

Full Length Research Article

Serum P53 Protein Level and Some Haematologic Parameters among Women of Reproductive Age Living with HIV Infection

*Akwiwu E.C.¹, Okafor A.O.², Akpan P.A.¹, Akpotuzor J.O.¹, Asemota E.A.¹, Okoroiwu H.U.¹, Anyanwu S.O.¹

¹Department of Medical Laboratory Science, College of Medical Sciences, University of Calabar, Nigeria.

²Department of Chemical Pathology, University of Calabar Teaching Hospital, Calabar, Nigeria

Summary: Human immunodeficiency virus (HIV) infection remains a health challenge in Nigeria, and women of reproductive age are disproportionately infected. P53 protein, D-dimer, serum ferritin, CD4 cell count, haemoglobin concentration and haematocrit levels were measured among non-pregnant women of reproductive age living with HIV infection in order to assess the impact of HIV infection on maternal health. A hundred and sixty-two subjects categorised into three groups of 54 persons each involving; newly diagnosed, subjects on highly active antiretroviral therapy (HAART) and apparently healthy control subjects were recruited. Blood samples were analyzed for haemoglobin concentration, haematocrit, CD4 cell count, serum ferritin, D-dimer and p53 protein levels by standard methods. The CD4 cell count, serum p53 protein, and Hb Conc. were significantly lower, while serum ferritin was higher in the newly diagnosed group ($p=0.001$), followed by the group on HAART ($p=0.001$) compared to the controls. D-dimer level was significantly lower in the control group ($2899.11\pm670.73\text{pg/ml}$) than both newly diagnosed ($4842.44\pm489.40\text{pg/ml}$) and HAART ($4660.31\pm519.83\text{pg/ml}$) groups, while significant decrease in haematocrit was observed between the newly diagnosed group ($0.336\pm0.071/l$) as against both treated ($0.378\pm0.041/l$) and control ($0.362\pm0.021/l$) groups. D-dimer correlated negatively with serum p53 protein level among the newly diagnosed subjects and with Hb Conc. among subjects undergoing treatment. The study concludes that women of reproductive age living with HIV infection showed higher D-dimer and lower tumour suppression protein levels as well as anaemia and reduced immune response. The newly diagnosed subjects were more affected.

Keywords: HIV infection, anaemia, tumour suppression, activated coagulation

©Physiological Society of Nigeria

*Address for correspondence: ecakwiwu@gmail.com; Tel: +234 8036777296

Manuscript received- April 2021; Accepted- June, 2021

INTRODUCTION

Several decades after the emergence of human immunodeficiency virus (HIV) infection, developing countries such as Nigeria still struggle from the health burden of HIV infection. From timely detection of HIV infection in the population to effective management of diagnosed cases, efforts to bring the scourge under control are ongoing in the country. Although the Nigerian national epidemiological data on HIV infection among persons aged 15-49 years of age shows a prevalence of 1.4%, the South-South geopolitical zone of the country has the highest prevalence of 3.1% in the country (United Nations Programme on HIV/AIDS, 2019). It is imperative that apart from the immediate concern of curbing spread and limiting prevalence, infected persons also require adequate care especially as it relates to the common complications of the infection. With HIV infection still a public health challenge in Nigeria, there is need to continue investigations in this direction.

Reports from previous studies within the studied population indicate a disproportionate age and gender distribution within the socio-demographic characteristics of persons living with HIV infection (Okafor *et al.*, 2016; Akwiwu *et al.*, 2017; Okafor *et al.*, 2019). Women of child-bearing age constitute a vulnerable group among adults infected with the virus. Added to this unfortunate situation, substantial proportions of HIV infection in children from

Calabar, Nigeria have been observed among those delivered by traditional birth attendants. A trend with implications for the eventual effective control of HIV infection in the locality as the risk of mother to child transmission could be heightened under these circumstances (Federal Ministry of Health, 2010; World Health Organization, 2010; Ugochi *et al.*, 2018). There is need for effective detection and management of people living with HIV infection, particularly women of reproductive age in order to break the transmission chain to the next generation. This concern has necessitated the present study on the possibility as well as magnitude of haemostatic and tumour suppression involvements in HIV infection as it affects women of reproductive age.

The immune deficiency that results from HIV infection is a consequence of the viral attack on the helper T-lymphocytes responsible for effective immune response. Reduction in CD4 cell count has been identified as a marker for severity of infection in HIV, and in its subsequent progression to acquired immune deficiency syndrome. Being the primary target in the viral invasion of host immunity, decline in CD4 cell count remains a relevant parameter in the assessment of the severity of immunosuppression. The CD4 cell count has also been relied on for its prognostic value as consistent decline has been associated with severity of anaemia, activated coagulation, increased risk of opportunistic infections and neoplasms (Biggar *et al.*, 2007; Cummins and Bradley,

2010; Riedel *et al.*, 2013; Coghill *et al.*, 2015; Panwar *et al.*, 2016). In fact, impaired immunity and the development of concurrent cancers culminate into a cycle that mediates HIV-related morbidity and mortality. It is thought that the pathogenesis of cancer derives from two basic mechanisms, which include the switching on of oncogenes and the suppression of suppressor genes. The tumour suppressor p53 gene and its protein take part in the early detection and deletion of tumour clones. Consequent upon this involvement, changes in the expression of p53 gene and its protein accompany associated conditions and contribute to HIV-associated mortality (Yoon *et al.*, 2015; Yang *et al.*, 2016).

Derangement in blood cell lineages have been among the haematologic complications of HIV infection. Particularly of interest with regards to disease progression is the occurrence of anaemia in association with HIV infection (Sullivan *et al.*, 1998; Gedefaw *et al.*, 2013). Anaemia has remained one of the major concerns in HIV infection especially as it is also a public health challenge in the general populations of developing countries such as Nigeria. Previous studies on the nature of this anaemia have so far revealed a derangement in the utilization of iron even in the face of excess available iron (Okafor *et al.*, 2016; Akwivu *et al.*, 2017). Anaemia of chronic infections is usually accompanied with a finding of iron imbalance. A situation that is attributed to the host body's defensive mechanism as body iron is sequestered from the infective agent. In fact, serum ferritin is reported to associate with immune deficiency and heightened inflammation in HIV infection (Walsh *et al.*, 2010; Ogbe *et al.*, 2012; Lopez-Calderon *et al.*, 2015). In view of the aforementioned concerns, this study aimed at assessing possible coagulation dysfunction, tumour suppression disturbance, immunosuppression and anaemia in women of reproductive age.

MATERIALS AND METHODS

Subjects: This study was carried out among non-pregnant women (between the ages of 21 and 43 years) living with HIV infection who were accessing healthcare for HIV diagnosis and management at University of Calabar Teaching Hospital, Calabar, Nigeria. Among the 108 HIV-infected subjects, 54 persons were newly diagnosed and were yet to be placed on highly active antiretroviral therapy (HAART). Another 54 subjects were already undergoing treatment, while an equal number of age-matched HIV seronegative apparently healthy women were recruited as controls.

Ethical consideration: Ethical approval was obtained from the Health Research and Ethics Committee of University of Calabar Teaching Hospital. Informed consent was obtained from each participant enrolled in the research and confidentiality was maintained.

Methods: Blood samples were collected into ethylene diamine tetra-acetic acid (at a concentration of 2 mg/mL of blood) and plain bottles.

Haemoglobin concentration and haematocrit were measured by automation using Sysmex KX-21N (Japan).

The Sysmex Kx-21N performs blood cells count by direct current detection method. Blood cells suspended in the diluted sample passing through the aperture, cause direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses.

The CD4⁺ cell count was analyzed using PartecCyflow Counter (Germany). When passing through a flow cuvette, cells are individually illuminated by light spot of a laser lamp. The scatter intensity is a measure of cell size and morphology. Additionally, due to the excitation, the dye molecules emit fluorescence of characteristic colour. This fluorescence light is separated into colour range by means of optical filters. The intensity of each colour range is analyzed for each single cell.

Serum ferritin was assayed using ELISA kit from BioCheck, Inc South San Francisco, USA. The ferritin quantitative test is based on a solid phase enzyme linked immunosorbent assay (ELISA). The assay system utilizes one rabbit anti-ferritin anti-body for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin anti-body in the anti-body-enzyme (horseradish peroxidase) conjugate solution. The concentration of ferritin is directly proportional to the color intensity of the developed solution. D-dimer and p53 protein were assayed using ELISA kits from Bioassay Technology Laboratory, China. For D-dimer assay, sample is added to wells pre-coated with D2D monoclonal antibody. After incubation a biotin-conjugated anti-human D2D antibody is washed away during a washing step. Following streptavidin-HRP and substrate solution additions, intensity of developed colour is measured as absorbance. Likewise, for P53 assay, sample is added to the wells pre-coated with P53/TP53 antibody. After incubation, unbound biotin conjugated anti-human P53/TP53 antibody is washed away during a washing step while the bound portion is estimated after colour development.

Statistical analysis: Statistical analysis was performed on Microsoft Excel (MS office 2010) for windows and SPSS version 20.0. One-way analysis of variance and Pearson's correlation were used in this study. Results were expressed as mean \pm standard deviation. The level of significance was set at $P \leq 0.05$.

RESULTS

Table 1 is the demographic representation of the study participants. These participants were literate and mostly married women of reproductive age. Table 2 captures variations in the mean values of CD4 cell count, D-dimer, serum p53 protein and ferritin levels as well as haemoglobin concentration (Hb Conc.) and haematocrit (HCT). The CD4 cell count, serum p53 protein, and Hb Conc. were significantly lower while serum ferritin was higher among the newly diagnosed group followed by the group on treatment compared to control group. For the D-dimer, both groups living with HIV infection had significantly higher mean values compared to the control group, while significant reduction in haematocrit was observed between the newly diagnosed against both treated and control groups.

Table 1.
Socio-demographic characteristics of study participants

Characteristics		Control subjects n =54 (100%)	Newly diagnosed subjects n =54 (100%)	Subjects on HAART n =54(100%)
Age range (years)	21-30	26 (48)	28 (52)	23 (43)
	31-40	18 (33)	19 (35)	17 (31)
	>40	10 (19)	7 (13)	14 (26)
Educational level	Secondary level	12 (22)	11 (20)	7 (13)
	Tertiary level	42 (78)	43 (80)	47 (87)
Marital status	Single	0 (0)	6(11)	3 (6)
	Married	54 (100)	48 (89)	51(94)
HIV status	Positive	0 (0)	54 (100)	54 (100)
	Negative	54 (100)	0 (0)	0 (0)
Treatment (HAART)	Commenced	0 (0)	0 (0)	54 (100)
	Yet to commence	0 (0)	54 (100)	0 (0)
	Not applicable	54 (100)	0 (0)	0 (0)

Table 2.
Mean values of the studied parameters among the participants

Parameters	Control subjects n=54	Newly diagnosed subjects n=54	Subjects on HAART n=54	p-Value
CD4 (cells/ml)	908.07±220.22 ^a	339.83±125.24 ^a	640.35±i60.31 ^a	0.001
D-Dimer (pg/ml)	2899.11±670.73 ^b	4842.44±489.40	4660.31±519.83	0.001
P53 (ng/l)	2041.93±482.77 ^a	1478.72±312.34 ^a	1834.28±400.61 ^a	0.001
Serum Ferritin (µg/l)	66.94±16.64 ^a	379.45±336.68 ^a	181.42±64.58 ^a	0.001
Hb Conc (g/l).	122.80±4.22 ^a	96.61±17.28 ^a	113.13±11.30 ^a	0.001
HCT (l/l)	0.362±0.02	0.336±0.07 ^b	0.378±0.04	0.001

Key:

a = significant difference across all groups; *b* = significant difference between identified group and the other groups
For routinely analysed parameters, reference range for women are as follows; Serum ferritin 15-200 µg/l, Hb Conc. 120-150 g/l and HCT 0.36-0.46 l/l.

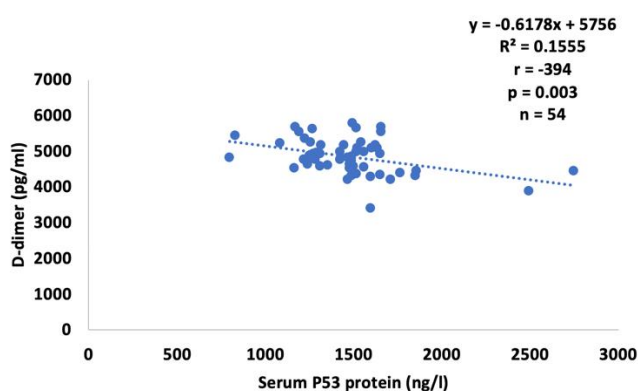


Figure 1
Correlation of Serum p53 protein and D-dimer among HAART-Naïve HIV-infected subjects

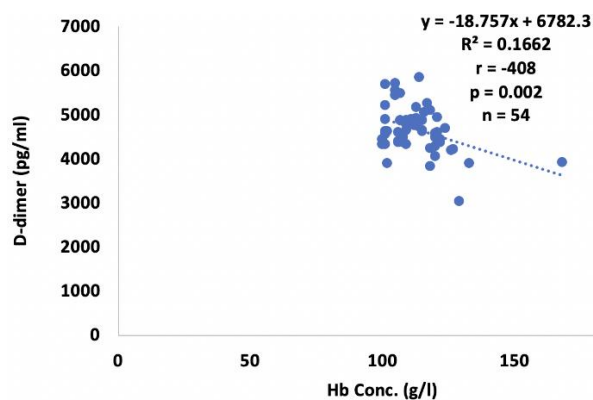


Figure 2
Correlation of Hb Conc. and D-dimer among HIV-infected subjects on HAART

Significant weak negative correlations between D-dimer and serum p53 protein level among the newly diagnosed as well as between D-dimer and Hb Conc. among subjects undergoing therapy (Figures 1 and 2 respectively) were observed.

DISCUSSION

Haemoglobin concentration and haematocrit are both consideration tools used for screening of anaemia, which are influenced by age and gender among other factors. Such physiological variations may mask the degree to which such indicators are deranged when general populations are

sampled. In view of the finding that women of reproductive age disproportionately carry the burden of HIV infection, it became necessary to segregate this group and investigate changes in some known and potential markers with regards to HIV infection and probable impact on reproductive health. Low Hb and elevated serum ferritin level were observed among persons living with HIV infection, particularly among the newly diagnosed followed by those undergoing antiretroviral therapy.

The latter group had more resolved values towards normalcy compared to the newly diagnosed. Interestingly, the values for Hb conc. would be expected to match the haematocrit across the groups, but the result revealed similar haematocrit values for both control and treated groups. This

observation in haematocrit values is at variance with the significantly lower value for Hb conc. of the group on treatment compared to controls. Increase in haematocrit for HIV-infected persons on treatment is attributable to both improvements in erythropoiesis as well as adverse effects from use of antiretroviral drugs that cause macrocytosis (Sullivan *et al.*, 1998; Okafor *et al.*, 2019). Imperatively, Hb conc. stands a better assessing parameter of anaemia for persons living with HIV, as haematocrit values alone may not be reliable.

Additionally, on effective erythropoiesis, serum ferritin serves as an indicator of stored iron but in chronic inflammatory conditions, it rather paints a picture of non-utilization of iron. Thus, the significant increase in serum ferritin observed in HIV infection as recorded in the present study supports the theory of iron sequestration commonly found in anaemia of chronic inflammation. The implication of this finding is that despite high iron stores, the release of iron for erythropoiesis remains insufficient. Although, being on antiretroviral therapy conferred some level of improvement, the infection itself remains an important aspect of HIV complication and a challenge in the management of the condition (Kyeyune *et al.*, 2014; Kallianpur *et al.*, 2016; Al-Kindi *et al.*, 2017; Okafor *et al.*, 2019).

The CD4 count and p53 level were significantly reduced, while elevated D-dimer level existed in HIV infection. Significant weak negative correlations were also seen between D-dimer versus serum p53 protein level and haemoglobin concentration among newly diagnosed subjects and those undergoing therapy respectively. The degree of immuno-competence versus immunodeficiency, mainly assessed by CD4 T-helper cell count, is an important aspect in the management of HIV infection. Indicative of disease progression, CD4 T-helper cell count is important in routine monitoring of patients even after commencement of antiretroviral therapy. D-dimer level attests to the presence or otherwise of activated coagulation beyond the information obtainable from platelets as part of full blood count. Its downward resolution among those undergoing antiretroviral therapy was not statistically different from the newly diagnosed, altogether both differed significantly from control mean value. Owing to the risk of poor pregnancy outcome in the event of activated coagulation, D-dimer measurement serves as a screening assessment for the possibility of venous thromboembolism (Choi and Krishnamoorthy, 2018; Zaini *et al.*, 2019). Therefore, a finding of elevated D-dimer values in a group of women of reproductive age is of concern, particularly as planned pregnancy and preconception maternal care are not very common in developing countries such as Nigeria. More so, activated coagulation correlated with anaemia among subjects undergoing therapy. In tallying to HIV-associated anaemia, there may be need to look into activated coagulation and also its possible mediation in HIV-related neoplasms more directly in the management of HIV infection among women.

Currently in developing countries, there is paucity of information on p53 assessment in disease diagnosis. The p53 gene and its protein regulate cell division and aid apoptosis. Regulation of p53 expression is necessary for immune competence, otherwise abnormal proliferations emanating during cell growth would be left unchecked

(Theoret *et al.*, 2008; Pinzone *et al.*, 2012). Unfortunately, cancer is among the AIDS-defining features in HIV infection. The observation of significant reduction in the p53 protein level among HIV-infected subjects suggests consumption of p53 protein possibly arising from increased tumour clones in HIV infection. The co-existence of activated coagulation and dwindling tumour suppression mechanisms among the newly diagnosed is a revealing observation capable of impacting reproductive health especially as conventional antenatal care is optional and influenced by socio-demographic dynamics. Anaemia on its own contributes immensely to maternal mortality, particularly, in developing countries. Added to the existing knowledge of HIV-associated anaemia, this study highlights heightened coagulation and diminishing tumour suppression indicators in HIV infection with the newly diagnosed being worse off. Unfortunately, it is not feasible to determine duration of infection prior to detection. It is also disturbing that maternal health in this region of the world is often considered in the context of pregnancy period with less attention on post-pregnancy period. Preconception aspect of maternal health for the general population is still evolving (Olowokere *et al.*, 2015; Ekem *et al.*, 2018; Akinajo *et al.*, 2019).

In addition to detecting anaemia and immunosuppression, this study has revealed ongoing coagulation disturbance as shown by higher D-dimer levels alongside lower tumour suppression protein levels among women of reproductive age living with HIV infection.

REFERENCES

- Akinajo, O.R., Osanyin, G.E., Okojie, O.E. (2019). Preconception care: Assessing the level of awareness, knowledge and practice amongst pregnant women in a tertiary facility. *J. Clin. Sci.* 16(3): 87-92.
- Akwivu, E.C., Akpotuzor, J.O., Okon, J.E., Egharevba, O. (2017). Iron studies of HIV-infected subjects in Calabar- A Nigerian perspective. *J. Med. Sci. Clin. Res.* 5: 19572-19577.
- Al-Kindi, S.G., Zidar, D.A., McComsey, G.A., Longenecker, C.T. (2017). Association of anisocytosis with markers of immune activation and exhaustion in treated HIV. *Pathog. Immun.*; 2: 138-150.
- Biggar, R.J., Chaturvedi, A.K., Geodert, J.J., Engels, E.A. (2007). AIDS-related cancer and severity of immunosuppression in persons with AIDS. *J. Natl. Cancer Inst.* 99: 962-972.
- Choi, H., Krishnamoorthy, D. (2018). The diagnostic utility of D-dimer and other clinical variables in pregnant and post-partum patients with suspected acute pulmonare embolism. *Int J Emerg Med.*; 11(1):10.
- Coghill, A., Shiels, M.S., Suneja, G., Engels, E.A. (2015). Elevated Cancer-specific Mortality among HIV-infected Persons in the US. *J Clin. Oncol.* 33:2376-83.
- Cummins NW, Badley AD (2010). Mechanisms of HIV-associated lymphocyte apoptosis. *Cell Death Dis.* 1:e99.
- Ekem, N.N., Lawani, L.O., Onoh, R.C., Iyoke, C.A., Ajah, L.O., Onwe, E.O., Onyebuchi, A.K., Okafor, L.C. (2018). Utilization of preconception care services and determinants of poor uptake among a cohort of women in Abakiliki Southeast Nigeria. *Journal of Obstetrics and Gynaecology* 38(6): 739-744.
- Federal Ministry of Health (2010). Nigeria National Guidelines on prevention of mother to child transmission of HIV in Nigeria. https://aidsfree.usaid.gov/sites/default/files/tx_nigeria_pmctc_2010.pdf
- Gedefaw, L., Yemane, T., Sahlemariam, Z., Yilma, D. (2013). Anemia and Risk Factors in HAART Naïve and HAART

- Experienced HIV Positive Persons in South West Ethiopia: A Comparative Study. PLoS ONE 8: e72202.
- Kallianpur, A.R., Wang, Q., Jia, P., Hulgán, T., Zhao, Z., Letendre, S.L., *et al.* (2016). Anemia and Red Blood Cell Indices Predict HIV-Associated Neurocognitive Impairment in the Highly Active Antiretroviral Therapy Era. *J Infect Dis.* 213: 1065–1073.
- Kyeyune, R., Saathoff, E., Ezeamama, A.E., Loscher, T., Fawzi, W., Guwatudde, D. (2014). Prevalence and correlates of cytopenias in HIV-infected adults initiating highly active antiretroviral therapy in Uganda. *BMC Infect Dis.* 14: 496.
- Lopez-Calderon, C., Palacios, R., Cobo, A., Nuno, E., Ruiz, J., Marquez, M. *et al.* (2015). Serum ferritin in HIV-positive patients is related to immune deficiency and inflammatory activity. *Inter J STD AIDS* 26: 393-397
- Ogbe, P.J., Idoko, O.A., Ezimah, A.C., Digban, K.A., Oguntayo, B.O. (2012). Evaluation of iron status in anemia of chronic disease among patients with HIV infection. *Clin. Lab. Sci.* 25:7-12.
- Okafor, A.O., Akwiwu, E.C., Akpotuzor, J.O. (2019). Prevalence of Anaemia after Initiation of Antiretroviral Therapy among HIV-infected Patients attending University of Calabar Teaching Hospital Calabar, Nigeria. *International Journal of Tropical Diseases and Health* 35: 1-7.
- Okafor, A.O., Usanga, E.A., Akwiwu, E.C. (2016). Iron related parameters of HIV-infected patients attending University of Calabar Teaching Hospital, Nigeria. *Journal of Dentistry and Medical Sciences* 15: 65-68.
- Olowokere, A.E., Komolafe, A., Owofadeju, C. (2015). Awareness, knowledge and uptake of preconception care among women in Ife Central Local Government Area of Osun State, Nigeria. *Journal of Community Medicine and Primary Health Care* 27(2): 83-92.
- Panwar, A., Sharma, S.C., Kumar, S., Sharma, A. (2016). A study of anemia in human immunodeficiency virus patients: Estimating the prevalence, analyzing the causative effect of nutritional deficiencies, and correlating the degree of severity with CD4 cell counts. *Med J DY Patil Univ* 9: 312-318.
- Pinzone, M.R., Fiorica, F., Di Rosa, M., Malaguanera, G., Malaguanera, L., Cacopardo, B., *et al.* (2012). Non-AIDS-defining cancers among HIV-infected people. *Eur. Rev. Med. Pharmacol. Sci.* 16: 1377-1388.
- Riedel, D.T., Mwangi, E.I., Fantry, L.E., Alexander, C., Hossain, M.B., Pauza, C.D. *et al.* (2013). High cancer-related mortality in an urban, predominantly African American, HIV-infected population. *AIDS* 27: 1109-1171.
- Sullivan, P.S., Hanson, D.L., Chu, S.Y., Jones, J.L., Ward, J.W. (1998). Epidemiology of anemia in human immunodeficiency virus (HIV)-infected persons: results from the multistate adult and adolescent spectrum of HIV disease surveillance project. *Blood* 91: 301-308.
- Theoret, M.R., Cohen, C.J., Nahvi, A.V., Ngo, L.T., Suri, K.B., Powell, D.J. Jr. *et al.*, (2008). Relationship between p53 overexpression on cancers and recognition by anti-p53 T cell receptor-transduced T cells. *Hum. Gene. Ther.* 19: 1219-1232.
- Ugochi V.E., Akwiwu E.C. & Akpotuzor J.O. (2018). Factors associated with HIV Transmission and Infection among Persons Aged 0-17 years in Calabar Metropolis of Nigeria. *Journal of Medical and Dental Science Research* 5 (5): 27-30.
- United Nations Programme on HIV/AIDS (2019). New survey results indicate that Nigeria has an HIV prevalence of 1.4%. <https://www.unaids.org>
- Walsh, C.M., Hattingh, Z., Veldman, F.J., Bester, C.J. (2010). Iron status and anaemia of chronic disease in HIV infected African women in Mangung, Bloefontein. *SA Family Practice* 52: 55-59.
- World Health Organization (2010). PMTCT strategic vision 2010-2015: Preventing mother-to-child transmission of HIV to reach the UNGASS and Millennium Development Goals. <https://www.who.int/hiv/pub/mtct/strategicvision.pdf>
- Yang, J., Su, S., Zhao, H., Wang, D., Wang, J., Zhang, F., *et al.* (2016). Prevalence and mortality of cancer among HIV-infected in patients in Beijing, China. *BMC Infect. Dis.*;16: 82
- Yoon, C.H., Kim, S.Y., Byeon, S.E., Jeong, Y., Lee, J., Kim, K.P., *et al.* (2015). P53-derived host restriction of HIV-1 replication by protein kinase R-mediated Tat phosphorylation and inactivation. *J Virol.* 89: 4262-4280
- Zaini, R., Al-Rehali, A., Kufia, R. (2019). Evaluation of plasma D-Dimer concentration among normal and complicated pregnancies, Saudi Arabia. *International Journal of Women's Health and Reproduction Sciences* 7(1):17-23.