Determination of CD4 T cells among children infected by HIV during anti-retroviral therapy (ART)

Hosna Ibrahim Ali Omer1, Ahmed Kamal Awad Alla2, Mohamed Elhassan Abakar3, Saeed Nasaddin4

1Al Neelain University, Faculty of Science

2Al Neelain University, Faculty of Medicine

3Jomo Kenyatta University for Science and Technology

Kenya-Nairobi

4Ahfad University, School of Medicine

Abstract

Mother-to-child transmission is by far the commonest source of HIV infection in children. Therefore, its necessary to have a policy which provides the frame work, response intervention in the prevention, care and support of these infected and affected by the epidemic and mitigation of its impact. This study is conducted on newly born babies to mothers infected with HIV/AID to determine levels of CD4 T cells in an attempt to find a correlation between these level and the Mother-to-child transmission process, so it can participate in the ongoing efforts of control using anti retroviral therapy (ART). Level of CD4 cell in infected mothers was determined by using rapid test (determine), immunocomb and PCR (DBS). The effect of ART on vertical transmission of neonates was evaluated by using CD4 and PCR, DBS. HIV analysis in mothers infected using rapid test (Determine) (RT) and immunocomb gave similar results. HIV analysis in infected mothers and their infected infants using either RT or immunocomb test revealed similar results and CD4 count was high in the majority of the study population indicating the effectiveness of ART. Our findings suggest that CD4 count following ART initiation have appreciably changed in the majority of the study population. However in few children CD4 count had not influenced by ART.

Introduction

HIV disease is a chronic progressive process that begins with infection, is often followed by a "primary HIV syndrome," and progresses in adults over a median period of more than 10 years to the late stage: AIDS. From the time of infection, the virus continuously and rapidly replicates, mutates, and as a result diversifies and evolves in response to selective pressure. HIV infections pass from person to person through sexual fluids; blood and breast milk but however giving antiretroviral treatment reduces transmission (WHO 2017). . HIV infection can also be transmitted from a mother to her child during pregnancy and during childbirth or from breast-feeding (Quinn *et al*., 2000). There is a 15-20% chance that infection will be transmitted to babies during breast-feeding. HIV infected adult with a CD_4 cell count greater than 500 cells/µl.

Progression is often accelerated in infants with prenatal HIV infection. Eventually the host become increasingly susceptible to and eventually dies as a result of complications of opportunistic infections and malignancies resulting from immune system dysfunction (Francioli *et al.*, 1982).

HIV, that causes the disease. HIV, a human retrovirus has a tropism for CD4 + T cell and monocytes, induces a decrease in T-cell counts. In early and Middle Stages of HIV Disease CD4+ count droped from normal levels to 200 to 300 cells/µL. Whereas in advanced HIV Disease patients have CD4+ counts below 200 cells/µL. As the disease advances further and the CD4+ count drops below 50 cells/µL.

Accordingly, this study was conducted on newly born babies to mothers infected with HIV/AID to reveal the level of the CD4 in an attempt to find a correlation between these level and the Mother-to-child transmission process, so it can participate in the ongoing efforts of control namely ART. Around two-thirds of Mother-to-child transmission (MTCT) occurs *in utero* and at delivery and one-third occurs during breast feeding. Long regimens of antiretroviral therapies (ART) have been shown to be highly effective in preventing MTCT but shorter, less complex and cheaper regimens have also been shown to reduce MTCT by half in infants who are not breast fed (UNAIDS. Mother., 2000). Implementing this intervention on a large scale in non-industrialised countries is complicated by the lack of safe alternatives to breast feeding, the issue of HIV testing and costs of the drug, service delivery and HIV tests.

Materials and Methods

Study design

This is a descriptive and cross sectional hospital based study. It was conducted in Ndjamena district Hospitals (Chad) during the study period. All files of patients admitted to hospital with HIV/AIDS infections were reviewed.

Ethical considerations

An approval of the research was obtained from the college research committee and permission was taken from the hospital authorities before starting collecting data from

them. A verbal consent was obtained from the patients who were enrolled in the research following an explanation to patients about the research and its objectives prior to filling the questionnaire and obtaining the wound swabs.

Study population

All HIV/ AIDs diagnosed mothers who gave birth to children at N' djamena hospitals AIDS and their new babies were enrolled as well in the study.

A total number of 160 pregnant mothers live with HIV included in this study, they transmit the virus vertically to their 160 children, the result of copy status proved this situation. The control groups include 40 mothers and their 40 children. Levels of depression of the CD4 was evaluated in all paticipants. The qualitative data described with frequency and percent while, the hypothesis tested using chi-square test (the test of frequency), and the quantitative data summarized in mean ± standard deviation and tested using independent sample t-test.

Data analysis

The data were collected analyzed using SPSS 21 with reference p-value for hypothesis tests (0.05), which means that, the confidence degree in this study (95%).

Alere Determine HIV – 1/2

Specimen collection for HIV diagnosis in almost all specimens serum plasma, and whole blood collected by veni-puncture EDTA, and collection tubes. Whole blood by Fingerstick was collected in a tube and 50 µLof the sample was applied to sample pad and after one minute one drop of chase buffer was applied to sample pad. Following that, when blood get absorbed into the sample pad then one drop of chase buffer was applied to the sample pad and read results were read in 15-60 minutes. Visible red bar in the patient window was interpreted as positive.

Immunocomb II

HIV 1&2 BiSpot

The ImmunoComb II HIV 1/2 BiSpot kit is an indirect solid-phase enzyme immunoassay (EIkA). The solid phase is a card with 12 projections ("teeth"). Each tooth is sensitized at three spots: upper spot-goat antibodies to human immunoglobulin (Internal Control) middle spot —HIV-2 synthetic peptides.
lower spot —HIV-1 synthetic peptides. The test was performed at room temperature.
In order to confirm that the test function properly and to demonstrate that the results are valid, the following three conditions must be fulfilled, 1. the positive control must produce three spots on the card tooth; 2. the negative control must produce an upper spots (internal control) and no other spots; 3. and each specimen tested must produce an upper spots (internal control). This will also conform that the specimen was added.

One Step Anti-HIV 1/2 Test

SD HIV 1 / 2 3.0

One step, Rapid, Immunochromatographic test for the detection of anti-HIV 1 / 2 in human serum, plasma or whole blood was performed

Test of CD4 count (Flow Cytometer)

BD FACS count CD4 reagent are used to enumerate the absolute count of CD4 T-lymphocytes, and determine the percentages of lymphocytes unlysed whole blood (CD4 count and CD4 percentages) and the reagents are intended for in vitro diagnostic use on a BD FACS count instrument.

Real-Time Polymerase Chain Reaction (PCR)

PCR used to detect, HIV proviral sequences and the results are reported as either
“ HIV-1 Detected” or “ Not detected” .

HIV testing using standard ELISA methods

This method is used for testing serum for antibodies to HIV with a standard ELISA (followed by a confirmatory of Western Blot) and is currently the most common, cost effective, and accurate method of screening for infection. HIV RNA tests are being used in research and clinical settings to diagnose primary HIV infection before the formation of detectable antibodies (Tropical Diseases January, 1986). Although HIV antibody tests are the most appropriate for identifying infection,

alternate technologies contribute to an accurate diagnosis, assist in monitoring the response to therapy, and can be used to effectively predict disease outcome. There for, the p24 antigen assay measures the viral capsid (core) p24 protein in blood that is detectable earlier than HIV antibody during acute infection. It occurs early after infection due to the initial burst of virus replication and is associated with high levels of viremia during which the individual is highly infectious (AIDS Sinfo March, 1987).

Timing of serological testing in infants

The most recent advances in EIA technology have produced ‘ combination assays’ , which allow for the simultaneous detection of p24 HIV antigen and HIV antibodies. This approach has further shortened the window period, i.e. the interval between HIV infection and detectable HIV antigen/antibodies. Rapid tests appear to offer similar performance characteristics but they detect antibody 2– 8 days later than third-generation EIAs.

Results

The mode of infection as shown by table (1) that 97% women get infected by sexual intercourse, while 1% of study population infected by HIV through intravenous drug abuse two women get the infection through blood transfusions.

HIV analysis in mothers infected using rapid test (Determine) (RT) shown in table (2) gave the following results: 79 (49.3%), 55 (34.4%), 26 (16.3%).

HIV analysis in infants infected using determine test (RT) shown by table (3).gave the following results: 111 (69.4%), 41 (25.8%), 8 (5%).

Detection of HIV-1 and HIV -2 using immunocomb among mothers infected shown in table (3)79 (49.3%), 55 (34.4%), 26 (16.3%).corresponding table (4).

HIV analysis in infants infected by mothers using immunocomb shown by table (5).gave the following results: 111 (69.4%) of age groups 0-4 months , 41 (25.6%) 4-8 months (5%) of age groups 8-12 months

The frequency of status of polymerase chain reaction (PCR) DBS among patient children shown table (6) 111 (69.4%), 4 8 (5%).

Descriptive statistics (mean ± standard deviation) of CD4 among infected mothers and infected children were shown by figure 1 and 2.

All non-infected mothers and their children using Rapid test (Determine) and immuncomb to detect HIV-1 and HIV-2 were negative for HIV.

All control mothers significantly with CD4 more than 500, (72.5%) while patient mothers divided into three groups of CD4, moreover, the more frequent CD4 group was from 200 to 500 of CD4 (26.3%) of them have low CD4 counts < 200 cell / µl

All control children significantly with CD4 more than 500, while patient children divided into three groups of CD4, moreover, the more frequent CD4 group was from more than 500 of CD4 while 12.5% with CD4 conts < 200 cell/µl

Table (1): The mode of the infection for known 160 infected mothers

Mode of Infection	Mothers	Percentage
Through sexual contact	155	97%
Trough intra venous drug abuse	2	1%
Trough blood transfusions	3	2%
Total	160	100%

Mostly all (97%) Hiv infected ladies think that it was through sexual contact

Table (2): HIV Analysis in mothers infected using Rapid test (Determine)

Age	Frequency	RT Determine	Valid Percent
15 to 25	26	Test Positive in Determine	16.3%
25 to 35	79	Test Positive in Determine	49.3%
35 to 45	55	Test Positive in Determine	34.4%

Number of mothers infected using first test determine (n=160)

Table (3): HIV Analysis infants infected using RT Determine

Age (months)	Frequency	RT Determine	Valid Percent
0 to 4	111	Test Determine Positive	69.4%
4 to 8	41	Test Determine Positive	25.6%

8 to 12	8	Test Determine Positive	5%

Number of infant infected by mothers (n=160)

Table (4): Detection of HIV-1 and HIV-2 in infected mothers using Immunocomb

Age	Frequency	Immunocomb to detected HIV-1 and HIV-2	Valid Percent
15 to 25	26	Test Positive in immunocomb detected HIV-1	16.3%
25 to 35	79	Test Positive in immunocomb detected HIV-1	49.3%
35 to 45	55	Test Positive in immunocomb detected HIV-1	34.3%

The confirmation test using Immunocomb test

Mothers infected (n=160)

Table (5) Immuncomb test for infants infected by mothers

Age (months)	Frequency	Immunocomb to detected HIV-1 and HIV-2	Valid Percent
0 to 4	111	Test Positive in immunocomb detected HIV-1	69.4%
4 to 8	41	Test Positive in immunocomb detected HIV-1	25.6%
8 to 12	8	Test Positive in immunocomb detected HIV-1	5%

Confirmed test of HIV-1 for infants infected by mother number of infants infected (n=160)

These results were correspondingly confirmed by Immunocomb test, which was detected HIV-1 in these infants infected by their mothers.

Table (6): the frequency of status of polymerase chain reaction (PCR) DBS among patient children.

Age (months)	Frequency	Genome positive	Valid Percent
0 to 4	111	Genome positive	69.4%
4 to 8	41	Genome positive	25.6%
8 to 12	8	Genome positive	5%

Genome wide profiling of RNA from dried blood spot.

Polymerase chain reaction (PCR) to detected HIV-1 DNA in pools of dried blood spot as an alternative to individual testing

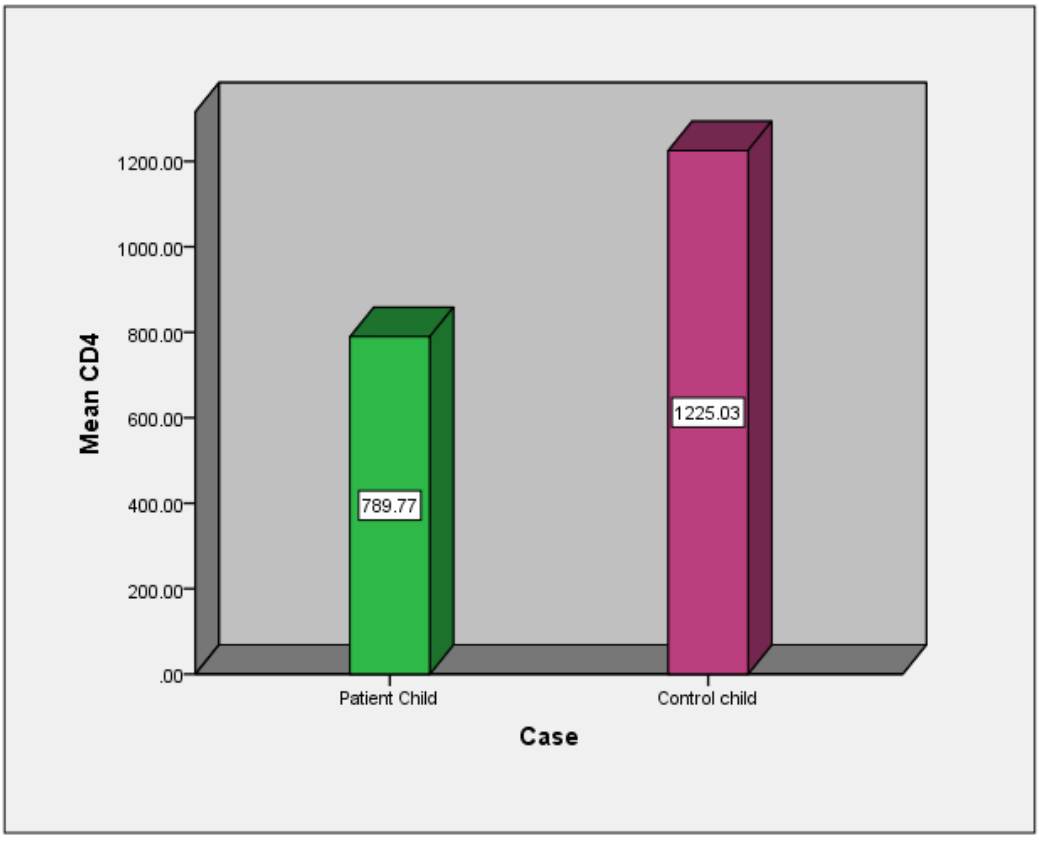

Figure (1) Comparison between the mean ± standard deviation of Patient children and Control children

Descriptive statistics (mean ± standard deviation) of CD4 among Control children were significantly higher (1225.03±492.1), than among Patient children (789.77±442). The P-value of independent sample =.000

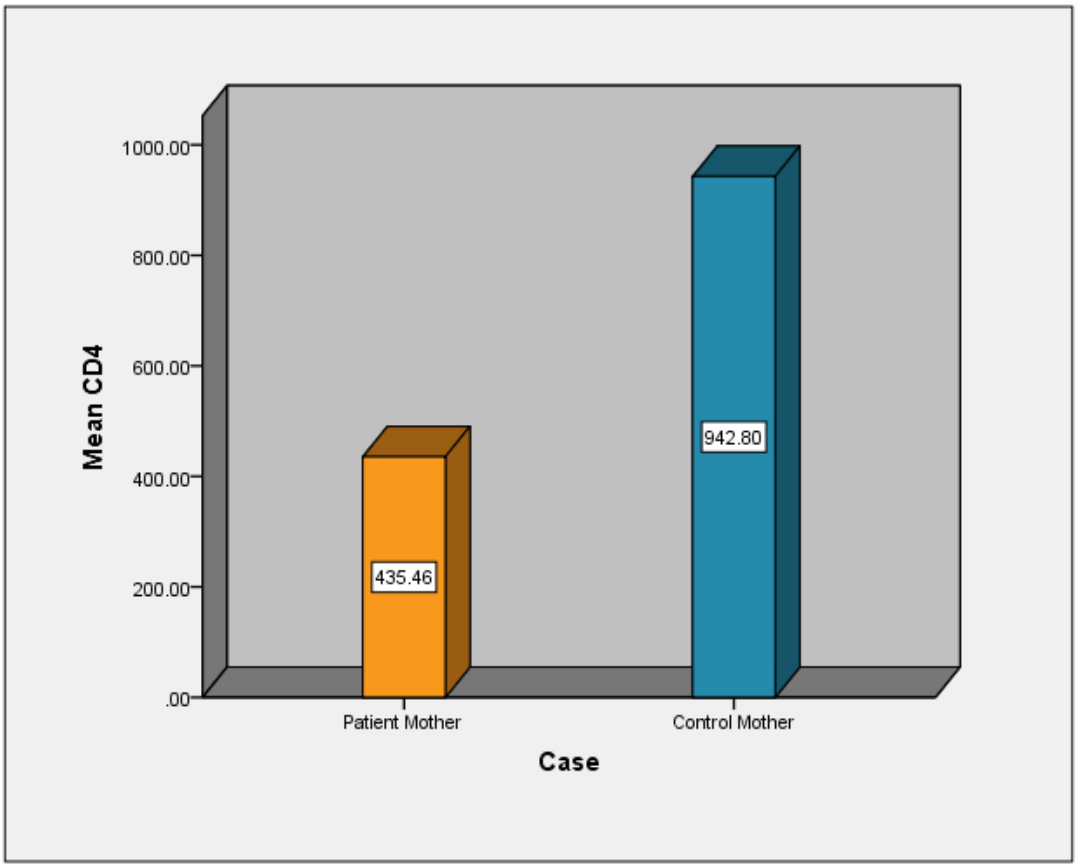

Figure (2) Comparison between the mean CD4 ± standard deviation of Patient mothers and Control mothers

Descriptive statistics (mean ± standard deviation) of CD4 among Control mothers were significantly higher (942±250.6), than among Patient mothers(435.46±300.7). The P-value of independent sample =.000

DISCUSSION

The rate of the tranmisstion of HIV to infants incraeses in parallel with clinical severity of maternal infection. Programs to prevent the transmission of HIV from mothers to

children can reduce rates of transmission by 92-99% (Kurth*et al.*, 2011). Long regimens of antiretroviral therapies (ART) have been shown to be highly effective in preventing Mother-to-child transmission (MTCT) but shorter, less complex and cheaper regimens have also been shown to reduce MTCT by half in infants who are not breast fed (UNAIDS, 2000).

In the current study serological tests and molecular tests revealed that ART had not influenced levels of CD4 cells in only few children on ART. In these children with low levels of CD4 despite they were in ART, little has been paid to the earliest stage and to HIV diagnosis. Or on the otherhand this finding suggests that a sizeable gap between ART eligibility and initiation has persisted in some regions. These results reinforce critical challenges to achieving the goal of an AIDS-free generation, even the most well-designed and well-supported ART programs will have limited capacity to maximize the health benefits of ART and prevent new infections if people continue to present to care during late stages of disease. Recent WHO recommendations to initiate ART for all persons with CD4 counts ≤500 cells/μL have spurred debate about the benefits of adopting this threshold as well as debate about the extent to which existing health systems can accommodate the expansion of treatment availability (Maman *et al*., 2012).

However the majority of our study population on ART have had reasonable levels of CD4. This indicating that, to reduce HIV-related morbidity and mortality at the population level, and to decrease secondary transmission, intensified efforts to increase demand for ART through active testing and facilitated referral should be a priority. Meanwhile, continued financial investments by multinational partners and the implementation of creative interventions to mitigate delays between presentation to care and ART initiation are needed.

HIV testing and referral may be undermined by a wide array of social forces such as generalized poverty. However the present study proved that simple rapid tests are suitable for use in diagnosis of HIV provided that adequate quality assurance procedures are in place. Taken together, as high risk groups play a major role in transmission of HIV, appropriate strategies should be developed to reduced the risk of HIV infection among specific high risk groups. And as HIV/AIDS being a social, cultural and economic problem, therefore, women and girls need extra consideration to protect themselves from the increased vulnerability to HIV infection in the various

social, cultural and economic environments as stipulated in the national policy and equity.

REFERENCES

UNAIDS (2000). Preventing mother-to-child transmission: technical experts recommend use of antiretroviral regimens beyond pilot projects [Press release]. Geneva: UNAIDS,

UNAIDS (2000). Mother-to Child Transmission. Summary Booklet of Best Practices. Geneva: UNAIDS,

Francioli. P (1982) acquired immunologic deficiency syndrome opportunistic infections and homosexuality. presentation of 3 cases studied in Switzerland schweizerische medizinische wochenschrift 112 (47):1682-1687

AIDS info (1987, 20 Match) ‘ Approval of AZT.

Quinn TC., Wawer Mj., N. and Swewank amb0 2000viral load and heterosexa;transmission of human immunodefeciency virus type1 342;921-999.

WHO 2017, infant feeding for the prevention of mother to child transmission of HIV