum and post-natal HIV transmission [66;67]. The role of innate immunity in protection against HIV acquisition and disease progression need not be over-emphasised among paediatric HIV infected infants whose adaptive immunity would not have fully developed. When it comes to immunity against retroviruses, mammalian cells have evolved powerful mechanisms to limit or restrict viral replication through the production of restriction factors (RFs), constituting the so called intrinsic immunity.

1.2.3. Intrinsic Immunity: Role of RFs

Host constitutively express cellular antiviral proteins called RFs. Examples of such proteins include apolipoprotein-B mRNA-editing enzyme catalytic polypeptide-like-3G (APOBEC3G), bone stromal tumour protein-2 (BST-2) or tetherin integral membrane protein, tripartite motif 5-alpha (TRIM5α) and sterile alpha motif histidine-aspartic domain-1 (SAMHD1). However, HIV has developed strategies to counter the antiviral activity byencoding accessory/regulatory proteins viral protein u (Vpu), viral protein r (Vpr), viral infectivity factor (Vif) and negative factor (Nef) to escape destruction from host RFs.Like the innate immune responses, the expression of RFs is also induced by interferons. Role of RFs in HIV infection for both mother and new-born pairs has so far received very little attention yet they constitute the very first line of immune defense, much before of both the innate and adaptive immune responses kick in. Few recently published articles suggest the key roles played by APOBEC3G,TRIM5α, tetherin and SAMHD1 in facilitating immediate response to viral infections[68-70]. The mechanisms by which RFs inhibit viral replication and their potential contribution to HIV pathogenesis remain poorly described. To improve our understanding of such underlying mechanisms, there is need to compare and contrast the innate and intrinsic immune development between HEI, HEU and HUU infants especially in resource poor settings where risks to infectious diseases are remarkably high during infancy. Greater understanding of intrinsic immunity may facilitate the development of safer and more effective pharmacological agents for the treatment of

viral infections. Also blocking viral replication pharmacologically is HAART that is offered to pregnant women to reduce maternal infectiousness including vertical transmission of HIV.

1.2.4 HAART in Pregnancy

HAART has had undoubted preventive benefits in reducing vertical transmission of HIV. Adherence to HAART in pregnancy is crucial to optimize efficacy and curtail vertical transmission. Despite these remarkable successes, HAART use in pregnancy has been associated with adverse birth outcomes such as preterm delivery, small for gestational age, stillbirths, anthropometric parameters and body composition anomalies including birth defects [71;72]. There is also need to explore possible etiologic role of HAART in cleft palate. HAART pose metabolic complications in HIV patients including pregnant women. Protease inhibitor (PI) use early in pregnancy has been associated with increased risk of prematurity and gestational diabetes mellitus development [73;74]. ARVs have also been demonstrated to damage the mitochondria. Studies have suggested that polymorphisms in the mitochondrial DNA (mtDNA) may predispose one to ART related metabolic complications. Mitochondrial toxicity due to ARVs may explain mt-related metabolic outcomes such as neuropathy, lipodystrophy, dyslipidaemia, hyperlactaemia and insulin resistance among ART experienced individuals. Pharmacogenomics studies have linked ART related metabolic complications and treatment side effects to genetic polymorphisms in genes encoding drug metabolising enzymes and drug transporters. Birth weights for gestational age norms for Botswana infants have been shown to be lower at term than norms for black American infants. Standard values for birth weight by gestational age are not generally available for SSA, where HIV infection, HAART and other *in utero* co-infections exposures may impact on birth outcomes, yet these parameters are necessary if incidence and risk factors for intrauterine growth retardation are to be assessed. HAART may cause adverse lipid profiles and increased risk for cardiovascular events due to complex interactions between traditional risk factors and HIV infection itself in terms of ongoing endothelial dysfunctional immune activation/inflammation and increased risk of thrombosis[75;76]. Both lipoatrophy and lipohypertrophy are associated with increased cardio-metabolic risk and in metabolic disorders that likely lead to premature aging reported in HIV infected patients [77]. Whether these complications are also experienced by the child and to what extent presents a knowledge gap. The need to quantify *in-utero* exposures to HAART may not be over-emphasized, hence the need to explore the impact of these chronically administered medications on pregnancy outcome and child development.

1.2.5 HEU Infants and exposure to HAART

After birth all HIV exposed infants must undergo HIV virological testing at the earliest possible opportunity to facilitate uptake of ART without any delay for infants with positive test results, in the process preserving their immune function and consequently reducing morbidity and mortality [78]. Marston and *et al*.; have demonstrated that failure to access treatment results in a 52% net survival at

one year among perinatally infected infants [79]. Thus the benefits of early infant diagnosis may not be overemphasised not to mention the reduced maternal psychological trauma associated with extended waiting time for the infant HIV diagnosis test results.

HAART exposure *in utero* or postpartum has been associated with significant anaemia and neutropenia, persistent decrease platelets and lymphocytes as well as an increased risk of transient lactic acidaemia, and mitochondrial deoxyribonucleic acid (DNA) depletion [80;81]. Mitochondrial dysfunctions following perinatal exposure to nucleoside analogues have been observed [82]. Studies have observed a greater neurodevelopmental delay among HEI and HEU compared with HUU infants/children in resource-poor settings [83]. Long-term effects of *in utero* and neonatal ART exposure on cognitive and academic development in HIV-exposed, uninfected school-age children are unknown. Other studies have shown that a greater proportion of HEI children receiving PI containing HAART to have hyperlactataemia, hyperlipidemia, high triglyceride concentrations compared to those not receiving ART [84;85]. Low-density lipoprotein cholesterol has been significantly higher in the cord blood of PI-exposed infants versus those not exposed to PIs *in utero* [86]. HIV-infected children have higher levels of biomarkers of vascular dysfunction than do HEU children. Unfavourable lipid profiles have been positively associated with interleukin (IL)-6, monocyte chemoattractant protein-1 (MCP-1), fibrinogen, and P- and E-selectin, whereas increased HIV viral load has been associated with markers of inflammation (MCP-1 and C-reactive protein, CRP) and endothelial dysfunction soluble intracellular cell adhesion molecule-1 and soluble vascular cell adhesion molecule-1 [87].HAART exposure in feotal and early neonatal life has also been linked to lower immunoglobulin G (IgG) titres [61]. The current extent of infant HAART exposure from breast milk remains unknown. All these observations call for the need for long-term follow-up of HEU and HEI infants with the aim of understanding the impact of maternal HAART treatment on infant toxicity, development of non-communicable diseases and HIV-1 resistance mutations.

1.3 Responses to Vaccines among HEU Infants

Following 6 months of HAART exposure, studies have also shown that more than half of previously immunized children still lacked positive measles antibody titres [88]. HEU infants have been shown to frequently exhibit a range of immunological abnormalities indicative of a deficiency in their ability to develop immunological memory following vaccinations [89;90]. Studies have shown that exposure to HIV *in utero* has been associated with significant alterations to CD4 and CD8T-lymphocyte responses to vaccines in infants[91]. Some vaccines effectiveness has been found to be similar among HEU and HUU infants, for example, the rotavirus vaccine [92]. However, some studies have shown impaired humoral immune responses to anti-tetanus and Bacille de Calmette et Guérin (BCG) vaccines in HEU infants[93-96]. Lower measles antibody quality and quantity following vaccination have raised challenges for ascertaining the long-term protection of these children [97]. Some studies support the need for a

second dose of the vaccine and for a booster dose of the diphtheria and polio or measles vaccines to maintain the necessary antibody concentrations in HEU and HEI African children[98;99].BCG-specific T-lymphocyte proliferation and interferon gamma (IFN-γ) concentration are reduced in HEU infants showing a delay in immune system maturation [95]. BCG vaccine administered soon after birth, before *in utero* and peripartum HIV infection exclusion may result in severe disease[100;101]. Studies have demonstrated that the immunogenicity of BCG vaccination among South African HEU infants is not compromised when delayed until 8 weeks of age[102]. Interestingly, obesity-associated factors have also been shown to interfere with vaccine immunogenicity and efficacy[103].Thus, the effectiveness of childhood standard vaccines in SSA with a high prevalence of HIV infection remain poorly described.

Foetuses can also be exposed to maternal co-infections that can modify immune system development and influence the new-born's immune response to other infections. High maternal exposure to coinfections may contribute to high rates of infections among HEU [104].There is paucity of data regarding the immunogenetics of HIV/tuberculosis (TB)/Human papilloma virus co-infections and how their co-existence may modify or modulate host immune response, HIV /TB acquisition, disease progression or responses to treatment. An integrated approach in dealing with the conditions is also warranted in view of the hepatotoxicity due to anti-tuberculosis drugs[105] that limit treatment options for patients co-infected with HIV and TB.

1.4 Viruses: Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV)

Liver diseases due to chronic HBV and HCV infections are now emerging as the leading causes of morbidity and mortality among HIV infected persons in SSA [106;107]. Prognosis of such patients co-infected with HIV and HBV or HCV is not well known not to mention the immunological, virological and biochemical including elastographic responses.HIV positive pregnant women co-infected with HBV/HCV have been shown to have an increased risk of liver enzyme elevation [108]. A Nigerian study found an HBV prevalence of 4% among HIV positive pregnant women that was associated with induced abortion, blood transfusion and elevated baseline transaminase[109]. HCV and/ or HBV can be transmitted from mother to child during pregnancy and delivery [110;111]. A 5% HBV prevalence and a 3% HBV/HIV co-infection prevalence has been reported among South African pregnant women which was associated with history of induced abortion [112].The overall rate of HCV MTCT among HIV-negative mothers has been estimated at around 5% [113]. However, co-infection with HIV raises this figure to 19% [114]. An Indian study showed HCV prevalence in pregnancy of 3% that was associated with surgical procedures and blood transfusion [115]. In the same study pregnancy complications such as pregnancy-induced hypertension and antepartum hemorrhage were more common in HCV-positive mothers. In Zimbabwe such viral co-infection burdens and pregnancy

outcomes are currently not known. The foregoing points to the fact that HCV/HBV/MTCT remains poorly described. In addition the trends and patterns of CMV infections warrant further investigation.

1.4.1 Viruses: Cytomegalovirus (CMV)

CMV infection is the leading infectious cause of congenital hearing loss and neurodevelopmental disability in developed countries. CMV together with other herpes viruses such as HSV, Varicella zoster virus (VZV) also cause of significant ocular morbidity. Maternal plasma CMV DNA has been shown to be a high risk factor that may result in death in 2 years following delivery. The possible effects of transplacental viral infections include feotal loss.HIV-1 and CMV are important pathogens which are both transmitted via breastfeeding. CMV infection increases T lymphocyte activation and apoptosis contributing to the rapid disease progression in co-infected infants [116].Maternal CMV DNA can be measured in cervical secretions, breast milk or dried blood spots at birth [117]. Infection of the foetus may cause congenital defects, mental retardation including cardiac anomalies or cerebral palsy, growth faltering and cognitive impairment [118;119].The prevalence of congenital CMV infection among HEU new-borns is around 3% in the USA. Maternal CMV sero-prevalence ranged from 84% to 100% in developed countries with CMV birth prevalence varying from 1% to 6%[120;121]. Studies have shown lower socio-economic status to be associated with CMV sero-positivity[122]. When CMV infection occurs early during gestation, it disturbs the neurogenesis of the central nervous system (CNS) while late-onset CMV infection affects brain growth and the development of the white matter, leading to leukomalacia and cyst formation[123]. CMV is one of the most common infections known to humans and is characterized by a self-limiting infection in healthy individuals. However, the emergence of HIV/AIDS has made it the leading cause of birth defects and developmental delays. CMV and HIV co-infections have been shown to increase infant morbidity, mortality and AIDS progression. Zambian HEU infants have shown poor growth and increased morbidity [124]. The birth of congenital CMV has been shown to be high despite prenatal ARV prophylaxis and is associated with advanced maternal immunosuppression and breast milk CMV levels. Studies have shown that CMV may facilitate *in utero* transmission of HIV-1 by activation of cord blood mononuclear cells [125]. Determinants of vertical CMV transmission in our setting of high maternal HIV-1 infection remain poorly-defined. More so being a neglected but growing problem with the advent of AIDS, congenital CMV infection leads to a wide spectrum of symptoms. The challenge is that these symptoms are not always obvious at birth.

1.5 Other STIs and Role of Cervico-vaginal /gut Microbiota

Healthy vaginal microbiota is an important biological barrier to pathogenic microorganisms playing a pivotal role in diseases acquisition. Changes in the vaginal microbiota have been associated with several adverse pregnancy outcomes including premature birth and acquisition of HIV infection [126]. Buve *etal* have hypothesised that variations in per sex-act transmission probability of HIV may in part be attributed to differences in the composition and function of the vaginal microbiota between high

and low income countries[127]. Microbial composition is affected by hormonal changes, vaginal douching practices including use of sex enhancing herbs/botanicals. A healthy vaginal microbiota is one predominated by hydrogen peroxide-producing *Lactobacillus spp*., mainly *Lactobacillus crispatus, Lactobacillus jensenii*, and *Lactobacillus iners*. Vaginal microbiota has been defined as BV when it is predominated by *Gardnerella vaginalis* and *Atopobium vaginae* including other anaerobic bacterial species. BV is associated with a distinct vaginal discharge syndrome, poor pregnancy outcomes, pelvic inflammatory disease, post-operative wound infections and endometritis after elective abortions [128;129]. Recent data have linked specific vaginal microbes and urogenital infection with preterm birth [130]. Additionally, BV predisposes women to infection by HIV as well as other STIs[131;132]. The aetiology of BV is unclear, though it is believed to involve loss of vaginal hydrogen peroxide-producing Lactobacilli and acquisition of complex bacterial communities that include fastidious BV-associated bacteria [133]. Cervico-vaginal microbiota dominated *Lactobacillus crispatus* and to a lesser extent *L. iners* has been associated with a lower prevalence of HIV/STIs and a lower likelihood of genital HIV-1 RNA shedding [134].Studies have shown that disturbances of the vaginal microbiome especially the presence of BV, increases the risk of acquisition of HIV infection more so, with use of Depo-Provera injectable contraception (DPIC)[135]. Interestingly DPIC use has also been associated with increased HSV-2 prevalence [136;137]. Contrary to the foregoing observations, some studies' findings suggest that hormonal contraception is associated with lower rates of BV [138].*Gardnerella vaginalis* has been significantly enriched in cases of HIV antepartum transmission compared with non-transmission [139]. A better understanding of the composition of the microbiota in healthy and diseased states is important in identifying the role of newly identified microbes. Vaginal microbiota may also play a role in mediating susceptibility to *Trichomonas vaginalis* (TV) [140]. If this relationship is causal, interventions that improve the vaginal microbiota could contribute to reductions in TV incidence. Maternal chlamydial infection, gonorrhoea, genital warts and syphilis are also associated with MTCT [141;142]. Chlamydia trachomatis, which is asymptomatic in most women, causes significant adverse effects for pregnant women and neonates. Studies have observed an unexplained high HIV-1 incidence among pregnant women who would have been sero-negative at the first antenatal visit but sero-convert later during antenatal and post-partum periods [143;144] and this may have serious consequences for both the mother and infant. Hence, screening and early treatment of maternal infections during pregnancy may reduce paediatric HIV infections. However, there is need to re-visit the current protocol of screening for HIV only once during pregnancy as this may miss women who sero-convert during pregnancy. The incidence of HIV and vertical transmission rates during the breastfeeding period remain poorly described in most resource poor settings. Different studies suggest that some bacteria present in the maternal gut could reach the mammary gland during late pregnancy and lactation through a mechanism involving gut monocytes [310]. Mammary dysbiosis, microbial imbalances may lead to mastitis, a condition that represents the first medical cause for

undesired weaning [145;146]. Thus, modulation of maternal gut microbiota during pregnancy could also have a direct effect on infant health.

1.5.1 Parasites

Helminthic and HIV-1 co-infections are common in SSA [6]. Thus, this convergent distribution of the HIV and helminths' infections is suggestive of a possible biologically plausible observation that persistent infection with helminths may exacerbates the HIV epidemic in the region [147;148].Helminths induced chronic immune-activation, altered immune cell distribution, strong T-helper-2 (Th2) bias and enhanced HIV replication facilitate faster progression to AIDS [149]. It is also important to note that activation of Th2 bias is critical for a successful pregnancy which predispose the pregnant women to infections. *Schistosoma hematobium* which affects almost 200 million people in SSA has been shown to act as a co-factor for HIV transmission where parasite lesions create open portals for HIV and inflammation in the genital area making transmission more efficient. This increases the risk of HIV acquisition three-fold [150;151]. Helminthiasis is a significant burden in pregnancy and it is also associated with anaemia [152].In SSA hookworms, malarial parasites and HIV are important factors implicated in anaemia of pregnancy [153]. Anaemia in pregnancy has been defined as haemoglobin<11 g/dl [154]. Infections and inflammation have been of great significance in the aetiology of anaemia among pregnant Ugandan women[155]. A Nigerian study has demonstrated that intestinal helminth infection has a negative correlation with haemoglobin and packed cell volume, contributing to anaemia [156]. In Peru *T. trichiura* infection was found to be a significant risk factor for anaemia in pregnant women [157].Maternal infections with parasites might affect the development of feotal immunity and susceptibility to post-natal infections independent of *in-utero* transmission of the pathogens [158;159].There is evidence that this may impact on the long-term responses to helminth and non-helminth antigens and to allergens [160;161].Prevalence of intestinal parasitic infections such as*Trichuris trichiura*, *Entamoeba histolytica/dispar/moshkovskii*, *Ascaris lumbricoides* and *Giardia duodenalis*, *Entamoeba spp., E. nana* and *Blastocystis hominis*remain poorly described among pregnant women in our setting. Parasitic intestinal infection with helminths and/or protozoa can lead to significant morbidity and mortality if not recognized and treated appropriately. There is also paucity of data regarding parasite(s) co-infection(s) with HIV and how their co-existence modify or modulate, host immune responses, HIV acquisition, disease progression and/or response to treatment in the background of a SSA population infested with a myriad of parasites. In addition to infections, food accessibility also contributes to the high rate of anaemia during pregnancy in our populations. The most common causes of anaemia are poor nutrition, deficiencies of iron and other micronutrients, malaria, hookworm disease, and schistosomiasis; HIV infection and haemoglobinopathies are additional factors. A Tanzanian study showed parasitic infections, vitamin D deficiency, low CD4 T-cell count and high erythrocyte sedimentation rate as the main predictors of iron deficiency in pregnancy and the postpartum period [162]. Severe anaemia is associated with increased incidence of

pre-term labour pre-eclampsia. Hence the need for nutritional education among pregnant women is critical.

1.6 Maternal nutritional Status

Studies have shown that 30% of SSA population is malnourished [8]. HIV infection further compromises the nutritional status of infected individuals. Malnutrition in turn worsens the effects of the disease by weakening the immune system consequently, hastening disease progression and early mortality [163;164]. Pregnant women in SSA are at risk of poor nutritional status and adverse pregnancy outcomes as a result of poverty, food insecurity, sub-optimal healthcare facilities, frequent infections and pregnancies. Subsequently, obstetric complications, including hypertension, anaemia, neural tube defects, night-blindness, and low birth weight, maternal and perinatal mortality are common [165].

Components leading to healthy pregnancy outcome include healthy pre-pregnancy weight, appropriate weight gain, physical activity during pregnancy, consumption of a wide variety of foods, appropriate vitamin and mineral supplementation, avoidance of alcohol, other harmful substances and safe food handling [166]. Nutritional assessment needs to encompass changes in anthropometric, biochemical and clinical indicators throughout pregnancy [166]. Due to some challenges associated with these measurements plasma insulin growth factor-1 (IGF-1) offers a potentially; simple but accurate single measurement of nutritional status alongside with total protein, creatinine, urea nitrogen, albumin, complement components C3/C4 and α-1 anti-trypsin[167]. Maternal anthropometry, haemoglobin concentration, serum iron, total iron binding capacity (TIBC) and serum albumin were assessed and the incidence of preterm labour was significantly higher in mothers with severe anaemia[168]. Anaemia is to be defined as Hb below 11.0 g dl^{-1} and severe anaemia as Hb below 7.0g dl^{-1} (WHO, 1991).Poor maternal bodyweight and low body mass index (BMI) have been associated with low birth weight in HIV-infected women [169;170]. Birth weight is largely determined by maternal factors other than hyperglycaemia, with the most significant influences being gestational age at delivery, maternal pre-pregnancy body mass index (BMI), maternal height, and pregnancy weight gain, the presence of hypertension, cigarette smoking and alcohol use. Anthropometric assessments of mid-upper arm circumference (MUAC) have also been shown to be an efficient, cost-effective screening tool for low birth weight in both HIV-infected and uninfected women [171]. Low MUAC has been associated with poor maternal health outcomes, including anaemia. [172]. Taking multivitamins during pregnancy may be an inexpensive and effective strategy to improve the health of the mother and baby. MUAC measurements of <11.0 cm among children has been associated significantly elevated risk of mortality[173]. An improvement in prenatal mean haemoglobin concentration linearly increased birth weight [174]. On the other hand maternal obesity is associated with increased risk of gestational hypertension, preeclampsia, gestational diabetes and feotal macrosomia, high birth weight (>4,000 grams).Accumulating evidence links vitamin D deficiency with abnormal glucose metabolism and

epidemiological studies have shown that women who develop gestational diabetes mellitus (GDM) are more likely to be vitamin D deficient [175].

1.7 Vitamin D status, GDM and Pregnancy

Vitamin D insufficiency has now reached epidemic proportions, even in healthy individuals living in the tropics [176]. Use of HAART has been a possible cause of hypovitaminosis D [176]. Recent evidence links not only low maternal vitamin D status with increased risk of severe preeclampsia or small-for-gestational age but also schizophrenia in the offspring. Other Foetal risks include intrauterine growth retardation, neonatal hypocalcemic seizures, impaired postnatal growth, rickets in infancy and cardiomyopathy with increased future risks of asthma and type 1 diabetes. Polymorphisms in the vitamin D receptor (VDR) gene may contribute to vitamin D-related disparities in feotal growth. Evidence from recent studies suggests an early prenatal influence of maternal vitamin D status on feotal skeletal development. [177]. Vitamin D status has also been shown to have a significant inverse association with TB incidence [178;179]. Vitamin D exerts its action through VDR and the gene polymorphisms associated with susceptibility or resistance to TB [180]. Vitamin D, selenium and zinc play fundamental roles in determining susceptibility to infections including accelerated HIV disease progression [181;182]. Currently little is known regarding the effects of HIV/AIDS and its treatment on the metabolism of vitamin D and pregnant income and susceptibility to infections. Being a potent immunomodulator, vitamin D's impact on morbidity and mortality among infants remains poorly described. There is a particular need for maternal education on healthy diet and for interventions which aim to limit over consumption of excess calories or insufficient nutrition during feotal vulnerable developmental periods appears to result in a lifelong predisposition to obesity and adult disease, such as cardiac diseases and type 2 diabetes. Maternal vitamin D levels exhibit a significant negative relationship with glycosylated haemoglobin (HbA1c) levels, supporting a potential role for this vitamin in maintaining glycaemic control [183]. Pregnancy is also a risk factor for impaired glucose metabolism. GDM is defined as glucose intolerance of variable degree with onset or first recognition during pregnancy. Carrying a male fetus has been associated with poorer maternal beta cell function and an increased risk of GDM [184]. Studies have given conflicting results regarding the contribution of PIs to impaired glucose tolerance and GDM in pregnant HIV-infected women. Betatrophin, also known as TD26/RIFL/lipasin/ANGPTL8/C19/f80, is a novel protein predominantly expressed in human liver. Betatrophin plays a significant role in the regulation of lipid metabolism and glucose homeostasis, positively correlating with weight gain during pregnancy, systolic blood pressure, very low-density lipoprotein levels , fasting insulin level and homeostatic model assessment insulin resistance [185;186].

GDM is associated with many adverse neonatal and maternal outcomes miscarriage, infants being large for gestational age (LGA) requiring a Caesarean delivery. A growing body of literature supports

a relationship between intrauterine exposure to maternal diabetes and risk of a metabolic syndrome childhood obesity, hypertension, dyslipidaemia, and glucose intolerance later in life. Glycosylated haemoglobin values (HbA1C) levels are associated with increased risk for malformation. HbA1C is a reliable measure of chronic glycemic control without the need for a fasting or timed sample. Infants born to mothers with diabetes have been shown to exhibit higher levels of cardiovascular risk biomarkers for endothelial damage and inflammation, higher leptin levels, BMI, waist circumference and systolic blood pressure but decreased adiponectin levels. Maternal GDM, low socioeconomic status and preterm infants have been associated with an increased risk of neurocognitive development especially the attention-deficit/hyperactivity disorder (ADHD) and compromised neurobehavioral functioning [187]. GDM birth anomalies also involve cardiovascular diseases (CVD). CVD complicates pregnancies with congenital heart disease being the most common pre-existing condition and hypertension as the most common acquired condition.

1.8 Hypertensive Disorders

Hypertensive disorders during pregnancy represent the major cause of maternal morbidity with chronic hypertension occurring in up to 5% of pregnant womenworldwide[188]. Mild to moderate hypertension in pregnancy is defined as systolic blood pressure of 140-159 mmHg or diastolic blood pressure of 90-109 mmHg. Hypertension during pregnancy is classified into three main categories: chronic hypertension, gestational hypertension, and preeclampsia (high blood pressure and proteinuria) with or without pre-existing hypertension. The occurrence of postpartum depression and anxiety is higher in pre-eclamptic women [188]. Chronic hypertension is defined as blood pressure >140/90 mm Hg that either precedes pregnancy or develops before 20 weeks gestation usually also persists beyond 42 days postpartum. Chronic hypertension is significantly higher among obese women [189].Hypertension during pregnancy is more likely in women with high baseline systolic blood pressure and those with higher MUAC [190]. Diet has been suggested to play a role in pre-eclampsia.Maternal serum concentration of the soluble receptor-1 of tumor necrosis factor-alpha (TNF-R1) a predictor of development of pre-eclampsia. Oxidative stress and endothelial cell dysfunction of the placenta have been implicated in the development of hypertension during pregnancy and vitamin intake can reduce oxidative stress and improve endothelial function [191;192].Endothelial dysfunction biomarkers include VonWillebrand factor (vWF), platelet derived micro particles (PMPs), and endothelin-1 that are easily analysed by ELISA. Other serum/plasma biomarkers associated with increased risk of hypertensive disorders of pregnancy that correlate with adverse pregnancy outcomes include soluble corin, vascular endothelial growth factor, matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 (TIMP-1). Haemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome is a risk factor for preeclampsia. HELLP is a life-threatening obstetric complication usually beginning during the third trimester. Itis characterized by

the presence of hypertension disorder plus microangiopathic haemolysis, elevated liver enzymes and low platelet count, less than 100000. Serum aspartate aminotransferase (AST) and lactic dehydrogenase lactic dehydrogenase levels of greater than 70 U/L, and 600 U/L, respectively. HELLP syndrome not only increases maternal morbidity and mortality but also that of the fetus. Thrombocytopenia is defined as a platelet count of less than 150 x 10^3 µl. It is commonly diagnosed and has attracted interest from the researchers in pregnant women especially among hypertensive pregnant women. Gestational thrombocytopenia defined as a platelet count of less than 150 x 10^3 µl is recognized as a major cause of thrombocytopenia particularly in hypertensive pregnant women during the third trimester. Hypertension during pregnancy increases feotal growth retardation, preterm deliveries, still births and perinatal deaths, yet its causes remain unclear [190]. Hypertension and pregnancy outcomes remain poorly described in our setting more so comparing and contrasting between HIV positive and negative pregnant women. HIV-infected women using HAART before conception have been shown to be at increased risk of developing preeclampsia/eclampsia during pregnancy among Latin American women[193]. Contradicting evidence exists concerning the relationship between HIV infection, HAART use and hypertensive disorders in pregnancy. In addition there are indications of hypertensive disorders of pregnancy being associated with high infant blood pressure. Moreover, the trends blood pressure changes across childhood remain poorly described.A history of pre-eclampsia or pregnancy induced hypertension is an important prognostic factor for micro- and macro-vascular complications later in life. CVD factors such as obesity, diabetes, and hypertension hyperlipidemia, and thrombophilia which are associated with increased risk of spontaneous abortion, preterm labour or premature rupture of membranes, and acute arterial or venous thromboses during pregnancy. Of course just like in any condition, host genetic factors also play a significant role.

1.9 Host genetics

A myriad of host and viral factors act in tandem to cause vertical transmission of HIV. Understanding the mechanisms, host and viral factors associated with vertical transmission of HIV will help to identify appropriate interventions and suitable antiretroviral chemoprophylaxis regimens to reduce or eliminate it. Increasing data support host genetic factors as important determinants of HIV-1 susceptibility, vertical transmission and disease progression with the CC chemokine CCL3 and HLA taking the centre stage [194-198]. Relative resistance to HIV infection has been associated with genetic polymorphisms. The killer cell immunoglobulin-like receptors (KIR) play a fundamental role in the innate immune system. KIR genes are incredibly polymorphic both in the number of genes an individual carries and in the number of alleles identified [199]. However, their interactions with HLA molecules, leading to the modulation of activity in NK cells, mainly related to killing pathogen-infected cells remain poorly described. Typing of HLA loci B, Cw, and killer cell KIR molecules has

shown that the reciprocal equilibrium between inhibitory and activatory NK receptors and their ligands favour NK activation among HEU adults [199]. A trend toward increased early HIV-1 acquisition among Kenyan infants presenting in HLA A*29 and increased late HIV-1 acquisition via breast milk for both Cw*07 and Cw*08. HLA B*18 may protect breast-feeding infants against both early and late HIV-1 acquisition, a finding that could have implications for the design and monitoring of HIV-1 vaccines targeting cellular immune responses against HIV-1[200]. Placental HLA-G1 expression has been shown to be up-regulated 4 times more in placentas of HIV-1 infected mothers with infected babies compared to uninfected babies [201]. Single nucleotide polymorphisms and copy number variations have been documented for defensins chemokines and RFs which are natural inhibitors of HIV infection [202-205]. These results demonstrate a significant relationship between genetic variants of beta-defensin-1 gene, RFs genes, viral load, and vertical transmission of HIV-1, thus supporting a critical role of innate immunity in paediatric HIV-1 infection. However, such studies are rare in the region.

1.10 HIV Genetic Diversity

HIV-1 genetic diversity in the HEI may also affect viral transmissibility, pathogenicity and responses to antiretroviral therapy. Variable region 3 (V3) of the envelope (env) gene for CCR5 and CXCR4 determination is a key determinant of vertical transmission [206;207]. HIV-1 env gp (glycoprotein)120 variable regions exhibit an unusual pattern with encoded amino acids (asparagine, serine and threonine) that leads to the creation of new N-linked glycosylation sites, which helps the virus to escape from the immune pressure [208;209]. Potential N glycosylation sites (PNGs) glycans and amino acid length polymorphism have been shown to play essential roles in disease progression with changes potentially affecting the capacity of the virus to replicate [210;211]. Studies have shown that at birth all HIV infected infants' *env* amino acid sequences are initially of the same length as the maternal ones with amino acid sequence length polymorphism observed from six months postpartum [212]. However, other studies have observed no clear trends in changes in neither the env PNGs nor median amino acid lengths with disease progression [213]. Genetic analyses revealed that rapid paediatric progressors receive and maintain a genetically homogeneous viral population throughout the disease course whilst slow progressors' exhibit low levels of variation [212] initially, but attain higher levels of diversity over time a phenomenon important in HIV transmission linkages. Phylogenetic and phylodynamic studies have identified clusters of new infections occurring along geographic routes and in different groups[214-216]. Late diagnosis of HIV remains prevalent and represents missed opportunities for early treatment. The combination of phylogenetic analyses of HIV sequences with patients' demographic data allows greater understanding of local HIV transmission and necessary knowledge for designing prevention strategies. [217]. The host immune response to HIV in early life is complex. Understanding the relationships between acquired immune responses to HIV, innate immune responses that include chemokines and their receptors, and host genetic influences that

affect these immune processes in HIV-exposed infants will help identify critical components of protective immunity. This helps in the identification of infants at risk of acquiring HIV-1 infection and/or at risk of more rapid disease progression. Results from such studies will vastly improve our knowledge of HIV-host interactions. Further elucidation of these processes will contribute to the rational design of vaccines and the development of novel preventative and therapeutic anti-HIV strategies for children exposed to HIV. Having discussed all the foregoing factors shortcomings associated with maternal psycho-social issues and being born in an HIV affected household may also play a pivotal role.

1.11 Maternal Psycho-Social Factors

Undiagnosed maternal HIV infection prior to conception, unplanned pregnancies, delays in accessing antenatal care, non-disclosure of the pregnant women's HIV status to her partner and low levels of education are some of the most significant maternal risk factors associated with vertical transmission and or infant death [218-220]. Directly disclosing a positive HIV sero-status to family members can have psychological and physiological health benefits [221]**.** Hence disclosure of HIV sero-status by women to their sexual partners is critical for the success of the PMTCT programme as an integrated service in antenatal care. Pregnant HIV-negative women and their unborn babies remained at risk of HIV infection owing to the resistance of their partners to go for HIV testing [222]. Poverty, lack of support, HIV, witchcraft and child illness have been identified as causes of worry in the perinatal period [223;224]. Adverse maternal health and feotal outcomes have been shown to be more prevalent in socio-economically-disadvantaged women [225]. Depression and its impact on maternal and child health has important implications with depressed mood being associated with alcohol use, less breastfeeding and low birth weight [226;227]. Smoking increases the risk of morbidity and mortality and is particularly harmful to pregnant mothers. Chronic maternal marijuana use has been associated with early sex in offspring [228].Studies have shown that alcohol use and HIV sero-status of partner predict high-risk sexual behaviour among patients receiving antiretroviral therapy [229;230].Alcohol-exposed pregnancy is a significant public health problem as alcohol consumption is associated with feotal abnormalities and long-term cognitive problems depending on the amount consumed, drinking pattern, and time of gestation. Feotal alcohol exposure is a leading cause of developmental disabilities and mental retardation [231]. Prenatal cocaine exposure has been linked to child behaviour problems[228]. Screening for alcohol use disorders and substance uses are important to reduce alcohol consumption during pregnancy and associated problems in infants. Intimate partner violence defined as actual or threatened physical, sexual, psychological and emotional abuse by current or former partners is a global public health concern [232;233].Hence there is need to manage health related stress. There is significant current interest in the degree to which prenatal exposures to stress influence infant outcomes. Such maternal psycho-socio and demographic factors also play a role in infant mortality and necessary interventions for common perinatal mental disorders may be required.

There is paucity of information regarding the impact of environmental factors related to being born in an HIV-affected household especially of low social economic status and how this impacts on child care practices. Limited data exists which explores the possible associations between the mothers' psychosocial and economic wellbeing during the prenatal and postnatal period and increased morbidity and mortality among HEU infants in the first year of life. It is against this background that in addition to investigating the abnormal immunity of HEU and the associated maternal genetic and environmental factors. For the holistic and integrative research, numerous other related sub-studies addressing other coinfections, NCD and maternal psycho social aspects will be developed for a full assessment of the common comorbidities. Each of these comorbidities in isolation and/or combination may be assigned characteristic immune biomarkers assayed from one blood plasma sample and such a development may lead to powerful tool to optimize and individualize treatment and care. Other sub studies linked to this study also seeking to determine the maternal psychological wellbeing such as stress and depression including other important co-infections such as Tb, herpes simplex virus (HSV), human papilloma virus (HPV), bacterial vaginosis, (BV) infections gut and vaginal microbiomes associated with high morbidity and mortality among HEU infants will be developed. In general, research questions will fall into seven thematic areas namely, co-infections, non-communicable diseases, nutrition, immunogenetics, pharmacokinetics/dynamics/genomics, psychosocial and child growth and development. See **Appendix 6.1**.

1.12 Justification

Viral, host immunogenetics and environmental factors associated with HIV non-acquisition and/or slow HIV disease progression among paediatric patients remain poorly described. The little that is currently known comes from HIV-1 subtype B studies. The role of innate immunity in protection against HIV infection and disease progression may not be over-emphasised among paediatric HIV infected individuals whose adaptive immunity may not have fully developed. Since HIV-infected children progress more rapidly than adults and have fewer background cofactors such as drug use and co-infections and the effects of host genetic factors on HIV-1 disease may be more clearly identified. Most current researches are done in "silos" (focused on single, isolated aspects of immune defense) downplaying systemic and interconnected nature of the arms of the immune system. Yet in real life situation disease acquisition, progression and even response to therapy are multi factorial with environmental and immunogenetic factors playing critical roles. There is need for a cohort to clearly discern the causes or/ and consequences of viral immune profiles which is impossible to determine with cross sectional studies. HEU and non-progressing HEI children offer hope that natural and effective HIV control is possible and can provide important insight to the development of the next generation of HIV/AIDS vaccines and immune-based therapies. There is need for a comprehensive,

integrative and a holistic approach to predict diseases outcomes.HIV infection and co-infections, immune profiles, nutritional status that may influence transmission, disease progression and immune development and these will be assessed from baseline.

2.0 Research Questions

1. Is the increased morbidity and mortality of HEU infants associated with increased exposure to maternal HIV load, other maternal co-infections (HBV, HCV, CMV, intestinal parasites) and how do these affect pregnant outcome and child growth and development?

2. Is the incidence of comorbidities, anaemia, diabetes, malnutrition and hypertensive disorders similar between HIV positive and HIV negative pregnant women and how do these affect pregnant outcome and child growth and development?

3. Is there a difference in biomarkers of inflammation and immune activation among mothers (HIV+/HIV-) and their (HUU/HEU/HEI) infants with different comorbidities?

2.1 Main Hypotheses

1. There is no difference in the prevalence and incidence of co-morbidities (anaemia, malnutrition, hypertension, diabetes, HCV, HBV, CMV) and immune profile of HIV+ and HIV- pregnant women and the impact of these comorbidities on pregnancy outcome and infant growth and development within 24 months post-delivery is similar.
2. There is no difference in biomarkers of inflammation and immune activation profiles among HIV+ and HIV- mothers with different comorbidities and their HEI, HUU HEU infants.

3.0 Project Goals:

1. The main goal of this study is to characterise co-morbidities, immunogenetics of pregnant women and their infants in Harare and ascertain their roles in disease acquisition and transmission
2. Contribute to the knowledge base on the role of HIV and human immunogenetics on acquisition and disease progression and co-infections in children.

3.1 Main Objective

This study aims to establish a birth cohort to investigate the role of HIV and comorbidities on vertical HIV transmission, immune development and disease progression in children 0-2 years, HEU, HEI and HUU as the control group.

Objectives

A. <u>All</u> Mothers Regardless of HIV Status

1. To determine the prevalence and incidence of coinfections (intestinal parasitic infections, HBV, HCV, CMV) malnutrition and non-communicable diseases (NCDs) (gestational diabetes mellitus, hypertensive disorders and CVD) risk factors over 24 months and relate all these comorbidities to:
    a. Pregnancy outcome, infant growth, development, morbidity and mortality
    b. Biomarkers of inflammation and immune activations

Infants

2. To determine vertically transmitted infections and incidence non-communicable diseases among infants.
3. To determine clinical and immunological profiles of HEI, HEU and HUU infants.

B. HIV+ Mothers: <u>Chronic HIV infections</u>

1. To determine vertical transmission rates of mothers with acute and chronic HIV infection on option B+
2. Determine any association(s) between different option B+ drug regimens, adverse pregnancy outcome, viral load suppression and CD4/CD8 counts on mothers and their infants

C. HIV+ Mothers; <u>Acute HIV infections,</u>

1. To determine the incidence of HIV in the postpartum period over the 2 years
2. To determine the viral set points, HIV suppression trends and biomarkers of immune activation
3. To determine the HIV and host genetic diversity and possible transmission linkages

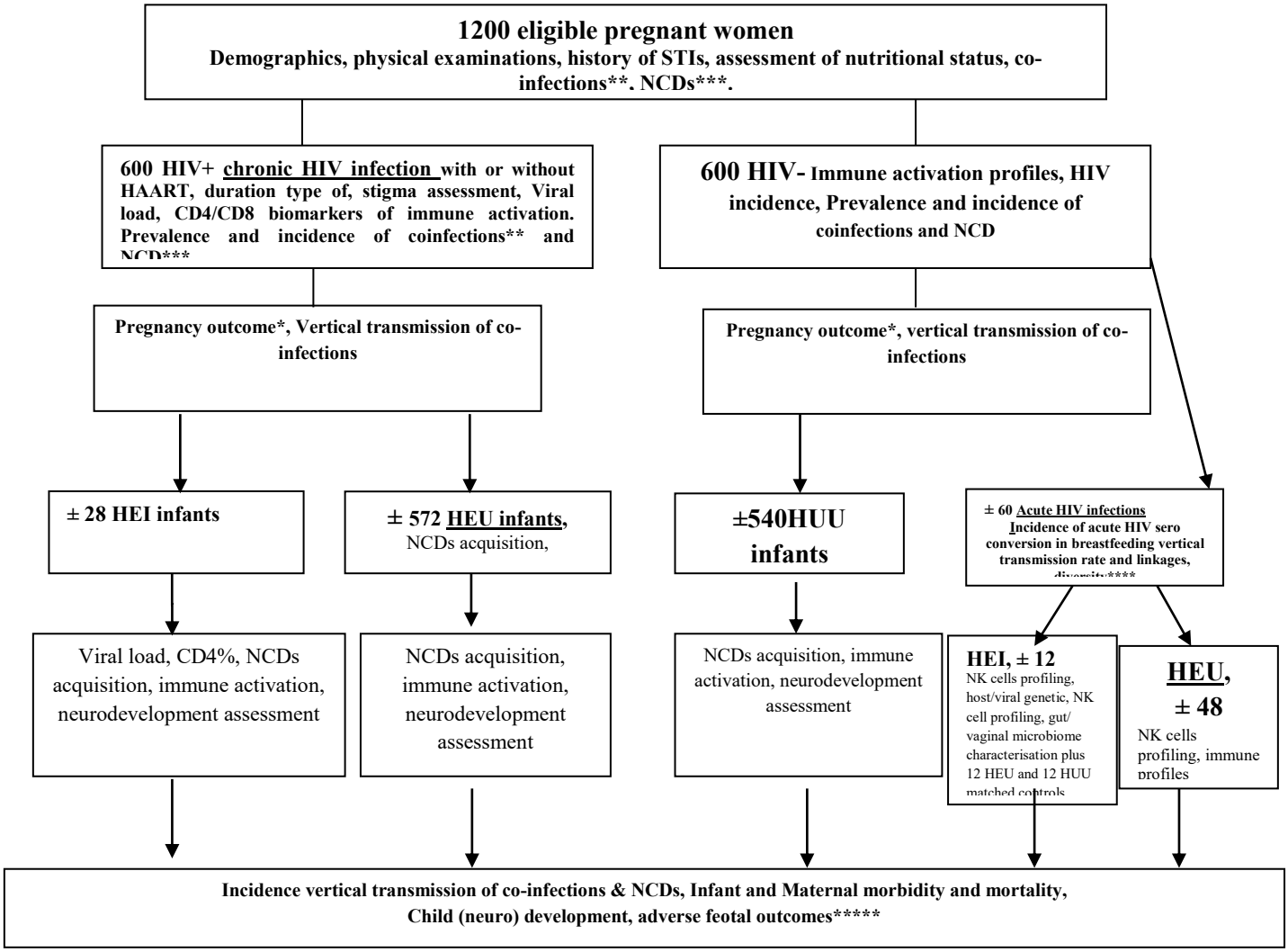
Infants

To characterise natural killer cell phenotype and function longitudinally from cord blood at birth up to 2 years and relate to infant HIV acquisition , morbidity and mortality

4.0 Methodology

4.1 Study Design

A prospective cohort study of 1200 pregnant women (600 HIV positive and 600 HIV negative).
Figure 1 below is the study flow diagram.



Viral load re-testing with be done for all Unsuppressed HIV load every 3 months

Figure 1: Study Flow Diagram

Key

*Pregnancy outcome include spontaneous abortions, birth defects, multiple births, low birth weights preterm infants, birth with prolonged hospitalization, birth requiring neonatal intensive care, maternal, perinatal and infant deaths.

**Coinfections include HBV, HCV, CMV and intestinal parasites

***NCD, non-communicable diseases (hypertensive disorders, malnutrition, diabetes)

**** For acute HIV infections ONLY

*****Adverse feotal outcomes include preterm birth (<37 weeks of gestation) low Apgar score<7, low birth weight, Fetal macrosomia, birth weight of more than 4,000 grams, birth defects, stillbirth (death of a fetus weighing ≥500g or ≥22 weeks gestation if weight is unavailable), early neonatal death (death between 0-6 days of life), neonatal death (death within the first 28 days of life), neonatal jaundice, infant death, intensive care unit admission, hospital admissions including documented local clinic treatments

4.2 Study Sites
City of Harare poly-clinics, that offer maternal and child health services.

4.3 Study Participants
ANC mothers registering at the ante-natal clinicsat 28-36 weeks gestation will be enrolled. Upon delivery, babies will be automatically recruited into the study and be followed up as mother-baby pairs for two years.

4.4 Inclusion Criteria
- Pregnant woman 18 years of age and above at 28-36 weeks gestation. The date of the last menstrual period (LMP) will be used to estimate gestational age.
- Planning to deliver at the study sites
- Able to give informed consent
    - Willing to be followed together with her baby from delivery up to two years
    - Willing to provide the required specimens

4.5 Exclusion Criteria
Women with severe obstetric complications and serious psychiatric disorders will be excluded.

4.6 Selection of the Participants
Potential participants will be identified during routine ante-natal care (ANC) visits at the City of Harare Polyclinics. The potential participants will be briefed about the study and those who verbally agree to participate in the study will be given the participant information sheet to allow voluntary participation and informed decision. All mothers with a positive HIV status will be encouraged to enrol in the study. HIV negative mothers will be recruited systematically.

4.7 Sampling Procedure
The whole population of HIV+ mothers and systematic sampling of HIV- mothers will done. For very HIV positive pregnant mother, the 10^{th} HIV negative mother will be recruited, taking cognisance of the 12% HIV prevalence in this population.

4.8 Mothers' Procedures
- A questionnaire will be administered at enrolment from which information regarding their socio-demographics, sexual behaviour and reproductive health issues including obstetrical records will be obtained. Antiretroviral use and regimen will be recorded for all the HIV+ mothers. Assessment of nutritional status/BMI will be done using standard anthropometric indices. A physical examination will be done at every visit by the study clinician. Mothers will be enrolled at 28-36 weeks gestation and followed up at delivery, 10 days and 6, 10 , 14, 24, 36, 48, 72 and 96 weeks postpartum thus totaling 11 visits during the study period. Data can still be collected in cases of missed visits provided the participant turns up within the seven day window period of the scheduled visits. The participant may be asked to discontinue if she consecutively fails to turn up for at least two successive scheduled study visits without any reason(s).

Biological specimens blood, cord blood, amniotic fluid, breast milk urine and stool will be collected at different visit with the respective quantities and purposes as shown in the **Table** 1 below.

Table 1: Types and total Volumes of Maternal Specimen Required

		Total Volume of Specimen Required					
Visit	**HIV Status**	**Blood* (mL)**	**Stool** (grams)**	**Breast Milk* (mL)**	**Urine *** (mL)**	**Amniotic Fluid (mL)**	**Cord Blood (mL)**
1 *In Pregnancy*	HIV+	**30**	**20**	**-**	**100**	**50**	
	HIV-	**25**	**20**		**100**	**50**	
2 *Delivery*	HIV+	**10**	**20**		**100**	**50**	**50**
	HIV-	**5**	**20**		**100**	**50**	**50**
3 ***7-10** days pp*	HIV+	-	If delivery visit is missed some of the samples are collected.	**10**	**100**		
	HIV-	-		**10**	**100**		
4 ***6** Weeks/1½Months pp*	HIV+	**20**	**20**	**10**	**100**		
	HIV-	**10**	**20**	**10**	**100**	**50**	
5 ***10** Weeks /**2½** Months pp*	HIV+	**20**	**20**	**10**	**100**		
	HIV-	**10**	**20**	**10**	**100**		
6 ***14** Weeks, **3½**Months pp*	HIV+	**20**	**20**	**10**	**100**		
	HIV-	**10**	**20**	**10**	**100**		
7 ***24** Weeks/**6** Months pp*	HIV+	**20**	**20**	**10**	**100**		
	HIV-	**10**	**20**	**10**	**100**		
8 ***36** Weeks/**9** Months pp*	HIV+	**20**	**20**	**10**	**100**		
	HIV-	**10**	**20**	**10**	**100**		
9 *48 Weeks/1 year pp*	HIV+	**20**	**20**	**10**	**100**		
	HIV-	**10**	**20**	**10**	**100**		
10 *72Weeks /1$^{1/2}$ years*	HIV+	**20**	**20**	**10**	**100**		
	HIV-	**10**	**20**	**10**	**100**		
11 *96 Weeks/2 years*	HIV+	**20**	**20**	**10**	**100**		
	HIV-	**10**	**20**	**10**	**100**		

*Generally blood samples, amniotic fluid cord blood and breast milk will be tested for trend in immune profiles and coinfections.

**Stool samples will be tested for intestinal parasites

*** Urine for urinalysis

Table 2: Type, Volume, intervals of collection and Purpose of Maternal Specimens Required

Procedure /Test	**Specimen Required (volume)**	***1*** **Volume/gram**	***2*** **labour**	***3*** **7-10 Day pp**	***4*** **6 wks pp**	***5*** **10 wks pp**	***6*** **14 wks pp**	***7*** **24 wks pp**	***8*** **36 wks pp**	***9*** **48 wks pp**	***10*** **72 wks pp**	***11*** **96 wks pp**
CMV / HBV/ HCV	**serum**(1 mL)	✓ (										✓
HIV testing/all negatives	**Plasma** (1 mL)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
STI serology												
Parasites At least 3 specimens	**Stool**(10 g) Anal swab	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
FBC/Differentials/platelets	**Plasma** (5mL)	✓			✓		✓			✓		✓
Markers of immune activation, cytokines levels and trends	**Blood** (10mL)	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
	Cord blood (10mL)		✓									
	Amniotic fluid (10mL)		✓									
	Breast milk(10 mL)			✓	✓	✓	✓	✓	✓	✓	✓	✓
Urine	**urinalysis**	✓			✓			✓				✓
Biochemistry Panel	**Plasma (5 mL)**	✓			✓		✓			✓		✓
	Sugar (3 mL)	✓			✓		✓			✓		✓
Restriction factors	**Cord blood**		✓									
	Breast milk			✓	✓	✓	✓	✓	✓	✓	✓	✓
FOR HIV POSITIVES ONLY												
T cell profile	**PBMCs**	✓						✓	✓	✓	✓	✓
HIV RNA load*	**Whole blood**	✓*										
HLA/KIR host genetics												
HIV genetic diversity for Transmission linkages**	**Whole blood**	✓										√

***Re-testing with be done for all Unsuppressed HIV load every 3 months**
****** **for all acute HIV infections**

4.9 Infants Procedures

From delivery infections, immunological profiles, co-infections patterns and trends, mortality will be determined and , feeding practices development , responses to standard vaccines, general health will be assessed. Types of specimen required and the test to be assayed are shown in **Tables 3 and 4.**

Table 3: Types and total Volumes of Infant Specimen Required

		Specimen Required				
Visits	**Baby Status**	**Blood***	**Stool****	**Urine*****	**PBMCs********	
1	HIV+					
	HIV-					
2	HIV+					
	HIV-					
3	HIV+					
	HIV-					
4	HIV+					
	HIV-					
5	HIV+					
	HIV-					
6	HIV+					
	HIV-					
7	HIV+					
	HIV-					
8	HIV+					
	HIV-					
9	HIV+					
	HIV-					
10	HIV+					
	HIV-					
11	HIV+					
	HIV-					

*Generally blood samples will be tested for trend in immune profiles and coinfections.
**Stool samples will be tested for intestinal parasites
*** Urine for urinalysis
****PBMC of infants born from mothers with acute HIV infection for NK cell phenotyping

Table 4: Type, Volume, intervals of collection and Purpose of Infant Specimens Required

Procedure /Test	Specimen Required (volume)	*1* Delivery	*2* 7-10 Day pp	*3* 6 wks pp	*4* 10 wks pp	*5* 14 wks pp	*6* 24 wks pp	*7* 36 wks pp	*8* 48 wks pp	*9* 72 wks pp	*10* 96 wks pp
CMV / HBV/ HCV	Serum (1 mL)	✓	✓	✓							✓
HIV testing/all exposed infants	**Plasma (2 mL)**	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Parasites	**Stool (5 grams)**	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
FBC/Differentials		✓		✓		✓			✓		✓
Markers of immune activation, cytokines levels and trends	**Blood,**	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urine dip stick	**Urine (5 ml)**		✓						✓		✓
Biochemistry	**ALT, AST, albumin,**		✓	✓		✓			✓		✓
	Sugar		✓	✓		✓			✓		✓
PMBC for infants born from Mothers with acute HIV infections											
HEI Infants only on First HIV positive sample, thereafter every 3 months											
T cell profile	**Whole blood**		✓	✓	✓	✓	✓	✓	✓	✓	✓
HIV RNA load*	**Whole blood**										
HIV genetic diversity for Transmission linkages**	**Whole blood**										√

All the tests will be done locally except for transmission linkages assay and NK cell phenotyping that will be done in South Africa.

4.10 Laboratory Methods

4.10.1 Sample Preparation and Storage

On the day of collection, clotted samples will be centrifuged for 5 minutes at 3000rpm. The supernatant will then be removed and placed in appropriately labelled serum cryo-tubes. All samples will be stored in the Department of Medical Laboratory Science at -70°C to -80°C for subsequent analysis.Full blood count, renal function, thyroid function, lipid profile, glucose levels, bone profile, and liver profile will be done at baseline, 6 weeks and thereafter every 6 months

4.10.2 Determination of Full Blood Count (FBC)

FBC report of white blood counts (WBC) and differentialswill give an idea about the presence of anaemia, infection, inflammation or blood disorders. Peripheral blood for FBC analysis will be sent to the laboratory in an EDTA tube and analysed using a Mindray Haematology Analyser, BC3600within six hours of collection. Results will be grouped into white and red cell parameters including platelets

4.10.2.1White Blood Cells Parameters

The FBC provides a total white cell count (WCC x 10^9)/white blood cell count (WBC) and an automated differential WCC which typically includes information the absolute and % of each type of white cell about neutrophils, lymphocytes, monocytes, eosinophils and basophils.

4.10.2.2Red Blood Cells Parameters

Iron deficiency anaemia will determined according to WHO guidelines where haemoglobin (Hb) level below 11gm/dl in pregnant women constitutes anaemia with haemoglobin below 7gm/dl indicating severe anaemia. Iron deficiency anaemia is suspected if the red cells show a *microcytic hypochromic* classification. An mean corpuscular volume (MCV) between 80 and 97 fL is normocytic, above 97 fl

is macrocytic and below 80 fl is microcytic. An mean corpuscular haemoglobin concentration (MCHC) between 31 and 34% is normochromic, above 36% is hyperchromic and below 31%, it is hypochromic. A diagnostic postpartum Hb level 10.0 g/dLwill be used.

4.10.2.3Platelet Counts

Gestational thrombocytopenia will be defined as a platelet count of less than 150 x 10^3 µL in hypertensive pregnant women during the third trimester. Platelet count of <100 000 µL will be an indicator ofincreased chance of pre-eclampsia in hypertensive pregnant women during the third trimester.

4.10.3 Biochemistry Determination

4.10.3.1Blood Glucose Determination

Blood will be collected in grey topped tubes (potassium oxalate and sodium fluoride) for HbA1C analysis and random blood glucose levels. A cutoff value of 6.5% will identify undiagnosed diabetes whilst individuals with A1C ≥5.6% will be assumed to have an increased risk for future diabetes according to the Glycohemoglobin standardization guidelines. The glucose levels will be analysed on Mindray BS200 Chemistry Analyser. All the blood tests will be conducted at Immuno-Path Diagnostic Laboratory Services.

4.10.3.2Kidney Function Profiles

Urea and electrolytes, essentially sodium and potassium including lactate dehydrogenase, will be done on mothers' and infants' serum or plasma samples baseline, 10 days, six weeks and every 6 months postpartum.

4.10.3.3Liver function Profiles

Albumin, bilirubin, hepatic enzymes; alanine transaminase (ALT), specific to the liver and aspartate transaminase more widely distributed are markers of damage to cells. Alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) are markers of cholestasis.

4.10.3.4Lipid profiles

Risk factors of CVDs of borderline and high risk ranges for cholesterol, triglycerides, high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc) cholesterol and cholesterol/HDL ratio at baseline will be determined using Mindray BS120 automated analyserat baseline, 10 days, six weeks and every 6 months postpartum.

4.10.3.5Bone Profiles

Longitudinal changes in ionised calcium, phosphorus, albumin and alkaline phosphatase levels in pregnancy (baseline), lactation (10 days pp, six weeks, 6 months) and weaning (earliest visit then after 6 months).Serum parathyroid hormone (PTH) including serum 1,25 $(OH)_2$ Vitamin D level will be

determined at baseline, 6 weeks, 6, 12 and 24 months postpartum. <25 nmol/L will be the cut off value for 25-OH Vitamin D deficient.

4.10.4 Determination of Serum prolactin Levels

Serum prolactin levels in pregnancy, 7-10 day pp, 6 weeks and thereafter 6 monthly will be determined using Sandwich (quantitative) Abcam^{R}. Prolactin Human ELISA Kit (ab108655) according to the manufacturer's instruction. The serum concentration of prolactin will be given in ng/mL which will be correlated with the reported breastfeeding patterns and frequencies.

4.10.5 Determination of Coinfections

HBs Ag/anti-HB/anti-HCV/anti-CMV will be screened at enrolment and at 24 month postpartum for the mothers using qualitative rapid immunochromatographic assays. These will coinfections will be tested in the peripheral blood, cord blood including amniotic fluid. Stool specimen will be examined for intestinal parasitic infections at the age of 7-10 days and six weeks old for the infants.

4.10.6 Determination of Intestinal Parasites

10 grams stool sample will be collected at enrolment, delivery six weeks and every six months for helminths and protozoa diagnosis. Sample will be processed with 24 hours into smears, stained appropriately and then examined under the light microscopy.

4.10.6.1 Helminths

Cestodes (tapeworms) *Taenia, Diphyllobothrium, Hymenolepis, Dipylidium, Echinococcus*, and *Spirometra* be diagnosed by collecting at least three stool samples and checking for ova and parasites.Enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) assay may help confirm a diagnosis; depending on the species involved. *Enterobius vermicularis* associated anal pruritus will be made through microscopic identification of ova and female pinworms from perianal swabs. *Round worm; Ascaris lumbricoides* diagnosis will be made by examination of stool ova and parasite under the microscopy. Hookworms, *Ancylostoma duodenale* and *Necator americanus* diagnosis will be made by means of stool ova and parasite examination. Intestinal trematodes*/Flukes, Fasciolopsis buski*, *Heterophyes heterophyes*, *Metagonimus yokogawai*, and *Echinostoma* diagnosis is will be made by means of stool ova and parasite examination. *Microsporidia* of*Encephalitozoon hellem* and *E intestinalis species* diagnosis will be made via stool microscopy. Schistosoma ova will also be examined including the determination of the serum circulating antigens

4.10.6.2 Protozoa

Balantidium coli, Dientamoeba fragilis, Giardia lamblia, Entamoeba histolytica, Cryptosporidium including Cystoisospora belli diagnosis will be made on at least 3 different smears of stool specimens

and will be examined for trophozoites, cysts or oocytes on a microscopy and complemented by antigen detection with ELISA and or PCR.

4.10.6.3Infants stool

Infant stool collected from delivery will be diagnosed for *E. coli* and Cryptosporidium infections.

4.10.7 Determination of soluble biomarkers of inflammation and immune activation

Several biomarker profiles association with different comorbidities may become a power tool for individualised treatment and care.

4.10.7.1Markers of inflammation

Hs CRP including inflammatory cytokinesIL-6, IL-10 and IL-17 will be determined.

4.10.7.2Markers of immune activation

Plasma INFγ and interferon gamma inducing protein 10 (IP 10) (CXCL10) chemokine whilst in breastmilk RANTES (CCL5) and MIP-1β (CCL4) chemokine will be assayed**.** For mothers with acute HIV infection HβD-2 ,IL-10, IL-12, IL-15, IL-17, IL12p70 determination from cervico-vaginal lavage and plasma IL-7.

4.10.7.3 Markers of Microbial translocation in coinfections

Lipopolysaccharide, soluble (s)CD14, endogenous endotoxin core antibody (EndoCAb) and bacterial 16S DNA complemented by marker of enterocyte damage, intestinal fatty acid binding protein (I-FABP) will be determined.

4.10.7.4Markers of Monocyte and macrophage activation

SCD14, sCD163, sCD40L and IL6 will be determined. Markers of tissue fibrosis and coagulation associated with CVD related comorbidities as fibrinogen**,** D-dimer, a fibrin degradation product associated with endothelial dysfunction and hyaluronic acid biomarker of tissue fibrosis will be determined.

4.10.7.5Cytokine level Determination, Flow Cytometry Method

IL-2,4,6 10, TNF IFN and IL17A will be determined in a single sample (50 µL) using the Becton, Dickinson (B cytometric Bead Array (CBA) human Th1/Th2/Th17 cytokine kit, catalogue number 560484 in either EDTA plasma or serum according to the manufacturer's instructions.

4.10.8 HIV Diagnosis

Qualitative rapid immunochromatographic assays, SD Bioline HIV-1/2 3.0 (Standard Diagnostics Inc., Kyonggi-do, South Korea) and Abbott'sDetermine®HIV-*1/2*will be used to detect HIV-1/-2

antibodies in mothers venipuncture whole blood specimens. Western blot test will be used as a tie breaker for any indeterminate test results.

4.10.9 HIV-1 RNA Load Determination

Maternal and infant plasma samples were quantified for HIV-1 RNA load using an automated TaqMan Roche Amplicor 1.5 Monitor Test (Cobas AmpliPrep/Cobas TaqMan, Roche Diagnostics, Branchburg NJ), according to the manufacturer's instructions. The detection level is 40 copies/mL.

4.10.10 Infants' Qualitative HIV-1 DNA PCR Test

Detection of infants' HIV-1 infection will be determined using a qualitative 1.5 Roche Amplicor HIV-1 DNA PCR kit (Roche Diagnostics Incorporation, Branchburg, New Jersey).This qualitative HIV-1 proviral DNA PCR tests will be done on all HIV exposed infants 'plasma samples/from birth up to 15 months past partum.

4.10.11 CD4 cell counts enumeration

Absolute and percentage $CD4^+$ T lymphocytes will be enumerated for all HIV positive mothers and infants using a Partec Cyflow counter (Cyflow, Partec, Munster, Germany) within 6 hours of blood collection..

4.10.12 Viral load Determination

Maternal and infant plasma viral load will be determined using automated Roche AmpliPrep COBAS AMPLICOR HIV-1 Monitor (Version 1.5) Ultrasensitive Assay according to the manufacture's instruction. All unsuppressed viral load based on a threshold of ≥ 1000 copies/mL will be re-run after 3 months.

4.10.13 Markers of immune activation and senescence at enrolment and exit whole blood sample

Determine maternal activation markers, profiling by flow cytometry.CD38 on both $CD4^+$ and $CD8^+$ T cells. Senescence marker: CD28-CD57+ will be determined.

4.10.14 NK cell phenotypic and functional analysis

Phenotype (CD56+ dim and bright) and function on PBMCs.

4.10.15 Maternal MUAC Measurements

MUAC will be used as an indicator or predictor of nutritional outcomes. The subject's left arm should be bent at the elbow at a 90 degree angle, with the upper arm held parallel to the side of the body. Measure the mid-point distance between the bony protrusion on the shoulder and the point of the elbow will be measured. Maternal MUAC cutoffs of <22 cm to ≤24 cm relative to the median of the sample will be used with the following assumption that;

1. If MUAC is <23.5 cm, BMI is likely to be <20 kg/m^2
2. If MUAC is >32.0 cm, BMI is likely to be >30 kg/ m^2

4.10.15.1 Infant Measurements

Among children 6-60 months old assessment for acute malnutrition will be done using the 2009, WHO and UNICEF recommendation of a MUAC cut off value of <11.5 cm

4.10.16 Determination of Hypertensive Disorders

Any one of the following will be enough to make a diagnosis of hypertension in pregnancy;

4.10.16.1 Chronic hypertension

- Preexisting hypertension: A woman diagnosed of hypertension either before she fell pregnant who may be already on treatment or first diagnosed after 20 weeks gestation and persisting after 12 weeks postpartum
- A woman with a blood pressure greater than or equal to 140/90mmHg on at least 2 separate occasions 4hours apart, anytime during the pregnancy. An Obstetrician will diagnose the following complications:

4.10.16.2 Increased chance of pre-eclampsia

BP ≥ 160/110mmHg, Proteinuria ≥ 2g/24 hrs, ≥ 2+ dipstick and platelets < 100 000 μL, urine protein (mg/dL)/creatinine (mg/dL) ratio ≥0.3, -elevated serum lactate dehydrogenase (LDH) level and activity, elevated serum transaminase levels-AST, ALT; persistence epigastric pain/headache/visual disturbances

4.10.16.3 Eclampsia Seizures

New onset of grand mal seizure activity or unexplained coma during pregnancy or postpartum period in a woman with hypertension in pregnancy or postpartum period.

4.10.16.4 HELLP syndrome

1. Hemolysis is associated with the following:
   - Total bilirubin >1.2 mg/dL
   - LDH >600 U/L
2. Elevated liver enzymes:
   - Aspartase aminotransferase (AST) >70 U/L
   - Alanine aminotransferase (ALT
   - LDH >600 U/L
3. A platelet count < 100,000/μL

Sub-classification of HELLP based on the severity of thrombocytopenia:

- Class 1 - Platelet count >50,000/μL
- Class 2 - Platelet count 50,000-100,000/μL
- Class 3 - Platelet count 100,000-150,000/μL

4.10.17 Transmission linkages

Mothers who seroconvert after enrolments will have host/viral genetics factors determined transmission linkages assessment. Peripheral blood mononuclear cells will be collected for these HUU, HEI and HEU infants for NK cell phenotype and functional analysis.

4.10.17.1 Nucleic acid extraction and DNA Amplification

Total RNA was extracted from plasma using the NucliSENS isolation kit, based on the Boom *et al.,* method Briefly samples are lysed in a lysis buffer containing a chaotropic agent, guanidine thiocyanate. Cells, bacteria and viruses in the sample are lysed whilst proteins such as nucleases are denatured and inactivated. DNA and RNA bind to silica particles and everything else is washed following several washing steps with the wash buffer. Finally the nucleic acids are eluted from the silica particles using the elution buffer. Purified DNA will be stored at -80 for genotyping.*Taq* polymerase is a thermostable DNA polymerase named after the hot spring *Thermusaquaticus* bacterium. The primary PCR amplified an approximately 800-base pair (bp) fragment spanning the V3 and V4 region of the envelope (positions 6948–7537) on the HIV-HXB2 genome using HIV primers ENV 2 and NY3. Secondary or nested PCR amplified a 535-bp env gene fragment using a PCR Thermal cycler. Detection and quantification of secondary PCR amplicons were done using a 1% agarose gel electrophoresed together with a standard mass ladder and then stained with SYBR safe stain.

4.10.18 Sample Size Determination

Pocock's formula was used to determine sample size (n) required:

- $n = \frac{p_1(1-p_1)+p_2(1-p_2)(Z_\alpha - Z_\beta)^2}{(p_1-p_2)^2}$
- Where p_1 and $p_2$are proportion of success in population 1 and 2
- Z_α and Z_β are values of standard normal distribution
- α= Significance level
- β = Power

3% incidence rate has been assumed to factor in for the a high risk of vertical transmission in the breastfeeding period for the few anticipated acute HIV infection[234].With the advent of cell phones the % loss to follow up may not be that bad and cannot be similar for the first and second year. Factored in the worst case scenario of a 15% and 20% loss to follow-up during the first and second year respectively, a minimum sample size required computed using **STATA** is 1200 (600 HIV+ and 600 HIV-) mothers. Assuming a 15% and 20% loss to follow up in the first and second year of the study respectively, should leave us with at least the 417 infants. The sample size has power to predict diseases outcomes of HEU children which must culminate in their improved management. The sample size has not factored in sample sizes of sub-studies linked to it in the **Appendices' Section 6.2**. Should there be sub-studies, they should fit within this parent protocol. These aim to address all the research gaps highlight throughout the literature review.

Table 5: Pictorial Depiction of the Stratification

	a) HIV Chronic Infection		b) HIV Acute Infection	c) HIV Negative	Outcomes
Comorbidities	**Treatment experienced**	**Treatment naive**			Prevalence and incidence of comorbidities, comorbidities trends, relating comorbidities to immunological profiles, pregnancy outcomes, vertical transmission of infections, patterns and transmission linkages, infant/child growth and neurodevelopment
	Immune Profiles for different comorbidities combinations				
HBV					
HCV					
CMV					
Anaemia					
Diabetes					
Nutritional Status					
Hypertensive disorders					

4.10.19 Ethics Statement

Ethical approval will be sought from the necessary institutional review boards, JREC and MRCZ. All study participants will give written informed consent. Permission for storage and future use of biological specimens, external shipping of specimens including genetic testing of blood samples will also be sought. This observational study involves minimal risks to both the mother and the baby hence one parental consent is sufficient. However, in case of death of the mother re-consenting will be done from either the father or any family member who shall have legal responsibility for the care and custody of the baby. In case of a child death, the mother will cease to continue participating in the study.

Permission has already been obtained from Harare City Health.The sensitivity of HIV infection and sexuality means that the mother's emotional response is a potential risk. Anxiety associated with sample testing and waiting for the results for HIV exposed children may also be experienced. Nevertheless the study will minimize such risks by providing the necessary education and counselling through the research nurses and where necessary, referring them to the appropriate clinics. Health education and counselling on pregnancy, delivery and childcare will be given to mothers on a continuous basis at every visit. The study will offer consultation and basic treatment to both the mother and child. Whenever necessary referrals will made through the clinic.

Each sub-study will apply for their respective ethical approvals to all the relevant ethical boards before they can access the data and/or specimens. However, some sub-studies may require separate consent forms should they entail collection of additional data from study participants. Those that rely on specimens and data collected through main study will need no additional informed consent.

4.10.20 Adverse Event Monitoring and Reporting

All Severe Adverse Events (SAEs): prolonged hospitalisation, life threatening, congenital birth defects and significant disabilities including deaths will be reported to MRCZ and sponsor within 24 hours of first becoming aware of it. Regular monitoring visits to the investigative sites will be conducted by UZ-CHS Research Support Centre (RSC) monitors in accordance with a site monitoring

plan which will be finalised by RSC before screening begins. The frequency and duration of visits will be sufficient to allow monitoring of:

1. Compliance with the informed consent process and documentation
2. Adequacy of maintenance of participant files
3. Accuracy of transfer of data from participant trial files into the CRFs
4. Compliance with the current version of the protocol
5. Ongoing assumption by the PI of primary responsibility for conduct of the study
6. Storage and handling of biological specimens.
7. Compliance with all regulatory requirements

4.10.21 Data Management Plan

Study data will be collected and managed using Research Electronic Data Capture(REDCap) tool hosted at the UZ RSC. Quality assurance on the accuracy of data entry will employed including independent double entries. Data analysis will be carried out using statistical packages such as; Stata version 13 and Statistical Package for the Social Sciences SPSS. Categorical data will be compared using chi squared test. Continuous variables will be compared between groups using analysis of variance (parametric) and sum rank (non-parametric) tests. Continuous variables will be correlated using regression models.

4.10.22 Data analysis

The data were collected and analyzed using SPSS (version17.0, Chicago, IL). Viral load values were $\log_{10}$ transformed. The Student's t-test was used to compare means. Regression analysis was used to investigate the associations Tests of statistical significance included the 95% confidence interval of unadjusted relative risks, two-sided p values based on Chi-square and Fisher's exact tests. Sequences were assembled using the Vector NTI Advance 10 program. Alignment was attained using Gene Doc, BioEdit, and Clustal X2 sequence alignment programs including manual editing to ensure that deletions or insertions did not alter the reading frame.

4.10.23 Budget

The baseline study will cost is 120 000 US dollars and enrolment is expected to start in July 2015 and the study will continue following children up to 24 months. The current budget should be sufficient for baseline surveys. However, additional funding for the study will be sourced as the study progresses. Please note that for sub-studies the respective MPhil or PhD students will bring or source own funding for to answer their specific research questions. Activities and task are shown in **Table 6** below.

Table 6: Tasks and Time Frames

TASK	WHEN	WHO	HOW LONG	RESOURCES	COST
Get ethical approval	Y1 m1-3	PI	3 months	PI	$700
Communication	Y1-2	Coordinator/ driver	24 months	Mobile phone hand set x 2, air time, data bundles	$1000
Training	Y1 M1	Team	2 weeks	Teas/ lunches	$1000
Meetings	Y1-2	Team	weekly	Teas	$ 1000
Recruit patients	Y1 m5-8	Midwives, Coordinator	3 months	Stationery & copying	$1000
Participant transport reimbursement	Y1-2	Research Nurse	24 months	$5/visit x 12 visits	$ 70 000
Transport (fuel, vehicle hire/ services)	Y1-2	Driver	24months		$5000
Sample preparation & storage	Y1-2	Team Members	24 Months	Tubes, pipettes, boxes, accessories, PMBCs , plasma for storage	$5000
Lab assays (local)	Y1-2	Team Members	24 Months	Kits, haematology, chemistry, urinalysis, lipid profile, T cell profiles, viral load	20 000
Data entry,	Y1-2	Biostatistician	24 Months	Laptops x 3, dongle, memory sticks.	$ 2500
Allowance	Y 1 M 1-6	PI	6 months	Research nurses, driver, secretary , coordinator and lab scientist, data clerks	$15 000
Conferences	Y2	PI/co-investigators		Travel and subsistence, tickets	$5 000
Publications	Y2	Team Members		Publication fees	$ 2000

Y: Year

4.10.24 Research Outcomes

- Characterisation of comorbidities of HIV negative and HIV positive mothers
- Immune activation profiles for different comorbidities
- HIV vertical transmission rate in the era of option B+ among mothers with chronic HIV infection
- HIV incidence and postpartum vertical transmission among mother with acute HIV infection
- Transmission linkages of acute HIV infection

Research outcomes will entail improved knowledge of HEU infants immunological abnormalities and genetics resulting in their better clinical management. There will also be technology transfer in

molecular biology and genetics techniques through the establishment of research networks both locally and internationally. The project will generate a critical mass of local professions with diverse expertise as they attain higher degrees, MPhils and PhDs from sub-studies. At least ten (10) papers will be published from the parent study material.

5.0 Reference List

(1) Lansky A, Brooks JT, DiNenno E et al.: Epidemiology of HIV in the United States. J Acquir Immune Defic Syndr 2010; 55 Suppl 2:S64-S68.
(2) Hunter DJ: AIDS in sub-Saharan Africa: the epidemiology of heterosexual transmission and the prospects for prevention. Epidemiology 1993; 4(1):63-72.
(3) Mapingure MP, Msuya S, Kurewa NE et al.: Sexual behaviour does not reflect HIV-1 prevalence differences: a comparison study of Zimbabwe and Tanzania. J Int AIDS Soc 2010; 13:45.
(4) Gisselquist D, Rothenberg R, Potterat J, Drucker E: HIV infections in sub-Saharan Africa not explained by sexual or vertical transmission. Int J STD AIDS 2002; 13(10):657-666.
(5) Lingappa JR, Lambdin B, Bukusi EA et al.: Regional differences in prevalence of HIV-1 discordance in Africa and enrollment of HIV-1 discordant couples into an HIV-1 prevention trial PLoS One 2008; 3(1):e1411.
(6) Karp CL, Auwaerter PG: Coinfection with HIV and tropical infectious diseases. II. Helminthic, fungal, bacterial, and viral pathogens. Clin Infect Dis 2007; 45(9):1214-1220.
(7) Tobian AA, Quinn TC: Herpes simplex virus type 2 and syphilis infections with HIV: an evolving synergy in transmission and prevention. Curr Opin HIV AIDS 2009; 4(4):294-299.
(8) Koethe JR, Chi BH, Megazzini KM, Heimburger DC, Stringer JS: Macronutrient supplementation for malnourished HIV-infected adults: a review of the evidence in resource-adequate and resource-constrained settings. Clin Infect Dis 2009; 49(5):787-798.
(9) Amuyunzu-Nyamongo M: Need for a multi-factorial, multi-sectorial and multi-disciplinary approach to NCD prevention and control in Africa. Glob Health Promot 2010; 17(2 Suppl):31-32.
(10) Ullrich A, Ott JJ, Vitoria M, Martin-Moreno JM, Atun R: Long-term care of AIDS and non-communicable diseases. Lancet 2011; 377(9766):639-640.
(11) Mayosi BM, Flisher AJ, Lalloo UG et al.: The burden of non-communicable diseases in South Africa. Lancet 2009; 374(9693):934-947.
(12) Lawn SD: AIDS in Africa: the impact of coinfections on the pathogenesis of HIV-1 infection. J Infect 2004; 48(1):1-12.
(13) Holtgrave DR, Hall HI, Prejean J: HIV Transmission Rates in the United States, 2006-2008. Open AIDS J 2012; 6:26-28.
(14) Gumbo FZ, Kurewa NE, Kandawasvika GQ et al.: Rising mother-to-child HIV transmission in a resource-limited breastfeeding population. Trop Doct 2010; 40(2):70-73.
(15) Shapiro RL, Lockman S: Mortality among HIV-exposed infants: the first and final frontier. Clin Infect Dis 2010; 50(3):445-447.
(16) Slogrove A, Reikie B, Naidoo S et al.: HIV-exposed uninfected infants are at increased risk for severe infections in the first year of life. J Trop Pediatr 2012; 58(6):505-508.
(17) Brahmbhatt H, Kigozi G, Wabwire-Mangen F et al.: Mortality in HIV-infected and uninfected children of HIV-infected and uninfected mothers in rural Uganda. J Acquir Immune Defic Syndr 2006; 41(4):504-508.
(18) Marinda E, Humphrey JH, Iliff PJ et al.: Child mortality according to maternal and infant HIV status in Zimbabwe. Pediatr Infect Dis J 2007; 26(6):519-526.
(19) Shapiro RL, Lockman S, Kim S et al.: Infant morbidity, mortality, and breast milk immunologic profiles among breast-feeding HIV-infected and HIV-uninfected women in Botswana. J Infect Dis 2007; 196(4):562-569.
(20) Cotton MF, Slogrove A, Rabie H: Infections in HIV-exposed Uninfected Children With Focus on Sub-Saharan Africa. Pediatr Infect Dis J 2014; 33(10):1085-1086.
(21) Afran L, Garcia KM, Nduati E et al.: HIV-exposed uninfected children: a growing population with a vulnerable immune system? Clin Exp Immunol 2014; 176(1):11-22.
(22) Hazra R, Siberry GK, Mofenson LM: Growing up with HIV: children, adolescents, and young adults with perinatally acquired HIV infection. Annu Rev Med 2010; 61:169-185.
(23) Wang A, Wang X, Dou L et al.: [Incidence of and related risk factors on preterm delivery among HIV-infected pregnant women in China]. Zhonghua Liu Xing Bing Xue Za Zhi 2015; 36(4):349-353.
(24) Slyker JA, Patterson J, Ambler G et al.: Correlates and outcomes of preterm birth, low birth weight, and small for gestational age in HIV-exposed uninfected infants. BMC Pregnancy Childbirth 2014; 14:7.
(25) Nielsen-Saines K, Komarow L, Cu-Uvin S et al.: Infant outcomes after maternal antiretroviral exposure in resource-limited settings. Pediatrics 2012; 129(6):e1525-e1532.
(26) Muhangi L, Lule SA, Mpairwe H et al.: Maternal HIV infection and other factors associated with growth outcomes of HIV-uninfected infants in Entebbe, Uganda. Public Health Nutr 2013; 16(9):1548-1557.
(27) Zash RM, Ajose-Popoola O, Stordal K et al.: Risk factors for mortality among human immunodeficiency virus-exposed and unexposed infants admitted to a neonatal intensive care unit in Botswana. J Paediatr Child Health 2014; 50(3):189-195.
(28) Duri K, Gumbo FZ, Kristiansen KI et al.: Antenatal HIV-1 RNA load and timing of mother to child transmission; a nested case-control study in a resource poor setting. Virol J 2010; 7:176.
(29) Kakkar F, Lamarre V, Ducruet T et al.: Impact of maternal HIV-1 viremia on lymphocyte subsets among HIV-exposed uninfected infants: protective mechanism or immunodeficiency. BMC Infect Dis 2014; 14:236.
(30) Pavlinac PB, John-Stewart GC, Naulikha JM et al.: High-risk enteric pathogens associated with HIV infection and HIV exposure in Kenyan children with acute diarrhoea. AIDS 2014; 28(15):2287-2296.
(31) Kourtis AP, Wiener J, Kayira D et al.: Health outcomes of HIV-exposed uninfected African infants. AIDS 2013; 27(5):749-759.

(32) Kinabo GD, van d, V, Msuya LJ et al.: Dynamics of nasopharyngeal bacterial colonisation in HIV-exposed young infants in Tanzania. Trop Med Int Health 2013; 18(3):286-295.
(33) Mussi-Pinhata MM, Motta F, Freimanis-Hance L et al.: Lower respiratory tract infections among human immunodeficiency virus-exposed, uninfected infants. Int J Infect Dis 2010; 14 Suppl 3:e176-e182.
(34) Bates M, Mudenda V, Mwaba P, Zumla A: Deaths due to respiratory tract infections in Africa: a review of autopsy studies. Curr Opin Pulm Med 2013; 19(3):229-237.
(35) Slogrove AL, Cotton MF, Esser MM: Severe infections in HIV-exposed uninfected infants: clinical evidence of immunodeficiency. J Trop Pediatr 2010; 56(2):75-81.
(36) Epalza C, Goetghebuer T, Hainaut M et al.: High incidence of invasive group B streptococcal infections in HIV-exposed uninfected infants. Pediatrics 2010; 126(3):e631-e638.
(37) Singh HK, Gupte N, Kinikar A et al.: High rates of all-cause and gastroenteritis-related hospitalization morbidity and mortality among HIV-exposed Indian infants. BMC Infect Dis 2011; 11:193.
(38) Fawzy A, Arpadi S, Kankasa C et al.: Early weaning increases diarrhea morbidity and mortality among uninfected children born to HIV-infected mothers in Zambia. J Infect Dis 2011; 203(9):1222-1230.
(39) Fox MP, Brooks DR, Kuhn L et al.: Role of breastfeeding cessation in mediating the relationship between maternal HIV disease stage and increased child mortality among HIV-exposed uninfected children. Int J Epidemiol 2009; 38(2):569-576.
(40) Asbjornsdottir KH, Slyker JA, Weiss NS et al.: Breastfeeding is associated with decreased pneumonia incidence among HIV-exposed, uninfected Kenyan infants. AIDS 2013; 27(17):2809-2815.
(41) Izadnegahdar R, Fox MP, Jeena P, Qazi SA, Thea DM: Revisiting pneumonia and exposure status in infants born to HIV-infected mothers. Pediatr Infect Dis J 2014; 33(1):70-72.
(42) Marquez C, Okiring J, Chamie G et al.: Increased Morbidity in Early Childhood Among HIV-exposed Uninfected Children in Uganda is Associated with Breastfeeding Duration. J Trop Pediatr 2014.
(43) Velilla PA, Montoya CJ, Hoyos A et al.: Effect of intrauterine HIV-1 exposure on the frequency and function of uninfected newborns' dendritic cells. Clin Immunol 2008; 126(3):243-250.
(44) Rich KC, Siegel JN, Jennings C, Rydman RJ, Landay AL: Function and phenotype of immature CD4+ lymphocytes in healthy infants and early lymphocyte activation in uninfected infants of human immunodeficiency virus-infected mothers. Clin Diagn Lab Immunol 1997; 4(3):358-361.
(45) Clerici M, Saresella M, Colombo F et al.: T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. Blood 2000; 96(12):3866-3871.
(46) Clerici M, Sison AV, Berzofsky JA et al.: Cellular immune factors associated with mother-to-infant transmission of HIV. AIDS 1993; 7(11):1427-1433.
(47) Kolte L, Rosenfeldt V, Vang L et al.: Reduced thymic size but no evidence of impaired thymic function in uninfected children born to human immunodeficiency virus-infected mothers. Pediatr Infect Dis J 2011; 30(4):325-330.
(48) Kolte L: Thymic function in HIV-infection. Dan Med J 2013; 60(4):B4622.
(49) Bunders MJ, van Hamme JL, Jansen MH et al.: Fetal exposure to HIV-1 alters chemokine receptor expression by CD4+T cells and increases susceptibility to HIV-1. Sci Rep 2014; 4:6690.
(50) Piconi S, Trabattoni D, Gori A et al.: Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy. AIDS 2010; 24(13):1991-2000.
(51) Lukwiya M, Rizzardini G, Trabattoni D et al.: Evaluation of immune activation in HIV-infected and uninfected African individuals by single-cell analysis of cytokine production. J Acquir Immune Defic Syndr 2001; 28(5):429-436.
(52) Kasahara TM, Hygino J, Blanco B et al.: The impact of maternal anti-retroviral therapy on cytokine profile in the uninfected neonates. Hum Immunol 2013; 74(9):1051-1056.
(53) Hygino J, Lima PG, Filho RG et al.: Altered immunological reactivity in HIV-1-exposed uninfected neonates. Clin Immunol 2008; 127(3):340-347.
(54) Hunt PW: Th17, gut, and HIV: therapeutic implications. Curr Opin HIV AIDS 2010; 5(2):189-193.
(55) Kuller LH, Tracy R, Belloso W et al.: Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med 2008; 5(10):e203.
(56) Shapiro RL, Holland DT, Capparelli E et al.: Antiretroviral concentrations in breast-feeding infants of women in Botswana receiving antiretroviral treatment. J Infect Dis 2005; 192(5):720-727.
(57) Delaugerre C, Chaix ML, Blanche S et al.: Perinatal acquisition of drug-resistant HIV-1 infection: mechanisms and long-term outcome. Retrovirology 2009; 6:85.
(58) Montesano C, Anselmi A, Palma P et al.: HIV replication leads to skewed maturation of CD8-positive T-cell responses in infected children. New Microbiol 2010; 33(4):303-309.
(59) Lambert JS, Moye J, Jr., Plaeger SF et al.: Association of selected phenotypic markers of lymphocyte activation and differentiation with perinatal human immunodeficiency virus transmission and infant infection. Clin Diagn Lab Immunol 2005; 12(5):622-631.
(60) Schenal M, Lo CS, Fasano F et al.: Distinct patterns of HIV-specific memory T lymphocytes in HIV-exposed uninfected individuals and in HIV-infected patients. AIDS 2005; 19(7):653-661.
(61) Bunders M, Pembrey L, Kuijpers T, Newell ML: Evidence of impact of maternal HIV infection on immunoglobulin levels in HIV-exposed uninfected children. AIDS Res Hum Retroviruses 2010; 26(9):967-975.
(62) Miyamoto M, Pessoa SD, Ono E et al.: Low CD4+ T-cell levels and B-cell apoptosis in vertically HIV-exposed noninfected children and adolescents. J Trop Pediatr 2010; 56(6):427-432.
(63) Gasper MA, Kunwar P, Itaya G et al.: Natural killer cell and T-cell subset distributions and activation influence susceptibility to perinatal HIV-1 infection. AIDS 2014; 28(8):1115-1124.
(64) Reikie BA, Adams RC, Leligdowicz A et al.: Altered innate immune development in HIV-exposed uninfected infants. J Acquir Immune Defic Syndr 2014; 66(3):245-255.
(65) Rodriguez-Garcia M, Oliva H, Climent N et al.: Human immature monocyte-derived dendritic cells produce and secrete alpha-defensins 1-3. J Leukoc Biol 2007; 82(5):1143-1146.
(66) Kuhn L, Trabattoni D, Kankasa C et al.: Alpha-defensins in the prevention of HIV transmission among breastfed infants. J Acquir Immune Defic Syndr 2005; 39(2):138-142.
(67) Trabattoni D, Caputo SL, Maffeis G et al.: Human alpha defensin in HIV-exposed but uninfected individuals. J Acquir Immune Defic Syndr 2004; 35(5):455-463.
(68) Biasin M, Piacentini L, Lo CS et al.: Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G: a possible role in the resistance to HIV of HIV-exposed seronegative individuals. J Infect Dis 2007; 195(7):960-964.

(69) Refsland EW, Harris RS: The APOBEC3 family of retroelement restriction factors. Curr Top Microbiol Immunol 2013; 371:1-27.
(70) Harris RS, Hultquist JF, Evans DT: The restriction factors of human immunodeficiency virus. J Biol Chem 2012; 287(49):40875-40883.
(71) Claudio CC, Patin RV, Palchetti CZ et al.: Nutritional status and metabolic disorders in HIV-exposed uninfected prepubertal children. Nutrition 2013; 29(7-8):1020-1023.
(72) Papp E, Mohammadi H, Loutfy MR et al.: HIV protease inhibitor use during pregnancy is associated with decreased progesterone levels, suggesting a potential mechanism contributing to fetal growth restriction. J Infect Dis 2015; 211(1):10-18.
(73) Sibiude J, Warszawski J, Tubiana R et al.: Premature delivery in HIV-infected women starting protease inhibitor therapy during pregnancy: role of the ritonavir boost? Clin Infect Dis 2012; 54(9):1348-1360.
(74) Watts DH, Williams PL, Kacanek D et al.: Combination antiretroviral use and preterm birth. J Infect Dis 2013; 207(4):612-621.
(75) Cerrato E, Calcagno A, D'Ascenzo F et al.: Cardiovascular disease in HIV patients: from bench to bedside and backwards. Open Heart 2015; 2(1):e000174.
(76) Young J, Xiao Y, Moodie EE et al.: The effect of cumulating exposure to abacavir on the risk of cardiovascular disease events in patients from the Swiss HIV Cohort Study. J Acquir Immune Defic Syndr 2015.
(77) Caron-Debarle M, Boccara F, Lagathu C et al.: Adipose tissue as a target of HIV-1 antiretroviral drugs. Potential consequences on metabolic regulations. Curr Pharm Des 2010; 16(30):3352-3360.
(78) Violari A, Cotton MF, Gibb DM et al.: Early antiretroviral therapy and mortality among HIV-infected infants. N Engl J Med 2008; 359(21):2233-2244.
(79) Marston M, Becquet R, Zaba B et al.: Net survival of perinatally and postnatally HIV-infected children: a pooled analysis of individual data from sub-Saharan Africa. Int J Epidemiol 2011; 40(2):385-396.
(80) Feiterna-Sperling C, Weizsaecker K, Buhrer C et al.: Hematologic effects of maternal antiretroviral therapy and transmission prophylaxis in HIV-1-exposed uninfected newborn infants. J Acquir Immune Defic Syndr 2007; 45(1):43-51.
(81) Dryden-Peterson S, Shapiro RL, Hughes MD et al.: Increased risk of severe infant anaemia after exposure to maternal HAART, Botswana. J Acquir Immune Defic Syndr 2011; 56(5):428-436.
(82) McComsey GA, Kang M, Ross AC et al.: Increased mtDNA levels without change in mitochondrial enzymes in peripheral blood mononuclear cells of infants born to HIV-infected mothers on antiretroviral therapy. HIV Clin Trials 2008; 9(2):126-136.
(83) Le DK, Bland R, Newell ML: Neurodevelopment in children born to HIV-infected mothers by infection and treatment status. Pediatrics 2012; 130(5):e1326-e1344.
(84) Hazra R, Cohen RA, Gonin R et al.: Lipid levels in the second year of life among HIV-infected and HIV-exposed uninfected Latin American children. AIDS 2012; 26(2):235-240.
(85) Vigano A, Cerini C, Pattarino G, Fasan S, Zuccotti GV: Metabolic complications associated with antiretroviral therapy in HIV-infected and HIV-exposed uninfected paediatric patients. Expert Opin Drug Saf 2010; 9(3):431-445.
(86) Melvin AJ, Kang M, Hitti J et al.: Cord blood lipids in infants born to HIV-1-infected women treated with combination antiretroviral therapy. Antivir Ther 2008; 13(3):349-355.
(87) Miller TI, Borkowsky W, DiMeglio LA et al.: Metabolic abnormalities and viral replication are associated with biomarkers of vascular dysfunction in HIV-infected children. HIV Med 2012; 13(5):264-275.
(88) Farquhar C, Wamalwa D, Selig S et al.: Immune responses to measles and tetanus vaccines among Kenyan human immunodeficiency virus type 1 (HIV-1)-infected children pre- and post-highly active antiretroviral therapy and revaccination. Pediatr Infect Dis J 2009; 28(4):295-299.
(89) Miles DJ, Gadama L, Gumbi A et al.: Human immunodeficiency virus (HIV) infection during pregnancy induces CD4 T-cell differentiation and modulates responses to Bacille Calmette-Guerin (BCG) vaccine in HIV-uninfected infants. Immunology 2010; 129(3):446-454.
(90) Gnanashanmugam D, Troy SB, Musingwini G et al.: Immunologic response to oral polio vaccine in human immunodeficiency virus-infected and uninfected Zimbabwean children. Pediatr Infect Dis J 2012; 31(2):176-180.
(91) Kidzeru EB, Hesseling AC, Passmore JA et al.: In-utero exposure to maternal HIV infection alters T-cell immune responses to vaccination in HIV-uninfected infants. AIDS 2014; 28(10):1421-1430.
(92) Groome MJ, Page N, Cortese MM et al.: Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study. Lancet Infect Dis 2014.
(93) Reikie BA, Naidoo S, Ruck CE et al.: Antibody responses to vaccination among South African HIV-exposed and unexposed uninfected infants during the first 2 years of life. Clin Vaccine Immunol 2013; 20(1):33-38.
(94) Besnard M, Sauvion S, Offredo C et al.: Bacillus Calmette-Guerin infection after vaccination of human immunodeficiency virus-infected children. Pediatr Infect Dis J 1993; 12(12):993-997.
(95) Mazzola TN, da Silva MT, Abramczuk BM et al.: Impaired Bacillus Calmette-Guerin cellular immune response in HIV-exposed, uninfected infants. AIDS 2011; 25(17):2079-2087.
(96) Abramczuk BM, Mazzola TN, Moreno YM et al.: Impaired humoral response to vaccines among HIV-exposed uninfected infants. Clin Vaccine Immunol 2011; 18(9):1406-1409.
(97) Nair N, Moss WJ, Scott S et al.: HIV-1 infection in Zambian children impairs the development and avidity maturation of measles virus-specific immunoglobulin G after vaccination and infection. J Infect Dis 2009; 200(7):1031-1038.
(98) Tejiokem MC, Gouandjika I, Beniguel L et al.: HIV-infected children living in Central Africa have low persistence of antibodies to vaccines used in the Expanded Program on Immunization. PLoS One 2007; 2(12):e1260.
(99) Tejiokem MC, Njamkepo E, Gouandjika I et al.: Whole-cell pertussis vaccine induces low antibody levels in human immunodeficiency virus-infected children living in sub-Saharan Africa. Clin Vaccine Immunol 2009; 16(4):479-483.
(100) Burgard M, Blanche S, Jasseron C et al.: Performance of HIV-1 DNA or HIV-1 RNA tests for early diagnosis of perinatal HIV-1 infection during anti-retroviral prophylaxis. J Pediatr 2012; 160(1):60-66.
(101) Mansoor N, Scriba TJ, de KM et al.: HIV-1 infection in infants severely impairs the immune response induced by Bacille Calmette-Guerin vaccine. J Infect Dis 2009; 199(7):982-990.
(102) Tchakoute CT, Hesseling AC, Kidzeru EB et al.: Delaying BCG Vaccination Until 8 Weeks of Age Results in Robust BCG-Specific T-Cell Responses in HIV-Exposed Infants. J Infect Dis 2014.
(103) Young KM, Gray CM, Bekker LG: Is obesity a risk factor for vaccine non-responsiveness? PLoS One 2013; 8(12):e82779.
(104) Cotton MF, Schaaf HS, Lottering G et al.: Tuberculosis exposure in HIV-exposed infants in a high-prevalence setting. Int J Tuberc Lung Dis 2008; 12(2):225-227.

(105) Lima MF, Melo HR: Hepatotoxicity induced by antituberculosis drugs among patients coinfected with HIV and tuberculosis. Cad Saude Publica 2012; 28(4):698-708.
(106) Dziuban EJ, Marton SA, Hughey AB et al.: Seroprevalence of hepatitis B in a cohort of HIV-infected children and adults in Swaziland. Int J STD AIDS 2013; 24(7):561-565.
(107) Puoti M, Moioli MC, Travi G, Rossotti R: The burden of liver disease in human immunodeficiency virus-infected patients. Semin Liver Dis 2012; 32(2):103-113.
(108) Huntington S, Thorne C, Newell ML et al.: Pregnancy is associated with elevation of liver enzymes in HIV-positive women on antiretroviral therapy. AIDS 2015.
(109) Ezechi OC, Kalejaiye OO, Gab-Okafor CV et al.: Sero-prevalence and factors associated with Hepatitis B and C co-infection in pregnant Nigerian women living with HIV infection. Pan Afr Med J 2014; 17:197.
(110) Kovacs A, Karim R, Mack WJ et al.: Activation of CD8 T cells predicts progression of HIV infection in women coinfected with hepatitis C virus. J Infect Dis 2010; 201(6):823-834.
(111) Okeke TC, Obi SN, Okezie OA et al.: Coinfection with hepatitis B and C viruses among HIV positive pregnant women in Enugu south east, Nigeria. Niger J Med 2012; 21(1):57-60.
(112) Thumbiran NV, Moodley D, Parboosing R, Moodley P: Hepatitis B and HIV co-infection in pregnant women: indication for routine antenatal hepatitis B virus screening in a high HIV prevalence setting. S Afr Med J 2014; 104(4):307-309.
(113) Benova L, Mohamoud YA, Calvert C, Abu-Raddad LJ: Vertical transmission of hepatitis C virus: systematic review and meta-analysis. Clin Infect Dis 2014; 59(6):765-773.
(114) Baldo V, Baldovin T, Trivello R, Floreani A: Epidemiology of HCV infection. Curr Pharm Des 2008; 14(17):1646-1654.
(115) Goyal LD, Kaur S, Jindal N, Kaur H: HCV and pregnancy: prevalence, risk factors, and pregnancy outcome in north Indian population: a case-control study. J Obstet Gynaecol India 2014; 64(5):332-336.
(116) Slyker JA, Rowland-Jones SL, Dong T et al.: Acute cytomegalovirus infection is associated with increased frequencies of activated and apoptosis-vulnerable T cells in HIV-1-infected infants. J Virol 2012; 86(20):11373-11379.
(117) Roxby AC, Atkinson C, Asbjornsdottir K et al.: Maternal valacyclovir and infant cytomegalovirus acquisition: a randomized controlled trial among HIV-infected women. PLoS One 2014; 9(2):e87855.
(118) Manicklal S, van Niekerk AM, Kroon SM et al.: Birth prevalence of congenital cytomegalovirus among infants of HIV-infected women on prenatal antiretroviral prophylaxis in South Africa. Clin Infect Dis 2014; 58(10):1467-1472.
(119) Mania A, Kemnitz P, Cudnoch K et al.: Clinical condition and transmission of coinfections with human cytomegalovirus in infants of HIV-1 infected mothers in the era of mother-to-child-transmission prophylaxis. Early Hum Dev 2013; 89(2):119-124.
(120) Bates M, Tembo J, Zumla A: Congenital cytomegalovirus infections in sub-Saharan Africa--a neglected and growing problem. Trop Med Int Health 2014; 19(8):996-998.
(121) Duryea EL, Sanchez PJ, Sheffield JS et al.: Maternal human immunodeficiency virus infection and congenital transmission of cytomegalovirus. Pediatr Infect Dis J 2010; 29(10):915-918.
(122) Cannon MJ, Schmid DS, Hyde TB: Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol 2010; 20(4):202-213.
(123) Tardieu M, Tejiokem M, Nguefack S: Virus-induced lesions and the fetal brain: examples of the transmission of HIV-1 and CMV from mother to offspring. Handb Clin Neurol 2013; 112:1103-1108.
(124) Gompels UA, Larke N, Sanz-Ramos M et al.: Human cytomegalovirus infant infection adversely affects growth and development in maternally HIV-exposed and unexposed infants in Zambia. Clin Infect Dis 2012; 54(3):434-442.
(125) Johnson EL, Howard CL, Thurman J et al.: Cytomegalovirus upregulates expression of CCR5 in central memory cord blood mononuclear cells, which may facilitate in utero HIV type 1 transmission. J Infect Dis 2015; 211(2):187-196.
(126) Srinivasan S, Fredricks DN: The human vaginal bacterial biota and bacterial vaginosis. Interdiscip Perspect Infect Dis 2008; 2008:750479.
(127) Buve A, Jespers V, Crucitti T, Fichorova RN: The vaginal microbiota and susceptibility to HIV. AIDS 2014; 28(16):2333-2344.
(128) Martin DH, Zozaya M, Lillis R, Miller J, Ferris MJ: The microbiota of the human genitourinary tract: trying to see the forest through the trees. Trans Am Clin Climatol Assoc 2012; 123:242-256.
(129) Danielsson D, Teigen PK, Moi H: The genital econiche: focus on microbiota and bacterial vaginosis. Ann N Y Acad Sci 2011; 1230:48-58.
(130) White BA, Creedon DJ, Nelson KE, Wilson BA: The vaginal microbiome in health and disease. Trends Endocrinol Metab 2011; 22(10):389-393.
(131) Sharma H, Tal R, Clark NA, Segars JH: Microbiota and pelvic inflammatory disease. Semin Reprod Med 2014; 32(1):43-49.
(132) Nardis C, Mosca L, Mastromarino P: Vaginal microbiota and viral sexually transmitted diseases. Ann Ig 2013; 25(5):443-456.
(133) Marrazzo JM: Interpreting the epidemiology and natural history of bacterial vaginosis: are we still confused? Anaerobe 2011; 17(4):186-190.
(134) Borgdorff H, Tsivtsivadze E, Verhelst R et al.: Lactobacillus-dominated cervicovaginal microbiota associated with reduced HIV/STI prevalence and genital HIV viral load in African women. ISME J 2014; 8(9):1781-1793.
(135) Mitchell CM, McLemore L, Westerberg K et al.: Long-term effect of depot medroxyprogesterone acetate on vaginal microbiota, epithelial thickness and HIV target cells. J Infect Dis 2014; 210(4):651-655.
(136) Borgdorff H, Verwijs MC, Wit FW et al.: The impact of hormonal contraception and pregnancy on sexually transmitted infections and on cervicovaginal microbiota in african sex workers. Sex Transm Dis 2015; 42(3):143-152.
(137) Guthrie BL, Introini A, Roxby AC et al.: Depot Medroxyprogesterone Acetate Use Is Associated With Elevated Innate Immune Effector Molecules in Cervicovaginal Secretions of HIV-1-Uninfected Women. J Acquir Immune Defic Syndr 2015; 69(1):1-10.
(138) Riggs M, Klebanoff M, Nansel T et al.: Longitudinal association between hormonal contraceptives and bacterial vaginosis in women of reproductive age. Sex Transm Dis 2007; 34(12):954-959.
(139) Frank DN, Manigart O, Leroy V et al.: Altered vaginal microbiota are associated with perinatal mother-to-child transmission of HIV in African women from Burkina Faso. J Acquir Immune Defic Syndr 2012; 60(3):299-306.
(140) Balkus JE, Richardson BA, Rabe LK et al.: Bacterial vaginosis and the risk of trichomonas vaginalis acquisition among HIV-1-negative women. Sex Transm Dis 2014; 41(2):123-128.
(141) Mwapasa V, Rogerson SJ, Kwiek JJ et al.: Maternal syphilis infection is associated with increased risk of mother-to-child transmission of HIV in Malawi. AIDS 2006; 20(14):1869-1877.
(142) Jarvis GA, Chang TL: Modulation of HIV transmission by Neisseria gonorrhoeae: molecular and immunological aspects. Curr HIV Res 2012; 10(3):211-217.

(143) Mbizvo MT, Kasule J, Mahomed K, Nathoo K: HIV-1 seroconversion incidence following pregnancy and delivery among women seronegative at recruitment in Harare, Zimbabwe. Cent Afr J Med 2001; 47(5):115-118.
(144) Munjoma MW, Mhlanga FG, Mapingure MP et al.: The incidence of HIV among women recruited during late pregnancy and followed up for six years after childbirth in Zimbabwe. BMC Public Health 2010; 10:668.
(145) Fernandez L, Langa S, Martin V et al.: The human milk microbiota: origin and potential roles in health and disease. Pharmacol Res 2013; 69(1):1-10.
(146) Mehta S, Manji KP, Young AM et al.: Nutritional indicators of adverse pregnancy outcomes and mother-to-child transmission of HIV among HIV-infected women. Am J Clin Nutr 2008; 87(6):1639-1649.
(147) Borkow G, Weisman Z, Leng Q et al.: Helminths, human immunodeficiency virus and tuberculosis. Scand J Infect Dis 2001; 33(8):568-571.
(148) Shapira-Nahor O, Kalinkovich A, Weisman Z et al.: Increased susceptibility to HIV-1 infection of peripheral blood mononuclear cells from chronically immune-activated individuals. AIDS 1998; 12(13):1731-1733.
(149) Lawn SD, Butera ST, Folks TM: Contribution of immune activation to the pathogenesis and transmission of human immunodeficiency virus type 1 infection. Clin Microbiol Rev 2001; 14(4):753-77, table.
(150) Lustigman S, Prichard RK, Gazzinelli A et al.: A research agenda for helminth diseases of humans: the problem of helminthiases. PLoS Negl Trop Dis 2012; 6(4):e1582.
(151) Kjetland EF, Leutscher PD, Ndhlovu PD: A review of female genital schistosomiasis. Trends Parasitol 2012; 28(2):58-65.
(152) K S, Radhika, R S, K K: Study of helminthiasis in pregnancy and its correlation with haemoglobin level. J Clin Diagn Res 2014; 8(10):OC07-OC09.
(153) Lee AI, Okam MM: Anaemia in pregnancy. Hematol Oncol Clin North Am 2011; 25(2):241-59, vii.
(154) Kefiyalew F, Zemene E, Asres Y, Gedefaw L: Anaemia among pregnant women in Southeast Ethiopia: prevalence, severity and associated risk factors. BMC Res Notes 2014; 7:771.
(155) Baingana RK, Enyaru JK, Tjalsma H, Swinkels DW, Davidsson L: The aetiology of anaemia during pregnancy: a study to evaluate the contribution of iron deficiency and common infections in pregnant Ugandan women. Public Health Nutr 2014;1-13.
(156) Obiezue NR, Okoye IC, Ivoke N, Okorie JN: Gastrointestinal helminth infection in pregnancy: disease incidence and hematological alterations. Iran J Public Health 2013; 42(5):497-503.
(157) Gyorkos TW, Gilbert NL, Larocque R, Casapia M, Montresor A: Re-visiting Trichuris trichiura intensity thresholds based on anaemia during pregnancy. PLoS Negl Trop Dis 2012; 6(9):e1783.
(158) Ndeffo Mbah ML, Kjetland EF, Atkins KE et al.: Cost-effectiveness of a community-based intervention for reducing the transmission of Schistosoma haematobium and HIV in Africa. Proc Natl Acad Sci U S A 2013; 110(19):7952-7957.
(159) Fernandes MA, Batista GI, Carlos JC et al.: Toxoplasma gondii antibody profile in HIV-1-infected and uninfected pregnant women and the impact on congenital toxoplasmosis diagnosis in Rio de Janeiro, Brazil. Braz J Infect Dis 2012; 16(2):170-1174.
(160) Mpairwe H, Tweyongyere R, Elliott A: Pregnancy and helminth infections. Parasite Immunol 2014; 36(8):328-337.
(161) Roka M, Goni P, Rubio E, Clavel A: Intestinal parasites in HIV-seropositive patients in the Continental Region of Equatorial Guinea: its relation with socio-demographic, health and immune systems factors. Trans R Soc Trop Med Hyg 2013; 107(8):502-510.
(162) Finkelstein JL, Mehta S, Duggan CP et al.: Predictors of anaemia and iron deficiency in HIV-infected pregnant women in Tanzania: a potential role for vitamin D and parasitic infections. Public Health Nutr 2012; 15(5):928-937.
(163) Peters BS, Wierzbicki AS, Moyle G, Nair D, Brockmeyer N: The effect of a 12-week course of omega-3 polyunsaturated fatty acids on lipid parameters in hypertriglyceridemic adult HIV-infected patients undergoing HAART: a randomized, placebo-controlled pilot trial. Clin Ther 2012; 34(1):67-76.
(164) Oliveira JM, Rondo PH: Omega-3 fatty acids and hypertriglyceridemia in HIV-infected subjects on antiretroviral therapy: systematic review and meta-analysis. HIV Clin Trials 2011; 12(5):268-274.
(165) Lindsay KL, Gibney ER, McAuliffe FM: Maternal nutrition among women from Sub-Saharan Africa, with a focus on Nigeria, and potential implications for pregnancy outcomes among immigrant populations in developed countries. J Hum Nutr Diet 2012; 25(6):534-546.
(166) Kaiser LL, Campbell CG: Practice paper of the Academy of Nutrition and Dietetics abstract: nutrition and lifestyle for a healthy pregnancy outcome. J Acad Nutr Diet 2014; 114(9):1447.
(167) Helle SI, Ekse D, Holly JM, Lonning PE: The IGF-system in healthy pre- and postmenopausal women: relations to demographic variables and sex-steroids. J Steroid Biochem Mol Biol 2002; 81(1):95-102.
(168) Abdel-Raoufabdel-Aziz AR, Ali DK, Talkhan HM: Pregnancy outcome and the effect of maternal nutritional status. J Egypt Soc Parasitol 2013; 43(1):125-132.
(169) Ezeaka VC, Iroha EO, Akinsulie AO, Temiye EO, Adetifa IM: Anthropometric indices of infants born to HIV-1-infected mothers: a prospective cohort study in Lagos, Nigeria. Int J STD AIDS 2009; 20(8):545-548.
(170) Banda Y, Chapman V, Goldenberg RL et al.: Influence of body mass index on pregnancy outcomes among HIV-infected and HIV-uninfected Zambian women. Trop Med Int Health 2007; 12(7):856-861.
(171) Ramlal RT, Tembo M, Soko A et al.: Maternal mid-upper arm circumference is associated with birth weight among HIV-infected Malawians. Nutr Clin Pract 2012; 27(3):416-421.
(172) Yang Z, Huffman SL: Review of fortified food and beverage products for pregnant and lactating women and their impact on nutritional status. Matern Child Nutr 2011; 7 Suppl 3:19-43.
(173) Myatt M, Khara T, Collins S: A review of methods to detect cases of severely malnourished children in the community for their admission into community-based therapeutic care programs. Food Nutr Bull 2006; 27(3 Suppl):S7-23.
(174) Haider BA, Olofin I, Wang M et al.: Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. BMJ 2013; 346:f3443.
(175) Alzaim M, Wood RJ: Vitamin D and gestational diabetes mellitus. Nutr Rev 2013; 71(3):158-167.
(176) Conrado T, Miranda-Filho DB, Bandeira F: Vitamin D deficiency in HIV-infected individuals: one more risk factor for bone loss and cardiovascular disease? Arq Bras Endocrinol Metabol 2010; 54(2):118-122.
(177) Dror DK: Vitamin D status during pregnancy: maternal, fetal, and postnatal outcomes. Curr Opin Obstet Gynecol 2011; 23(6):422-426.
(178) Arnedo-Pena A, Juan-Cerdan JV, Romeu-Garcia A et al.: Vitamin D status and incidence of tuberculosis among contacts of pulmonary tuberculosis patients. Int J Tuberc Lung Dis 2015; 19(1):65-69.

(179) Rahman S, Rehn A, Rahman J et al.: Pulmonary tuberculosis patients with a vitamin D deficiency demonstrate low local expression of the antimicrobial peptide LL-37 but enhanced FoxP3(+) regulatory T cells and IgG-secreting cells. Clin Immunol 2015; 156(2):85-97.
(180) Selvaraj P, Harishankar M, Afsal K: Vitamin D: Immuno-modulation and tuberculosis treatment. Can J Physiol Pharmacol 2015;1-8.
(181) Kibirige D, Mutebi E, Ssekitoleko R, Worodria W, Mayanja-Kizza H: Vitamin D deficiency among adult patients with tuberculosis: a cross sectional study from a national referral hospital in Uganda. BMC Res Notes 2013; 6(1):293.
(182) Wellinghausen N: Immunobiology of gestational zinc deficiency. Br J Nutr 2001; 85 Suppl 2:S81-S86.
(183) Bennett SE, McPeake J, McCance DR et al.: Maternal vitamin D status in type 1 diabetic pregnancy: impact on neonatal vitamin D status and association with maternal glycaemic control. PLoS One 2013; 8(9):e74068.
(184) Jaskolka D, Retnakaran R, Zinman B, Kramer CK: Sex of the baby and risk of gestational diabetes mellitus in the mother: a systematic review and meta-analysis. Diabetologia 2015.
(185) Erol O, Ellidag HY, Ayik H et al.: Evaluation of circulating betatrophin levels in gestational diabetes mellitus. Gynecol Endocrinol 2015;1-5.
(186) Fu Z, Zhao L, Aylor KW et al.: Angiotensin-(1-7) recruits muscle microvasculature and enhances insulin's metabolic action via mas receptor. Hypertension 2014; 63(6):1219-1227.
(187) Lindstrom K, Lindblad F, Hjern A: Preterm birth and attention-deficit/hyperactivity disorder in schoolchildren. Pediatrics 2011; 127(5):858-865.
(188) Rigo J, Jr., Kecskemeti A, Molvarec A et al.: [233-POS]: Postpartum depression and anxiety in hypertensive disorders of pregnancy. Pregnancy Hypertens 2015; 5(1):117-118.
(189) Wortman AC, Hernandez JS, Holcomb DS et al.: Effect of body mass index on maternal morbidity following peripartum hysterectomy. Clin Obes 2015.
(190) Shapiro RL, Souda S, Parekh N et al.: High prevalence of hypertension and placental insufficiency, but no in utero HIV transmission, among women on HAART with stillbirths in Botswana. PLoS One 2012; 7(2):e31580.
(191) Merchant AT, Msamanga G, Villamor E et al.: Multivitamin supplementation of HIV-positive women during pregnancy reduces hypertension. J Nutr 2005; 135(7):1776-1781.
(192) Kolstad E, Gilhus NE, Veiby G et al.: Epilepsy and eating disorders during pregnancy: Prevalence, complications and birth outcome. Seizure 2015.
(193) Machado ES, Krauss MR, Megazzini K et al.: Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. J Infect 2014; 68(6):572-580.
(194) Takeshita LY, Gonzalez-Galarza FF, dos Santos EJ et al.: A database for curating the associations between killer cell immunoglobulin-like receptors and diseases in worldwide populations. Database (Oxford) 2013; 2013:bat021.
(195) Little K, Thorne C, Luo C et al.: Disease progression in children with vertically-acquired HIV infection in sub-Saharan Africa: reviewing the need for HIV treatment. Curr HIV Res 2007; 5(2):139-153.
(196) Singh KK, Spector SA: Host genetic determinants of human immunodeficiency virus infection and disease progression in children. Pediatr Res 2009; 65(5 Pt 2):55R-63R.
(197) Tiemessen CT, Kuhn L: Immune pathogenesis of pediatric HIV-1 infection. Curr HIV /AIDS Rep 2006; 3(1):13-19.
(198) Huang S, Dunkley-Thompson J, Tang Y et al.: Deficiency of HIV-Gag-specific T cells in early childhood correlates with poor viral containment. J Immunol 2008; 181(11):8103-8111.
(199) Guerini FR, Lo CS, Gori A et al.: Under representation of the inhibitory KIR3DL1 molecule and the KIR3DL1+/BW4+ complex in HIV exposed seronegative individuals. J Infect Dis 2011; 203(9):1235-1239.
(200) Farquhar C, Rowland-Jones S, Mbori-Ngacha D et al.: Human leukocyte antigen (HLA) B*18 and protection against mother-to-child HIV type 1 transmission. AIDS Res Hum Retroviruses 2004; 20(7):692-697.
(201) Moodley S, Bobat R: Expression of HLA-G1 at the placental interface of HIV-1 infected pregnant women and vertical transmission of HIV. Placenta 2011; 32(10):778-782.
(202) Ricci E, Malacrida S, Zanchetta M et al.: Role of beta-defensin-1 polymorphisms in mother-to-child transmission of HIV-1. J Acquir Immune Defic Syndr 2009; 51(1):13-19.
(203) Milanese M, Segat L, Arraes LC, Garzino-Demo A, Crovella S: Copy number variation of defensin genes and HIV infection in Brazilian children. J Acquir Immune Defic Syndr 2009; 50(3):331-333.
(204) Tomescu C, Abdulhaqq S, Montaner LJ: Evidence for the innate immune response as a correlate of protection in human immunodeficiency virus (HIV)-1 highly exposed seronegative subjects (HESN). Clin Exp Immunol 2011; 164(2):158-169.
(205) Refsland EW, Hultquist JF, Luengas EM et al.: Natural Polymorphisms in Human APOBEC3H and HIV-1 Vif Combine in Primary T Lymphocytes to Affect Viral G-to-A Mutation Levels and Infectivity. PLoS Genet 2014; 10(11):e1004761.
(206) Duri K, Soko W, Gumbo F et al.: Genotypic analysis of human immunodeficiency virus type 1 env V3 loop sequences: bioinformatics prediction of coreceptor usage among 28 infected mother-infant pairs in a drug-naive population. AIDS Res Hum Retroviruses 2011; 27(4):411-419.
(207) Melendez-Guerrero LM, Arroyo MA, Vega ME et al.: Characterization of HIV isolates from Puerto Rican maternal-infant pairs reveal predominance of non-syncytium inducing (NSI) variants with CCR5 genotype. Cell Mol Biol (Noisy -le-grand) 2001; 47 Online Pub:OL39-OL47.
(208) Bosch ML, Andeweg AC, Schipper R, Kenter M: Insertion of N-linked glycosylation sites in the variable regions of the human immunodeficiency virus type 1 surface glycoprotein through AAT triplet reiteration. J Virol 1994; 68(11):7566-7569.
(209) Duri K, Gumbo F, Kristiansen K et al.: Phylogenetic analysis of human immunodeficiency virus type 1 subtype C env gp120 sequences among four drug-naive families following subsequent heterosexual and vertical transmissions. AIDS Res Hum Retroviruses 2012; 28(8):885-893.
(210) Duri K GFKKMMRSS-PBaM. HIV-1 Subtype C Envelope (Env) Gp120 Region Amino Acid Length Polymorphism and Potential N Glycosylation Sites (PNGs) Variations; Association with Markers of 6 Long term non-progressing Pediatric Patients. Hebert Open Access Journals: Virology Discovery Journal . 2013.
Ref Type: Journal (Full)
(211) Yoo PD, Shwen HY, Ng J et al.: Hierarchical kernel mixture models for the prediction of AIDS disease progression using HIV structural gp120 profiles. BMC Genomics 2010; 11 Suppl 4:S22.
(212) Zhang H, Hoffmann F, He J et al.: Characterization of HIV-1 subtype C envelope glycoproteins from perinatally infected children with different courses of disease. Retrovirology 2006; 3:73.
(213) Tso FY, Hoffmann FG, Tully DC et al.: A comparative study of HIV-1 clade C env evolution in a Zambian infant with an infected rhesus macaque during disease progression. AIDS 2009; 23(14):1817-1828.

(214) Eaton JW, Hallett TB: Why the proportion of transmission during early-stage HIV infection does not predict the long-term impact of treatment on HIV incidence. Proc Natl Acad Sci U S A 2014; 111(45):16202-16207.
(215) Buchbinder SP, Liu AY: CROI 2014: new tools to track the epidemic and prevent HIV infections. Top Antivir Med 2014; 22(2):579-593.
(216) Braunstein SL, Udeagu CC, Bocour A, Renaud T, Shepard CW: Identifying the correlates of membership in HIV-serodiscordant partnerships in New York City. Sex Transm Dis 2013; 40(10):784-791.
(217) Dennis AM, Hue S, Hurt CB et al.: Phylogenetic insights into regional HIV transmission. AIDS 2012; 26(14):1813-1822.
(218) Nozyce ML, Huo Y, Williams PL et al.: Safety of In Utero and Neonatal Antiretroviral Exposure: Cognitive and Academic Outcomes in HIV-exposed, Uninfected Children 5-13 Years of Age. Pediatr Infect Dis J 2014; 33(11):1128-1133.
(219) Jasseron C, Mandelbrot L, Dollfus C et al.: Non-disclosure of a pregnant woman's HIV status to her partner is associated with non-optimal prevention of mother-to-child transmission. AIDS Behav 2013; 17(2):488-497.
(220) Zaba B, Whitworth J, Marston M et al.: HIV and mortality of mothers and children: evidence from cohort studies in Uganda, Tanzania, and Malawi. Epidemiology 2005; 16(3):275-280.
(221) Fekete EM, Antoni MH, Duran R et al.: Disclosing HIV serostatus to family members: Effects on psychological and physiological health in minority women living with HIV. Int J Behav Med 2009; 16(4):367-376.
(222) Rujumba J, Neema S, Byamugisha R et al.: "Telling my husband I have HIV is too heavy to come out of my mouth": pregnant women's disclosure experiences and support needs following antenatal HIV testing in eastern Uganda. J Int AIDS Soc 2012; 15(2):17429.
(223) Stewart RC, Umar E, Gleadow-Ware S, Creed F, Bristow K: Perinatal distress and depression in Malawi: an exploratory qualitative study of stressors, supports and symptoms. Arch Womens Ment Health 2015; 18(2):177-185.
(224) Villar-Loubet OM, Illa L, Echenique M et al.: Prenatal and mental health care among trauma-exposed, HIV-infected, pregnant women in the United States. J Assoc Nurses AIDS Care 2014; 25(1 Suppl):S50-S61.
(225) Ugwuja EI, Akubugwo EI, Ibiam UA, Obidoa O: Maternal sociodemographic parameters: impact on trace element status and pregnancy outcomes in Nigerian women. J Health Popul Nutr 2011; 29(2):156-162.
(226) Tomlinson M, O'Connor MJ, le Roux IM et al.: Multiple risk factors during pregnancy in South Africa: the need for a horizontal approach to perinatal care. Prev Sci 2014; 15(3):277-282.
(227) Hartley M, Tomlinson M, Greco E et al.: Depressed mood in pregnancy: prevalence and correlates in two Cape Town peri-urban settlements. Reprod Health 2011; 8:9.
(228) De GN, Goldschmidt L, Richardson GA: Prenatal cocaine exposure and age of sexual initiation: direct and indirect effects. Drug Alcohol Depend 2014; 145:194-200.
(229) Bajunirwe F, Bangsberg DR, Sethi AK: Alcohol use and HIV serostatus of partner predict high-risk sexual behavior among patients receiving antiretroviral therapy in South Western Uganda. BMC Public Health 2013; 13:430.
(230) Peltzer K, Mlambo G: Sexual HIV risk behaviour and associated factors among pregnant women in Mpumalanga, South Africa. BMC Pregnancy Childbirth 2013; 13:57.
(231) Heberlein A, Leggio L, Stichtenoth D, Hillemacher T: The treatment of alcohol and opioid dependence in pregnant women. Curr Opin Psychiatry 2012; 25(6):559-564.
(232) Ntaganira J, Muula AS, Siziya S, Stoskopf C, Rudatsikira E: Factors associated with intimate partner violence among pregnant rural women in Rwanda. Rural Remote Health 2009; 9(3):1153.
(233) Ntaganira J, Muula AS, Masaisa F et al.: Intimate partner violence among pregnant women in Rwanda. BMC Womens Health 2008; 8:17.
(234) De SC, Mabunda N, Ferreira OC et al.: High HIV incidence in the postpartum period sustains vertical transmission in settings with generalized epidemics: a cohort study in Southern Mozambique. J Int AIDS Soc 2014; 17:18808.