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# CLINICAL EFFECT OF *MORINGA OLEIFERA* ON BODY MASS INDEX,TRIGLYCERIDE AND HIGH DENSITY LIPOPROTEIN IN SUBJECTS TAKEN TENOFOVIR COMBINATION REGIMEN

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**ABSTRACT**: *Antiretroviral drugs can have adverse effects. Most are manageable, but some can be serious. The aim of this clinical study is to evaluate the potential benefits of taking Moringa oleifera on body mass index (BMI), blood triglyceride and High density lipoprotein (HDL) level in patient taking Tenofovir/Lamivudine/efavirenz (TLE) combination. The study was designed as a Longitudinal Randomized Comparative Trial (LRCT) involving 140 HIV adult subjects (56 males, 84 females) who have been on Tenofovir/Lamivudine/efavirenz (300/300/600mg) TLE combination for at least 6 months prior to the study. They were recruited from a Teaching Hospital in Nigeria. Moringa oleifera capsules (200mg) were administered by the subjects to be used beginning from the first day of visit 0, through visit 1 (after four weeks) and 2 (after 12 weeks). Blood samples of subjects were collected at each visit (visit 0, 1 and 2) and analyzed for triglyceride and HDL level.There was no significant reduction in serum HDL level (P<0.01) of subjects in visit 1 but there was significant (P<0.01) increase on visit 2 when compared to visit 0. There was also significant improvement in blood triglyceride level (P<0.01) in visit 1 and 2 compared to visit 0 of tenofovir/Lamivudine/Efavirenz (TLE) moringa combination. Results from the study suggests that Moringa oleifera may be useful in improving triglyceride and cholesterol level of patients receiving TLE combination.*

**KEYWORD**: Moringa oleifera, blood, glucose, tenofovir, triglyceride

## INTRODUCTION

HIV is a retrovirus that targets the immune system, which is the system that fights off infection and disease. The virus damages or destroys white blood cells called CD4 cells. This makes it difficult for the body to fight off illness (Deeks, Lewin, Havlir, 2013; Adedapo, Mogbojuri, Emikpe 2009). Antiretroviral therapy prevents the virus from multiplying, which reduces the amount of HIV in the body (Adias et al., 2013). This gives the immune system a chance to produce more CD4 cells. Although antiretroviral therapy cannot completely remove HIV from the body, it keeps the immune system strong enough to combat infections and some HIV-related cancers. The aim of antiretroviral therapy is to reduce the amount of HIV in the blood to very low levels. Viral



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suppression occurs when the count reaches fewer than 200 copies of the virus per milliliter of blood (Moore, Chaisson, 1999; Adusi-Poku et al., 2008).

HIV drugs have improved over the years, and serious side effects are less likely than they used to be. However, HIV drugs can still cause side effects (Akashi,Traver, Kondo, 1999; Bai et al., 2013). Some are mild, while others are more severe or even life-threatening. A side effect can also get worse the longer a drug is taken. It's possible for other medications to interact with HIV drugs, causing side effects. Other health conditions can also make the side effects from HIV drugs worse (Bai et al., 2013). For these reasons, when starting any new drug, people with HIV should tell their healthcare provider and pharmacist about all the other medications, supplements, or herbs they're taking (Moore, Chaisson, 1999;Bai et al., 2013).

Antiretroviral formulations blocks HIV at certain stages of the viral "life cycle" (Builder., Anzaku. and Joseph, 2019). Processes such as "binding, fusion and entry, reverse transcription and integration, proviral transcription, cytoplasmic expression" are involved in the viral cycle (Da Silva et al., 2010), replication, assembly and budding, release, maturation. Moringa oleifera Lam (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics (Moore, Chaisson, 1999. The Moringa leaf are prepared for consumption either fresh, dried, or as extract of an aqueous solution (Fauci, Folkers 2012; Chukwuebuka, 2015; Von Maydell, 1986). Some populations consume it in their daily diet, whereas others use as a nutritional supplement and for medicinal purposes, mainly for diabetes. Common ailments such as malaria, typhoid fever, swellings, cuts, hypertension and diabetes are treated with the leaves (Guidelines 2015). They are also used to bring about milk production in lactating women (Guidelines et al., 2016; Logie, Gadalla, 2009; Calza,, Manfredi, Chiodo, 2004a), sediment impurities of water (Tsai et al., 1990), detoxifies the system of free radicals (Romanelli, Smith, Hoven 2004; Murray et., 2010), improves immunity (to manage HIV/AIDS and treat related symptoms). The aim of this study is to evaluate the clinical effect of taking Moringa oleifera with Tenofovir/Lamivudine/efavirenz (300/300/600mg) (TLE) regimen on blood cholesterol and triglyceride level.

## MATERIALS AND METHOD

The study designed was a Longitudinal "Randomized Comparative Trial" (LRCT) as applicable in clinical investigation involving two or more patient treatment groups, over a time frame. This study is designed in line with a part of the FDA (Food and Drug Administration)/WHO Phases during "randomized controlled clinical trials" (RCCT) of drugs. However, details about the application of RCCT have been clarified by FDA/WHO which made the purpose of such investigation explicit; stating that it was designed to affirm and or set aside hypothetical clinical claims (Junod and Beaver, 2013) of administrable substances. Groups were analyzed in 3 phases as baseline (commencement) 4weeks follow-up and 12 weeks post

commencement of supplements (conclusion of administration).

**Recruitment procedure**

Subjects were recruited at the out-patient department of a Teaching Hospital HIV-clinic. Prospective participants were officially and properly informed prior to the exercise, doubts were cleared and benefits x-rayed to the patients. The Longitudinal Randomized Comparative Trial (LRCT) was employed and used.

**Procedure**

The study was designed as a Longitudinal Randomized Comparative Trial (LRCT) involving a total of 140 HIV adult subjects (56 males, 84 females) who have been on Tenofovir/Lamivudine/efavirenz (300/300/600mg) TLE combination for at least 6 months. Subjects were categorized into groups as underweight, normal weight, over weight and obese. On visit 0, blood samples of the subjects already on TLE regimen (without moringa or any supplements) for at least 6 month were taken for analysis. Moringa oleifera capsules (200mg) were given to each subject to be taken from commencement (baseline) to 12 weeks post commencement of study. Blood samples of subjects were collected at each visit (visit 1 and 2) and analyzed for HDL and triglyceride level.

**Data collection**

Anthropometric parameters (weight and height) and blood samples were determined for eligible patients (participants) distributed into the various categories; after duly signed consent forms were retrieved. Blood samples were analyzed at the UPTH Hematology research lab within the hospital premises.

**Blood Sample**

Analysis of samples was done at the hematology laboratory of the University of Port Harcourt Teaching Hospital (UPTH), Rivers state, Nigeria. "Computerized clinical chemistry analyzer" (VS10) (Vitro Scient) operating with the principle guided by "Beer-lambert's law" was used to determine concentration of biochemical parameters under study. Parameters as analysed were;

**1.** Triglyceride (TG; 0.9-1.03mmol/l); 1000ul of reagent as well as 10ul of serum were incubated for four (4) minutes at room temperature, at a wavelength of 460-540nm.

**2.** High Density Lipoproteins (HDL; 0.78-2.06mmol/l): 5ul of serum and 480ul of reagent were incubated for four (4) minutes at room temperature. And therefore 160ul of reagent was incubated for four (4) minutes and then read at 540nm wavelength.

**Data analysis**

Data was presented in tables using SPSS (IBM® version 23) and MATLAB (version 17). Descriptive statistics was used to express variable characteristics (with continuous data stated as mean (S.D) while categorical data as frequency [%]). Dunnette T3 Post Hoc test of



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multiple comparisons was used to compare means, while binary logistic regression was used to predict factors contributing to the changes in variables. Variable interactions were tested at 95% confidence level; with P≤0.05 taken to be significant.

**Ethical consideration**

**Ethical approval**

Ethical approval was granted by the "University of Port Harcourt Research Ethics Committee"referenced as UPH/R&D/REC/---

**Patient consent**

In line with the ethical requirement documented by Didia (2008), the following ethical issues were considered while carrying out the study:

i. Beneficence, (the duty to do good, and with due consideration of the best interests of the subjects).
ii. Non-maleficence, (the obligation of avoidance of harm to the subjects; when possible).
iii. Respect for persons, (Giving the deserved respect to all subjects).
iv. Justice and confidentiality (ensure fairness and unconditional privacy protection)

Individual who did not want to participate were not compelled nor forced. Volunteer subjects gave informed consent prior to the experiment. This was done following the Revised "Council for International Organization of Medical Sciences (CIOMS) International Ethical Guidelines, Utrecht, Netherlands, June 2016". However, all relevant statutory requirements were followed to the later and where necessary.

## RESULT

**High density lipoprotein (HDL) and triglyceride level of ART subject taking TLE on visit day 0**

Underweight subjects were found to be 6 subjects, normal weight were 76 subjects, overweight were 44 subjects while obese were found to be 14 subjects (table 1).

**Effect of Moringa oleifera on ART patient taking TLE on visit day 1**

There was no significant differences (P<0.001) in HDL level observed in mean values of TLE/Moringa subjects between visit 0 and visit 1. There was significant (P<0.001) decrease in the level of triglyceride in visit 1 when compared to visit 0. Also, there was no significant difference between TLE/Moringa (visit 1) and TLE/Non Moringa (visit 1) in the level of serum triglyceride of the subjects (table 2,3,4,5 and 6)

**Effect of Moringa oleifera on ART patient taking TLE on visit day 2**

There was statistically significant (P<0.001) different in mean values of the TLE/Moringa subjects between visit 0 and visit 2 in HDL and triglyceride levels, while there was no significant

differences (P<0.001) between TDF/Non Moringa (visit 2) and TDF/Non Moringa (visit 0) in the level of serum HDL and triglyceride (table 2, 3, 4,5 and 6).

**Table 1:** Socio-demographic and anthropometric characteristics of the study population

| **Sex** | | **N** | **Mean±S.D** | **S.E** |
|---|---|---|---|---|
| **Age (yrs.)** | **Male** | 54 | 39.11±10.46* | 1.43 |
| | **Female** | 86 | 35.63±8.33 | 0.89 |
| | **Total** | **140** | **36.01±9.41** | **0.77** |
| **Weight (kg)** | **Male** | 54 | 69.00±9.76 | 1.3 |
| | **Female** | 86 | 66.43±12.1 | 1.25 |
| | **Total** | **140** | **67.38±11.3** | **0.92** |
| **Height (m)** | **Male** | 54 | 1.71±0.09** | 0.01 |
| | **Female** | 86 | 1.64±0.08 | 0.01 |
| | **Total** | **140** | **1.66±0.09** | **0.01** |
| **BMI (kgm-$^2$)** | **Male** | 54 | 23.77±3.26 | 0.44 |
| | **Female** | 86 | 24.79±4.60 | 0.47 |
| | **Total** | **140** | **24.41±4.17** | **0.35** |

**Table 2:** The descriptive characteristics and test of mean differences of metabolic profile of the HIV patients on tenofovir based ART at Visit 0 (Baseline)

| | **Descriptive statistics** | | | | **T-test of mean difference** | | |
|---|---|---|---|---|---|---|---|
| **PARAMETERS** | **Sex** | **N** | **Mean±S.D** | **S.E** | **t-value** | **P-value** | **Inf** |
| **TG (mmol/l)** | **Male** | 56 | 1.43±0.49 | 0.07 | 2.305 | **0.023** | **S** |
| | **Female** | 84 | 1.24±0.47 | 0.05 | | | |
| | **Total** | **140** | **1.32±0.48** | | | | |
| **HDL (mmol/l)** | **Male** | 56 | 1.38±0.44 | 0.06 | 0.526 | 0.600 | NS |
| | **Female** | 84 | 1.34±0.56 | 0.06 | | | |
| | **Total** | **140** | **1.36±0.51** | | | | |

**Note:** TG=Triglyceride, HDL=High density lipoprotein N=Distribution, S.D=Standard deviation, S.E=Standard error of mean, Min=Minimum, Max=Maximum, P-value=Probability value, t-value=t-test calculated value, Inf=Inference (S=Significant, NS=Not Significant).



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**Table 4:** The descriptive characteristics of metabolic profile of the HIV patients on tenofovir with Moringa (TDF/M) and tenofovir alone (TDF/NM) at Visit 1 (4 weeks of administration)

| GROUPS | | T.G (mmol/l) | HDL (mmol/l) |
|---|---|---|---|
| **Male (N=56)** | **Mean±S.D** | 1.25±0.40 | 1.27±0.50 |
| | **S.E** | 0.05 | 0.06 |
| | **Range (Min – Max)** | 0.42 - 2.17 | 0.39 - 2.27 |
| **Female (=84)** | **Mean±S.D** | 1.27±0.37 | 1.32±0.49 |
| | **S.E** | 0.04 | 0.05 |
| | **Range (Min– Max)** | 0.24 - 2.21 | 0.4 -2.25 |
| **Total (N=140)** | **Mean±S.D** | **1.26±0.38** | **1.30±0.49** |
| | **S.E** | 0.03 | 0.04 |
| | **Range(Min – Max)** | 0.24 - 2.21 | 0.39 - 2.27 |

**Table 5:** The descriptive characteristics of metabolic profile of the HIV patients on tenofovir with Monringa (TDF/M) and tenofovir alone (TDF/NM) at Visit 2 (12 weeks of administration)

| GROUPS | | T.G (mmol/l) | HDL (mmol/l) |
|---|---|---|---|
| **Male (N=56)** | **Mean±S.D** | 1.09±0.55 | 1.87±0.57 |
| | **S.E** | 0.07 | 0.08 |
| | **Range (Min – Max)** | 0.02 – 3.25 | 1.17 – 4.27 |
| **Female (N=84)** | **Mean±S.D** | 1.15±0.47 | 1.42±0.46 |
| | **S.E** | 0.05 | 0.05 |
| | **Range (Min– Max)** | 0.23 - 3.18 | 0.17 - 3.35 |
| **Total (N=140)** | **Mean±S.D** | **1.13±0.50** | **1.60±0.55** |
| | **S.E** | 0.04 | 0.05 |
| | **Range(Min – Max)** | 0.02 - 3.25 | 0.17 - 4.27 |



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**Table 6:** Post Hoc (Dunnette T3) multiple comparison of the metabolic profile of HIV patients on TDF NOT taking moringa (TDF) supplement across the various visits

| Parameters | Visits | Mean±S.D | Min | Max | S.E | 95% C.I for Mean | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| T.G (mmol/l) | Visit 0 | 1.24±0.47 | 0.30 | 2.30 | 0.05 | 1.14 | 1.35 |
| | Visit 1 | 1.27±0.37 | 0.24 | 2.21 | 0.04 | 1.19 | 1.35 |
| | Visit 2 | 1.15±0.47 | 0.23 | 3.18 | 0.05 | 1.05 | 1.25 |
| | **Total** | **1.22±0.44** | **0.23** | **3.18** | **0.03** | **1.17** | **1.28** |
| HDL (mmol/l) | Visit 0 | 1.34±0.56 | 0.28 | 2.31 | 0.06 | 1.22 | 1.46 |
| | Visit 1 | 1.32±0.49 | 0.40 | 2.25 | 0.05 | 1.21 | 1.42 |
| | Visit 2 | 1.42±0.46 | 0.17 | 3.35 | 0.05 | 1.32 | 1.52 |
| | **Total** | **1.36±0.50** | **0.17** | **3.35** | **0.03** | **1.30** | **1.42** |

**Table 7:** Post Hoc (Dunnette T3) multiple comparison of the metabolic profile of HIV patients on TDF taking moringa (TDF+M) supplement across the various visits

| Parameters | Visits | Mean±S.D | Min | Max | S.E | 95% C.I for Mean | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| T.G (mmol/l) | Visit 0 | 1.43±0.49*^ | 0.58 | 2.83 | 0.07 | 1.30 | 1.56 |
| | Visit 1 | 1.25±0.40' | 0.42 | 2.17 | 0.05 | 1.14 | 1.35 |
| | Visit 2 | 1.09±0.55 | 0.02 | 3.25 | 0.07 | 0.94 | 1.23 |
| | **Total** | **1.26±0.50** | **0.02** | **3.25** | **0.04** | **1.18** | **1.33** |
| HDL (mmol/l) | Visit 0 | 1.38±0.44*^ | 0.24 | 2.14 | 0.06 | 1.26 | 1.50 |
| | Visit 1 | 1.27±0.50' | 0.39 | 2.27 | 0.07 | 1.14 | 1.41 |
| | Visit 2 | 1.87±0.57 | 1.17 | 4.27 | 0.08 | 1.72 | 2.02 |
| | **Total** | **1.51±0.57** | **0.24** | **4.27** | **0.04** | **1.42** | **1.59** |



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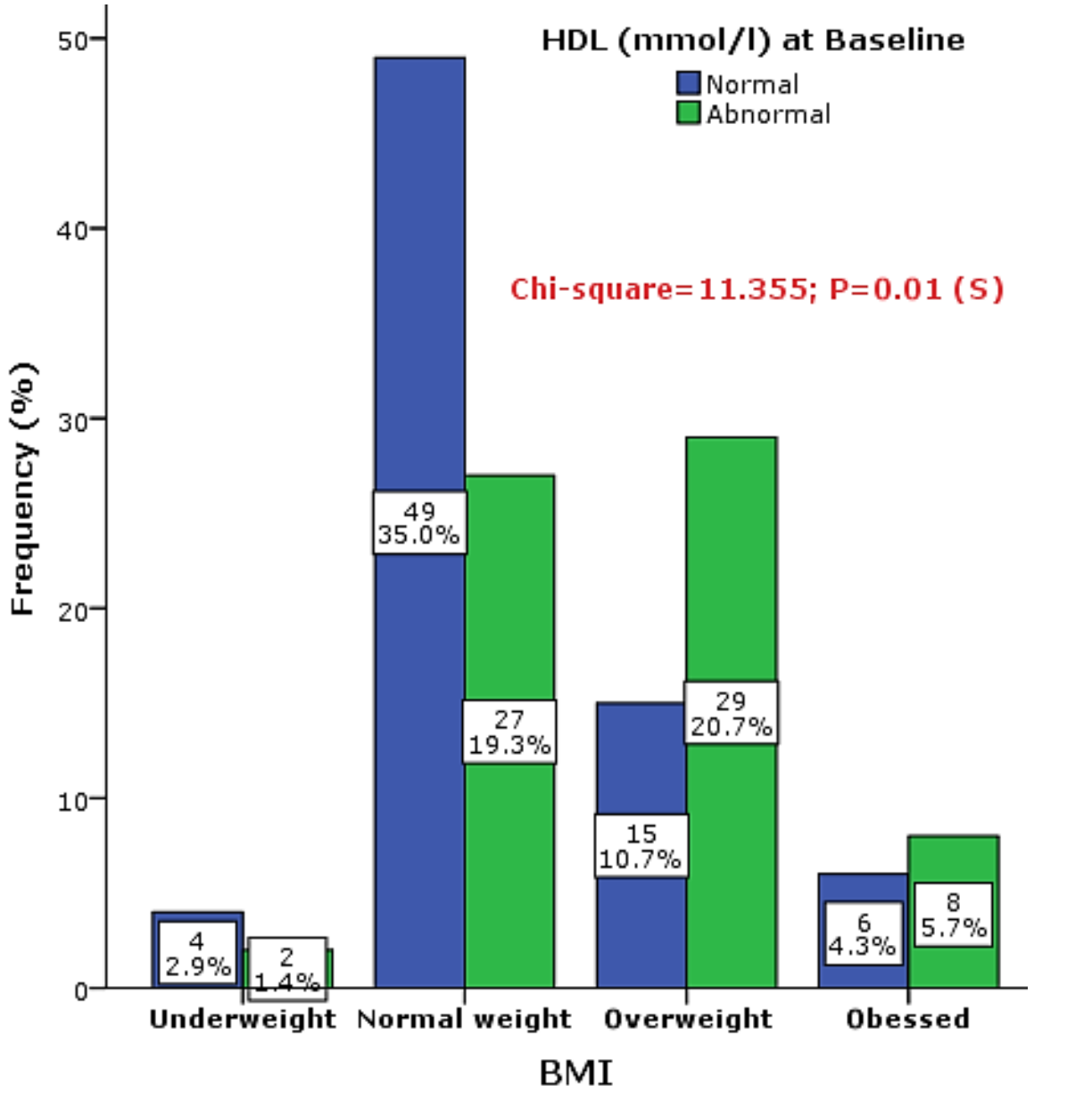


**Figure 1:** BMI associated high density lipoprotein (HDL) classification and distribution at Visit 0 (Baseline)



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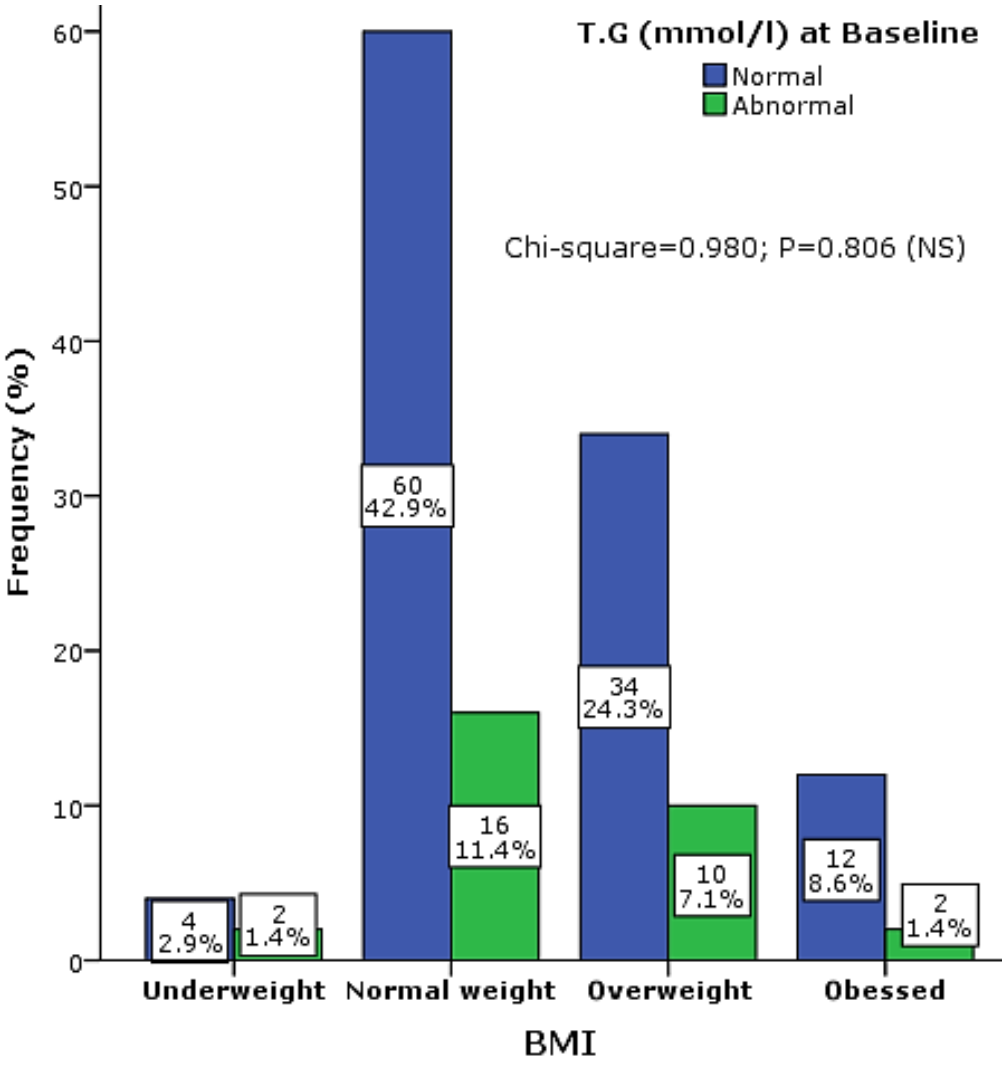


**Figure 2:** BMI associated triglyceride classification and distribution at Visit 0 (Baseline)

## DISCUSSION

The primary goal of antiretroviral therapy for human immunodeficiency virus (HIV) infection is suppression of viral replication (Savarino, Shytaj et al., 2015;WHO, 2008). Evidence indicates that the optimal way to achieve this goal is by initiating combination therapy with two or more antiretroviral agents. This therapy helps keep the body healthy and prevent infections (Lieberman-Blum, Fung, Bandres, 2008; LaRosa, Grundy, Waters, 2005). Specifically, successful antiretroviral therapy prevents people from developing advanced HIV and makes it impossible to transmit the virus to others. Although the study was abinitio designed to investigate the effect of M. oleifera supplementation on TDF dependent HIV patients; however various reports of ARV

therapy associated metabolic abnormalities (Meraiyebu et al., 2014; Calza et al., 2004; Moore, Chaisson, 1999) informed this investigation. Also the increased prevalence of these abnormalities necessitated the evaluation of the relationship TDF based regimen has with abnormal presentation of selected metabolic profile. Prevalent abnormal metabolic abnormalities were higher in proportion with abnormal BMI; mostly overweight and obesed; as proportion of HIV patients with HDL-C and hypertriglyceridemia was almost 2 times that observed in normal body weight HIV patients. This trend have been observed by Joseph et al. (2019); although in normal individual.

Tradomedical practices and other scientific researches (mostly on animal models) have suggested that Moringa significantly (positively) affect abnormal metabolic profile induced by various physiologic factors (Sabastine., 2019; Samson et al., 2019; Perk 2012; Joseph et al., 2019; Kansal, Kumari, 2014; Rathi et al., 2006; Bais et al., 2014). Therefore it would be worthwhile to determine how effective an already established Moringa supplement is in ameliorating some metabolic abnormalities induced by ART drug such as TDF base regimen.

Despite lower HDL levels, at visit 1 (4 weeks after administration of Moringa supplement, no significant increase was observed; However, a significant decrease in both the mean values as well as proportion of subjects with high TG and significant increase HDL-C was observed at Visit 2; when compared to the TDF-NM group which had a reverse result, is an indication of positive gradual effect of M. oleifera supplement. These observations are in accord with those of Joseph et at., 2019, Oyebadejo, et al. 2019, Young et al., 2007, Reynell, Trkola 2012, Modupe, Oyepata, and Akpobome, 2019) which they reported the "hypocholesterolemic and hypoglycemic" effect of M.oleifera. The "antilipidemic effect of Moringa"in this study is in accord with the findings of Siegfried et al., 2011) and Horvath et al., (2009); as they mentioned that the presence of a bioactive phyto-constituents,that is β-sitosterol played the significant role. Different parts of the MO tree have been established as being good sources of unique glucosinolates, flavonoids and phenolic acids, carotenoids, tocopherols, polyunsaturated fatty acids (PUFAs), highly bioavailable minerals, folate etc. most of these compound have established to excercised various pharmacological activity (Kansal, Kumari, 2014;Builder, Anzaku and Joseph, 2019;. Doughari et al., 2009; Evans, 2007; Edema, 2012; Akashi, Traver, Kondo, 1999).

Adusi-Poku et al., (2008) observed that M.oleifera consumed in dietary form lowered the serum CHOL, PHOSLIPID, TG, VLDL, LDL, cholesterol to "phospholipid ratio and atherogenic index", but increased the "HDL/HDL-total cholesterol ratio". The "antilipidemic effect of Moringa"in this study is in accord with the findings of Adedapo et al. (2009) and Adias et al., (2013); as they mentioned that the presence of a bioactive phyto-constituents,that is β-sitosterol played the significant role. M.oleifera appears not to have much effect on serum level of high density lipopolysaccharide at the early stage (first 4 weeks) but after 12 weeks of administering the drug there was significant improvement in the HDL level when compared to first visit of commencement of therapy. This suggests that for proper clinical improvement in metabolic profile of patient on antiretroviral drug regimen, there may be the need to take MO for a prolong period of time.



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## CONCLUSION

Result from this work suggests that consumption of Moringa oleifera, may improve the metabolic parameters in patients on antiretroviral regime over a sustained period of time. Further study may be necessary understand molecular and pharmacology activity and mechanism of action of this plant in improving the metabolic profile of patient on HIV drugs.

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**Conflict of interest**
There is no conflict of interest

**Reference**

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