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Full Length Research Paper

Impact of low dose naltrexone (LDN) on antiretroviral therapy (ART) treated HIV+ adults in Mali: A single blind randomized clinical trial

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To implement an immuno-regulatory approach for reducing or preventing the onset of AIDS symptoms in HIV+ individuals we conducted a single blind nine-month randomized clinical trial to evaluate the impact of low-dose naltrexone (LDN) on asymptomatic HIV+ Mali adults undergoing antiretro virial (ART) treatment with CD4 counts below 350 cell/mm^3. We measured differences between groups in CD4 count, CD4%, hemoglobin, viral load, interferon alpha, and standard chemistry panel five times during the clinical period. The random mixed model and restricted maximum likelihood method for estimating slopes for repeated measures on subjects were used to predict CD4 counts and CD4%. The improvement in CD4 count in the treatment group (51 subjects) was significantly greater than the control group (49 subjects) at 6 months (p = 0.041) and marginally at 9 months (p = 0.067). Improvement in CD4% in the treatment group also was observed throughout the clinical period but these increases were not significant relative to the control group. Since, for this period of time, the combination of LDN + ART appears to be more effective in increasing CD4 count, and since LDN is inexpensive, easy to administer and without side effects, further exploration of LDN together with ARV treatment is recommended.

Key words: HIV+, LDN, CD4-count, CD4%, immuno-enhancement, ART.

INTRODUCTION

The number of people living with HIV/AIDS by end of 2008 was estimated at 33.4 million. In developing and transitional countries, 9.5 million people are in immediate need of life-saving AIDS drugs but only 4 million (42%) are receiving ART treatment. In sub-Saharan Africa, where the majority of new HIV cases occur, an estimated 1.8 million people became infected in 2009. An estimated 370,000 of these were infants and children infected during the perinatal and breastfeeding period. At just 26%, antiretroviral therapy for children in sub-Saharan, Africa is slightly below the global average (UNAIDS, 2010). In addition ARV medications are costly and

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Abbreviations: LDN, Low dose naltrexone.

complex to administer, particularly for children (Cross Continents Collaboration for Kids, 2008). Moreover, the great diversity of HIV strains and the emergence of resistant hybrids limit this therapeutic arsenal (Bennett et al., 2008). As a consequence, new therapeutic approaches are being explored, including immuno-regulatory molecules that may have the potential to delay or prevent the onset of AIDS symptoms in HIV positive individuals (Gekker et al., 2001; Steele et al., 2003, Zagon 2011). The search for immuno-regulatory treatments led to the identification of "low-dose naltrexone" (LDN), an opioid antagonist previously prescribed for autoimmune patients and drug addicts (Smith et al., 2007; Roy and Loh, 1996; McCarthy et al., 2001). LDN has shown the capacity for immuno-enhancement in HIV infected subjects with no significant side effects, thus preventing or delaying the progression of the disease (Bihari et al., 1988; Mathews et al., 1983; Puente et al., 1991). These encouraging results suggested that it was time to conduct a quantitative controlled evaluation of LDN in the treatment of HIV+ individuals in most countries affected by the illness. Dr. Bernard Bihari conceived such study in 2004; the present program is a fulfillment of his foresight (Bihari et al., 1988). Because of naltrexone's non-toxicity, ease of administration, low cost and potentially preventive effects on the progression of the disease, it seemed particularly important to carry out a clinical trial in a country where AIDS is endemic--such as Mali-- in order to test its safety and efficacy in preventing the advancement of HIV+ to full-blown AIDS.

MATERIALS AND METHODS

With the approval of the National Ethics Committee of Mali, the program was conducted as a single blind randomized, two-group clinical trial during the period of March 2008 to March 2010. The control group received the standard ART medication plus a placebo, while the treatment group received 3.0 mg of LDN daily in addition to the standard ART medications. All patients enrolled in this study signed informed consent forms required by the Mali Ethics Committee and were covered by health insurance during the study period. The objective of the present study was to evaluate the efficacy of LDN when used in conjunction with ART medication for HIV+ adults.

Study sites

Clinical evaluations were conducted at three Bamako sites: the National Hospital of Point G (HNPG), the National Center to Support the Fight against Disease (CNAM) and the Center for the Care, Facilitation and Support of HIV+ patients (CESAC). These three sites are the primary recruitment and treatment centers for HIV/AIDS in Bamako, the capitol of Mali and by far its largest urban center. Point G Hospital is the primary treatment facility for infectious diseases in Mali and was where the study began to recruit patients; CNAM, which specializes in infectious diseases and the skin diseases that are common in HIV/AIDS patients, was added to increase the pace of enrollment in the study; finally, CESAC, a major Mali NGO, was devoted to the treatment of HIV/AIDS and was added, after the clinical program commenced, to complete the enrollment in the time span specified by the study.

Study population and follow-up

Subjects were recruited from one of the three above sites from a population of HIV positive adults, all of whose CD4 counts were below 350 cells/mm^3, and none of whom had exhibited any symptoms of AIDS. This CD4 level for initiating ART treatment is the current suggested guideline of the World Health Organization. Beside the specified CD4 count, the enrollment criteria included: age between 18 and 60 and hemoglobin level greater than 8 g/dl. Exclusion criteria included: clinical signs of AIDS, TB, pregnancy, nursing mothers, patients receiving ARV therapy, and patients with impaired renal or hepatic functions, physical or mental conflict with monitoring or other serious pathologies that would interfere with monitoring. All patients had to give informed consent in writing. One hundred and fourteen patients were enrolled in the study with one and half randomly assigned to each of the two groups.

The sample size of 114 (57 for each group) was established using available best estimates of expected dropout rates and the variability of CD 4 count measurements with 95% confidence and 80% power as the goal in drawing statistical conclusions. Randomization was achieved by assigning qualified participants alternately to the two groups. Strictly speaking, only the subjects were "blinded" during the study, since the overall clinical coordinator (FFCK) knew the coded identification of all the participants. Subjects were enrolled for a nine-month clinical period in staggered fashion, starting in March of 2008. The last few patients who were enrolled in September of 2009 completed their nine-month clinical evaluation in March of 2010. Testing was conducted for all subjects starting with the first day in the program (baseline) and then on the 15th day of the first month, and the end of the first, third, sixth and ninth months. The impact of LDN+ART was evaluated in immunological, virological and toxicological terms by measuring the absolute CD4 count, CD4 percentage, viral load, hemoglobin, interferon-alpha, creatinine, alanine aminotransferase (ALT), asparate aminotransferase (AST) and body mass index (BMI). Onset of AIDS symptoms or pregnancy was conditions requiring removal of the subject from the clinical study (with treatment initiated as appropriate). (Since this population was very small, no survival analysis was performed on these patients.)The time needed to gather the required population for adequate statistical analysis extended beyond our expectations because: (1) The HIV/AIDS stigma in Mali was and is a powerful force inhibiting potential HIV+ candidates; (2) The simultaneous requirements for a CD4 count below 350 and non-appearance of any AIDS symptoms limited to the available population; and (3) The nine-month commit-ment, travel implications and extensive testing involved was a daunting challenge for many of the potential candidates. A diagrammatic flow chart of the number of patients participating at each time of testing, from recruitment to the end of the program is given in Figure 1.

Laboratory procedures

The diagnosis of HIV+ infection was established by the detection of antibodies to HIV by at least two different tests (Immuno Coombs II and Genie II) according to the Mali national guidelines. CD4 percentages were measured directly by conventional flow cytometry and the CD4+ count was done by the FasCount (Beckton Dickinson).

The Elisa technique was used to estimate the level of the interferon-alpha obtained from the enrolled subjects. The kit was purchased from Prestka Biomedical Laboratories (Verikine TM Human IFN-α Kit, Piscataway, NJ, USA) and the test was done

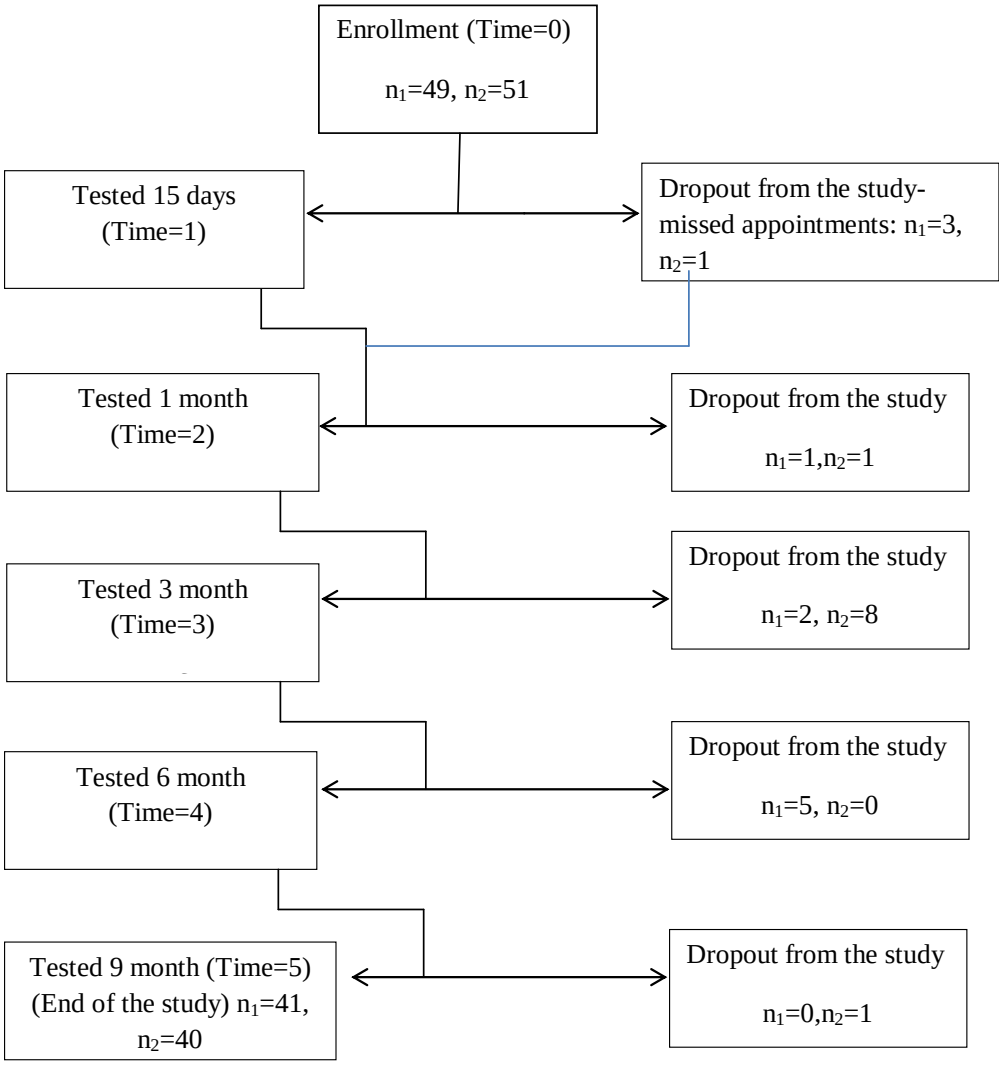

Figure 1. The follow-up and dropout of the intensive clinical survey of the patients over time. The subscripts 1 and 2 refer to the control and treatment groups, respectively: LDN's Impact on ART treated HIV+, Bamako, Mali, March 2008-March 2010.The minimum sample size required was 38 patients for each group with 80% power and 5% risk error for the study. We started with 100 patients in both groups anticipating 20% of drop out or noncompliance.

using plate reader DiaMed Euro Gen (Belgium).

To determine the possible toxicity of LDN, the blood chemistry of all subjects was checked by measuring the liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), as well as blood creatinine and hemoglobin using Lisa 300, Lisa 3D and Diana 5, respectively.

The Viral load was estimated using the BioMérieux nuclear EASY Q and Abbott M2000RT (Abbott).

Statistical analysis

For both groups, the amount of change in CD4 count and CD4 percentage during the study period were used to estimate the impact of LDN on the standard ART treatment in two separate models. To obtain an unbiased inference about the longitudinal changes in CD4 absolute count and CD4 percentage for repeated measurements, the mixed random effect models and restricted maximum likelihood method for slopes estimation was utilized. This allowed all the subjects' data to be used according to their actual participation in the study. The population demographics (age, gender, marital status, and body mass index (BMI)), and the immunological and virological data (viral load, hemoglobin, and interferon-alpha) were included in the models. The significance of all covariates over time was explored by including interaction terms for the predictors with time. The intercept was treated randomly and was allowed to vary between subjects, which resulted in a good fit at the time when it was used as the random variable in the models (all $p > 0.2$). The correlations between measurements at each time for CD4 count and also for CD4% were not only very high but also quite similar to each other. This motivated the use of a component symmetry correlation approach in both random mixed models. The correlations from these models were 79.06 and 73.87% for the CD4 count and CD4%, respectively. After graphically and numerically analyzing the residuals for distribution assumptions, it was found that a square root transformation of the CD4 count was needed;

Table 1. Demographics at baseline for control and treatment groups, LDN's Impact on ART treated HIV+ adults, Mali.

Parameter	Control group (ART + Placebo), n=49		Treatment Group (ART+LDN), n=51		
	Mean	**Std Dev**	**Mean**	**Std Dev**	**P-value**
CD4 Count	186.327	99.236	188.549	106.062	0.914
CD4 Percentage	12.594	8.322	12.010	7.150	0.707
BMI	20.861	3.982	20.737	3.485	0.869
Hemoglobin	11.416	1.467	10.959	1.516	0.129
Age	36.878	9.068	34.941	9.673	0.305
Log Viral load[a]	10.810	1.622	11.680	1.849	0.016[c]
Interferon Alpha[b]	0.703	0.152	0.776	0.186	0.059
	n	**Percentage**	**N**	**Percentage**	
Gender					
Male	18	36.73	12	23.53	0.149
Female	31	63.27	39	76.47	
Marital status					
Unmarried	9	18.37	12	23.53	
Married	24	48.98	27	52.94	0.667
Divorced	6	12.24	6	11.76	
Widows	10	20.41	6	11.76	

a: n=47 for control group and 49 for treatment group; b: n=40 for both groups; c: The two groups were not similar for viral load only, with the mean being higher in the treatment group.

however, no transformation was needed for CD4 percentage. The final models of CD4 count and CD4% fit as well as their prospective saturated model with p = 0.21 and p= 0.998, respectively. The log of viral load and the interferon-alpha (adjusted by the density and concentration of the plates) were used in the study as other HIV infection markers to assess their relative utility compared with CD4 and CD4% now considered as the best markers for HIV-induced immune impairment (Hulgan et al., 2005; Pirzada et al., 2006) .

RESULTS

Of the 114 patients originally enrolled, 8 patients (14.03%) and 6 patients (10.53%) were excluded from the control and treatment groups, respectively, because of missing clinical information after their recruitment at baseline (9 patients), or missing BMI (5 patients). This exclusion did not exceed the anticipated dropout and or loss of follow up rate of 30% (17 subjects in each group) used in determining the original sample size. Among the remaining one hundred patients who were included in the study (n = 49 for the control group and n = 51 for the treatment group), 18 (n = 7 and n = 11 for the control and treatment groups, respectively) were missing at different times as the study progressed for non-compliance (not following the protocol correctly), pregnancy or voluntary withdrawal from testing. The total follow up time was equivalent to 726.04 person-months, with 365.37 person-months for the control group and 360.67 person-months for the treatment group. The mean follow up time for patients in each group was similar (7.46 months and 7.06 months for the control and treatment groups, respectively, p =0.777). Table 1 shows the demographic and baseline measurements for both groups. There was no difference between the groups for any variable at baseline except for viral load where the treatment group's mean was higher, p = 0.016. At the end of the study, three patients in the control group and two in the treatment group were known to have died (due to extremely low CD4 counts in four cases that led to secondary infections and nasopharyngeal cancer in the fifth case), 95 were known to be alive and 14 had lost contact with the clinical program. At no time did any of the subjects report or exhibit adverse reactions to their daily dosage of LDN. There is no reason to suggest that LDN had anything to do with the two deaths in the treatment group, since the safety of this medication has been demonstrated in a related Mali study with patients undergoing daily dosage, as well as in other studies (Smith et al., 2007). Table 2 summarizes the descriptive statistics at each of the six testing times for CD4 count and CD4 percentage over the nine-month period of the study. The mean CD4 count and CD4% increase over time, with the amount of increase being higher at each time for the treatment group compared to the control group in both predicted models. Full details of the final models are presented in Table 3 and the more significant results are highlighted in Table 4.

Although there was no significant difference between the two groups for the entire clinical period, we did observe a significant increase in the number of CD4 + cells between months 3 and 6 (p = 0.041) in the treatment group. Between months 6 and 9 this increase

Table 2. CD4 progression statistics for control and treatment groups, LDN's Impact on ART treated HIV+ adults, Mali.

Measure time (code)		Control group (ART+Placebo)			Treatment group (ART+LDN)		
		N	Mean	Std Dev	N	Mean	Std Dev
Baseline(0)	CD4 count	49	186.327	99.236	51	188.549	106.062
	CD4 percentage	49	12.594	8.322	51	12.010	7.150
0.5 month (1)	CD4 count	46	247.174	131.721	50	272.800	161.728
	CD4 percentage	46	16.713	9.501	50	17.782	8.978
1 month (2)	CD4 count		306.063	147.072	49	310.673	154.612
	CD4 percentage	48	19.280	9.032	49	20.161	9.428
3 months(3)	CD4 count	46	321.978	145.183	41	324.024	143.480
	CD4 percentage	46	20.434	9.863	41	21.100	9.987
6 months(4)	CD4 count	41	319.049	149.066	41	375.122	172.222
	CD4 percentage	41	22.056	10.808	41	22.969	8.987
9 months(5)	CD4 count	41	318.073	150.116	40	372.625	199.109
	CD4 percentage	41	21.726	9.726	40	23.237	10.217

Table 3. Fitted random mixed models for CD4 and CD4%, LDN's Impact on ART treated HIV+ Adults, Mali.

	CD4 count model [a]			CD4 percentage model		
	Slope	Standard error	P-Value	Slope	Standard error	P-Value
Baseline	3.564	2.582	0.171	9.773	1.521	<.0001
Time=5	4.415	2.121	0.038	9.087	1.020	<.0001
Time=4	5.936	2.122	0.005	9.436	1.020	<.0001
Time=3	5.661	2.084	0.007	7.819	0.983	<.0001
Time=2	4.211	1.860	0.024	6.468	0.969	<.0001
Time=1	1.549	1.795	0.389	4.474	0.981	<.0001
Treatment	4.066	3.249	0.214	-0.301	1.809	0.868
Control(Ref)	-	-	-	-	-	-
T5*Treat	1.321	0.720	0.067	1.740	1.444	0.229
T4*Treat	1.423	0.692	0.041	0.996	1.439	0.489
T3*Treat	0.166	0.658	0.802	0.955	1.413	0.499
T2*Treat	0.180	0.618	0.771	1.655	1.362	0.225
T1*Treat	0.598	0.617	0.334	1.309	1.364	0.338
Widows	NI			6.227	2.281	0.008

Table 3. Contd.

Divorced	NI			4.609	2.543	0.073
Unmarried	NI			5.368	2.053	0.010
Married(Ref)	-	-	-	-	-	-
BMI	0.447	0.120	0.000	NI		
BMI*Treat	-0.192	0.150	0.201	NI		
BMI*T5	-0.043	0.093	0.643	NI		
BMI*T4	-0.104	0.094	0.269	NI		
BMI*T3	-0.084	0.093	0.367	NI		
BMI*T2	-0.027	0.085	0.754	NI		
BMI*T1	0.025	0.083	0.762	NI		

[a] the square root of CD4 was used in the model, NI signifies not Improving the model. The BMI is significantly associated with the predicted values of CD4 count but the effect is not different in the two groups as shown by BMI's interaction term with group. The change of BMI over time is not significant as shown by its interaction terms with time. The amount of change in the CD4 count for the treatment group is significantly higher than for the control group at 6 months (p=0.041) and marginally significant at 9 months (p=0.067). For CD4 percentage, the model shows no difference in the magnitude of change between the two groups (all interaction terms had p- value > 0.2).

Table 4. Highlights of CD4 and CD4% progression for treatment group, LDN's Impact on ART treated HIV+ adults, Mali.

Measure time in months	Treatment group (ART+LDN)			
	Increased CD4 count cells/mm3 (%)	p-value	Increased CD4 percentage (%)	p-value
0.5 – 1	0.76 (0.63)	0.771	1.47 (21.92)	0.225
1 – 3	1.14 (0.84)	0.802	1.25 (15.96)	0.499
3 – 6	49.67 (37.06)	0.041	1.50 (15.83)	0.489
6 – 9	42.09 (31.62)	0.067	2.1 (22.95)	0.229

The amount of change in the CD4 count for the treatment group is significantly higher than for the control group at 6 months (p=0.041) and marginally significant at 9 months (p=0.067). For CD4 percentage, the model shows no difference in the magnitude of change between the two groups (all interaction terms had p value > 0.2).

remained relatively stable, with marginal significance (p = 0.067). In contrast, the control group did not show a significant increase in CD4+ count between months 3 and 6 (Table 4). When CD4% was used as the immunological marker, we did not observe a significant increase during the clinical period (Table 4).

Taken together, those results suggest that the combination of ART and LDN was able to maintain the CD4 count of patients more effectively than ART alone, particularly after three months of treatment. However, in regard to CD4%, there was no significant difference in the change over time between the two groups. Although CD4% is generally accepted as a useful measure of the progression of HIV infection, its usefulness decreases with the CD4 count, particularly when the latter is below 350 cells/mm^3, which is the upper limit for all patients in this study (Pirzada et al., 2006).

The BMI was the only covariate that improved the CD4 count model and was significantly associated with the predicted CD4 count, p = 0.003 for both groups. For each unit changes in BMI, the

adjusted predicting CD4 count; increases significantly by 0.45 cells/mm^3, $p < 0.001$, confirming the expected improvement in CD4 count with the maintenance of body weight. For CD4%, accounting for marital status improved the model and was also significantly associated with CD4 percentage ($p = 0.009$) for both groups in the study. The increase in the adjusted predicted values of CD4% for married patients was significantly lower by 5.37 and 6.23% ($p = 0.01$ and $p = 0.008$, respectively) compared to the unmarried and widowed patients, respectively. Given the relatively short time of our study (nine months), the CD4 % is perhaps a more stable but less sensitive measure for evaluating the effects of short term treatment differences on the immune system (Hulgan et al., 2005; Pirzada et al., 2006; Duvignac et al., 2008). Figures 2a and b show the predicted mean at each testing time for CD4 count and CD4 percentage with the fitted line joining the means over time and the 95% confidence interval represented by the vertical ranges at each time. The means are higher in the treatment group for both models but, as already indicated, the amount of change is large and significant only for the CD4 count model from six months onward.

Figures 2a and b also show the predicted mean of log of viral load for the two groups. The decrease is significant in both groups, with all $p < 0.0001$ at all testing times compared to the baseline. In the control group the mean (when converted to the original scale) decreased from 101806.72 copies/ml at baseline to 122.48 copies/ml at nine months. In the treatment group the viral load decreased from 324416.55 at baseline to 260.54 copies/ml at nine months. However the amount of change between the two groups was similar at all times: $p = 0.537$, 0.988, 0.282, 0.070 and 0.386 at 0.5, 1, 3, 6 and 9 months, respectively. Since the mean viral loads were significantly different at baseline and higher in the treatment group, the decrease might have been significantly greater in the treatment group if the two means of viral load had been comparable at the baseline.

The relationships among interferon alpha, viral load, CD4 count and CD4% were evaluated using the Pearson and Spearman correlation (after controlling for time and groups effects) between the latter two variables and interferon alpha (adjusted for the concentration and density of the plates) and the log viral load. The results, as shown in Table 5, indicate that there is no significant correlation (not significantly different from zero) between the interferon alpha and any of the other three markers: CD4 count, CD4% and log viral load. Interferon alpha explains less than 1% of the total variation of the three markers with all $p > 0.6$. If a normal distribution for the markers is not assumed, these results are not altered, as shown by the Spearman Correlation in Table 5. The percentage of the total variation of the CD4 count and CD4% explained by log viral load is significant, 9.15 and 5.75% for CD4 count and CD4%, respectively, with all $p < 0.0001$. However these percentages are relatively small compared to the two CD4 measures themselves (53.77% with $p < 0.0001$).

DISCUSSION

Antiretroviral therapy (ART) has been shown to be effective in decreasing the risk of opportunistic infections following suppression of HIV replication in conjunction with reconstitution of CD4 counts, but not all individuals in ART treatment reconstitute CD4 at the same rate or to the same extent. In this single randomized clinical study, which was conducted from March 2008 to March 2010, we evaluated whether low dose naltrexone (LDN) has an impact on the immune system recovery of patients undergoing ART treatment over a nine month period. We also examined the association of other independent predictors such as age, BMI, gender, marital status, hemoglobin, and baseline viral load. Only BMI was significantly (and positively) associated with the increase in CD4 count in the linear mixed model, while married patients showed a significantly slower immune system recovery relative to other relational states in the CD4% model. However, neither of these variables showed a significant difference between the two groups in the study over the entire clinical period. Since the BMI has been shown to be significantly related to the CD4 change over time (Duvignac, 2008; Fahey et al., 1990), these results suggest—not surprisingly--that those patients with a higher BMI (due primarily to better nutrition and general health) will exhibit a faster and higher CD4 recovery rate. Since marital status was not a focus of the present study and both groups demonstrated a similar trend, we will not speculate on why our results turned out differently from the majority of studies that show married couples generally fare better regarding the onset and treatment of HIV/AIDS (UNAIDS, 2009; Shisana et al., 2004; Songok et al., 1990).

Conclusions

It has been demonstrated that a treatment program which used three anti-retrovirals reduced progression to AIDS or death by about 40% when compared with a treatment program that used a placebo and only two anti-retrovirals (Jordan et al., 2002). We found that adding LDN to the three-antiretroviral regime used in our study improved the recovery rate of CD4 count significantly by 37.06% at six months and marginally by 31.62% at nine months ($p = 0.041$ and $p = 0.067$, respectively). The CD4% also improved, but not significantly by 15.83 and 22.95% at six and nine months, respectively. Although modest, these immune system benefits of LDN are encouraging, especially for the CD4 count marker, and they suggest that further exploration of LDN as part of an HIV+ treatment regime is warranted.

Table 5. Time and group effect correlations among interferon alpha, viral load, CD4 and CD4%, LDN's Impact on ART treated HIV+ adults, Mali.

Variable	With Variable	N	Pearson partial correlation		Spearman partial correlation	
			Correlation	P- Value	Correlation	P-Value
CD4 Count	CD4 percentage	397	0.73330	<.0001	0.7652	<.0001
CD4 Count	Interferon Alpha	397	-0.01895	0.7075	-0.05288	0.2946
CD4 Count	Log Viral load	397	-0.30259	<.0001	-0.24009	<.0001
CD4 percentage	Interferon Alpha	397	0.02605	0.6059	0.00386	0.939
CD4 percentage	Log Viral load	397	-0.23988	<.0001	-0.18063	0.0003
Interferon Alpha	Log Viral load	397	0.000693	0.9891	0.02426	0.631

The correlation squared is the coefficient of determination or the percentage of variation that explained one variable relative to the other variable. For example, CD4 count explains 53.77 % of the total variation of CD4 percentage after controlling for time and group effects.

(a)

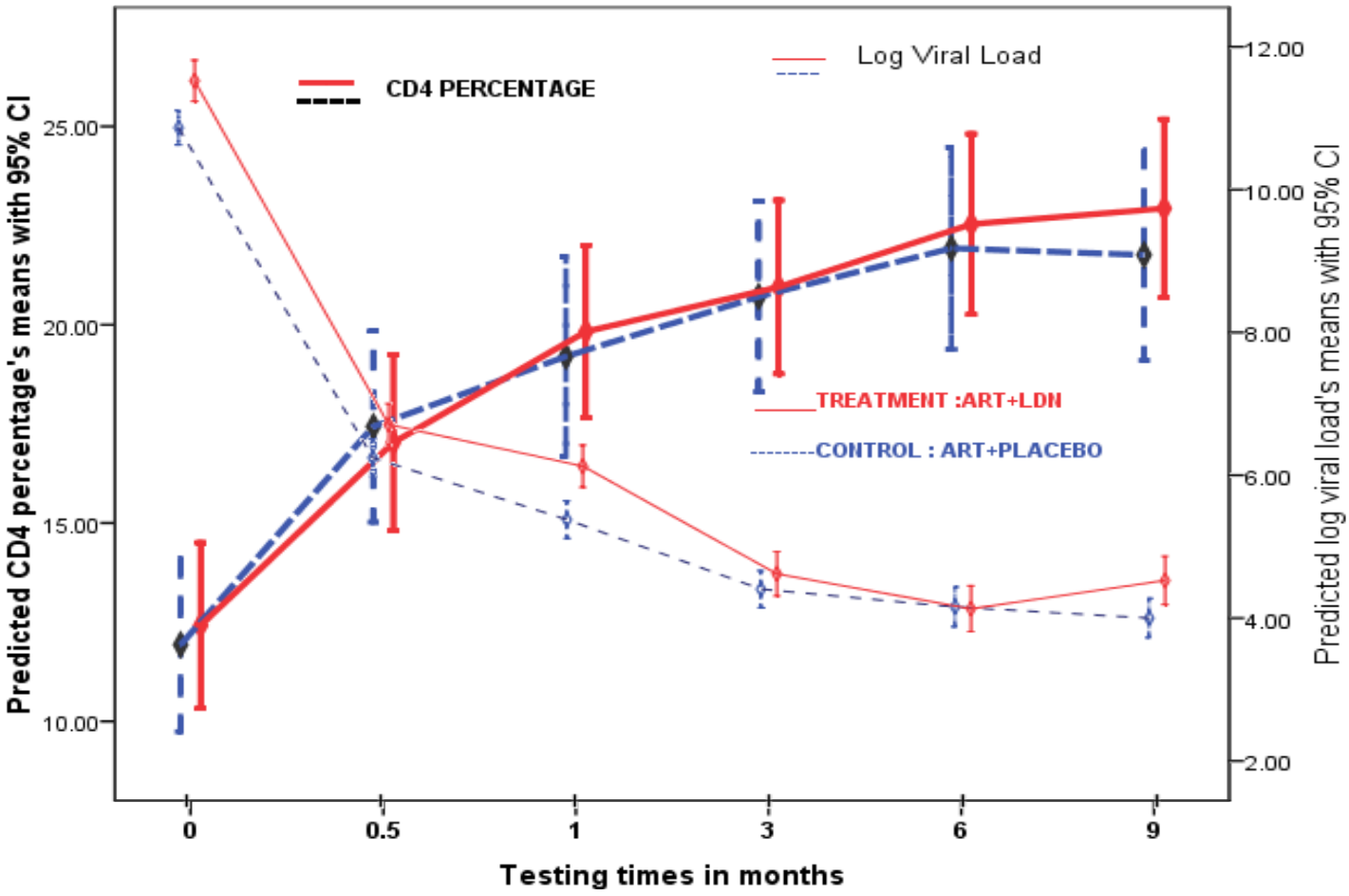

Figure 2. Fitted line and 95% confidence interval (CI) on Predicted mean of CD4 count and Log of Viral load (2a) and the predicted mean of CD4 percentage and Log viral load (2b) by group, LDN's Impact on ART treated HIV+, Bamako, Mali, March 2008-March 2010. The line across each bar joins the predicted meansof CD4 count and the two limits on each bar are the lower and the upper limits of 95% confidence interval around the predicted means at each time. The means are higher in the treatment group for both models but the amount of change is large and significant only for the CD4 count model from six months onward. In the control group the mean of viral load (when converted to original scale) decreased from 101806.72 copies/ml at baseline to 122.48 copies/ml at nine months. In the treatment group it decreased from 324416.55 at baseline to 260.54 copies/ml at nine months. However the amount of change between the two groups was similar at all times, p= 0.537, 0.988, 0.282, 0.070 and 0.386 at 0.5, 1, 3, 6 and 9 months.

b

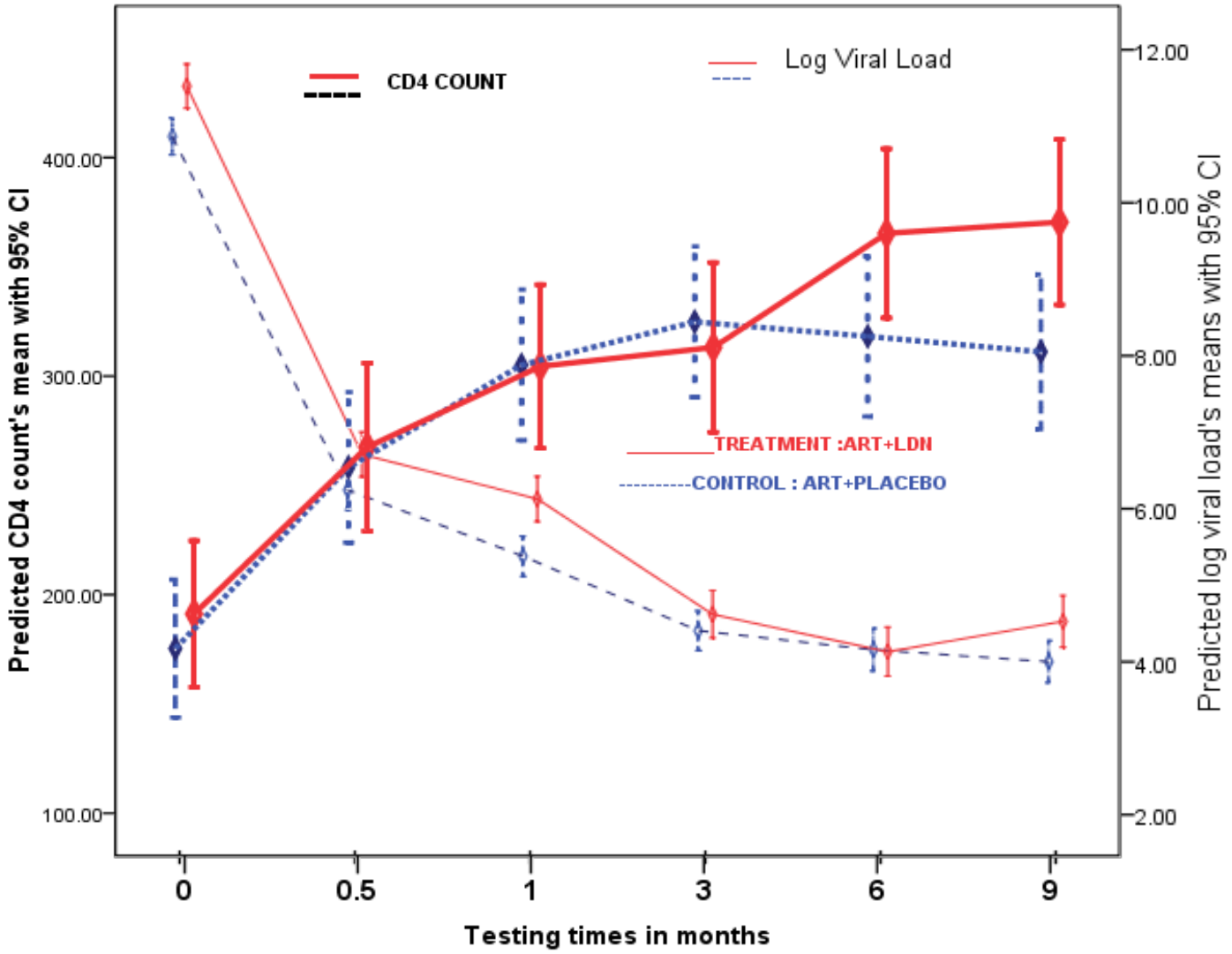

Figure 2. Contd.

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