Scientific Inquiry and Review (SIR)
Volume 6 Issue 2, 2022
ISSN (P): 2521-2427, ISSN (E): 2521-2435
Homepage: https://journals.umt.edu.pk/index.php/SIR

Article QR

Title:	**Estimation of CD4 Count and HIV Viral Load among HIV/AIDS Patients: An Analysis of Antiretroviral Therapy in Punjab, Pakistan**
Author (s):	Samavia Siddique[1], Hasnain Javed[2], Huma Shafique[1]
Affiliation (s):	[1]Lahore Garrison University, Lahore, Pakistan [2]Punjab AIDS Control Program, Lahore, Pakistan
DOI:	https://doi.org/10.32350/sir.62.05
History:	Received: August 11, 2021, Reviewed: June 12, 2022, Accepted: June 18, 2022
Citation:	Siddique S, Javed H, Shafque H. Estimation of CD4 count and HIV viral load among HIV/AIDS patients: An analysis of antiretroviral therapy in Punjab, Pakistan. *Sci Inquiry Rev.* 2022;6(2):00-00. https://doi.org/10.32350/sir.62.05
Copyright:	© The Authors
Licensing:	This article is open access and is distributed under the terms of Creative Commons Attribution 4.0 International License
Conflict of Interest:	Author(s) declared no conflict of interest

A publication of
The School of Science
University of Management and Technology, Lahore, Pakistan

Estimation of CD4 Count and HIV Viral Load among HIV/AIDS Patients: An Analysis of Antiretroviral Therapy in Punjab Pakistan

Samavia Siddique[1], Hasnain Javed[2], Huma Shafique[3*]

[1,3] Department of Biology, Lahore Garrison University, Lahore, Pakistan
[2] Punjab AIDS Control Program, Lahore, Pakistan

* humashafique@lgu.edu.pk

ABSTRACT

HIV/AIDS is a global health problem of the world. HIV type 1 mostly cause HIV infection in Asia and Europe and is also predominant in underdeveloped countries like Pakistan. HIV type 1, increases morbidity and mortality due to progressive damage of CD4 T cell. Approximately, 2 million people were infected with HIV type 1 infection through sexual activities globally. Hence, the life expectancy of people living with HIV was increased by using combined antiretroviral therapy (CRT). In HIV prognosis, CD4 count is a very crucial marker of prognosis of HIV/AIDS. In the current research, we analyzed CD4 count of 118 HIV/ AIDS patients, who were taking antiretroviral treatment to evaluate their immune system. HIV viral load testing was done on the same number of patients through HIV quantitative PCR to evaluate the viral load. Statistical analysis was also done to check the percentage of viral load and association of CD4 count with the viral count. According to the results, there were a greater percentage of males (55.5%) than females (44.4%) who developed AIDS which is most devastating form of HIV infection. High viral load (>100000 copies/ml) was also observed higher in males (65.5%) than females (31.4%).

Keywords: art drugs (antiretroviral drugs), HIV, CD4 count, viral load.

INTRODUCTION

HIV (human immunodeficiency virus) belongs to family of human retroviruses of lentivirus subfamily [1]. HIV infection is categorized by a progressive destruction of the body's immune system, which in turn becomes the reason in causing an opportunistic infection, immunological, and hematological complications [2]. According to United Nations Program on HIV/AIDS, in 2020, there were nearly 37.7 million people living with HIV. Some HIV infected patients would develop AIDS (acquired immunodeficiency syndrome). AIDS is a systematic disorder in which

severe impairment and progressive damage of both cellular and humeral immune responses occur [3].

The causes of increased spread of HIV infection in different areas of the world are different. Men who have sex with men (MSM) and female sex workers (FSW) contribute significantly to HIV transmission in most countries of the world. In China the epidemic of HIV is caused by the high prevalence among MSM. It calls for improved, hands-on, and operative HIV prevention interventions. About 1.5 million people are living with HIV in Kenya. The main cause of it is men who having sex with men (MSM) and female sex workers (FSW) [4, 5].

T cell plays a key role in several diseases. T lymphocytes expresses a T-cell receptor (TCR) for the recognition of specific peptide antigen within the major histocompatibility complex (MHC) binding groove [6]. T cells mature in the thymus, express TCR (T cell receptor), and it could also express either CD8 glycoprotein on their surface which is called CD8+ T cells (cytotoxic) or CD4 glycoprotein termed as CD4 cells (helper T cells). CD4+ subsets play a critical role in the immune and effector response functions of T cells [7]. HIV infects thymus-derived cells (T cells) by means of a glycoprotein-120 (gp 120) embedded in its envelope [8]. The incidence of opportunistic infections also depends upon the level of immune suppression (occurs when CD4 count is $<200/mm^3$ and total lymphocyte count is $<1200/mm^3$) [9]. A basic role of CD4 effector T cells is the production of cytokines. On the basis of cytokines, they expressed, CD4 T cells which could be classified into different subsets having distinct functions [10]. CD4 T cell lymphocyte count is the important parameter in the evaluation of immune function of HIV/AIDS patients. CD4 count also guide us about disease prognosis, antiretroviral treatment eligibility, and clinical management of treated patients [11]. The delay in the availability of CD4 test could delay the adequate patient assessment and information about the stages of HIV infection. It could also lead to delay in initiating the ATV and prophylaxis in newly diagnosed patients [12]. There are different number of approaches to control HIV. The primary prevention method includes sexual health education, behavior improvements, use of condoms to male circumcision, prevention of mother-to-child transmission, use to clean needles and many others. The primary purpose of this research was to evaluate the CD4 count, concentration of viral load, and association of CD4 count with viral load among different genders of AIDS patients.

2. MATERIALS AND METHOD

2.1 Sample Collection

Samples were collected from HIV/AIDS patients that were registered (Known Positive) in different treatment centers of Punjab AIDS Control Program located in Lahore, DG Khan, Faisalabad, Kotmomin, Multan, Rahim Yar Khan, Sargodha, and Sheikhupura in Punjab, Pakistan. 5ml venous blood samples were collected in EDTA (Ethylenediamine tetra-acetic acid) anticoagulant vials and stored at 4 °C [13].

2.2 CD4 Count Estimation

CD4 count was done on Alere Pima^{TM} following all the instructions given in its manual [14]. Whole blood was used after mixing on mixer by using sample collector, a cartilage, and an analyzer. 25ul sample was added in sample collector attached to the cartilage with the help of micropipette. The sample collector was removed and cartilage was capped. Capped cartilage was inserted into the analyzer to run the test after adding specific lab ID that was given to the sample. The result was recorded and samples stored at 4°C for the future use [14].

2.3 Viral RNA extraction

Extraction of RNA was done by using QIAGEN manual extraction kit [15]. All the reagents including AW1, AW2 buffer, and QIAGEN proteases (QP) was prepared according to the instructions. RNAs were extracted from 200-µl liquid plasma of arranged and labeled samples following the manufacturer's instructions and were eluted in elution buffer. Carrier RNA with 310ul of elusion buffer was taken and short spin and then added in 33ml of lysis buffer. 20ul of this solution along with 200ul plasma and 25ul of protease enzyme were added in micro-tubes, vortex, and short spined in centrifuge. This mixture incubated at 56 ^{0}C for 15 minutes. Incubated mixture was short spined and 250ul of ethanol was added. The whole mixture was centrifuged for 1 min at 8000rpm. Receiver tubes were discarded and filter column tubes were transferred into new receiver tubes. 500ul of wash buffer (AW1) was added in each tube. It was centrifuged for 1 min at 8000rpm. The receiver tubes were again discarded and filter column tubes were placed again inside the new receiver tubes. 500ul of wash buffer (AW2) was then added. All tubes were centrifuged for 1 min at 8000rpm. 500ul of ethanol was added in each tube again after discarding waste from all receiver tubes. All tubes were then dry spined to dry ethanol.

Then all filter column tubes were placed and labelled as Eppendorf tubes. 50ul of elusion buffer was added in each tube. All tubes were centrifuged for 1 min. Then filter column tubes were discarded. The eluted (RNA) was then saved at 4 ^{0}C in a labelled box or rack for the further process [15].

2.4 Amplification

Commercially prepared QIAamp Kit [(Master mix A, B, Internal control (IC), standard, and non-template control (NTC)] was used for amplification. After thawing of reagents at room temperature, prepared working master mix by adding given master mix B to master mix A. Then 25ul IC (internal control) was added into working master mix. Pour 30ul of working master mix into every well. Add 20ul of sample up to 69 wells, standard in 4 wells, and NTC in a single well and mix by pipette 2-3 times [16].

2.5 Data analysis

Analysis of patient samples was done to check the percentage of viral load and CD4 count and association of CD4 and HIV load by using SPSS version 28.0.

3. RESULTS

All of the selected samples (n=118) included in the current study had HIV, which were registered (already tested positive patients) in PACP treatment centers. Collected samples from different treatment centers includes, 74% males, 24% females, and 2% transgender (TG) as shown in the **Figure 1**.

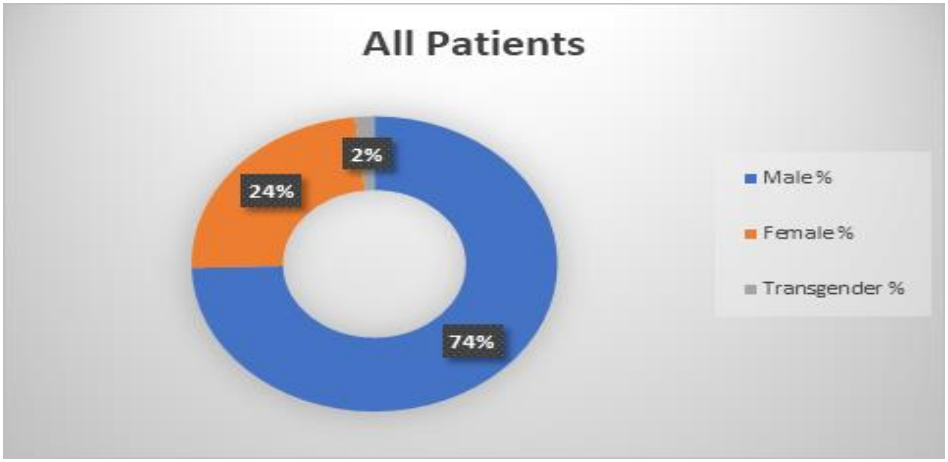

Figure 1. Percentage of HIV male, female and TG

3.1 CD4 count estimation

The CD4 count estimated through Pima Alere^{TM} of each sample, which was processed within 18 to 20 minutes and results which were showed on the screen were recorded. Among all the patients 6% of patients had CD4 count <50, 9% had CD4 count from 50-200, 39% had 201-500, 46% had >500 as shown in **Table 1**.

Table 1: Analysis of Gender/No of patients with CD4 Count

CD4 count	Male	Female	TG	Total patients
<50	5	4	0	9
50-200	10	4	0	14
201-500	40	5	1	46
>500	35	13	1	49

Graphical representation of CD4 count was shown in **Figure 2**. Analysis of all the included patients was done according to their age range with CD4 values, results showed that >15 patients: Majority patients had normal CD4 count and lower no of AIDS patients.

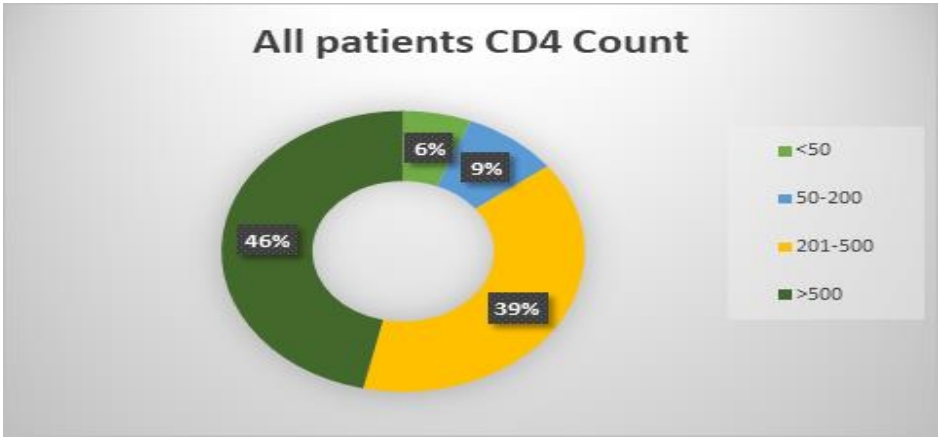

Figure 2. CD4 Count of patients

Age group 15-24: in this range several numbers of patients having normal range. The number of patients decreased as the CD4 counts decreased. Age group 25-34: in this category most of patients having normal

CD4 count but significant number of patients in this age range developed AIDS. Age range 34-44/45-54 included a greater number of patients which had CD4 count between 201-500 (at risk of developing AIDS) and most of the patients of age >55 had normal CD4 count. Analysis of male, female, and transgender with CD4 count showed that there are more females who developed AIDS (CD4 <200) than males, while TG in our data did not develop AIDS.

3.2 Polymerase Chain Reaction (PCR)

PCR results showed that 37% of HIV patients had ND viral load, 11% had <1000, 10% had between 1001-1000, 12% had between 10001-10000, and 30% had >100000 as shown in **Table 2**.

Table 2. Analysis of Gender/Number of Patients with Viral Load

Viral Load	Male	Female	TG	Total patients
ND	32	11	1	44 (37%)
<1000	13	0	0	13 (11%)
1001-10000	10	2	0	12 (11%)
10001-100000	12	2	0	14 (12%)
>100000	23	11	1	35 (30%)

Graphical representation of viral load is shown in **Figure 3**. Only 37% patients had non- detectable viral load due to effectiveness of antiretroviral therapy. Analysis of all the age ranges with PCR values showed that patients having viral load >100000 were among the 25-34 and 35-44 age groups. A huge number of patients had non-detectable viral load in PCR in all age ranges due to antiretroviral therapy. Analysis of male, female, and TG with HIV PCR values was also done. Therefore, males had more viral load then females, while TG had very anomalous behavior of showing ND or viral load >100000.

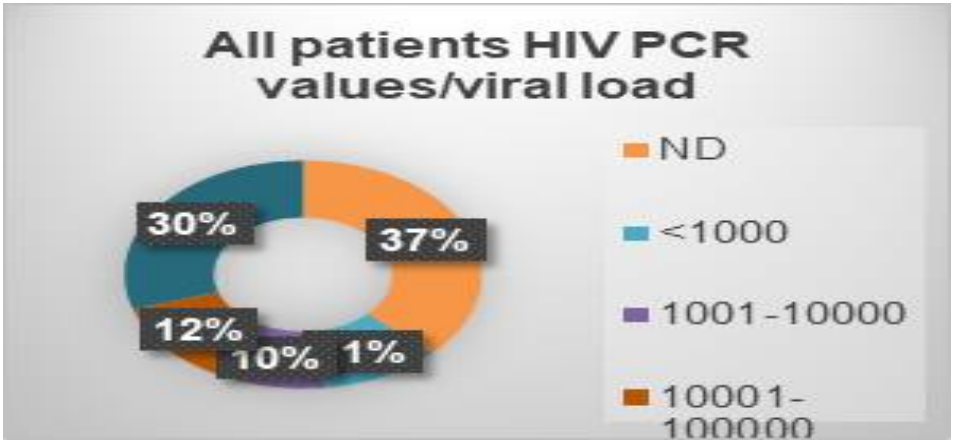

Figure 3. Percentages of patients having different viral loads

Relationship of CD4 count with viral load is shown in **Table 3**. Hence, it shows that as the viral load increases the number of patients having normal CD4 count also increases.

Table 3: Relationship of CD4 count with Viral Load

CD4 count	ND	<1000	1001-10000	10001-100000	>100000
<50	0	1	0	1	7
50-200	4	2	1	1	6
201-500	14	4	9	7	12
>500	26	6	2	5	10

As the CD4 count increases the ND viral load in patients' increases. All patients taking antiretroviral therapy mostly had ND viral load, while 30% of patients who were taking antiretroviral therapy still had viral load >100000. Males had more viral load then females, while TG had very anomalous behavior of showing ND or viral load >100000.

4. DISCUSSION

In HIV patients, CD4 count is considered as a marker of immune senescence. It predicts mortality rate in most of the HIV patients [17]. Patients who had CD4 count less than 100 cells/ml were stout predictor of impermanence, instead of the patients who had higher CD4 levels chances

of endurance [18]. Conventionally, antiretroviral therapy (ART) is initiated for all HIV patients having CD4 count 500 cells per µl or less, while for the children who were under the age of 5 or patients who had any kind of secondary or co-infection abrupt initiation of ART were certainly recommended [19]. Patients having CD4 count <200 cells per ul were suffering from AIDS [20]. Late diagnosis was demarcated as having a CD4 count lesser than 350 cells per mm^3 within 3 months of diagnosis [21]. Initiation of ART regardless to CD4 count could increase the number of individuals treated because the main objective of ART is to suppress viral load and thwart inflammation and immune deficiency [22]. The effect of immediate antiretroviral therapy (ART) was estimated by a significant increase in CD4 count in those patients

Elevated viral load shows the treatment failure and ineffectiveness of ART. That's why World Health Organization (WHO) guidelines insisted on expanding the viral load estimation testing [23]. This is a challenging criterion especially in developing or under developed countries because sample transport, the requirement for infrastructure, multifaceted equipment, training of lab workers, cold chain, and instrument maintenance is difficult [24] Assessment of viral load (VL) also guides us about the HIV transmission potential and future rate of new infections in a particular community. Recent studies showed that males have higher mean viral load as compared to females. Therefore, our research also highlighted the increased percentage of viral load among male patients [25].

In Pakistan, patients who had undetectable viral load (ND) within or after 6 months of ART were considered virally suppressed. However, the patients who had viral load above 1000 after six months avowed to have treatment failure. According to the previously collected data, only 40% of the total HIV infected patients in Pakistan have viral suppression. This is because most of the patients lost follow up to treatment [26]. To eradicate HIV, UNAIDS proposed "90-90-90" criteria which means by 2020. 90% of HIV patients knew their status (viral load, CD4 count and any other abnormalities), 90% of the patients who knew their status must get access to treatment (antiretroviral therapy) and 90% of the patients who were getting treatment had viral suppression. In Pakistan there are so many barriers in achieving these criteria like socio-economic disparities, lack of proper health care system, and organized surveillance. The first and

foremost strategy to upgrade this situation is to improve HIV surveillance system [27].

5. CONCLUSION

According to our results, there were a greater percentage of males who developed AIDS than females and males had more viral load than females. All patients taking antiretroviral therapy, most of them had ND viral load, while 30% of patients who were taking antiretroviral therapy still have viral load >100000. In conventional systems CD4 count suggests, when to start antiretroviral therapy and underlying immune status of the patients. Viral load suggests the prognosis of the disease and its burden in a particular community. Significance of this study is that there is more need to emphasize on man's health in Pakistan with respect to HIV infection. Male gender is more detected and higher viral load in our study than female. There will be lots of reasons like social factors, poverty, and other modes of transmission like injecting drug users or sexual lifestyle etc. In future this research could be done on a larger cohort or different populations with different approach like, to study the viral suppression in patients and their regular updates for the further analysis.

REFERENCES

1. Yomi J, Ngano OS, Minlo J, Pagbe JJ. Traitement des cancers associés au VIH/SIDA: Notre expérience de 25 ans à l'Hôpital Général de Yaoundé. *Health Sci. Dis*. 2015;16(4):1-4.
2. Gouda S, Udayaraj A, Solanke PV. Clinical Spectrum of Pulmonary Tuberculosis in HIV Patients. *Int J Contemp Med*. 2016;4(1):87-93. https://doi.org/10.5958/2321-1032.2016.00019.X
3. Katemba C, Muzoora C, Muwanguzi E, Mwambi B, Atuhairwe C, Taremwa IM. Hematological abnormalities in HIV-antiretroviral therapy naïve clients as seen at an immune suppression syndrome clinic at Mbarara Regional Referral Hospital, southwestern Uganda. *J Blood Med*. 2018;9:e29983600. https://doi.org/10.2147/JBM.S157148
4. Lou J, Cheng J, Li Y, et al. Comparison of different strategies for controlling HIV/AIDS spreading in MSM. *Infec Dis Model*. 2018 ;3:293-300.
5. Bershteyn A, Akullian AN, Klein DJ, et al. Hotspots by random Chance: Small community size and isolation can explain "Patchiness" in HIV epidemics. Poster exhibited at: the 22nd International AIDS

Conference;2018. https://programme.aids2018.org/PAGMaterial/eposters/7503.pdf
6. Campbell C, Andersen L, Mutsikiwa A, Madanhire C, Nyamukapa C, Gregson S. Can schools support HIV/AIDS-affected children? Exploring the 'ethic of care'amongst rural Zimbabwean teachers. *PloS one*. 2016;11(1):e0146322. https://doi.org/10.1371/journal.pone.0146322
7. Golubovskaya V, Wu L. Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy. *Cancers*;*8*(3):e36. https://doi.org/10.3390/cancers8030036
8. Ifeanyichukwu OM, Brigh, EO, Meludu SC, Okeke CO. Effect of HIV infection on some haematological parameters and immunoglobulin levels in hiv patients in benin city, Southern Nigeria. *J HIV Retro Virus*. 2016;2:e2.
9. Kumari R, Kumar M, Mohapatra SC. Clinico-demographic profile of HIV patients at ART centre of a tertiary care referral hospital in North India. *Int J Community Med Public Health*. 2017;4(9):3166-3173. http://dx.doi.org/10.18203/2394-6040.ijcmph20173632
10. Waickman AT, Ligons DL, Hwang S, et al. CD4 effector T cell differentiation is controlled by IL-15 that is expressed and presented in trans. *Cytokine*, 2017;99, 266-274. https://doi.org/10.1016/j.cyto.2017.08.004
11. Zeh C, Rose, CE, Inzaule S, et al. Laboratory-Based performance evaluation of PIMA CD4+ T-lymphocyte count point-of-care by lay-counselors in Kenya. *J Immunol Methods*. 2017;448:44-50. https://doi.org/10.1016/j.jim.2017.05.006
12. Herbert S, Edwards S, Carrick G, et al. Evaluation of PIMA point-of-care CD4 testing in a large UK HIV service. *Sex Transm Infect*. 2012;88(6):413-417. http://dx.doi.org/10.1136/sextrans-2012050507
13. Tendulkar S, Wiebe C, Woko C, et al. Assessing community engagement (ACE)@ The Dimmock center: A community-based Participatory Research (CBPR) Project. *Undergraduate J Serv Learn Commun-Based Res*. 2015;4:1-26.
14. Faye B, Mbow M, Seck MC, et al. Evaluation of PIMATM CD4 system for decentralization of immunological monitoring of HIV-infected patients in Senegal. *PloS one*. 2016;11(5):e0154000. https://doi.org/10.1371/journal.pone.0154000

15. Gupta S, Taylor T, Patterson A, et al. A robust PCR protocol for HIV drug resistance testing on low-level viremia samples. *BioMed Res Int*. 2017;2017: e4979252. https://doi.org/10.1155/2017/4979252
16. Yamazaki S, Kondo M, Sudo K, et al. A Qualitative Real-time PCR assay for HIV-1 and HIV-2 RNA. *Jpn J Infect Dis*. 2016;69(5):367-372. https://doi.org/10.7883/yoken.JJID.2015.309
17. Fuster F, Vargas JI, Jensen D, et al. CD4/CD8 ratio as a predictor of the response to HBV vaccination in HIV-positive patients: A prospective cohort study. *Vaccine*. 2016;34(16):1889-1895
18. Farahani M, Price N, El-Halabi S, et al. Trends and determinants of survival for over 200 000 patients on antiretroviral treatment in the Botswana National Program: 2002–2013. *AIDS (London, England)*. 2016;30(3):477-485. https://doi.org/10.1097/QAD.0000000000000921
19. Ford N, Meintjes G, Pozniak A, et al. The future role of CD4 cell count for monitoring antiretroviral therapy. *Lancet Infect Dis*. 2015;15(2):241-247. https://doi.org/10.1016/S1473-3099(14)70896-5
20. Macías J, Téllez F, Rivero-Juárez A, et al. Early emergence of opportunistic infections after starting direct-acting antiviral drugs in HIV/HCV-coinfected patients. *J Viral Hepat*. 2019;*26*(1):48-54. https://doi.org/10.1111/jvh.13003
21. May MT. Better to know: the importance of early HIV diagnosis. *Lancet Public Health*. 2017;2(1): e6-e7. https://doi.org/10.1016/S24682667(16)30038-X
22. Eholié SP, Badje A, Kouame GM, et al. Antiretroviral treatment regardless of CD4 count: the universal answer to a contextual question. *AIDS Res Ther*. 2016;13(1): e27. https://doi.org/10.1186/s12981-016-0111-1
23. Myer L, Essajee S, Broyles LN, et al. Pregnant and breastfeeding women: a priority population for HIV viral load monitoring. *PLoS Med*. 2017;14(8): e1002375. https://doi.org/10.1371/journal.pmed.1002375
24. Gous N, Scott L, Berrie L, Stevens W. Options to expand HIV viral load testing in South Africa: Evaluation of the GeneXpert® HIV-1 viral load assay. *PloS one*. 2016;11(12):e0168244. https://doi.org/10.1371/journal.pone.0168244
25. Tanser F, Vandormael A, Cuadros D, et al. Effect of population viral load on prospective HIV incidence in a hyperendemic rural African community. *Sci Transl Med*. 2017;9(420):eaam8012. https://doi.org/10.1126/scitranslmed.aam8012

26. Assir MZK, Ahmad F, Riaz SH, Adil A, Rashid T. Viral suppression and loss to follow up in HIV/AIDS patients on antiretroviral therapy in Pakistan. *Int J Infect Dis*. 2018;73(Suppl):e249. https://doi.org/10.1016/j.ijid.2018.04.3982
27. Joulaei H, Shooshtarian S, Dianatinasab M. Is UNAIDS 90-90-90 target a dream or a reality for Middle East and North Africa region on ending the AIDS epidemic? A review study. *AIDS Rev*. 2018;20(2):83-93. https://doi.org/10.24875/AIDSRev.M18000020