Protocol Title

High-Dose Vitamin D3 in the Treatment of Human Immune Deficiency Virus Patients, A Double-Blind Randomized Control Trial

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Abstract

Human immunodeficiency virus is a key challenge for global health. Vitamin D deficiency is common in people living with HIV infection. Antiretroviral therapy may create unique risk factors for vitamin D insufficiency, including alterations of vitamin D metabolism by ART.

The objective of the study is, to know the effect of high doseVitamin D, in the treatment of HIV patients with antiretroviral therapy. It will be a randomized controlled trial,in which 104patients visiting Said Mitha Teaching Hospital Lahore will be included.

The patients will be divided into two groups. Group-A patients will receive ARV + Vitamin D supplements and patients in Group-B will receive only antiretroviral therapy and placebo.

Methods/design: A randomized placebo double blind control trails ofpatients with age 19-50 years will be the participants of our study at VCCT center of said MithaTeaching Hospital, located in walled city of Lahore,Pakistan. Participants will be randomly allocated to receive 25 hydroxyvitamin D supplementation (n=) or placebo (n=) with the start of ART.

The primary outcome will be achieved with high doseofserum 25-hydroxyvitamin D level at 24weeks. Secondary outcome will include decrease in viral load, increase mean CD4 count to reduce prevalence of clinical symptoms (elevated levels of LFTs,decrease HB level& serum calcium level).Purposive sampling will be carried out.

Data will be collected through Performa, which will be entered and statistically analyzed, using SPSS version 24.0, This Performa is designed by the Punjab AIDS Control Programme including socio-demographic information, Pretest

information,posttest information & follow up chart. Chi-square test will be used to estimate the association among qualitative variables.

Discussion: High dose Vitamin D supplementation in HIV-infected patients has not previously been investigated in Pakistan and it is unknown whether increasing levels is associated with improved clinical outcome or not. Therefore, it is significant to conduct a study to know the effect of vitamin D in the treatment of HIV patients with antiretroviral therapy.

From the public health point of view, it is also important to know the effectiveness of high dose of 25-hydroxy Vitamin-D level in positive human immune deficiency patients.

Trial Registration: The trial will be registered with clinical trials..

Keywords: AIDS, HIV, anti-retroviral therapy, high dose 25-hydroxy Vitamin-D level, CD4 count, viral load.

1. INTRODUCTION

1.1 Background

Human immunodeficiency virus (HIV) is retrovirus; it infects immune system cell and impairs their function. This virus causes infection that leads to worsening the immune system, resulting in immune deficiency. There are two types of HIV i.e. HIV 1 and HIV 2. HIV 1 is more virulent easily **transmit**. HIV in majority is caused by HIV 1 while HIV 2 is less easily transmitted and it is mostly confined to West Africa. HIV 2 is also called non-**progresive,** which means the chance of progression into AIDS is less (Nyamweya et al., 2013; Abbas, Naveed, Qamar, Zehra& Jawed, 2016).

Human immunodeficiency virus is a global health problem (Mansueto, Seidita, Vitale, Gangemi, Iaria&Cascio, 2015)and a major health concern which remains incurable (Imran, **Nasi&Riaz,** 2018). Globally, about 36.7 million people are living with HIV. HIV infection causes AIDS and is epidemic throughout the world. In 2016; about 1.8 million were reported as newly infected and 1 million died of AIDS-related illnesses in the same period (Khan, Wali, Fatima, Yaqoob& Aziz, 2019).

The HIV infection is becoming a prevalent disease in Pakistan, and its death toll has been steadily increasing each year since 1987 (Hussain et al., 2018).In Pakistan, HIV epidemic trend has increased from “low prevalence, high risk” to “concentrated” epidemic particularly in high risk populations. According to World Health Organization and National AIDS Control Program, an estimated 91,340 people were living with HIV in 2014. Majority of HIV-infected population belonged to high risk groups including

injection drug users (IUD), transgender, males and female sex workers. Also, the previous studies have shown prevalence rate of 27.2%, 5.2%, 1.6% and 0.6% respectively in these high risk groups (Shaukat et al., 2018).

1.2 Study Medication

The introduction of antiretroviral therapy (ART) has not only improved the prognosis of HIV patients by reducing their mortality and morbidity, but also has revealed some complications related to the therapy (Grana et al., 2019).Vitamin D deficiency is common in people living with HIV infection (Hileman, Overton&McComsey, 2016; Hsieh & Yin, 2018).HIV infection and exposure to certain anti-retroviral might contribute to altered levels of25-hydroxy vitamin D **25**(OH)D).The preferred and most commonly used parameter for assessment of vitamin D status is serum 25(OH)D concentration. 25(OH)D, which is the major circulating metabolite of vitamin D and reflects the vitamin D inputs from cutaneous synthesis and dietary intake(Lips, 2010; Deshwal&Arora, 2019). Most current guidelines define vitamin D deficiency and insufficiency as a serum vitamin D value 25 (OH) D) ˂30 nmol/L (˂12 ng/ml) and between 30–50 nmol/L respectively (12–20 ng/ml) (Penner et al., 2018).The prevalence of vitamin D deficiency in HIV infected individuals ranges from 60% to 90% and is associated with female gender, black ethnicity and antiretroviral use (Tiraboschi et al., 2016).

HIV infection may create unique risk factors for vitamin D insufficiency such as chronic inflammation, and both the protease inhibitor and non-nucleoside reverse transcriptase inhibitor classes of antiretroviral agents may enhance vitamin D metabolism via modulation of the cytochrome P450 system and vitamin D hydroxylation. Additionally, in HIV-infected persons, vitamin D insufficiency has been

associated with lower CD4+ T lymphocyte counts (Aziz et al., 2013; Coelho et al., 2015).

The main role of vitamin D is to maintain the function of monocytes and macrophages which are linked to human innate immunity to certain infectious agents. This role is very important in body's natural defense against infection in which macrophagesplays a vital role in pathogenesis. Vitamin D acts by combining the nuclei receptor on affected cells so both abnormality in receptor function and structure or low vitamin D level alter the immunity against HIV (Nnoaham& Clarke, 2008; Afzal, Rathore, Butt &Randhawa, 2018).

Vitamin D is obtained in its natural form by exposing the skin to sun rays that converts 7-dehydrocholesterol (pro-vitamin D3) into Cholicalceferol (vitamin D3) through which the bloodstream passes, where it joins the D-binding protein (VDBP) and is transported to the liver. The second source of vitamin D is via oral intake, through the transformation of ergo sterol (vitamin D2) in plants and fungi. Vitamins D3 and D2 are both metabolized in the liver to 25-hydroxicholecalciferol and are transformed in kidneys into its active form, 1,25dihydroxyvitamin D, which is a steroid hormone vital forcalcium and bone metabolism (Conrado, de Barros Miranda-Filho & Bandeira, 2010).

1.3 Rationale for the Proposed Study

Human immunodeficiency virus is a leading health problem that exacerbates the quality of life and a major cause of mortality among patients. In Pakistan, HIV infection is also increasing rapidly while injection drug users, transgender and sex workers are high risk groups. Vitamin D deficiency is common in people living with HIV infection. It has recently been confirmed that Vitamin D supplementation reduces the progression of

disease and prevents HIV-infected individuals from mortality (Anderson et al., 2010; Mehta et al., 2010; Barbosa et al., 2014). Present study evaluates the effect of vitamin D in the treatment of HIV patients with antiretroviral therapy.

2. RESEARCH OBJECTIVE

2.1 Primary objectives:

1. To determine whether proposed oral high dose vitamin D3 is sufficient for achieving the physiological concentration in HIV positive patients on ART. (primary outcome)

2.2 Secondary objectives:

1. To determine whether this intervention also improves CD4 count, viral load count, liver enzyme in study participants. (Efficacy outcome).
2. To discover whether this interventional regimen is safe and well tolerated by study participants. (Safety outcome).
3. To ascertain socio-demographics factors responsible for decreased level of vitamin D3 in HIV positive patients on ART.

2.3 Tertiary objectives:

1. To conclude the effects of total leucocytes count in HIV patients with vitamain D supplementation.
2. To determine the effects of total neutrophil count in HIV patients with vitamain-D supplementation.
3. To investigate the effects of total basophil count in HIV patients with vitamain-D supplementation.
4. To determine the effects of total eosinophil count in HIV patients with vitamain-D supplementation.
5. To conclude the effects of total monocyte count in HIV patients with vitamain-D supplementation.
6. To establish the effects of total platelets count in HIV patients with vitamin-D supplementation.
7. To find out the effects of total hemoglobin count in HIV patients with vitamin-D supplementation.
8. To determine the effects of total lymphocytes count in HIV patients with vitamin-D supplementation.

3. Study Population

Patients who visited the VCCT center of GOVT Said Mitha Teaching Hospital Lahore and the treatment center of Jinnah hospital HIV clinic were the study population and they were HIV positive and on ART. The study population consisted of HIV patients ranging in age from 19 to 50 years. Patients with severe vitamin D3 deficiency were seen at the multi-centric outpatient HIV department.

3.1 Eligibility Criteria

Inclusion criteria:

- Age at enrolment between 19-50 years old
- Vitamin D deficiency level>20ng/ml
- Had not been taking any kind of vitamin D supplementation mega doses for since six months
- Written consent was signed by participants voluntarily

Exclusion criteria

- Pregnant women
- Vitamin D level<20ng/ml or taking vitamin D supplementation mega doses since last three months were not included

4. STUDY PROCEDURES

4.1 Study Population

Patients visiting Said Mitha Teaching Hospital Lahore will be the study population and having diagnosed with HIV positive patients.

4.2 Sampling technique:

Random list was generated for the double blind purpose. This list was generated through Excel sheet.

4.3 Ethical Consideration:

The study will be conducted after approval from the Punjab University Ethical Review Board. Permission will also be obtained from Punjab AIDS Control Programme to use health facilities and this study will be registered atClinical Trail.gov, to use the health facilities and services of paramedics staff for data collection. Written consent of parents and guardians will be taken before starting the study.

4.4 Data Collection:

All staff involved in the study will be trained by the principal investigator before start of data collection. Trained laboratory technician will screen the patients aged 19 years to 50 years of age in(VDT) diagnostic center of hospital visiting outpatients department in their community and will refer the positive patients to treatment center of AIDS Control Programme clinic of Mayo Hospital Lahore, After receiving their prescribed ART, the two positive groups would be divided into two equal groups, one group will receive 25hydroxy vitamin –D and other halves will receive placebo at the same time once weekly for 12 weeks. Patients will be called for follow-up after every

4 weeks. At the end, we will perform posttest and check the level of 25 hydroxy in both groups and other parameters and by using logistic regression we will evaluates the results of effectiveness of 25 hydroxyin blood levels of HIV patients

4.5 Randomization:

The random allocation sequence will be generated in an excel spread sheet by a statistician who will be independent of the study. Consecutive numbers will be assigned to active vs. placebo in equal numbers. No restrictions (e.g. stratification, block size) will be applied. This sequence will be used by the study pharmacy to label pairs of syringes containing active and placebo medication with a study number assigned to active and placebo arms, respectively. These staff will assign the consecutive ID numbers to participants according to the sequence in which they are enrolled, and the hospital pharmacy then supply syringes of placebo active medication bearing this ID number.

4.6 Blinding:

Patients of all study participants will be blinded to allocation, as will be the doctors and the staff nurse who will enrol participants and perform study assessments. Active and placebo medication will be presented identically in 1 ml syringes, and will have identical appearance and texture.

4.7 Interventions:

All participants will be treated with vitamin D supplements (Annexure attached) provided by AIDS Control Programme at outpatients department according to WHOguidelines.Vitamin D and calcium will be supplied to HIV positive patients according to the study (Annexure attached) on a weekly basis by suitably trained staff

who will provide information regarding benefits of vitaminD advise as to how it should be taken. Participants who will be randomised to the intervention arm of the trial will receive one oral dose of 100,000 IU (10 mg) vitamin D3 (cholecalciferol) in 1 ml olive oil, will administer via a syringe at 5 and 12 weeks' post-initiation of vitamin D3 supplementation. Participants who will randomised to the control arm of the trial will receive one oral dose of placebo (1 ml extra virgin) via a syringe at 5 and 12 week's post-initiation of vitamin D3 supplementation. After screening further dose of RDA Vitamin-D will be provided for next 5 weeks to complete 24 weeks of study.

5. LAB ANALYSIS AND COLLECTION OF BLOOD SAMPLE:

25-hydroxyvitaman D concentration will be measured 0 and 60 days to assess baseline deficiency and then effect of supplementation,3ml blood sample will be taken in gel tube after clot formation will be centrifuged. Serum or plasma may be stored for up to 3 days at 2-8 centigrade. Initially **Alere** HIV combo strips will be used for screening of HIV and syphilis, then on positive result we will use second screening on uni-gold HIV strip. Then on third step we will use SD HIV ½ 3.0 strip for final confirmation of screening step and finally we perform RT-PCR at last.

The haemoglobin level will be determined at 0 days and 60 days to assess the anaemia and effect of supplementation in all selected participants. Blood sample will be drawn from patients who will give their consent voluntarily.

6. STUDY SCHEDULE

Schedule of clinical assessment and procedure:

	Screening	**Treatments**			
	V 1 Screening & Assessment	**V 2 Randomization & 1st Dose**	**V 3 2nd dose**	**V 4 3rd dose**	**V 5 Post Assesments**
	-7~1	**1 Day**	**5 week**	**12 week**	**24 week**
ICF	×				
Eligibility Criteria	×	×			
Baseline Questionnaire	×				
Weight	×		×	×	×
Height	×		×	×	×
Vital Signs	×	×	×	×	×
Vit-D	×			× V4+1 Day	
CD4 count	×			× V4+1 Day	
Viral load	×			× V4+1 Day	
PCR	×			× V4+1 Day	
CBC	×			× V4+1 Day	
Study Medication		×	×	×	
AE Monitoring		×	×	×	×
RDA					×
End of Study					×

6.1 STUDY PROCEDURES AND ASSESSMENTS:

Visit Schedule:

Visit 1 (Screening, Days -3 to 1)

Subjects who were informed about the study and voluntarily gave written consent to participation had the following tests.

a. Informed consent and screening number assignment

b. Demographics

c. Information on exposure to HIV infection

d. Medical history

e. Previous HIV lab test if any

f. RT-PCR

g. Laboratory tests

h. Prior medications and therapies

i. Physical examination (investigator questioning)

j. Symptoms

k. Body weight measurement

l. Assessment of the inclusion/exclusion criteria

Visit 2 (Randomization, Baseline, Day 1):

a. Medical history

b. Prior medications and therapies

c. Physical examination (investigator questioning)

d. RT-PCR

e. Laboratory tests

f. Symptoms

g. Assessment of the inclusion/exclusion criteria

h. Randomization

i. Prescription of test drug or placebo

Visit 3 (Day 4)

a. Concomitant medications and therapies

b. Physical examination (investigator questioning)

c. RT-PCR

d. Symptoms

e. Treatment adherence.

Visit 4 (Day 7):

a. Concomitant medications and therapies

b. Physical examination (investigator questioning)

c. RT-PCR

d. Laboratory tests

e. Symptoms

Visit 5 (Day 14)

a. Concomitant medications and therapies

b. Physical examination (investigator questioning)

c. RT-PCR

d. Laboratory tests

e. Symptoms

f. Body weight measurement

g. Adverse events

Visit 6 (Day 28):

a. Physical examination (investigator questioning)

b. Laboratory tests

c. AEs

d. Collection of clinical treatment information

Even if a subject was withdrawn, unless the subject withdrew the consent for safety follow-up, the safety follow-up was conducted at the scheduled visits (Visits 3, 4, 5, and 6) to collect the following clinical treatment information.

e. Treatment adherence

Unscheduled Visit:

If a subject arrived at the site for medical treatment on an unscheduled date due to an AE, a change in concomitant drugs, withdrawal status, or measurement data acquired during the research, all relevant facts were documented as thoroughly as possible in the SD. Unplanned visits did not interfere with a planned study schedule. Unscheduled visits may had happen at the subject's solicitation or when the examiner considered it fundamental. There had been no limitations on the tests done during impromptu visits, and the specialist embraced tests that were reasonable and needed for the subject.

7. STATISTICAL CONSIDERATION

Statistical analyses will be conducted using SPSS VERSION 23.Qualitative data will be presented in form of frequency and percentage and quantitative data will be presented in form of mean ± S.D. For the comparison of both treatments (randomly allocated to patients) Wilcoxon test will be applied to compare effectiveness of both treatment plans. P-value $\leq$ 0.05 will be considered as significant, Baseline characteristics of trial participants will be summarised separately for both randomised group. For each group dichotomous and categorical data of participants will be presented as number and percentages.

The primary outcome, effect of allocation on continuous outcomes that will assessed both at baseline and at the end of study(e.gVitamin-D supplementation at24 weeks will be assessed using linear regression, adjusting for the baseline values. The effect of allocation of categorical outcomes variables that will be assessed both at baseline and at the end of study (clinical outcome)will be analysed with generalized linear regression with a log link and binomial distribution to yield a risk ratio adjusted for the baseline

values with 95% CI and p value(**cummings**,2009).. Serum concentration of 25{OH} D will be measured at baseline,05 03 months at baseline, Statistical significance will be inferred where p< 0.05.

Allocated to patients) Wilcoxon test will be applied to compare effectiveness of both treatment plans. P-value ≤ 0.05 will be considered as significant.

8. ETHICAL CONSIDERATION

The study will be conducted after approval from the Punjab University Ethical review board. Permission will also be obtained from Punjab AIDS control programme to use health facilities and this study will be registered at CLICAL TRAIL.gov, to use the health facilities and services of paramedics staff for data collection. Written consent of parent and guardian will be taken before starting the study.

8.1 Data Collection

A Performa will be prepared by researcher and finalized after pre-testing. Each patientvisiting Said Mitha Teaching Hospital Lahore will be assessed by the researchers and their demographic information will be noted on the pre-designed Performainitially. Data is collected from all those patients whom would be registered in our 03 months of study after approval of synopsis.

8.2 Human Immunodeficiency Virus

The human immunodeficiency virus (HIV) infects cells of the immune system, destroying or impairing their function. Infection with the virus results in progressive deterioration of the immune system, leading to "immune deficiency."

8.3 Standard Antiretroviral Therapy

Standard antiretroviral therapy (ART) consists of the combination of at least three antiretroviral (ARV) drugs to maximally suppress the HIV virus and stop the progression of HIV disease.

9. ANNEXURE 1: ABBREVIATIONS

AIDS:	Acquired immunodeficiency syndrome
25(OH)vitamin-D:	25 Hydroxy Vitamin-D
RDA:	Recommended daily allowances.
ART:	Anti-retroviral therapy.
HIV:	Human-immune deficiency virus
WHO:	World health organization.
PACP:	Punjab AIDS control programme.
CD4 count:	Cluster of differentiation

10. WORK PLAN

Weeks	**Months**					
	1	**2**	**3**	**4**	**5**	**6**
Synopsis writing and approval						
Data collection						
Data analysis						
Thesis writing						
Thesis submission						

11. References

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Research Performa

1. Biographic Data

Registration No. _____ Date: _____

Group-A (ARV + Vit.-D) ☐ Group-B (ARV+ Placebo) ☐

Name_____ Age _____ (years)

Gender ☐

Male ☐ Female ☐

Education ☐

Illiterate ☐ Under matric ☐

Matric and above

Employed ☐

Unemployed ☐

Family monthly income (Rs.) ☐

Marital Status

Married ☐

Unmarried ☐

Occupation

BMI

Underweight ☐

Overweight ☐

Obese ☐

2. Exposure to sunlight

Yes ☐

No ☐

3. Serum vitamin D level ng/ml

4. Vitamin D deficiency

Yes ☐ No ☐

5. Consent Form Taken

Yes ☐ No ☐

6. Ethical committee minutes of meetings

7.Presentingcomplaint

_____

8.Brief Physical Examination (Vital Sign & Brief History)

BP ☐ Temp ☐

RR ☐ Pulse ☐

9. Inclusion Criteria ☐

(All patients which are not taking vitamin-D Supplementation from last 6 months & having deficiency)

10.Exclusion Criteria ☐

1. pregnant women
2. Patients on active TB medicines
3. Patients do not sign consent form freely

Follow up visits:

_____

_____

_____

11. Treatment obtained: Yes No

12.Adverse effects of medicine: Yes No

13. Compliance of medicine Yes No

14. Outcome of achievement:: Yes No

Investigator signature:

_____