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Application of paired student t-test on impact of Anti-retroviral therapy on CD4 cell count among HIV Seroconverters in serodiscordant heterosexual relationships: A case study of Nyanza region, Kenya.

Bernard K. Rono [1, 2], George Orwa [1], Joseph Mungatu[1], Antony Wanjoya[1],

1. Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O Box 62000-00200, Nairobi, Kenya
2. Kenya Medical Research Institute (KEMRI), P.0 Box 54840-00200, Nairobi, Kenya

* E-mail of the corresponding author: brono36@gmail.com

Abstract

Human immunodeficiency virus (HIV) infection leads to rise in HIV-RNA resulting in CD4 T-cell decline leading to AIDS-related illness. Knowing the effect of Antiretroviral Therapy (ART) on CD4 cell count is vital in assessing the progression of the disease and treatment planning for treatment. This study sought to apply paired t-test distribution to assess the effect of CD4 cell count just before and after initiation of ART among HIV infected individuals. The target populations were HIV sero-converters enrolled in a prospective randomized placebo controlled trial in Nyanza region, Kenya. CD4 cell count was measured at the time of sero-conversion and subsequently after very six months of follow up. Participants were referred for initiation of ART at patient support centre once the criteria for initiation was met and report back the ART regime they were put on and the date they were started on . We applied paired t-test to assess the change in CD4 cell count after initiating ART. Use of ART within a median time of 9 months resulted rise in CD4 cell count by 241 cells per ul, 95% CI (60-422) which confirms the effect of ART in protecting depletion of CD4 cell count.

Keywords: Sero-converters, Progression, ART, HIV, CD4 cells, t-test.

1. Introduction

Globally, the number of new HIV infections is estimated to range between 1.9 million to 2.7 million as at 2012 (UNAIDS, 2013). Approximately 1,192,000 persons are currently living with HIV infection in Kenya with highest prevalence of about 15% in Kisumu County (KAIS, 2012) which is at least three folds higher than other regions of the country. Studies have shown 70-80% of the HIV infected experience intermediate disease progression resulting in development of AIDS-related illnesses within 6-10 years of acquiring HIV (Pantaleo and Fauci, 1996). A number of vitro studies and clinical trials (St Clair et al., 1995; Pagano and Chong,1995; Markowitz et al., 1995; Stein et al., 1996; Hammer et al., 1997; Collier et al.,1996; Cameron et al., 1997) and findings of observational studies (Egger et al., 1997;Chiasson et al., 1997; Hogg et al., 1998; Detels et al., 1998) have demonstrated the effectiveness of treating HIV-infected individuals with combinations of Anti-retroviral Therapy (ART). This has been estimated by a decrease in viral load to levels below the detection limits. The scale-up of ART represents an unprecedented effort to provide access to life-saving drugs to HIV-infected patients in resource-limited settings.

The average rate of decline of CD4 cells ("CD4 slope") according to Mellors et al,(1997); Vlahov et al. (1998) is about 50/mm ^{3}per year and the average viral burden (without therapy) is 30,000 to 50,000 copies/mL. Few records of sero-converter cohorts exist in low-income and middle-income countries, and the number of

participants enrolled is relatively small and so has resulted in collaborative analyses of individual participant data from several studies (Stewart and Tierney, 2002). Also a limited number of studies have estimated CD4 cell loss in ART-naive individuals in African countries (Deuffic-Burban et al., 2007; Jaffar et al., 2004; Morgan et al., 1997) and a few have directly compared this to estimates derived from high-income countries (Meyer et al.,2007; Keller et al., 2009; Lewden et al., 2010; Porter et al., 2000). Fewer still have used data from individuals with well-estimated dates of HIV sero-conversion (Anon, 1992; Fauci and Lane, 2005).

According to UNAIDS (2010) majority of HIV-infected people live in low and middle income countries, particularly sub-Saharan Africa but current treatment guidelines are largely based on data from high-income countries. Knowledge of effect of ART on CD4 cell count in low income setup would aid in evaluation and planning for the response to the HIV epidemic and to inform HIV policy within the government. The information is also believed to contribute to the wider body of knowledge on promotion of HIV testing and counseling and to form a basis upon which other related studies can be anchored. The study therefore sought to apply paired t-test to assess the impact of ART on CD4 cell count among sero-converters in heterosexual relationship in Kisumu County, Kenya.

1.1 Objectives of the study

The objective of this study was to determine the effect of ART on CD4 cell count prior and after initiation of antiretroviral therapy among sero-converters in heterosexual relationship in Kisumu Kenya.

1.2 Assumptions of the Study

ART is a combination of three drugs which varied in some individuals. We assumed that each different combination have equal impact on CD4 cell count although this may not be true.

2. Literature review

Sometimes in studying the difference between two means it is possible to use pairs or matched samples advantageously. Paired t test is often quite effective in partially eliminating the effects of extraneous factors. Matching is commonly used in case-control studies where patients are matched by gender and age and is often used in surgical experiments where two surgical treatments are used on the opposite sides of animals such as rats to compare the outcomes (Olive and Virgina ,2009).

The t-test requires that observations are drawn from a normally distributed population and the two-sample t-test requires that the two populations have the same variance. According to Siegel (1956) traditional parametric tests should not be used with extremely small samples, because these tests have several strong assumptions underlying their use and that these assumptions cannot be tested when the sample size is small. Campbell et al(1995) estimated sample sizes required in two-group comparisons and concluded that N = 5 per group may be suitable as long as one accepts very low statistical power. Siegel (1957) stated that there is no alternative to using a nonparametric statistical test for samples as small as 6 unless the nature of the population distribution is known exactly. A simulated study showed that there is no fundamental objection to using a regular t-test with extremely small sample sizes. According to results from simulated study by Winter 2013 assessing the application of t-test in extremely small sample sizes, a sample size as small as 2 did not pose problems and Type I error rate did not exceed the nominal value of 5% concluding that paired t-test to be feasible with extremely small sample sizes, particularly when the within-pair correlation coefficient is high.

The non parametric alternative to paired t test is wilcoxon test. Wilcoxon test according to Posten (1982) gives bigger power compared to t-test for smaller samples while according to Bridge and Sawilowsky (1999) the t-test is more powerful than the Wilcoxon test under relatively symmetric distributions. In this test the sum of Type I and Type II errors can be minimized according to Mudge et al. (2012) recommendation by adjusting the

significance level.

3. Model Development

3.1 Standard normal (Z- test)

There are two specific statistical tests used for hypotheses concerning means: the z test and the t test (Bluman, 2009). The z test is a statistical test for the mean of a population. It can be used when n ≥30, or when the population is normally distributed and standard deviation σ is known. Its formula is as shown by (3.1).

$$z = \frac{\bar{x} - \mu}{\sigma/\sqrt{n}} \tag{3.1}$$

Where

$\bar{x}$ - The sample mean,

μ - Hypothesized population mean

σ - Population standard deviation

n - The sample size.

The denominator in (3.1) is the standard error of the mean.

3.2. Student t- test

When the population standard deviation is unknown, the z test is not normally used for testing hypotheses involving means. A different test, called the t test, is used. The t distribution was derived by W.S Gosset(1876-1937) and published under the pseudonym of 'Student' and published in 1908 and frequently referred to student t distribution (Armitage et al.,2002).The distribution of the variable should be approximately normal. The t distribution is similar to the standard normal distribution in that it is bell-shaped, symmetric about the mean, the mean, median, and mode are equal to 0 and are located at the center of the distribution and that the curve never touches the x axis. The difference with the standard normal distribution is that the variance is greater than 1. The t distribution is a family of curves based on the degrees of freedom, which is a number related to sample size. As the sample size increases, the t distribution approaches the normal distribution. The formula for t test is

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}} \tag{3.2}$$

The degrees of freedom d.f is n-1 and s is the sample standard deviation. When comparing two means by using the t test, the researcher must decide if the two samples are independent or dependent. Independent t test is used to test the difference between means when the two samples are independent and when the samples are taken from two normally or approximately normally distributed populations. Samples are independent samples when they are not related. The t test formula (3.3) is for testing difference in two independent means.

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{(\frac{s_1^2}{n_1^2} + \frac{s_2^2}{n_2^2})}} \quad (3.3)$$

Where the degrees of freedom are equal to the smaller of $n_1 - 1$ and $n_1 - 1$
It follows the format of

$$Test\ value = \frac{(observed\ value - expected\ value)}{standard\ error.} \quad (3.4)$$

Where $\bar{x}_1 - \bar{x}_2$ is the observed difference between sample means and where the expected value μ1-μ2 is equal to zero when no difference between population means is hypothesized. The denominator in (3.3) is the standard error of the difference between two means.

Samples are considered to be dependent samples when the subjects are paired or matched in some way. An example is where one wants to see whether a drug will affect the reaction time of its users. To test this hypothesis, the researcher must pretest the subjects in the sample first and test again after using the drugs. When the samples are dependent, a special t test for dependent means is used. This test employs the difference in values of the matched pairs (3.5). We first, find the differences of the values of the pairs of data and we will denote it with D where

$$D = \bar{x}_1 - \bar{x}_2 \quad (3.5)$$

It is assumed that the D has a normal distribution (Ambrosius, 2007). The formula (3.6) shows mean the difference where n is the number of data pairs.

$$\bar{D} = \sum \frac{D}{n} \quad (3.6)$$

The formula (3.7) shows the standard deviation of the differences while (3.8) is the estimated standard error S_D of the differences.

$$S_D = \sqrt{\frac{n\sum D^2 - (\sum D)^2}{n(n-1)}} \quad (3.7)$$

$$S_{\bar{D}} = \frac{S_D}{\sqrt{n}} \quad (3.8)$$

The test value t formula is (3.9) with d.f. n-1.

$$t = \frac{\bar{D} - \mu_D}{S_D/\sqrt{n}} \quad (3.9)$$

The expected value μ_D is zero if the hypothesis is $\mu_D = 0$. The confidence interval of the difference in the paired mean difference is (3.10)

$$\bar{D} - t_{\alpha/2}\frac{S_D}{\sqrt{n}} < \mu_D < \bar{D} + t_{\alpha/2}\frac{S_D}{\sqrt{n}} \tag{3.10}$$

The alternative non parametric test for paired t test is Wilcoxon Signed Rank Test. These derived equations are based on Ambrosius (2007), Bluman (2009) and petrie and Sabin (2005)

4. Data analysis and results

4.1 Statistical analysis

We used paired t test to compare CD4 cell count at most recent visit before ART initiation and at the final CD4 measurement before exiting the study after using ART. Shapiro-Wilk test was used to test the assumption of normal distribution. Study results was interpreted to be significant at P<0.05. Data were analyzed with Stata 12 (Stata Corporation College Station, Texas, USA).

Table1. Baseline Clinical and Socio demographic characteristics of sero-converters in the study

		Progressed to ART initiation?		
Characteristic		**No(n=24)**	**Yes(n=39)**	**Total(n=63)**
Age Median (IQR)		27.14 (22.24-38.78)	33.21(26.24-47.93)	30.20(25.35-45.44)
Baseline CD4- Median(IQR)		603 (549-785)	414(351-588)	521(377-644)
Children with study partner -Median(IQR)		0 (0-0)	0(0-2)	0(0-1)
Number of living children- Median(IQR)		1(0-2)	2(1-5)	2(1-4)
Income	no	17(70.83%)	21(3.85%)	38(60.32%)
	yes	7(29.17%)	18(46.15%)	25(39.68%)
Sex	Female	11(45.83%)	18(46.15%)	29(46.03%)
	Male	13(54.17%)	21(53.85%)	34(53.97%)
Marital status	Cohabiting	0(0%)	3(7.68%)	3(4.76%)
	Married	24(100%)	36(92.31%)	60(95.24%)
Age category	18-25 years	9(37.50%)	6(15.38%)	15(23.81%)
	25-36 years	8(33.33%)	17(43.59%)	25(39.68%)
	>36 years	7(29.17%)	16(41.03%)	23(36.51%)
Level of	primary	14(58.33%)	27(69.23%)	41(65.08%)
education	Secondary	8(33.33%)	11(28.21%)	19(30.16%)
	Tertiary	2(8.33%)	1(2.56%)	3(4.76%)
WHO Staging	Stage 1	24(100%)	38(96.15%)	62(98.41%)
	Stage 2	0(0.00%)	1(3.85%)	1(1.59%)
Baseline CD4	<500	2(8.70%)	26(68.42%)	28(45.90%)
category	501-600	9(39.13%)	3(7.89%)	12(19.67%)
	601-700	2(8.70%)	6(15.79%)	8(13.11%)
	701-800	5(21.74%	2(5.26%)	7(11.48%)
	>800	5(21.74%	1(2.63%)	6(9.84%)

4.2 Results

4.2.1 Study participants

Among 1161 individual who were enrolled as HIV negative partners in sero-discordant hetero-sexual relationship, 63(5.4%) got infected with HIV i.e sero-converted and all were followed up. A total 39 (61.9%) out of the 63 sero-converters met the eligibility of ART initiation within a median time of 6 months where 7/39 (17.9 %) were eventually started on ART. Table 1 shows the demographic characteristics of the sero-converters where the baseline median age in years of the cohort was 30.20, IQR (25.35-45.44) (Table1). A total of 34/63 (53.97 %) of the cohort were male; 60/63(95.25%) were married while 15/63(23.8%), 25/63(39.68%) and 23/63(36.51%) were within the age categories of 18-25, 26-36 and >36 years respectively. The median number of children with study partner was 0; IQR (0-1), while the median number of living children born to sero-converter was 2; IQR (1-4). A total of 25/63(39.68%) reported earning some monthly income.

On education, 41/63(65.06), 19/63(30.16%) and 3(4.76%) attained Primary, Secondary and Tertiary levels of education respectively.

The baseline median CD4 cell count/ul was 521 IQR (377-644). The CD4 categories with most individuals of 28/63(45.90%) was <500 cell/ul; 12/63(19.67%) within (501-600) cells/ul; 8/63(13.11%) within (601-700) cells/ul ; 7/63(11.48%) within (701-800) cells/ul while 6/63(9.84%) within >800 cells/ul category. A part from one participant at WHO stage2 of clinical symptoms, the rest 62/63 (98.41%) were at WHO stage 1 at the time of sero-conversion(Table 1)

4.2.2. Paired t test results

Thirty nine individuals (62%) progressed to ART initiation eligibility out of which 7(17.9%) of those were eventually initiated on ART. Reasons for not initiating ART were however not documented. We compared measurement of CD4 cell count for the 7 individuals with CD4 cell count measurement taken just before initiation of ART and the last CD4 measurement taken before exiting the study having used ART using paired t-test method. The median time of using ART for the seven participants was 9 months, IQR (9-30).

Table 2. Paired t test of CD4 cell count before and after initiation of ART

Variable	n	Mean	Standard Error	Standard Deviation	95% Confidence intervals	
CD4 After	7	683.43	116.98	309.5	397.19	969.67
CD4 Before	7	442.29	88.96	235.37	224.61	659.97
Difference	7	241.14	73.94	195.63	60.25	422.07

Table 3 Paired t-test statistical results.

```
    mean(diff) = mean(CD4_after_ART - CD4_before_ART)          t =   3.2613
Ho: mean(diff) = 0                              degrees of freedom =        6

Ha: mean(diff) < 0          Ha: mean(diff) != 0          Ha: mean(diff) > 0
Pr(T < t) = 0.9914        Pr(|T| > |t|) = 0.0172         Pr(T > t) = 0.0086
```

The paired t test results (Table2) showed a mean CD4 cell cell/ul before and after initiating ART as 442 and 683 respectively which give mean difference (D) as 683-442=241 cells/ul. The test statistic (t) =3.26 with degree of freedom (d.f).f of n-1 i.e. 7-1=6. Table 3 results test the null hypothesis that there is no impact of ARV on CD4 cell count among seroconverters i.e P (|T|>|t|) =0. In our case P=0.0172 (Table 3) suggest evidence of difference in mean CD4 cell count before and after initiation of ART. We however needed to test the assumption of

normality in the paired difference in observation before we could reject the null hypothesis.

We therefore tested for this assumption by generating a variable which we abbreviated as CD4_Diff by finding the difference in CD4 cell count/ul before and after initiation of ART. We then tested this variable for normality using Shapiro-Wilk. The paired difference in CD4 cell count did not violate the assumption of normality; W 0.892, P=0.29329. We therefore reject the null hypothesis and interpreted the results that in a median time of 9 months ART treatment resulted in elevation of mean CD4 cell count by a 241 cells/ul; 95% CI (60-422). Each observation experienced at least some increase in CD4 cell count after being started on ART.

5. Discussion

The participants who were initiated on ART were followed up for a median time of 9 months. A significant increase in mean CD4 cell count of 241 cells/ul, 95%CI(60-422), P=0.0172 was evident after using ART for the period confirming the effectiveness of ART in reducing the depletion of CD4 cells by HIV hence helping in fighting opportunistic infections. The median CD4 cells/ul increase was 235. Few studies on HIV sero-discordant heterosexual relationships have assessed the effect of ART on CD4 cell count hence this finding are significant as future reference for similar studies. Multiple studies have provided strong support for this finding (St Clair et al., 1995; Pagano and Chong, 1995; Hogg et al., 1998; Chiasson et al., 1997) although majority of these studies are from middle income countries. A study in India (Dravid et al., 2011) which followed patients on ART for 2 years experienced a median rise n CD4 cell count by 220 cells/within 2 years. Although the increase in median CD4 cell count was higher in our study and within a shorter follow-up time; the Indian study median baseline CD4 was however smaller i.e. 137 cells/ul compared to ours of 346 cells/ul. This suggests initiating ART at higher CD4 values would enable quicker recovery in CD4 cell count. A study carried out in a Uganda and Kenya cohorts has shown delayed initiation of ART after meeting eligibility (Mujugira et al. 2014) hence the benefit of ART may still not be realized. This would therefore call for identifying and addressing issues surrounding the delay in initiation of ART. The size of our sample could not allow stratification for various groups and hence recommend assessing these groups with bigger samples in future similar studies.

6. Conclusion

ART is effective in elevating the CD4 cell count among HIV infected individuals initially in sero-discordant relationship. There is need to address issues surrounding delays initiation of ART to allow maximum benefit i.e quick recovery of CD4 cells which are known to aid in fighting opportunistic infections.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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