Full Length Research Paper

Single cohort study of the effect of low dose naltrexone on the evolution of immunological, virological and clinical state of HIV+ adults in Mali

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To implement an immuno-regulatory approach for reducing or preventing the onset of AIDS symptoms in HIV+ individuals a single prospective cohort study was conducted to evaluate the effect of low-dose naltrexone (LDN) on HIV infected, asymptomatic, otherwise untreated Mali adults with CD4 levels between 350 and 600 cell/mm^3. We measured changes in CD4 count, CD4%, BMI, hemoglobin, viral load, interferon alpha, and standard chemistry panel five times over a nine-month period. Linear regression mixed models were used with maximum likelihood as the estimation method for repeated measures on subjects. Of 55 subjects followed, 71% completed the full program without indications of clinical AIDS symptoms, side effects or enough loss of CD4 count to warrant initiation of ART medication. The decrease of CD4 count was marginally significant over the full testing period (p=.066) and became significant as the cohort aged (37.73 cells/mm^3 with p=0.027 and 52.94 cells/mm^3 with p=0.003, respectively, at six and nine months). In contrast, the estimated mean CD4% did not show significant decrease over the entire study (p=0.842). No other covariates were associated significantly with the results. These findings support the therapeutic potential of LDN in treating HIV+ in its early stages and suggest further studies are indicated.

Key words: HIV, LDN, CD4+, CD4+%, Immuno-regulatory, ART.

INTRODUCTION

The number of people living with HIV/AIDS by end of 2009 was estimated at 33.3 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

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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. In addition ARV medications are costly and complex to administer, particularly for children. Moreover, the great diversity of HIV strains and the emergence of resistant hybrids limit this therapeutic arsenal (UNAIDS, 2010). As a consequence, new therapeutic approaches are being explored that involve 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). The search for immunoregulatory treat-ments 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., 1992). These encouraging results suggested that it was time to conduct a quantitative, controlled evaluation of LDN in the treatment of HIV+ individuals. Dr. Bihari conceived of such a 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 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, this program consisted of a single group cohort prospective study conducted during the period, March 2008 to October 2009. They agreed with the decision to forgo the use of a control group in this study for ethical reasons. To ask HIV+ individuals to participate in a clinical study for the prevention of AIDS symptoms and then give them a placebo, seemed clearly inappropriate. Fortunately, in the absence of a control group, a recent Ivory Coast study (Duvignac et al., 2008), provides useful comparisons with a similar cohort of untreated HIV+ adults. The objective of this study was to evaluate the effect of low-dose naltrexone (LDN) on HIV infected adults in Mali without AIDS symptoms and undergoing no ART treatment.

Study sites

The 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, devoted to the treatment of HIV/AIDS, was added after the clinical program started 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 count were between 350 and 600 cells/mm^3, and none of whom had exhibited any symptoms of AIDS. Pregnancy and TB were additional reasons for exclusion from the study. This "CD4 window" was chosen because it represents the transition region between normal CD4 count levels and the level below which ART treatment is strongly indicated. The sample size of 57 was established using available best estimates of expected dropout rates and the variability of CD4 count measurements with 95% confidence and 80% power as the goal in drawing statistical conclusions. All patients enrolled in this study signed extensive release forms required by the Mali Ethics Committee and were covered by health insurance during the study period. All subjects received 3.0 mg of LDN daily but no other treatment for their HIV infections. The subjects were enrolled for a nine-month clinical period in staggered fashion, starting in March, 2008. The last few patients who were enrolled in February, 2009 completed their nine-month clinical evaluation in October, 2010. Testing was conducted for all subjects starting with the first day in the program (baseline) and then again on the 15th day of the first month, and the end of the first, third, sixth and ninth months. Effectiveness of LDN was evaluated in immunological, virological and toxicological terms by measuring the absolute CD4 count, CD4 percentage, viral load, hemoglobin, interferon-alpha, creatininemia, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and body mass index (BMI). Onset of Tuberculosis, AIDS symptoms, too low a CD4 count or pregnancy were conditions requiring removal of the subject from the clinical study (with treatment initiated as appropriate). 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 narrow CD4 range chosen made it difficult to find subjects; and 3) The nine-month commitment, travel implications and extensive testing involved was a daunting challenge for many of the potential candidates.

Laboratory procedures

The diagnosis of HIV+ infection was established by the detection of antibodies to HIV by at least two different tests (ImmunoCoombs II and Genie II). CD4 percentages were measure directly by conventional flow cytometry and the CD4+ count was done by the FasCount method of BD (Beckton Dickinson). The dosage of LDN chosen for the study was 3.0 mg, the low end of the "optimum" range of 3.0 mg- 4.5 mg recommended by Dr.Bihari in his 1988 presentation (Bihari et al., 1988). 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 using plate reader DiaMed Euro Gen (Belgium). To determine the possible toxicity of LDN, the blood chemistry of all subjects was checked by measuring liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), as well as blood creatinine and hemoglobin using Lisa 300, Lisa 3D and Diana 5. The Viral load was estimated using the BioMérieux nuclear EASY Q and Abbott M2000RT.

Statistical analysis

Changes in CD4 count and CD4 percentage from the baseline during the study period were used to estimate the effect of LDN in two separates models. To obtain unbiased inferences 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 and, therefore made more effective use of the cohort sample. The population demographics (age, gender, and marital status) and the immunological, virological and toxicological variables (viral load, hemoglobin, interferon-alpha, creatinine level, alanine aminotransferase (ALT), aspartate aminotransferase (AST)) and body mass index (BMI) were included in the models. The significance of all covariates over time was accessed by including interaction terms for the predictors with time. The intercept was treated as random and was allowed to vary between subjects, since the model did not converge using time as a random variable in the model. Finally, the random mixed model with random intercept and fixed slope was used to predict the CD4 count and CD4 percentage change with time. The correlations between measurements for CD4 count were very high in general and particularly higher for times which were adjacent compared to times more distant. This was the case also for CD4 percentage values and motivated the use of an autoregressive correlation order
(1) in the random mixed model, which turned out to be 52.65 and 70.54% for the CD4 count and CD4 percentage respectively. After graphically and numerically analyzing the residuals for distribution assumptions, we found there was no need to transform the predicted variables CD4 count and CD4 percentage. A linear time variable for the model predicting CD4 count was the only significant variable (p=0.006) versus all other ortho-polynomials orders. For the model predicting CD4 percentage all the ortho-polynomial orders turned out to be not significant. Only time as the dummy variable fit the data in both regression models. Finally, all the interaction terms with time for all the covariates turned out to be not significant and time as the single dummy variable fit as well as the random mixed regression models' full main effects for CD4 count
(and CD4 percentage), p=0.48 (and p=0.21). 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 count and CD4 percentage, now considered as the best markers in HIV-induced immune impairment (Hulan, 2005; Pirzada et al, 2006; Fahey et al., 1990).

RESULTS

Initially fifty seven subjects started the clinical program but two (3.5%) were excluded from the cohort because no other data became available after their recruitment at baseline. This exclusion did not exceed the anticipated dropout and/or loss of follow up rate of 30% (17 subjects) in setting the original sample size. Fifty five patients were included in the study and followed up during 376 person-months (mean of 6.84 months per patient), among them, sixteen were removed at different times as the study progressed for a variety of reasons such as non-compliance (not following the protocol correctly), very low CD4 count and a need to start antiretroviral therapy, pregnancy, and voluntary withdrawal from testing. There were no cases of Tuberculosis in the study. Table 1 shows the baseline characteristics of the 55 patients included in the analysis. We note that the distributions of social and demographic parameters are similar to Malian HIV infected patients in general, which lends further credence to our cohort.

One patient who left the study needing ART medication was known to have died by the end the study, fifty were known to be alive, and contact was lost with the remaining four. None of the thirty nine subjects who completed the full clinical study (70.9%) reported any indications of clinical AIDS symptoms or a large enough loss of CD4 count to warrant initiation of ART medication (because of the strict Mali health policies). At no time did any of the participants report or exhibit adverse reactions to their daily dosage of LDN. Table 2 shows the distribution of the test measures at baseline for the patients who completed the full nine month study and those who did not. There was no difference at baseline between these two groups for all the parameters except log of viral load (p=.048), where the means were marginally comparable and the interferon alpha (p<0.0001), where the means at baseline were significantly higher in patients who were success-fully able to complete the full nine months of the study. Table 3 shows descriptive statistics over the nine-month clinical period for the CD4 count and CD4 percentage.

The mean CD4 count seems to decrease from baseline in a roughly linear pattern with small differences in means relative to the high variability demonstrated by their standard deviations. In contrast the mean CD4 percentage appears essentially constant over time with very small fluctuations relative to their standard deviations. Table 4 shows the random mixed models with all covariates predicting CD4 count and CD4 percentage. As shown in this Table 4, none of the factors were significantly associated with the predicted variables for both models. All interactions among the covariates and time were not significant, indicating there were no significant changes over time for these predictors. Table 5 and Figures 1a and 1b show the linear mixed models with time as the only explanatory (dummy) variable for the two mixed models. These simple models fit the data as well as the corresponding full main effects models (p=0.48 for CD4

Table 1. Baseline Information for patients, LDN single cohort study with HIV+ adults, Mali, Febuary 2008-March 2010.

Patients information	N	Mean	Median	Std Dev	Percentage
Age	55	34.636	34.000	8.914	-
BMI	55	23.096	22.600	4.796	-
CD4 count	55	456.855	454.000	64.252	-
CD4 percentage	55	24.187	23.800	8.079	-
Hemoglobin	55	12.120	11.900	1.710	-
ALT	55	23.527	23.000	10.685	-
AST	55	31.272	29.000	13.303	-
Creatinine	55	88.960	90.500	18.171	-
Log viral load	52	9.934	10.434	2.314	-
Interferon alpha	47	0.756	0.741	0.190	-
Gender					
Male	18	-	-	-	32.7
Female	37	-	-	-	67.3
Marital status					
Unmarried	11	-	-	-	20.0
Married	30	-	-	-	54.5
Divorced	6	-	-	-	10.9
Widows	8	-	-	-	14.5

The distributions of social demographic parameter are similar to Malian HIV infected patients in general.

Table 2. Baseline information for complete and incomplete patients, LDN Single Cohort Study with HIV + adults, Mali.

	Failed to complete nine months of the study (n=16)		Completed nine months of the study (n=39)		P Value
	Mean	Std Dev	Mean	Std Dev	
Age	33.438	9.993	35.128	8.523	0.528
BMI	21.943	3.334	23.570	5.245	0.257
CD4 count	450.438	77.477	459.487	58.929	0.640
CD4 percentage	21.450	6.167	25.310	8.560	0.108
Hemoglobin	11.869	2.046	12.223	1.569	0.490
ALT	21.688	9.925	24.282	11.017	0.419
AST	33.625	14.118	30.308	13.021	0.406
Creatinine	90.681	20.572	88.254	17.332	0.657
Log viral load	10.878	1.835	9.5148[a]	2.402	0.049[d]
Interferon alpha	0.588[b]	0.143	0.834[c]	0.157	<0.0001[e]
Gender	n	Percentage	n	Percentage	
Male	5	31.25	13	33.33	0.881
Female	11	69.75	26	66.67	0.586
Marital status					
Unmarried	5	31.25	6	15.38	
Married	7	43.75	23	58.97	
Divorced	2	12.50	4	10.26	
Widows	2	12.50	6	15.38	

[a] n=36, [b] n=15, [c] n=32. [d] Marginal comparability of the means of log viral load for the two groups. [e] There is a significant difference between the Interferon alpha means at baseline. The mean at baseline was significantly higher in patients who were able to complete the nine months of the study without any indications of clinical symptoms or a large enough loss of CD4 count to warrant the initiation of ART medication.

Table 3. CD4 count and CD4% over time, LDN Single Cohort Study with HIV+ adults, Mali.

Parameter	N	CD4 count Mean (Std Dev)	CD4 percentage Mean (Std Dev)
Baseline (time =0)	55	456.85 (64.252)	24.19 (8.079)
15 days (time=1)	52[a]	435.56(111.592)	23.56(8.211)
1 month(time=2)	54	436.22(128.444)	24.12(7.940)
3 months(time=3)	49	430.73(143.259)	24.01(8.741)
6 months (time=4)	44	416.48(147.864)	23.88(9.309)
9 months (time=5)	39	409.62(108.394)	24.29(7.622)

[a]Three patients missed the first test evaluation and were enrolled with the second test
The mean of CD4 count decreased in somewhat of a linear pattern but the mean of CD4 percentage remained the same over time with only minor differences.

Table 4. Random mixed models for CD4 and CD4%: full effect, LDN Single Cohort Study with HIV+ adults, Mali.

Parameter	Effect on the CD4 count				Effect on the CD4 percentage			
	Slope	P value	95% CI		Slope	P Value	95%CI	
			Lower	Upper			Lower	Upper
Baseline	507.09	<.0001	321.32	692.86	22.481	0.0017	8.8794	36.0827
time=1	-25.8672	0.1035	-57.0467	5.3122	-0.5771	0.4943	-2.238	1.0838
time=2	-26.8698	0.0914	-58.1063	4.3667	-0.08839	0.9168	-1.7543	1.5775
time=3	-31.3298	0.0564	-63.5166	0.857	-0.5487	0.5304	-2.2693	1.1719
time=4	-36.5307	0.0294	-69.3751	-3.6862	-0.9013	0.3137	-2.6601	0.8574
time=5	-53.4249	0.0026	-88.016	-18.8338	-0.3389	0.7187	-2.1904	1.5127
Age	-0.6816	0.6804	-3.9868	2.6236	0.003391	0.9786	-0.2492	0.256
Male	-10.1596	0.7458	-72.793	52.4737	-3.8402	0.1122	-8.6112	0.9308
Female	Ref	.	.	.	.	.	.	.
Unmarried	-61.5218	0.1948	-155.57	32.526	-1.1694	0.7443	-8.3351	5.9962
Married	-29.9326	0.4453	-108.1	48.236	2.2635	0.4487	-3.6924	8.2194
Divorced	-7.1951	0.8851	-106.74	92.3524	0.599	0.8749	-7.0069	8.2048
Widows	Ref	.	.	.	.	.	.	.
BMI	1.2604	0.633	-3.9332	6.454	0.1648	0.3848	-0.2082	0.5378
Hemoglobin	1.2131	0.2658	-0.9295	3.3557	-0.01054	0.8573	-0.1259	0.1048
Creatinine	-0.1559	0.1663	-0.3771	0.06533	-0.01969	0.0013a	-0.03162	-0.00777
ALT	-0.3097	0.674	-1.7583	1.1389	0.05359	0.1849	-0.02581	0.133
AST	-0.5291	0.3571	-1.6588	0.6007	-0.04504	0.1513	-0.1067	0.0166

For example: for one unit change in BMI, the CD4 count (CD4 percentage) increased by 1.26cell/mm^3 (0.165%) at any time after controlling for others covariates with a p-value 0.633(0.385). BMI and CD4 counts are positively but not significantly associated. In addition, the interaction of BMI with time is not significant (not shown here).

[a] The creatinine level was significantly associated with the CD4 percentage (P value=.0013) but the change over time was not si gnificant (not shown here). For each unit change of creatinine level the CD4 percentage decreased by 0.0197.

count and p=0.21 for CD4 percentage). According to these random mixed effects models, the mean at baseline of CD4 count (CD4 percentage) was 456.85 cells/mm^3 (24.19%). The estimated decrease of CD4 count was marginally significant over the entire study period (p =0.066). However, the estimated loss of CD4 count became significant as the cohort aged with values of 37.73cells/mm^3 (p = 0.027) and 52.94 cells/mm^3 (p = 0.003) at six and nine months, respectively. In contrast, the estimate mean of CD4 percentage did not show any significant decrease over the entire clinical period (p = 0.842). Thus, for this study patients showed a marginally significant loss of CD4 count over the full clinical period with significant decreases from six months onward but

Table 5. Random mixed model of CD4 and CD4%: dummy time effect, LDN Single Cohort Study with HIV+ adults, Mali.

Parameter	Effect on CD4 count				Effect on CD4 percentage[a]			
	Slope	P value	95%CI		Slope	P Value	95%CI	
			Lower	Upper			Lower	Upper
Baseline	456.85	<.0001	424.73	488.98	24.1873	<.0001	21.9761	26.3985
time=1	-25.9709	0.1027	-57.2033	5.2615	-0.7848	0.3628	-2.4806	0.911
time=2	-20.4325	0.1938	-51.3243	10.4593	-0.1116	0.8958	-1.7888	1.5657
time=3	-27.5273	0.0902	-59.4003	4.3457	-0.6194	0.4818	-2.3514	1.1126
time=4	-37.7255	0.0252	-70.7213	-4.7296	-1.0181	0.2647	-2.8122	0.776
time=5	-52.9426	0.0026	-87.2547	-18.6306	-0.7859	0.4076	-2.6522	1.0804

The baseline mean CD4 count was 456.85; 15 days after baseline (time=1) the CD4 count dropped by 25.97 cells/mm3 with a non s ignificant p- value of 0.103. At nine months (time=5), the estimated drop of CD4 count is 52.943 cell/mm3 with a significant p–value of 0.003. The baseline mean of CD4 percentage is 21.98%; at 15 days after baseline (time=1) the CD4 percentage drops by 0.785% with a non-significant p- value of 0.363. At nine months (time=5), the estimated drop of CD4 percentage is 0.786 % with a non-significant p –value of 0.407.
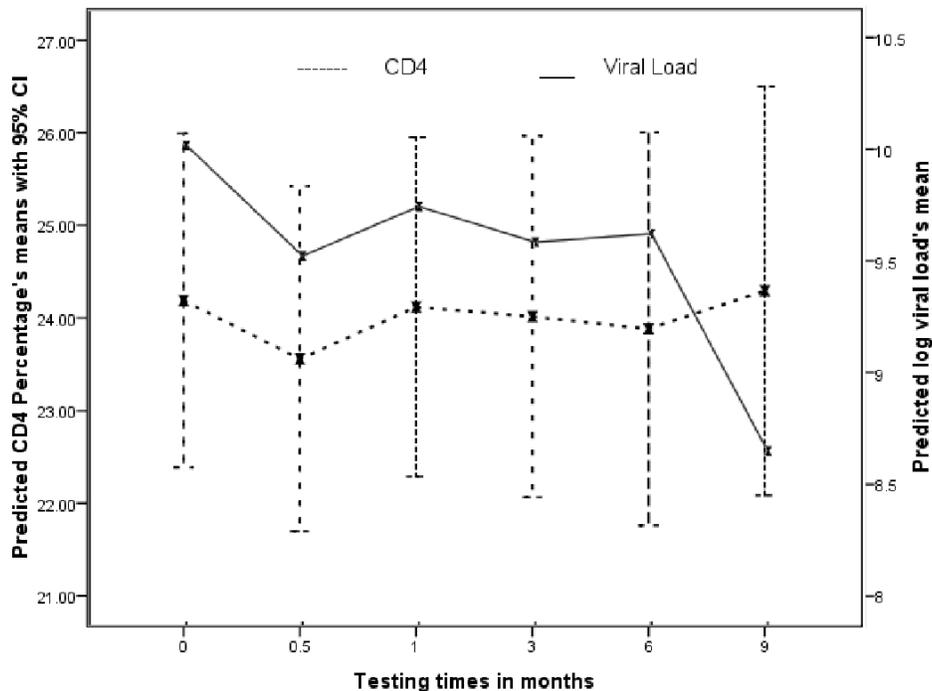[a] the change of CD4 percentage is not significant at any time.
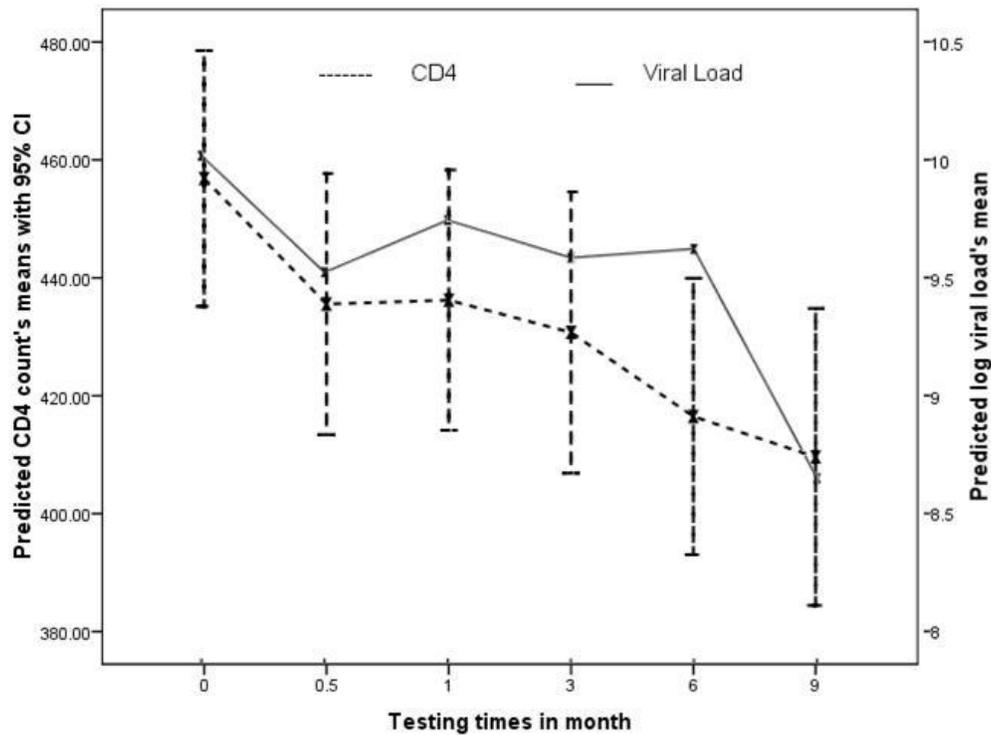
Figure 1a. Fitted line and 95% confidence interval (CI) on predicted mean of CD4 count and CD4 percentage LDN Single Cohort Study in HIV- Infected, Bamako, Mali, February 2008-March 2010. The line across each bar joins the predicted meansof CD4 count and CD4% and the two limits oneach bar are the lower and the upper limits of 95% confidence Interval around the predicted means at each time.
The mean of viral load decreased significantly (p=0.012) over nine months from the baseline (in the original scale it decreased from 50060.39 to 13634.69 copy/ml).

the CD4 percentage remained virtually stable over the full clinical period with no significant decreases at any time. This behavior of the CD4 percentage is encouraging in the light of recent reports that recommend the use of CD4 percentage as a more stable marker of immune system strength, particularly when the absolute CD4 levels are

Figures 1b. Fitted line and 95% confidence interval (CI) on predicted mean of CD4 count with the predicted mean of Log viral load, LDN Single Cohort Study in HIV- Infected, Bamako, Mali, February 2008-March 2010. The line across each bar joins the predicted meansof CD4 count and CD4% and the two limits oneach bar are the lower and the upper limits of 95% confidence Interval around the predicted means at each time. The mean of viral load decreased significantly (p=0.012) over nine months from the baseline (in the original scale it decreased from 50060.39 to 13634.69 copy/ml).

lower than in healthy individuals (Hulgan et al., 2005; Perzada, 2006).

To evaluate the relationships among interferon alpha, viral load, CD4 count and CD4 percentage, we used the Pearson correlation (after controlling for time) between the latter two variables and interferon alpha (adjusted for concentration and density of the plates) and the log viral load. The results are summarized in Table 6 and clearly show there is no significant correlation (not significantly different from zero) between the interferon alpha and any of the other three markers: CD4 count, CD4 percentage and log viral load. Interferon alpha explains less than 1% of the total variation of each the three markers; the percentage explained is 0.026, 0.52 and 0.74% with p=0.812, 0.285 and 0.086 for CD4 count, CD4 percentage and log of viral load, respectively. Although the percentage of the total variation of the CD4 count and CD4 percentage explained by log viral load is relatively low: 3.59 and 10.31% for CD4 count and CD4 percentage, respectively, the p–values were significant (0.0045 and <0.0001, respectively). If a normal distribution for the markers is not assumed, these results are not altered, as

shown by Spearman Correlation in (Table 6). Moreover, as shown also in Figures 1a and 1b, the predicted mean of log viral load decreased over time and was significantly different at nine months compared to the baseline value (p = 0.012). Baseline mean of log viral load was 10.02 (50060.39 copies/ml) and the mean of log viral load at nine month was 8.65 (13634.69 copies/ml).

DISCUSSION

Although previous researchers have shown the promise of LDN in the treatment of HIV infection (Bihari et al., 1988; Mathews et al., 1983; Puente et al., 1992), to the best of the authors' knowledge, the present study is the first quantitative, statistically designed and analyzed clinical evaluation of the effects of LDN on a single cohort of HIV positive adults. A similar untreated cohort has been studied, however, (Duvignac et al., 2008) and, since our single cohort study had no control group for the reasons already described, it is important to note that the "natural loss" of CD4 count for untreated HIV positive

Table 6. Correlations over time between interferon alpha, viral load, CD4 and CD4%, LDN Single Cohort Study with HIV+ adults, Mali.

Variable	With variable	N	Pearson partial correlation		Spearman partial correlation	
			Correlation	P Value	Correlation	P Value
CD4 Count	CD4 percentage	223	0.4316	<.0001	0.40086	<.0001
CD4 Count	Interferon alpha	223	-0.01605	0.8122	-0.0282	0.6764
CD4 Count	Log viral load	223	-0.18947	0.0045	-0.21028	0.0016
CD4 percentage	Interferon alpha	223	-0.07208	0.2853	-0.0328	0.6273
CD4 percentage	Log viral load	223	-0.32107	<.0001	-0.31811	<.0001
Interferon alpha	Log viral load	223	0.08585	0.2028	0.10196	0.13

The Correlation squared is the coefficient of determination or the percentage of variation explained by one variable relative to the other variable. For example, CD4 count explains 18.63 % of the total variation of CD4 percentage after controlling for time effect.

patients in this recent Abidjan study showed a significant drop in CD4 count after 12 months of 69 cells/mm^3 and a significant loss of CD4 percentage of 1.7% for patients with mean BMI 20.4 kg/m^2 at baseline. In our cohort, the BMI was positively but not significantly associated with CD4 count, as was the case in the Abidjan Study. In contrast and, despite the slight (but not significant) drop of BMI over time, the CD4 percentage was stabilized in our cohort (Figure 1), unlike the untreated subjects in the Abidjan Study. This is encouraging, since (as mentioned above) CD4 percentage is seen to reflect the evolution of infection in cohort studies more accurately than CD4 count (Duvignac et al., 2008; Pirzada et al., 2006; Hason et al., 1995). Thus our results both affirm the use of CD4 percentage as a marker for therapeutic decisions (along with the absolute CD4 count) and, if confirmed, suggest that LDN might have a positive effect in delaying the onset of AIDS symptoms for Sub-Saharan populations similar to that used in our and the Abidjan studies. Our results for interferon alpha suggest that this marker is far less useful in describing the evolution of HIV infection and in predicting CD4 and CD4% levels for HIV+ adults— in agreement with recent literature (Puente et al., 1992). Although viral load was significantly correlated with CD4 and CD4% values and did decrease significantly by the ninth month from baseline, it accounted for only a small percentage of the total variation in the latter two markers and is, therefore, of relatively less significance in describing long-term immune system behavior.

Additional information about this study, including further background material on the therapeutic use of LDN can be found on the two US authors' web site: www.ldnafricaaids.org.

The strengths of the present study include: a) It is the first of its kind, and contributes multiple immunological, virological and clinical testing of the effects of LDN on the course of HIV infection for patients in Sub-Saharan Africa; b) It is an "incident" study with all new data, rather than a prevalent one, which makes it more informative and unbiased in describing the course of HIV

infection; and c) We used recent developments in statistical methods in order to maximize the information drawn from our cohort sample, and minimize the bias from missing information and patient drop-out.

However, this study had limitations: a) The necessary foregoing of a formal control group, nevertheless means that other unmeasured variables might have affected the predicted results besides LDN; b) Since the sample was based on a specific narrow CD4 count range, our results might not be replicable for patients at others stages of HIV infection; c) The study involved testing patients for only a nine month period and so the longer term effects of LDN were not evaluated.

Conclusions

The extensive medical evaluations and clinical testing (standard metabolic chemistry tests including liver enzymes) and other parameters (hemoglobin, creatinine) conducted during this cohort study support the generally accepted characterization of LDN as "a safe medication with no side effects" (Smith et al., 2007: Roy and Loh, 1996; McCarthy et al., 2001; Bihari et al., 1988). Had the dosage of 4.5 mg of LDN--now widely recommended in treating adults for a wide variety of autoimmune illness-- been used instead of the smaller dosage, the results observed might have been even more encouraging. In any event it is clear that further study of the efficacy of LDN in strengthening the immune systems of HIV+ individuals is clearly warranted. This seems particularly important for the treatment of infants and young children for whom ART treatment is challenging, even when it is available. LDN might offer a simple, relatively safe, inexpensive and easily monitored treatment alternative.

Abbreviation: LDN, low dose naltrexone.

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