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Relationship Of Th1/Th2 Cytokine Ratio and Haart Type in Pregnant Women with Intermediate Vaginal Flora in Kisumu, Kenya.

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ABSTRACT

*Most studies have linked protease inhibitor (PI) based highly active antiretroviral therapy (HAART) to spontaneous preterm birth (sPB) without explaining how. We hypothesized that alteration in genital tract flora caused by HAART may cause changes in local cytokine profile which may further result in sPB. The aim of this study was to determine association between Non-efavirenz based HAART, particularly PI based HAART, and Th1/Th2 cytokine ratio in pregnant women with intermediate vaginal flora (IVF) in Kisumu, Kenya. The study was carried out at Lumumba sub county hospital. Willing 76 pregnant women who started HAART on or before the first trimester were enrolled after signing informed consent forms. Using formatted questionnaire participants were interviewed on demographic, clinical and behavioral information. High vaginal swabs for diagnosis of abnormal vaginal flora (AVF), venous blood and lavage for IL 1β and IL 6 assay were collected in the second trimester and at the same time from each participant. Fisher's Exact Chi square test and multinomial logistic regression were used to determine the association of AVF with HAART and participants characteristics. Eleven (11) participants out of the 76 had IVF and in 4 of the 11 there was positive independent association between IVF and Non-efavirenz based HAART. The 4 were cases and the remaining 7 control. Enzyme linked immunoassay (ELISA) was used to assay IL 1β and IL 6 in serum and cervico-vaginal lavage (CVL) in the 11 participants. Th1/Th2 cytokine ratio was taken as ratio of IL 1β to IL 6 concentration and Mann-Whitney U test used for comparison of Th1/Th2 cytokine ratio and concentration of IL 1β and IL 6 in HAART categories. In CVL IL1β concentration was statistically significantly higher in pregnant women on non efavirenz HAART than the pregnant women on efavirenz HAART (*U = *1.00,* p = *0.012). This was replicated in PI based HAART against efavirenz based HAART (*U = *0.00,* p = *0.017). On the other hand Th1/Th2 cytokine ratio in CVL was significantly statistically higher in pregnant women on non efavirenz based HAART than the pregnant women on efavirenz based HAART (U = 3.00, p = 0.042). Which was again replicated in PI based HAART against efavirenz based HAART (U = 0.00, p = 0.017). Since sPB is mostly correlated to local genital biomarkers compared to systemic biomarkers, studying Th1/Th2 cytokine ratio in genital tract may better explain what triggers sPB than studying systemic cytokine profiles.*

Keywords: Intermediate vaginal flora, Highly active antiretroviral therapy, Spontaneous preterm birth, Protease inhibitor (PI), Efavirenz, Th1/Th2 cytokine ratio.

1.0 INTRODUCTION

1.1 Background information

Highly Active Antiretroviral Therapy (HAART) is a recommended treatment for adult HIV-infected population which uses at least 3 antiretroviral drugs(1). Abnormal vaginal flora (AVF) is a condition in which the normal vaginal microflora of Lactobacilli is severely reduced and anaerobic bacteria predominate along with the facultative organism, G. vaginalis and includes BV and IVF (2). Spontaneous preterm birth (sPB) is live birth before 37 weeks of gestation which follows spontaneous preterm labour with or without preterm premature rupture of membrane (PPROM)(3).

The public health burden of preterm birth has been compounded by the fact that preterm birth rate have been reported to be increasing globally (1, 2, 3, 4, and 5) while efforts to stop or reduce its prevalence have been largely futile (5). The use of HAART in pregnancy and breast feeding to prevent mother to child transmission of HIV (8) has further complicated this matter. Studies in developed countries have shown that protease inhibitor (PI) based HAART is associated with preterm birth (9) although with lack of consensus (10). Data from Europe and the UK (10) have shown positive association while that from United States (11), Latin American and the Caribbean (12) as well as a recent meta-analysis of 14 perinatal studies (13) did not show any significant association. Some studies have also reported increased risk of preterm birth (<37 weeks gestational age) with PIs (12, 13, and 7), whereas others have not found this association (12).

In Africa few studies have linked HAART to low birth weight (16) however experience with HAART during pregnancy in Africa has been relatively limited and information about pregnancy outcomes lacking (17). Studies in developing nations have also suggested that association between HAART and adverse pregnancy outcome is not confined to PI-based HAART (18). For example a Ugandan randomized trial comparing lopinavir-ritonavir and efavirenz-based HAART found no significant difference in preterm delivery between regimens (19). However Li et al., 2016 (20) linked efavirenz based HAART to preterm birth. Women who received efavirenz based HAART had higher preterm delivery rates than those receiving nevirapine based HAART (RR 1.45, 95% CI, 1.01–2.07).

Factors associated with preterm birth have been linked to preterm birth through inflammation (21) and markers of inflammation known as biomarkers of preterm birth (22). In the same way attempts have been made to try and link PI based HAART and BV to preterm birth. Fiore *et al.* (23) showed that HAART increases the level of IL-2 (Th1) and decreases the level of IL-10 (Th2) in peripheral blood mononuclear cells (PBMCs) although this shift was not linked to preterm birth (23).

On the other hand a number of studies done in United Kingdom, United States, Finland, Jakarta, Indonesia and Australia have linked BV to preterm birth. This is seen as increased risk of preterm delivery (24) and pregnancy loss or miscarriage between 14 -22 weeks. Bacterial vaginosis is associated with adverse effects in the first, second and third trimester of pregnancy (25).

Bacterial vaginosis may be linked to preterm birth through inflammation. The inflammatory link is seen as increased concentrations of IL-lβ and IL-8 in vaginal fluid of women with BV (26) including pregnant ones (27) or a Th1 shift of local vaginal inflammatory response (28). Bacterial vaginosis has also been shown to cause partial activation of systemic cytokine network (29). Recently a study suggested that HIV does not actually have a major impact on vaginal concentrations of proinflammatory cytokines if the presence of bacterial vaginosis is controlled for (30). A number of studies have shown that HAART have no influence on genital tract inflammation (29, 30, 31, and 32) while preterm birth in human being have been solely link to changes in genital tract inflammation rather than changes in systemic inflammation.

Animal studies have shown that genital local inflammation models mimics more closely what is observed clinically in sPB than systemic illness or inflammation (35). Most preterm labour occur without signs or symptoms of systemic illness. Animal models link systemic inflammation to preterm birth only in cases where there are conditions which increase bacteremia or sepsis like pneumonia or pyelonephritis while most preterm labour occur without significant increases in white blood cell counts, C-reactive protein or temperature (34, 35, and 36). Studies have also demonstrated an association between intrauterine infection or inflammation and preterm birth (37, and 38). Intrauterine inflammation shown by histological chorioamnionitis is evidenced in more than 85% sPB at less than 28 weeks (39, and 34) and about 65% of pregnancies delivered at less than 37 weeks (42).

This study is therefore designed to determine if the link between HAART and sPB could be changes in local genital inflammation due persistent BV during the use PI based HAART therapy.

2.0 MATERIALS AND METHODS

2.1 Study Site

The study was conducted at Lumumba sub county hospital in Kisumu, Kenya. Lumumba Sub County hospital is the Kisumu's busiest municipal maternity facility. FACES is a family-focused, comprehensive HIV prevention, care, and treatment program working collaboratively with the Kenyan Ministries of Health (MOH) to build sustainable HIV care systems including prevention of mother to child transmission of HIV/AIDS. It was launched in Kisumu in March 2005 at Lumumba Health Centre, the now Lumumba Sub County hospital. It therefore has facilities and experienced staff for this study plus being a catchment for a population with high prevalence of HIV and adverse pregnancy outcome.

Kisumu town is within Kisumu County which lies within longitudes 33° 20'E and 35° 20'E and latitudes 0° 20'south and 0° 50'south. The County is bordered by Homa Bay County to the South, Nandi County to the North East, Kericho County to the East, Vihiga County to the North West and Siaya County to the West. The County covers a total land area of 2,009.5 km^2 and another 567 km^2 covered by water. The catchment area for this facility is Kisumu county and to a lesser extent the neighboring counties.

The prevalence of HIV in this region is the highest in Kenya and more than doubles the national average of about 6%. The prevalence of each county is as follows: Homa bay 20.7%, Siaya 21.0%, Kisumu 16.3% and Migori13.3%. Approximately 50% of all adults living with HIV in the country are in Nairobi, Homa Bay, Kisumu, Siaya, Migori, Kiambu, Kakamega and Mombasa counties while 50% children 0-14 years living with HIV are from 7 out of the 47 counties, namely, Homa Bay, Siaya, Kisumu, Nairobi, Migori, Kakamega and Nakuru. (43). As early as 1987 Premature labour accounted for 55.3% of the 15.0% incidence of LBW in Nyanza Provincial General Hospital, Kisumu (44). Nyanza Provincial General Hospital is the now Jaramogi Oginga Odinga Teaching and Refferal Hospital and with the rising prevalence of

adverse pregnancy outcomes this figure could be much higher now.

2.2 Study Population

The study recruited expectant female participants, aged 18 to 39 years, seeking care at Lumumba sub county hospital under FACES program. FACES is a family-focused, comprehensive HIV prevention, care, and treatment program working collaboratively with the Kenyan Ministries of Health (MOH) to build sustainable HIV care systems including prevention of mother to child transmission of HIV/AIDS. It was launched in Kisumu in March 2005 at Lumumba Health Centre, now Lumumba sub county hospital. Lumumba sub county hospital is the Kisumu's busiest maternity facility.

2.3 Inclusion Criteria

Pregnant women who initiated HAART on or before first trimester and were willing to participate in the study.

2.4 Exclusion criteria

Must not be suffering from any conditions associated with adverse infant outcome like cardiovascular conditions, obstetric risk factors and factors which could influence the level of interleukins in the serum and genital tract other that the factors being investigated.

2.5 Study Design

Willing 76 pregnant women, aged 18 to 39 years, and started HAART on or before the first trimester were enrolled after signing informed consent forms. Using formatted questionnaire participants were interviewed on demographic, clinical and behavioral information. High vaginal swab for diagnosis of AVF, venous blood and lavage for ELISA were collected in the second trimester and at the same time from each participant. Fisher's Exact Chi square test and multinomial logistic regression were used to determine the association of HAART and participants characteristics with AVF as reported in Awiti et al 2022(45). Eleven (11) participants out of the 76 had IVF and in 4 of the 11 there was positive independent association between IVF and Non-efavirenz based HAART. Since the aim of this study was to determine the association between Non-efavirenz based HAART, particularly PI based HAART, and Th1/Th2 cytokine ratio in pregnant women with IVF in Kisumu, Kenya we identified the remaining 7 with IVF as control while the 4 participants remained the cases. ELISA was used to assay IL 1β and IL 6 in serum and CVL in the 11 participants. Th1/Th2 cytokine ratio was determined as explained in Rhodus et al. 2007(46) while Mann-Whitney U test was used for comparison of CVL and serum IL 1β, IL 6 and Th1/Th2 cytokine ratio in the HAART categories.

2.6 Study Procedures

2.6.1 Sample collection and processing

Lavage and venipuncture were performed on the same day higher vaginal swab for the diagnosis of AVF by Gram stain (Nugent's scoring system) was performed.
Lavage was performed using10 ml of RPMI 1640 medium into the vagina, allowing one minute pooling and aspirating the suspension. Presence of blood contaminants was tested using reactive strips, Bayer, multistic-10 visual; while the absence of red blood cells and sperm was confirmed using a microscope (47).

Venipuncture was used to collect blood using heparin as an anticoagulant. The lavage fluid was centrifuged for 10 min at 80g and the supernatant collected. The blood sample was also centrifuged to obtain serum. The supernatant and serum were cryopreserved in liquid nitrogen at UNIM in Lumumba Sub county hospital until use. The supernatant of CVL and serum was used to assay IL 1β and IL 6.

2.6.2 Assay of supernatants and serum for IL-1β and IL-6 by ELISA assay

The supernatant and serum were used for the measurement of IL-1β and IL-6 levels in CVL and blood serum. Measurement of IL-1β and IL-6 levels was done via an ELISA kit (Dldevelop © 2016; A3-South, 100#; shuigoutou,renminxi; Rd,Wuxi,Jiangsu,214031,PRC. www.dldevelop.com) as per the manufacturer's instructions. The optical densities were read using the Multiskan™ FC Microplate Photometer Thermofisher scientific *(*168 Third Avenue. Waltham, MA USA).

Briefly, 7 wells for diluted standard, 1 well for blank and remaining wells for samples were determined in pre-coated 96-well strip plate. Into appropriate wells 100µL each of standard, blank and samples were added sealed and incubated at 37^0C for 2 hours. Liquid was removed from each well without washing and 100µL of detection reagent A working solution (1:100) added to each well and incubated for 1 hour at 37^0C. This was followed by 3 times aspiration/washing using 300µL of 1 x wash solution. Addition of 100µL of detection reagent B working solution (1:100) to each well then followed, incubated for 1 hour at 37^0C. After 5 times aspiration/washing using 300µL of 1 x wash solution 90µL of substrate solution was added to each well and incubated for 15-25 minutes at 37^0C. Finally 50µL of stop solution was added to each well and OD measurements taken at 450nm.

The duplicate readings for each standard, control and sample were averaged, then the average zero standard optical density subtracted. A standard curve was then constructed for each run by plotting the mean O.D. and concentration for each standard and a best fit curve drawn through the points, where the sample reading

was outside the standard curve, the sample was serially diluted to obtain values in the range of the reference curve.

The intra and inter-assay coefficients of variation for the IL-1β assay were <10% and <12%, and for the IL-6 assay were <10% and <12% respectively. The lower detection limit for human IL-1β was 31.2 pg/mL and for IL-6 it was 7.81 pg/mL; a zero value was assigned to samples below these limits, but there was no sample which had value below detection limit (48 and 49).

2.6.3 Determination of Th1/Th2

Th1/Th2 cytokine ratio was determined as explained in Rhodus et al. 2007(46). Briefly, after taking the average concentration of IL-1β and IL-6 in CVL and serum, the concentration of IL 1β was divided by that of IL 6.

2.7 Ethical Consideration

2.7.1 Benefits

The benefit to the participants included free laboratory diagnosis of BV by Nugents criteria as per the guidelines by the Ministry of Health.

2.7.2 Potential discomforts, inconveniences, injuries, harm, risks or stress.

Potential risks to subjects included the discomfort from the pelvic examinations (insertion of the speculum) and/or discomfort during the interview due to the sensitive nature of the questions including loss of privacy, but safeguards was implemented to minimize this risk. Extreme care was taken to minimize patient discomfort during high vaginal swabs procedures which involves insertion of the speculum. Procedures for minimizing risk and discomfort from the interview included using well trained clinicians with a lot of experience in STI clinics to establish rapport with the subjects and to place them at ease during the interview. Well trained and experienced laboratory staff help in reducing the risk during pelvic procedures. The participants were told to skip any questions they did not want to answer and terminate the interview at any time without any consequence.

2.7.3 Confidentiality

All records were identified by codes only, to maintain participant confidentiality; participant's names or other personal identifiers were not recorded. Completed study forms were kept in locked files, in an access-limited room at the study site. The participants were given opportunity to review the records and delete any portion they were not comfortable with. Only the investigator had access to personal identifiers and all questionnaires were stored in locked cabinets. Passwords and back-up ensured limited access to the computer containing data on participants.

2.7.4 Participation

Participants who were willing to take part in the study signed informed consent forms. Participants were allowed to decline participation or withdraw from the study at any time without penalty or loss of benefits to which they were otherwise entitled to. Ethical clearance was obtained from Maseno University Ethics and Review Committee.

2.8 Data Management

2.8.1 Data entry

Date entry was done by manual double entry in SPSS version 20 (SPSS inc., Chicago, IL) program for window.

2.8.2 Data analysis.

Data was analysed using SPSS version 20.0 (SPSS inc., Chicago, IL) program for windows. Demographic and behavioral data were compared between participants on efavirenz and non efavirenz based HAART among those having IVF by Fisher's exact chi square test and Mann-Whitney U test, where appropriate. Mann-Whitney U test was also used to compare IL 1β, IL 6 and Th1/Th2 cytokine ratio between participants on efavirenz and non efavirenz based HAART.

3.0 RESULTS

Only 11 participants, out of 76, who were diagnosed with IVF were included in this analysis, four who showed positive independent association between non-efavirenz based HAART and IVF were cases while the remaining seven were the control.

3.1 Participant's characteristics

The demographic and behavioral characteristics of the two groups of women, as well as the relevant P values, are shown in table 3.1. There was no significant difference among the two groups with regard to any characteristics. Moreover all the 11 participants were Christians, non-smokers, not on antibiotic at the time of specimen collection, not taking alcohol and not using water to clean their anus after defecation.

3.2 Cervicovaginal and serum IL 1β and IL 6 in non efavirenz and efavirenz based HAART among pregnant women with IVF in Kisumu, Kenya

Cervicovaginal and serum concentration of IL 1β and IL 6 was determined by ELISA. In cervico-vaginal fluid IL1β concentration was statistically significantly higher in pregnant women on non efavirenz than the pregnant women on efavirenz ($U = 1.00, p = 0.012$) while in serum IL1β concentration was higher in pregnant women on efavirenz than the pregnant women on non efavirenz although it was not statistically significant ($U = 11.00, p = 0.648$). In cervico-vaginal fluid IL6 concentration was equally higher in those on non efavirenz than those on efavirenz however it did not

reach statistical significance ($U = 11.00$, $p = 0.648$) while in serum IL6 concentration was equally higher in those on non efavirenz than those on efavirenz however it did not reach statistical significance ($U = 8.00$, $p = 0.315$) (Table 3.2).

Table 3.1: Participant's characteristics between Non efavirenz and Efavirenz HAART categories

Variable	Category	HAART category Count (% within each row variable)		P value (Fisher's exact/ Mann Witney U test)
		Non efavirenz	Efavirenz	
Demographic factors				
Age		7.38	5.21	0.339
Age category	Upto 24	0(0.0)	1(100.0)	1.000
	25 to 34	3(37.5)	5(62.5)	
	35 and above	1(50.0)	1(50.0)	
Education	Below secondary	3(37.5)	5(62.5)	1.000
	Upto secondary	1(33.3)	2(66.7)	
Occupation	House wife	1(33.3)	2(66.7)	1.000
	Self employed	1(33.3)	2(66.7)	
	Educators	1(50.0)	1(50.0)	
	Unemployed	0(00.0)	1(100.0)	
	Others	1(50.0)	1(50.0)	
Marital status	Single	0(00.0)	1(100.0)	1.000
	Married	3(37.5)	5(62.5)	
	Others	1(50.0)	1(50.0)	
Last birth	Successful	2(25.0)	6(75.0)	0.491
	Not successful	2(66.7)	1(33.3)	
Medical history				
Start HAART	Before conception	4(40.0)	6(60.0)	1.000
	After conception	0(0.0)	1(100.0)	
Life style behavior				
Sex partner in the last two months	One	2(28.6)	5(71.4)	0.576
	None	2(50.0)	2(50.0)	
Vaginal douching	Earlier than two weeks to specimen collection	0(00.0)	1(100.0)	1.000
	This week	0(00.0)	1(100.0)	
	Never	4(44.4)	5(55.6)	
Dry sex	Yes	0(00.0)	1(100.0)	1.000
	No	4(40.0)	6(60.0)	
Clinical history				
Candiasis	Positive	0(00.0)	1(100.0)	1.000
	Negative	4(40.0)	6(60.0)	

Table 3.2: Cervicovaginal and serum IL 1β and IL 6 in non efavirenz and efavirenz based HAART among pregnant women with IVF in Kisumu, Kenya

Variables	Category	HAART category	N	Mean Rank	U	P
IL 1β concentration	CVL	Non efavirenz based HAART	4	9.25	1.00	0.012
		Efavirenz based HAART	7	4.14		
	Serum	Non efavirenz based HAART	4	5.25	11.00	0.648
		Efavirenz based HAART	7	6.43		
IL 6 concentration	CVL	Non efavirenz based HAART	4	6.75	11.00	0.648
		Efavirenz based HAART	7	5.57		
	Serum	Non efavirenz based HAART	4	7.50	8.00	0.315
		Efavirenz based HAART	7	5.14		

3.3 Cervicovaginal and serum IL 1β and IL 6 in PI and efavirenz based HAART among pregnant women with IVF in Kisumu, Kenya

Table 3.3: Cervicovaginal and serum IL 1β and IL 6 in PI and efavirenz based HAART among pregnant women with IVF in Kisumu, Kenya

Variables	Category	HAART category	N	Mean Rank	U	P
IL 1β concentration	CVL	PI based HAART	3	9.00	0.00	0.017
		Efavirenz based HAART	7	4.00		
	Serum	PI based HAART	3	5.33	10.00	1.00
		Efavirenz based HAART	7	5.57		
IL 6 concentration	CVL	PI based HAART	3	6.00	9.00	0.833
		Efavirenz based HAART	7	5.29		
	Serum	PI based HAART	3	6.67	7.00	0.517
		Efavirenz based HAART	7	5.00		

In cervico-vaginal fluid IL1β concentration was statistically significantly higher in pregnant women on PI than the pregnant women on efavirenz (U = 0.00, p = 0.017) while in serum IL1β concentration was higher in pregnant women on efavirenz than the pregnant women on PI although it was not statistically significant (U = 10.00, p = 1.000)

In cervico-vaginal fluid IL 6 concentration was equally higher in those PI than those on efavirenz however it did not reach statistical significance (U = 9.00, p = 0.833) while in serum IL6 concentration was equally higher in those on PI than those on efavirenz however it did not reach statistical significance (U = 7.00, p = 0.517).

3.4 Cervicovaginal and serum Th1/Th2 cytokine ratio in non efavirenz and efavirenz based HAART among pregnant women with IVF in Kisumu, Kenya.

Th1/Th2 cytokine ratio was determined as explained in Rhodus et al. 2007(46). Briefly, the average concentration of IL 1β was divided by that of IL 6.

Table 3.4: Cervicovaginal and serum Th1/Th2 cytokine ratio in non efavirenz and efavirenz based HAART among pregnant women with IVF in Kisumu, Kenya

Variables	Categories	HAART category	N	Mean Rank	U	P
Th1/Th2 cytokine ratio	CVL	Non efavirenz based HAART	4	8.75	3.00	0.042
		Efavirenz based HAART	7	4.43		
	Serum	Non efavirenz based HAART	4	4.75	9.00	0.412
		Efavirenz based HAART	7	6.71		

In CVL Th1/Th2 cytokine ratio was significantly statistically higher in pregnant women on non efavirenz based HAART than the pregnant women on efavirenz based HAART (U = 3.00, p = 0.042). In serum Th1/Th2 cytokine ratio was higher in pregnant women on efavirenz based HAART than the pregnant women on non efavirenz based HAART however it did not reach statistical significance (U = 9.00, p = 0.412).

3.5 Cervicovaginal and serum Th1/Th2 cytokine ratio in PI and efavirenz based HAART among pregnant women with IVF in Kisumu, Kenya.

Table 3.5: Cervicovaginal and serum Th1/Th2 cytokine ratio in PI and efavirenz based HAART among pregnant women with IVF in Kisumu, Kenya

Variables	Category	HAART category	N	Mean Rank	U	P
Th1/Th2 cytokine ratio	CVL	PI based HAART	3	9.00	0.00	0.017
		Efavirenz based HAART	7	4.00		
	Serum	PI based HAART	3	4.67	8.00	0.667
		Efavirenz based HAART	7	5.86		

In CVL Th1/Th2 cytokine ratio was significantly statistically higher in pregnant women on PI based HAART than the pregnant women on efavirenz based HAART (U = 0.00, p = 0.017). In serum Th1/Th2 cytokine ratio was higher in pregnant women on efavirenz based HAART than the pregnant women on PI based HAART however it did not reach statistical significance (U = 8.00, p = 0.667).

4.0 DISCUSSION

HIV infection impairs body's immune functions by lowering systemic Th-1 and raising systemic Th-2 cytokine (42, and 43) . HAART restores this balance thereby modulating systemic cytokines. (44, and 45).
However HAART has no effect on genital levels of inflammatory markers (31) and usually fails at completely normalizing systemic immune activation (46, and 47). Studies have shown that systemic proinflammatory cytokine have short half-life (32) and therefore may not reach the uterus leave alone the lower genital tract. Other studies have also shown that the systemic proinflammatory cytokines do not cross the placenta (31, and 32).

The source of local female genital inflammation must therefore be the genital tract itself. Studies have shown that foetus or infants may be the source of these cytokines (56) as a response to hostile intrauterine environment like inflammation in chorioamniotic membranes and decidua (49, 50, and 51). It has been shown that even the very preterm infants are capable of developing Th1 and Th2 response (60). But these are possible cases in the absence of HAART therapy.

HAART is supposed to normalize inflammatory response (44, and 45) after imbalance caused by HIV (42, and 43), however there are several studies showing persistent local genital inflammation even when participants are on HAART. Mirmonsef et al linked such persistent genital inflammation despite HAART therapy to chronic bacterial vaginosis (61) likely to be left untreated because most women usually remain asymptomatic or due to inappropriate practices promoting local irritation, inflammation and loss of balance of the vaginal microbiota (62), such as vaginal cleansing (63).

However there are instances where inflammation in genital tract during HAART therapy has been associated with some HAART categories and not others. For example PI based HAART has been associated with local female genital inflammation during HAART therapy as opposed to others like nucleoside reverse transcriptase inhibitors (64). Highly active antiretroviral therapy enters the extravascular compartment by facilitated diffusion, active transport and passive diffusion. Passive diffusion is less efficient than active transport or facilitated diffusion. Since protease inhibitor's high protein affinity does not allow it to pass through biological membranes it uses passive diffusion. Protease inhibitor is therefore likely to achieve low concentrations in biological fluids, compared to others like nucleoside reverse transcriptase inhibitors which uses facilitated diffusion or active transport (65).

Low concentration of PI in vaginal fluid may make it less effective in having impact in the vaginal microbiome (64). Specific combinations of HAART have been shown to have antimicrobial properties which may result to dramatic effects on microbiome while others are catabolized by microbes mainly bacteria (66). The possible explanation therefore is: PI based HAART is not metabolized by bacteria but has antimicrobial effects since its concentration in genital tract is negatively associated with BV. Some female genital tract microbiomes have also been found to be associated with low concentration of vaginal antiretroviral concentrations (67). From this we can concur with Ondoa et al that most inflammations in female genital tract may not primarily be related to genital tract viral load (31). Marques et al actually observed an increased frequency of BV in women on PI based HAART with undetectable viral load (64).

Bacterial vaginosis has been found to alter local cervico-vaginal cytokine rather than systemic cytokine. Studies have shown increased concentrations of IL-lβ and IL-8 in vaginal fluid of women with BV (26) including pregnant ones (27) but only partial activation of systemic cytokine network (29). Recently a study suggested that HIV does not actually have a major impact on vaginal concentrations of proinflammatory

cytokines if the presence of bacterial vaginosis is controlled for (30).

In the current study we found statistically significant elevated level of IL1β in cervico-vaginal fluid and not serum in pregnant women on non efavirenz compared to those on efavirenz. This was replicated in pregnant women on PI compared to those on efavirenz. IL1β concentration was statistically elevated in cervico-vaginal fluid and not serum in pregnant women on PI compared to those on efavirenz. The difference in the level of IL1β in serum and IL 6 in both serum and CVL remained statistically non-significant in all the groups.
Our finding is not unique since studies have shown that correlates of BV and intermediate vaginal flora may be the same (68) .The elevated IL1β level in CVL could then be due to intermediate vaginal flora arising from use of non efavirenz based HAART manly PI based HAART. In this group of participants intermediate vaginal flora was positively statistically significantly associated with non efavirenz based HAART mainly PI (45).

IL–1beta is a member of the IL–1 family, which includes the classical IL–1alpha (IL–1F1) and IL–1beta (IL–1F2) cytokines. IL–1alpha is mainly intracellular, whereas IL–1beta can be secreted outside cells in the extracellular fluids, thus it is commonly found in serum and secretions. IL–1β plays a prominent role in the regulation of the inflammatory response (69).

Both IL-1 cytokines activate T-helper cells to produce and liberate IL-2 which is a cytokines of the Th1-type which enhance cell mediated immune responses (28).
Various reports have also demonstrated elevated levels of local IL1β in women with BV or abnormal vaginal microflora in pregnancy (64,66,67 and 68). However Wasiela et al (72)found out that IL 6 concentration was high in women with BV than those with normal vaginal flora although it did not reach statistical significant. Hedges et al found significantly higher concentration of IL1β in vaginal wash of women with either intermediate vaginal flora or BV than did women with normal flora (68).

Several studies have actually associated IL-1β with bacterial vaginosis (26). A study showed that levels of IL-4, IL-6, and IL-10 showed no significant difference compared to control participants after 12 months of therapy ($P > 0.05$) (52). Our study equally found out non significant difference in IL 6 concentration between those on efavirenz and those on non efavirenz. Some observations have indicated that addressing the role of individual cytokines during immune response may be far more informative than using the blanket Th1/Th2 descriptors (74). Yet some observations have shown the importance determining Th1/th2 cytokine ratio shifts instead of the traditional Th1/Th2 ratio by flow cytometry (26, and 41). Since we collected lavage instead of cells using cytobrush it would be easy to determine Th1/th2 cytokine ratio using ELISA instead of the traditional Th1/Th2 ratio by flow cytometry. Studying Th1/Th2 cytokine ratio has also become relevant because of the discovery of some cytokines which are not secreted by CD4+ T cells but promoted the development of either Th1 or Th2 cells (74). For example, TNF-alpha, IL-1, and IL-8 as Th1- associated cytokines and IL-6 as Th2-associated cytokines have been assigned in some investigations (67 , and 68).
Studying role of individual cytokines and Th1/Th2 cytokine ratio rather the traditional Th1/Th2 ratio may provide accurate information on pathway that trigger preterm birth since local vaginal environment may be more linked to uterine environment than systemic environment which has a control at the blood vascular barrier.

After determining the level of IL-lβ and IL 6 by ELISA, we proceeded to determine Th1/Th2 cytokine ratio as explained in Rhodus et al. 2007(46).

We found statistically significantly higher Th1/Th2 cytokine ratio in cervico-vaginal fluid and not serum in pregnant women on non efavirenz compared to those on efavirenz. This was replicated in pregnant women on PI compared to those on efavirenz. Th1/Th2 cytokine ratio was statistically significantly higher in cervico-vaginal fluid and not serum in pregnant women on PI compared to those on efavirenz. The difference in magnitude of Th1/Th2 cytokine ratio remained statistically non-significant in serum.

Fiore *et al.* (23) had shown that HAART increases the level of IL-2 (Th1) and decreases the level of IL-10 (Th2) in PBMCs although this shift was not linked to preterm birth (23). This could have been due to the fact that systemic cytokines do not cross the placenta(31, 32) or have short half life and therefore cannot reach the uterus (32). This study did not even show the influence of HAART on genital microbiota.

We have shown that determining Th1/Th2 ratio in genital tract is not easy because of the type of sample easily collected in the lower genital tract. Lavage may contain very few immune cells for flow cytometry.
Although there are few studies so far on cervico-vaginal Th1/Th2 cytokine ratio Anton et al showed a Th1 shift of local vaginal inflammatory response during BV infection in participants who were not on HAART therapy (28). Since sPB is mostly correlated to local genital biomarkers compared to systemic biomarkers studying Th1/Th2 cytokine ratio in genital tract may better explain what triggers sPB than studying systemic cytokine profiles. Local cytokine profile is associated with abnormal vaginal flora (AVF) (45). So by treating AVF we will reduce local cytokine profile thus reducing sPB.

Limitations:

The limitations of this study included the small sample size which was attributable to our reliance on convenience sampling, little finance and the fact that non efavirenz HAART is not preferred in developing nations. Long storage may have also affected the outcome. Long storage was occasioned by COVID 19 outbreak which raised the cost of kits because of shipping restriction during COVID 19. COVID 19 emerged just immediately after my data collection and this forced me to store samples for long to wait for shipping to normalize and cost of reagents and kits to come down.

REFERENCES

1. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach. 2010 Revision. Heal (San Fr. 2010;

2. Hill GB. The microbiology of bacterial vaginosis. Am J Obstet Gynecol. 1993;169(2 PART 2):450–4.

3. Lumley J. Defining the problem: The epidemiology of preterm birth. BJOG An Int J Obstet Gynaecol. 2003;110(SUPPL. 20):3–7.

4. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, et al. The worldwide incidence of preterm birth: A systematic review of maternal mortality and morbidity. Bull World Health Organ. 2010;88(1):31–8.

5. Goldenberg R, Culhane J, Iams J, Romero R. Epidemiology and causes of preterm birth. 2008. p. 75–84.

6. Plunkett J, Muglia LJ. Genetic contributions to preterm birth: Implications from epidemiological and genetic association studies. Ann Med. 2008;40(3):167–79.

7. Menon R. Spontaneous preterm birth, a clinical dilemma: Etiologic, pathophysiologic and genetic heterogeneities and racial disparity. Acta Obstet Gynecol Scand. 2008;87(6):590–600.

8. Arendt V, Ndimubanzi P V. AMATA study: effectiveness of antiretroviral therapy in breastfeeding mothers to prevent post-natal vertical transmission in Rwanda [TuAX102]. In: Program and abstracts of the 4th IAS Conference on HIV Pathogenesis,Treatment and Prevention. Sydney, Australia: International AIDS Society; 2007.

9. Cotter AM, Garcia AG, Duthely ML, Luke B, O'Sullivan MJ. Is antiretroviral therapy during pregnancy associated with an increased risk of preterm delivery, low birth weight, or stillbirth? J Infect Dis. 2006;193(9):1195–201.

10. Thorne C, Patel D, Newell ML. Increased risk of adverse pregnancy outcomes in HIV-infected women treated with highly active antiretroviral therapy in Europe. Aids. 2004;18(17):2337–9.

11. Tuomala R, Shapiro D, Mofenson L, Bryson Y, Culnane M, Hughes M, et al. Antiretroviral Therapy During Pregnancy and The Risk of an Adverse Outcome. N Engl J Med. 2002;346(24):1863–70.

12. Szyld EG, Warley EM, Freimanis L, Gonin R, Cahn PE, Calvet GA, et al. Maternal antiretroviral drugs during pregnancy and infant low birth weight and preterm birth. Aids. 2006;20(18):2345–53.

13. Kourtis A, Schmid C, Jamieson D, Lau J. Use of antiretroviral therapy in pregnant HIV-infected women and the risk of premature delivery: A meta-analysis. Aids. 2007;21(13):1831–2.

14. Thorne C, Rudin C, Newell ML, Kind C, Hug I, Gray L, et al. Combination antiretroviral therapy and duration of pregnancy. Aids. 2000;14(18):2913–20.

15. European Collaborative Study I of child H university C of LUK. Exposure to Antiretroviral Therapy in Utero or Early Life: the Health of Uninfected Children Born to HIV - Infected Women. JAIDS Volume 3 No 4; 2003.

16. Ekouevi DK, Coffie PA, Becquet R, Tonwe-Gold B, Horo A, Thiebaut R, et al. Antiretroviral therapy in pregnant women with advanced HIV disease and pregnancy outcomes in Abidjan, Côte d'Ivoire. Aids. 2008;22(14):1815–20.

17. Masaba R, Ndivo R, Nyangau I, Achola K, Zeh C, Thigpen M. Comparison of adverse fetal outcomes in HIV-1-infected antiretroviral-naive pregnant women who have received combivir and either nevirapine or nelfinavir for prevention of mother-to-child transmission antenatally. Boston, USA [abstract 640]; 2008. p. 3–6.

18. Chen JY, Ribaudo HJ, Souda S, Parekh N, Ogwu A, Lockman S, et al. Highly active antiretroviral therapy and adverse birth outcomes among HIV-infected women in botswana. J Infect Dis. 2012;206(11):1695–705.

19. Koss CA, Natureeba P, Plenty A, Luwedde F, Mwesigwa J, Ades V, et al. Risk factors for preterm birth among HIV-infected pregnant ugandan women randomized to lopinavir/ritonavir-or efavirenz-based antiretroviral therapy. J Acquir Immune Defic Syndr. 2014;67(2):128–35.

20. Li N, Sando MM, Spiegelman D, Hertzmark E, Liu E, Sando D, et al. Antiretroviral therapy in relation to birth outcomes among HIV-infected women: A cohort study. J Infect Dis. 2016;213(7):1057–64.

21. Goldenberg RL, Culhane JF, Johnson DC. Maternal infection and adverse fetal and neonatal outcomes. Clin Perinatol. 2005;32(3):523–59.

22. Chan RL. Biochemical markers of spontaneous preterm birth in asymptomatic women. Biomed Res Int. 2014;2014.

23. Fiore S, Newell ML, Trabattoni D, Thorne C, Gray L, Savasi V, et al. Antiretroviral therapy-associated modulation of Th1 and Th2 immune responses in HIV-infected pregnant women. J Reprod Immunol. 2006;70(1–2):143–50.

24. Hay P, Ugwumadu A, Jeffrey I, Manvonda J. Oral clindamycin prevents spontaneous preterm birth and mid trimester miscarriage in pregnant women with bacterial vaginosis. Int J STD AIDS; 2001. p. 70–1.

25. Romero R, Gómez R, Chaiworapongsa T, Conoscenti G, Kim JC, Kim YM. The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol. 2001;15(SUPPL. 2):41–56.

26. Sturm-Ramirez K, Gaye-Diallo A, Eisen G, Mboup S, Kanki PJ. High levels of tumor necrosis factor-α and interleukin-1β in bacterial vaginosis may increase susceptibility to human immunodeficiency virus. J Infect Dis. 2000;182(2):467–73.

27. Platz-Christensen JJ, Mattsby-Baltzer I, Thomsen P, Wiqvist N. Endotoxin and interleukin-1α in the cervical mucus and vaginal fluid of pregnant women with bacterial vaginosis. Am J Obstet Gynecol. 1993;169(5):1161–6.

28. Anton G, Rid J, Mylonas I, Friese K, Weissenbacher ER. Evidence of a TH1-shift of local vaginal inflammatory response during bacterial vaginosis. Infection. 2008;36(2):147–52.

29. Bogavac M, Brkić S, Simin N, Grujić Z, Božin B. Do bacterial vaginosis and chlamydial infection affect serum cytokine level? Srp Arh Celok Lek. 2010;138(7–8):444–8.

30. Mitchell CM, Balkus J, Agnew KJ, Cohn S, Luque A, Lawler R, et al. Bacterial vaginosis, not HIV, is primarily responsible for increased vaginal concentrations of proinflammatory cytokines. AIDS Res Hum Retroviruses. 2008;24(5):667–71.

31. Ondoa P, Gautam R, Rusine J, Lutter R, Jurriaans S, Kootstra N, et al. Twelve-month antiretroviral therapy suppresses plasma and genital viral loads but fails to alter genital levels of cytokines, in a cohort of HIV-Infected rwandan women. PLoS One. 2015;10(5):1–16.

32. Vedder H, Schreiber W, Yassouridis A, Gudewill S, Galanos C, Pollmächer T. Dose-dependence of bacterial lipopolysaccharide (LPS) effects on peak response and time course of the immune-endocrine host response in humans. Inflamm Res. 1999;48(2):67–74.

33. Reisenberger K, Egarter C, Kapiotis S, Sternberger B, Gregor H, Husslein P. Transfer of erythropoietin across the placenta perfused in vitro. Obstet Gynecol. 1997;89(5):738–42.

34. Aaltonen R, Heikkinen T, Hakala K, Laine K, Alanen A. Transfer of proinflammatory cytokines across term placenta. Obstet Gynecol. 2005;106(4):802–7.

35. Elovitz MA, Mrinalini C. Animal models of preterm birth. Trends Endocrinol Metab. 2004;15(10):479–87.

36. Yoon BH, Romero R, Park JS, Kim CJ, Kim SH, Choi JH, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. Am J Obstet Gynecol. 2000;182(3):675–81.

37. Turhan NÖ, Karabulut A, Adam B. Maternal serum interleukin 6 levels in preterm labor: Prediction of admission-to-delivery interval. J Perinat Med. 2000;28(2):133–9.

38. Yoon BH, Jun JK, Park KH, Syn HC, Gomez R, Romero R. Serum C-reactive protein, white blood cell count, and amniotic fluid white blood cell count in women with preterm premature rupture of membranes. Obstet Gynecol. 1996;88(6):1034–40.

39. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. 2000;

40. Gonçalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev. 2002;8(1):3–13.

41. Yoon BH, Romero R, Moon J Bin, Shim SS, Kim M, Kim G, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 2001;185(5):1130–6.

42. Üstün C, Koçak I, Bariş S, Uzel A, Saltik F. Subclinical chorioamnionitis as an etiologic factor in preterm deliveries. Int J Gynecol Obstet. 2001;72(2):109–15.

43. National AIDS Control Council, National AIDS Control Council (NACC). Kenya HIV Estimates Report 2018. Kenya HIV Estim. 2018;1–28.

44. Were EO, Karanja JK. Low birth weight deliveries at the Nyanza General Hospital Kisumu, Kenya. 1994;10(71):667–70.

45. Awiti CA, Benard G, Majiwa M. Intermediate vaginal flora is associated with highly active antiretroviral therapy in HIV positive pregnant women on highly active antiretroviral therapy in Kisumu, Kenya. 2022;

46. Rhodus NL, Cheng B, Ondrey F. Th1/Th2 cytokine ratio in tissue transudates from patients with oral lichen planus. Mediators Inflamm. 2007;2007:15–8.

47. Zara F, Nappi RE, Brerra R, Migliavacca R, Maserati R, Spinillo A. Markers of local immunity in cervico-vaginal secretions of HIV infected women: Implications for HIV shedding. Sex Transm Infect. 2004;80(2):108–12.

48. Cauci S, Guaschino S, Driussi S, De Santo D, Lanzafame P, Quadrifoglio F. Correlation of local interleukin-8 with immunoglobulin A against Gardnerella vaginalis hemolysin and with prolidase and sialidase levels in women with bacterial vaginosis. J Infect Dis. 2002;185(11):1614–20.

49. Cauci S, Guaschino S, de Aloysio D, Driussi S, De Santo D, Penacchioni P, et al. Interrelationships of interleukin-8 with interleukin-1β and neutrophils in vaginal fluid of healthy and bacterial vaginosis positive women. Mol Hum Reprod. 2003;9(1):53–8.

50. Hernández JC, Stevenson M, Latz E, Urcuqui-Inchima S. HIV type 1 infection up-regulates TLR2 and TLR4 expression and function in vivo and in vitro. AIDS Res Hum Retroviruses. 2012;28(10):1313–28.

51. Kedzierska K, Crowe SM. Cytokines and HIV-1: Interactions and clinical implications. Antivir Chem Chemother. 2001;12(3):133–50.

52. Osuji FN, Onyenekwe CC, Ahaneku JE, Ukibe NR. The effects of highly active antiretroviral therapy on the serum levels of pro-inflammatory and anti-inflammatory cytokines in HIV infected subjects. J Biomed Sci. 2018;25(1):1–8.

53. Musa F, Shaviya N, Mambo F, Abonyo C, Barasa E, Wafula P, et al. Cytokine profiles in highly active antiretroviral treatment non-adherent, adherent and naive HIV-1 infected patients in Western Kenya. Afr Health Sci. 2021;21(4):1584–92.

54. French MA, King MS, Tschampa JM, Da Silva BA, Landay AL. Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+ T Cells. J Infect Dis. 2009;200(8):1212–5.

55. Lederman MM, Calabrese L, Funderburg NT, Clagett B, Medvik K, Bonilla H, et al. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. J Infect Dis. 2011;204(8):1217–26.

56. Skogstrand K, Hougaard DM, Schendel DE, Bent NP, Sværke C, Thorsen P. Association of preterm birth with sustained postnatal inflammatory response. Obstet Gynecol. 2008;111(5):1118–28.

57. Romero R, Espinoza J, Gonçalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007;25(1):21–39.

58. Romero R, Espinoza J, Gonçalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. Semin Fetal Neonatal Med. 2006;11(5):317–26.

59. Romero R, Gomez R, Ghezzi F, Bo Hyun Yoon, Mazor M, Edwin SS, et al. A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. Am J Obstet Gynecol. 1998;179(1):186–93.

60. Zhang B, Ohtsuka Y, Fujii T, Baba H, Okada K, Shoji H, et al. Immunological development of preterm infants in early infancy. Clin Exp

Immunol. 2005;140(1):92–6.

61. Mirmonsef P, Krass L, Landay A, T. Spear G. The Role of Bacterial Vaginosis and Trichomonas in HIV Transmission Across The Female Genital Tract. Curr HIV Res. 2012;10(3):202–10.

62. Ma B, Forney LJ, Ravel J. Vaginal microbiome: Rethinking health and disease. Annu Rev Microbiol. 2012;66:371–89.

63. Ruiz RJ, Jallo N, Murphey C, Marti CN, Godbold E, Pickler RH. Second trimester maternal plasma levels of cytokines IL-1Ra, Il-6 and IL-10 and preterm birth. J Perinatol. 2012 Jul;32(7):483–90.

64. Marques MPC, Pinto AC, Soares LC, Macedo JMB, dos Santos DF, de Oliveira MAP. Protease inhibitor effects on prevalence of bacterial vaginosis and human papillomavirus-related lesions. J Obstet Gynaecol Res. 2020;46(6):899–906.

65. Bennetto-Hood C, Johnson VA, King JR, Hoesley CJ, Acosta EP. Novel methodology for antiretroviral quantitation in the female genital tract. HIV Clin Trials. 2009;10(3):193–9.

66. Pinto-Cardoso S, Klatt NR, Reyes-Terán G. Impact of antiretroviral drugs on the microbiome: Unknown answers to important questions. Curr Opin HIV AIDS. 2018;13(1):53–60.

67. Carlson RD, Sheth AN, Read TD, Frisch MB, Mehta CC, Martin A, et al. The Female Genital Tract Microbiome Is Associated with Vaginal Antiretroviral Drug Concentrations in Human Immunodeficiency Virus-Infected Women on Antiretroviral Therapy. J Infect Dis. 2017;216(8):990–9.

68. Hedges SR, Barrientes F, Desmond RA, Schwebke JR. Local and systemic cytokine levels in relation to changes in vaginal flora. J Infect Dis. 2006;193(4):556–62.

69. Baggia S, Gravett MG, Witkin SS, Haluska GJ, Novy MJ. Interleukin-1 beta intra-amniotic infusion induces tumor necrosis factor-alpha, prostaglandin production, and preterm contractions in pregnant rhesus monkeys. 1996;5576(96):0–5.

70. Spandorfer S, Neuer A, Giraldo P, Rosenwaks Z, Witkin S. Relationship of abnormal vaginal flora, proinflammatory cytokines and idiopathic infertility in women undergoing IVF. The Journal of Reproductive Medicine, 01 Sep 2001, 46(9):806-810; 2001. p. 806–10.

71. Kalinka J, Wasiela M, Brzezińska-Błaszczyk E. Impact of threatened preterm labor and lower genital tract biocenosis on the concentrations of selected proinflammatory cytokines in cervicovaginal fluid of pregnant women. Ginekologia Polska, 01 Oct 2003, 74(10):1118-1125; 2003. p. 1118–25.

72. Wasiela M, Krzeminski Z, Kalinka J, Brzezinska-Blaszczyk E. Correlation between levels of selected cytokines in cervicovaginal fluid of women with abnormal vaginal bacterial flora. Med Dosw Mikrobiol 2005; 57: 327–333; 2005. p. 327–333.

73. Mattsby-Baltzer I, Platz-Christensen JJ, Hosseini N, Rosén P. IL-1β, IL-6, TNFα, fetal fibronectin and endotoxin in the lower genital tract of pregnant women with bacterial vaginosis. Acta Obstet Gynecol Scand. 1998;77(7):701–6.

74. Gor DO, Rose NR, Greenspan NS. TH1-TH2: A procrustean paradigm. Nat Immunol. 2003;4(6):503–5.

75. Diehl S, Rincón M. The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol. 2002;39(9):531–6.

76. Babu S, Nutman TB. Proinflammatory Cytokines Dominate the Early Immune Response to Filarial Parasites. J Immunol. 2003;171(12):6723–32.