Statistical Analysis Plan (SAP)

High Dose Vitamin-D3 Supplementation In The Treatment Of Human Immune Deficiency Virus Patients, A Double-Blind Randomized Control Trial (HDVDS-HIVT)

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Abbreviations

ART	Anti-Retro Viral Therapy
CD4	Cluster Of Differentiation 4
HCT	Hematocrit
LFTs	Liver Function Test
CBC	Complete Blood Count
TLC	Total Leukocytes Count
ART	Anti-Retroviral Therapy
HIV	Human –Immune Deficiency Virus
AIDS	Acquired Immune Deficiency Syndrome
VCCT	Voluntary Confidential Counseling and Treatment
Hb	Hemoglobin
ALP	Alkaline Phosphatases
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase.
PACP	Punjab AIDS Control Program
25(OH)	25 Hydroxy
PI	Protease Inhibitors
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NTI	Nucleoside Transcriptase Inhibitors
NACP	National AIDS Control Program
WHO	World Health Organization
UNAIDS	United Nation Acquired Immune Deficiency Syndrome
HAART	Highly Active Anti-Retroviral Therapy
DHHS	Department Of Health And Human Services
CDC	Communicable Disease Control
Ng/Ml	Nano Gram/Microliter
IU	International Unit
RCT	Randomized Control Trial
PI	Principal investigator
TG	Transgender
MSM	Male Sex Worker
WSW	Women Sex Worker
SW	Sex Worker
IDU	Intravenous Drug Users
RDA	Recommended Daily Allowances
PCR	Polymerase Chain Reaction
VL	Viral Load
CMIA	Chemiluminescence

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1. **Introduction**:

2. Human immunodeficiency virus (HIV) is a key challenge for global health. Vitamin D deficiency is common in people living with HIV infection. Antiretroviral therapy (ART) may create unique risk factors for vitamin D insufficiency, including alterations of vitamin D metabolism by ART. Vitamin D supplementation in HIV-infected patients have not previously been investigated in Pakistan and it is unknown whether sufficient physiological levels is associated with improved clinical outcome or not.This statistical analysis plan (SAP) will give more detailed descriptions of the endpoints in the study and the corresponding analyses.

3. **Study design**: We have designed this study with the hypothesis, that the addition of high dose of vitamin D supplementation would be effective in elevating serum 25-hydroxy vitamin D concentration into normal physiological range in patients with HIV infection and this would improve their clinical outcome over the period of 6 months' treatment. The primary outcome of the study will be the sufficient elevating level of serum 25-hydroxyvitamin D at 24weeks. Secondary outcome of the study will be, the increase means CD4 count, decrease in viral load and improvement in liver function test and to reduce the severity of clinical symptoms. This is a double-blind randomized placebo-controlled trial.114, patients aged (19-50) years with diagnosed HIV disease will be recruited by purposive sampling technique from VCCT treatment centre of Government Said Mitha Teaching Hospital located in the walled city of Lahore, Pakistan. The patients will be divided into two arms. Arm-A patients will receive recommended ARV + Vitamin D supplements and patients in Arm-B will receive recommended ARV and placebo. Participants will be randomly allocated to receive 25 hydroxy vitamin D supplementation (n=) or placebo (n=) with the start of ART

	Screening	Treatments			
	V 1 **Screening & Assessment**	**V 2** **Randomization & 1st Dose**	**V 3** **2nd dose**	**V 4** **3rd dose**	**V 5** **Post Assessments**
	-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					×

3.1 Sample size calculation

Assuming that 60% of patients in the control arm would attain > 30% of their baseline vitamin-D status at 6 months, we calculated that a total of 94 participants (47 per arm) would need to complete follow-up in order to detect a 25% absolute increase (to 85%) in the proportion of patients attaining > 30% vitamin-D level at 6 months in the intervention arm with 80% power at the 5% significance level. (Lake, Vitamin D in HIV-Infected Patients on HAART, 2014).

This number was inflated to a total of 114 to allow for attrition due to death and loss to follow-up. No interim analysis was performed. (Shriver, 2019)

4. Aims and objectives:

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)

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.

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 determine the effects of total eosinophil count in HIV patients with vitamain-D supplementation.
4. To conclude the effects of total monocyte count in HIV patients with vitamain-D supplementation.
5. To establish the effects of total platelets count in HIV patients with vitamin-D supplementation
6. To find out the effects of total hemoglobin count in HIV patients with vitamin-D supplementation
7. To determine the effects of total lymphocytes count in HIV patients with vitamin-D supplementation
8. To see if the effects of total HCT count in HIV patients with vitamin-D supplementation

5. Outcomes

This section will present the outcomes investigated to answer the study aims and objectives. The analyses are described in section 6 Analyses.

5.1 Primary outcome:

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)

5.2 Secondary outcomes:

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.

5.3 Tertiary outcome:

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 determine the effects of total eosinophil count in HIV patients with vitamain-D supplementation.
4. To conclude the effects of total monocyte count in HIV patients with vitamain-D supplementation.
5. To establish the effects of total platelets count in HIV patients with vitamin-D supplementation
6. To find out the effects of total hemoglobin count in HIV patients with vitamin-D supplementation

7. To determine the effects of total lymphocytes count in HIV patients with vitamin-D supplementation
8. To see if the effects of total HCT count in HIV patients with vitamin-D supplementation

Other blood laboratory parameters

Vitamin-D level,CD4 count, viral Load,PCR, CBC

5.4 Safety outcomes

Adverse events

Adverse events are reported from the visit 1 till Visit 5.

Concomitant medications
Usage of medications during study period will be recorded.

6. Populations and subgroups to be analysed

6.1 Populations

Intention-to-treat (ITT)
All randomised study subjects. This will be seen as the primary population for the analysis.

Per Protocol (PP)
All randomised study subjects completing the whole study period (complete cases). For a specific analysis, study subjects with missing data on any of the variables in the model will be excluded from the analysis. Analyses of this population is seen as a sensitivity analysis to investigate whether conclusions are sensitive to assumptions regarding the pattern of missing data.

6.2 Groups:

Patients with with vitamin-D deficiency are divided into two groups i.e placebo group and vitamin-D group.

Placebo group: out of total 95 participants 47 participants were on placebo arm

Vitamin-D group:

Similary out of total 95 participants 48 participants were on vitamin-D arm

Vitamin-D deficiency:
All randomized study subjects will be divided into two subgroups according to having vitamin-D deficiency at baseline visit.

Division of groups:

All randomised study subjects will be divided into two subgroups according to treatment.

7. Analyses

All outcomes will be presented using descriptive statistics. As the sample size is low so we will check the normality of each variable on screening (Visit 1) and according to the normality check we will determine the mean difference change in Placebo and active treatment arm.

In descriptive analysis we will mention the mean value of each variable with lower and upper bound with 95% confidence interval, 5% trimmed mean, median, Std. Deviation as per the each group ; normally distributed data by the Mean, Variance, Standard deviation (SD), lower and upper values of the variables to see the outliers if any.

In analytical analysis we will apply T-Test for two independent samples to see the difference between two arms. In case any variable doesn't follow the normality assumptions, we will use non-parametric test.

For the safety objective we will use only frequency/number of events to present it.

SPSS 21 will be used for all statistical analysis.

7.1 Primary outcome

The primary outcome analysis will be performed to check the difference change in Placebo group and Active Treatment group (Vitamin-D Group) through comparing mean difference from Visit 1 to Visit 4. This test will identify the mean increase or decrease in the Vitamin-D levels in the study Participants.

7.2 Secondary Outcomes

The secondary outcome analysis will be performed to check the difference change in Placebo group and Active Treatment group (Vitamin-D Group) through comparing mean difference from Visit 1 to Visit 4. This test will identify the mean increase or decrease in the respective variables including CD4 count, PCR detection, Viral Load and Liver Enzymes change.

For the safety objective we will use only frequency/number of events to present it.

For the Socio-Demographic factors association or correlation chi square test or bivariate correlation will be checked with one constant variable (Low Vitamin-D Level on Visit 1).

7.3 Tertiary Outcomes

In all tertiary objectives, we will check the mean change in the blood counts from Visit 1 to Visit 4 with 95% Confidence Interval by using two independent samples T-Statistics or Non-Parametric test Mann-Whitney U.