Clinical Study on the Effect of *Moringa oleifera* on Serum Level of Glucose and Tryglyceride in Subjects Taken Tenofovir, Lamivudine and Efavirenz Combination Regimen

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Abstract

Introduction/ Aim: Acquired immune deficiency syndrome (AIDS) is a disorder caused by human immune-deficiency virus. There are various life-long regimens that are currently used by HIV Patients to suppress and manage this disease. Tenofovir/Lamivudine/efavirenz (300/300/600mg) is one of the most available combinations that are frequently prescribed and dispensed to HIV patients. The aim of this clinical study is to evaluate the potential benefits of taking *Moringa oleifera* with Tenofovir/Lamivudine/efavirenz (TLE) on blood glucose and triglyceride level. **Method:** The study was designed as a Longitudinal Randomized Comparative Trial (LRCT) involving 140 HIV adult subjects (56 males, 84 females) who have been on TLE combination for at least 6 months. They were recruited from a Teaching Hospital in Nigeria. On visit 0, blood samples of the subjects were taken for analysis. *Moringa oleifera* capsules (200mg) were administered by the subjects to be used beginning from the 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 1 and 2) and analyzed for glucose and triglyceride level. **Result:** The analysis of each visits were compared and it was observed that there was no significant reduction in blood glucose level ($P<0.01$) of subjects in visit 1 compared to visit 0. There was significant improvement in blood glucose level ($P<0.01$) in visit 2 compared to visit 0, when subjects received tenofovir/Lamivudine/Efavirenz (TLE) combination without *Moringa oleifera.* It was also observed that there was no significant difference in the blood triglyceride level ($P<0.01$) of subjects in visit 1 and visit 2, compared to visit 0. **Conclusion:** Results from the study revealed that *Moringa oleifera* may be useful in improving blood level of glucose of patients administering TLE combination with no observable effects on triglyceride level of HIV patients on the drug regimen.

Keywords: Moringa oleifera, Blood, Glucose, Tenofovir, Triglyceride

Introduction

There was sudden rise of Kaposi Sarcoma in 1981, observed among young homosexuals (CDC, 1982) with infrequent lung Pneumocystis Carinii Pneumonia (PCP) discovered among homosexual men and drug abusers in that same period (Masur et al., 1981; Gallo et al., 1984) The initiator for these conditions was "Acquired Immunodeficiency Syndrome (AIDS)" which was the "terminal stage infection" by a retrovirus called the Human Immunodeficiency Virus (HIV) (NIH, 2010; Leone et al., 2015). HIV is of two types namely; HIV 1 and HIV 2. They are two different viruses. HIV 1 accounts for 95% of all infectious cases worldwide. HIV 2 is mainly seen in a few West African countries. Though HIV 2 progresses slowly than HIV 1 some antiretrovirals such as nevirapine and efavirens do not work against HIV 2. The management of HIV/AIDS normally includes the use of multiple antiretroviral drugs in an attempt to control HIV infection. Antiretroviral formulations blocks HIV at certain stages of the viral "life cycle"(Estrella, Mantaring and David, 2000). Antiretroviral therapy treats HIV by suppressing the virus activity in the body. For most people who take them, these medications are very effective at keeping HIV under control. Treatment helps to improve quality of life, and it can ensure that a person with HIV has a similar life expectancy to a person without the virus. Suppressing viral activity results in a low viral load and a reduced risk of developing other illnesses. According to the United States Department of Health and Human Services (HHS), the main goal of antiretroviral therapy is to reduce a person's viral load to undetectable levels. The drugs can have serious side-effects which can lead to harm as well as keep patients from taking their medications regularly. This effects include lactic acidosis, liver steatosis, peripheral neuropathy, myopathy, lipoatrophylipodystrophy,

elevated triglycerides and elevated risk of heart attack. *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. The Moringa leaf are prepared for consumption either fresh, dried, or as extract of an aqueous solution (Siddhuraju and Becker, 2003; 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 (Siddhuraju and Becker, 2003). They are also used to bring about milk production in lactating women (Olsen, 1987; Calza, Manfredi, Chiodo, 2004b; Calza,, Manfredi, Chiodo, 2004a), sediment impurities of water (Anwar *et al*., 2007), detoxifies the system of free radicals (Ete et al., 2004; Kumar and Mandapaka, 2013), 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 glucose 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 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 glucose 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. Random Plasma Glucose (RPG; 4.4-7.8 mmol/l): Determined using a glucometer. The routine screening processes were undertaken by the physician.

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 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 $P \leq 0.05$ 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

Glucose 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 significant differences ($P<0.001$) observed in mean values between TLE/Moringa (visit 1) and TLE/Non Moringa (visit 1) in the level of serum glucose of the subjects. 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 and 5)

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

The differences observed in mean values between TLE/Moringa (visit 2) and TLE/Non Moringa (visit 0) were statistically significant ($P<0.001$) for the subjects used, while there was no significant differences ($P<0.001$) between TDF/Moringa (visit 2) and TDF/Non Moringa (visit 0) in the level of serum triglyceride (table 2, 3, 4 and 6).

Table 1: The descriptive characteristics and test of mean differences of glucose and triglyceride level 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
GLU (mmol/l)	Male	56	7.86±2.35	0.31	3.010	*0.003*	*S*
	Female	84	6.74±2.00	0.21			
	Total	*140*	*7.18±2.21*				
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*				

Table 2: Post Hoc (Dunnette T3) multiple comparison of the metabolic profile of HIV male patients on TLE taking moringa supplement across the various visits

Parameters	Visits	Mean±S.D	Min	Max	S.E	95% C.I for Mean	
						Lower Bound	Upper Bound
GLU (mmol/l)	Visit 0	7.86±2.35*^	4.22	18.00	0.31	7.23	8.49
	Visit 1	6.20±1.03ˡ	4.10	8.71	0.14	5.92	6.47
	Visit 2	5.59±0.80	4.08	8.05	0.11	5.38	5.81
	Total	*6.55±1.82*	*4.08*	*18.00*	*0.14*	*6.27*	*6.83*
T.G (mmol/l)	Visit 0	1.43±0.49*^	0.58	2.83	0.07	1.30	1.56
	Visit 1	1.25±0.40ˡ	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*

Note: GLU=Serum glucose, TG=Triglyceride,TLE/M= Tenofovir/Lamivudine/efavirenz with moringa, *TDF/NM=* Tenofovir/Lamivudine/efavirenz without moringa
*S.D=Standard deviation, S.E=Standard error of mean, Min=Minimum, Max=Maximum, *^ˡPost Hoc (Dunntte T3) multiple comparison (* Visit 0≠Visit 1, ^ Visit 0≠Visit 2, ˡ Visit 1≠Visit 2; *^ˡP<0.05).*

Table 3:Post Hoc (Dunnette T3) multiple comparison of the metabolic profile of HIV (female) patients on TLE taking moringa supplement across the various visits

Parameters	**Visits**	**Mean±S.D**	**Min**	**Max**	**S.E**	**95% C.I for Mean**	
						Lower Bound	**Upper Bound**
GLU (mmol/l)	**Visit 0**	6.74±2.00*^	4.09	13.30	0.22	6.31	7.18
	Visit 1	6.09±1.35	3.99	11.80	0.15	5.80	6.38
	Visit 2	6.06±0.73	5.01	8.25	0.08	5.90	6.22
	Total	***6.30±1.48***	***3.99***	***13.30***	***0.09***	***6.11***	***6.48***

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***

Table 4: The descriptive characteristics of metabolic profile of the HIV patients on tenofovir with Moringa (TLE/M) and tenofovir not on Moringa (TLE/NM) at Visit 0 (Baseline)

GROUPS		GLU (mmol/l)	T.G (mmol/l)
TLE/M (N=56)	Mean±S.D	7.86±2.35	1.43±0.49
	S.E	0.31	0.07
	Min	4.22	0.58
	Max	18	2.83
TLE/FM (N=84)	Mean±S.D	6.74±2.00	1.24±0.47
	S.E	0.22	0.05
	Min	4.09	0.3
	Max	13.3	2.3
Total (N=140)	Mean±S.D	7.19±2.21	1.32±0.48
	S.E	0.19	0.04
	Min	4.09	0.3
	Max	18	2.83

Table 5: The descriptive characteristics of metabolic profile of the HIV patients on tenofovir with Moringa (TLE/M) and tenofovir alone (TLE/NM) at Visit 1 (4 weeks of administration)

GROUPS		GLU (mmol/l)	T.G (mmol/l)
TLE/M (N=56)	Mean±S.D	6.20±1.03	1.25±0.40
	S.E	0.14	0.05
	Range (Min – Max)	4.1 - 8.71	0.42 - 2.17
TLE/FM (=84)	Mean±S.D	6.09±1.35	1.27±0.37
	S.E	0.15	0.04
	Range (Min– Max)	3.99 - 11.8	0.24 - 2.21
Total (N=140)	*Mean±S.D*	*6.13±1.23*	*1.26±0.38*
	S.E	*0.1*	*0.03*
	Range(Min – Max)	*3.99 - 11.8*	*0.24 - 2.21*

Table 6: The descriptive characteristics of metabolic profile of the HIV patients on tenofovir with Monringa (TLE/M) and tenofovir alone (TDF/NM) at Visit 2 (12 weeks of administration)

GROUPS		GLU (mmol/l)	T.G (mmol/l)
TLE/M (N=56)	Mean±S.D	5.59±0.11	1.09±0.55
	S.E	0.11	0.07
	Range (Min – Max)	4.08 – 8.05	0.02 – 3.25
TLE/FM (N=84)	Mean±S.D	6.06±0.73	1.15±0.47
	S.E	0.10	0.05
	Range (Min– Max)	4.18 - 8.25	0.23 - 3.18
Total (N=140)	*Mean±S.D*	*5.87±0.79*	*1.13±0.50*
	S.E	0.07	0.04
	Range(Min – Max)	4.08 - 8.25	0.02 - 3.25

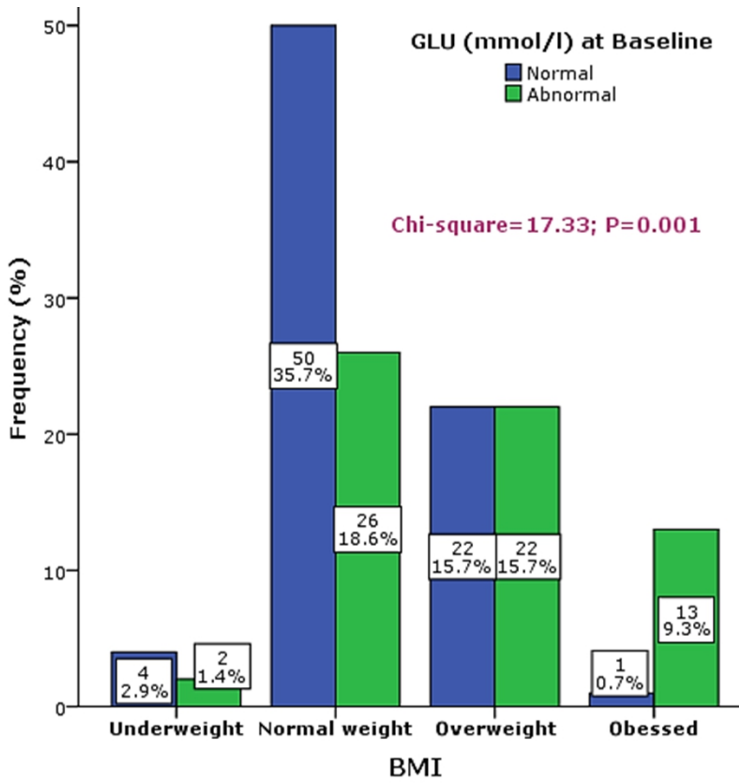

Figure 4.2: BMI associated glucose classification and distribution at Visit 0 (Baseline)

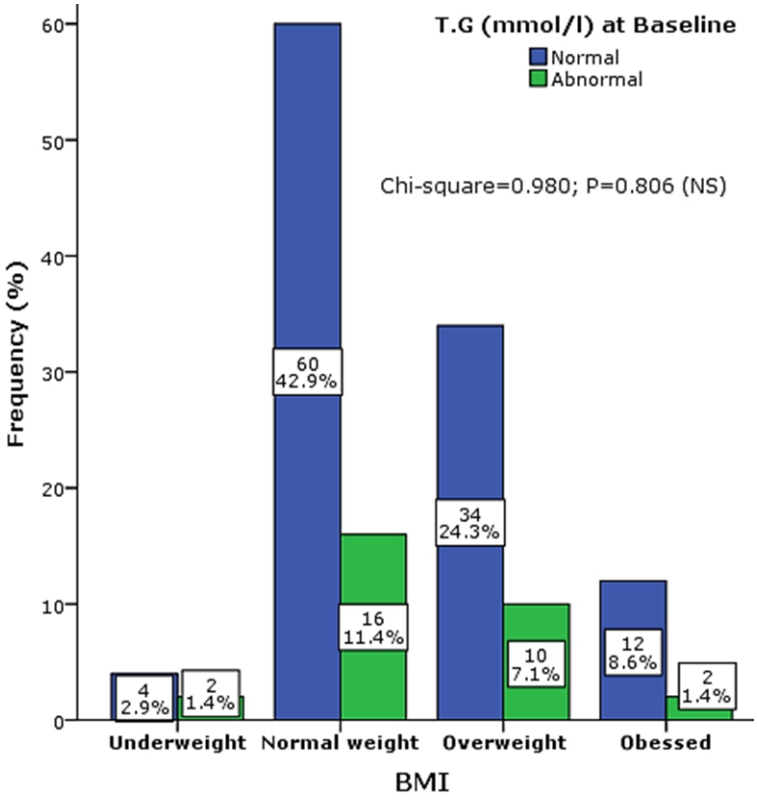

Figure : BMI associated triglyceride classification and distribution at Visit 0 (Baseline)

Discussion

Although the study was abinitio designed to investigate the effect of *M. oleifera* supplementation on TLE dependent HIV patients. However, various reports of ARV therapy associated metabolic abnormalities (Kar, Choudhary, and Bandyopadhyay, 2003; Meraiyebu, Ogunwole, and Izuchukwu, 2014; Ndong, Uehara, Katsumata, and Suzuki, 2007) 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. It should be known that HIV patients in the current study have been on TDF ART for at least six months and there are strong suggestions that the type of ARV-T, duration and application is significantly associated with "the severity of metabolic syndromes" (Mbikay, 2012; Moore and Chaisson, 1999; Eisinger, Dieffenbach and Fauci, 2019; Fauci and Folkers, 2012)). Moringa has been reported to have antioxidant and immune boosting effects (Deeks, Lewin and Havlir, 2013; Walensky et al., 2013).

Blood glucose level difference was mostly affected by the *Moringa* at Visit 2 (12 weeks after administration) with values and proportion of the

subjects being closer to normal when compared to the baseline data. Studies have suggested that *Moringa* contains niazirin (=4-(α-L-rhamnopyranosyl) phenylacetonitrile, niazicin (=methyl N-{4-[(4′-O-acetyl-α-L-rhamnopyranosyl) benzyl]}thiocarbamate, methyl N-{4- [(α-Lrhamnopyranosyl) benzyl]} carbamate, and methyl N-{4-[(4′-O-acetyl-α-Lrhamnopyranosyl) benzyl]} carbamate (Horn, 2012; Priyanka et al., 2015; Beardsley, 1998; Thompson et al., 2012) and the combination of the compound at specific yield values at 100 ppm can significantly stimulated insulin release which increase glucose uptake and significantly reduce the free glucose molecules in circulation (Beardsley, 1998; Thompson et al., 2012). The time and effect on M.oleifera on ART dependent HIV patients may differ owing to the counteracting effect of both HIV and the ART on the progresses action of *Moringa*; However, Kar *et al.* (2012) and Meraiyebu *et al.* (2005) established that after a week administration of single doses of *Moringa* leaves extract in increased dosage (250, 300, 400mg.kg^{-1} BW) there was 1/2 reduction in glucose level diabetic rats. Using rat models (Meraiyebu *et al.* 2005; Austin, Hokanson, Edwards, 1998; Sarwar et al 2007; Hulley, Rosenman, Bawol, Brand, 1980) also established that in normoglycaemic conditions *Moringa* activities may not be quite noticeable when compared glucose uptake compromised rats. The reason for such activities have been associated with certain compounds like isothiocyanates found in Moringa decreases the effect on insulin resistance and hepatic gluconeogenesis. Also Phenolic acids and flavanoids, have been reported to induce glucose homeostasis by affecting on some cellular signaling pathways in tissues known to undertake gluconeogenesis (Consensus Conference, 1984; NIH, 1983).

The study showed there was no significant reduction in the level of triglyceride in visit 1 and 2 when compared to parameter measured in visit 0. A long-standing association exists between elevated triglyceride levels and cardiovascular disease (CVD) (Carroll et al., 2005). Hypertriglyceridemia that result from either increased production or decreased catabolism of triglyceride-rich lipoproteins (TRLs; ie, chylomicrons and very low-density lipoproteins) directly influences LDL and HDL composition and metabolism and artherogenicity (Flegal, Carroll, Ogden, Johnson, 2002; Oyepata, Jude and Opeyemi, 2018; Daniels et al 2005; Johnson et al., 2009; NCEP, 2001). Increase level of triglyceride possibly induced by taking tenofovir, lamivudine and efivarenz drugs was not reduced or controlled by moringa. This is an indication that moringa plant may not have effect in reducing or reversing cardiovascular effect induced by hyperglyceridenemia.

Conclusion

Results from the study suggests that *Moringa oleifera* alongside other benefits, may be useful in controlling blood glucose level of patients taking tenofovir/lamivudine/efavirenz and maybe other tenofovir base regimen, but may be of little to no benefit in reducing serum level of triglyceride which is usually triggered by the use of tenofovir regime.

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