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Research Article

Anology of CD4 Cells, CD8 Cells and CD4/CD8 Ratio in Human Immunodeficiency Virus-1 (HIV-1) Infected Individuals Treated with Phytochemical Therapy

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ABSTRACT

Due to the development of highly active antiretroviral therapy in 1996, the life expectancy of HIV infected people in all over the world improved dramatically. However, it is undesirable unless the virus is eliminated completely from the body of infected people. Since the ART compounds interact with virus-specific enzymes, the emergence of drug-resistant viruses may not be avoidable during long-term drug treatment. The ideal antiviral drug regimen would be one that induces strong and persistent suppression of virus replication, gives prolonged immunologic and clinical benefits without toxicity can be administered at infrequent dosage intervals, is affordable and easy to store. Thus, more effective and less toxic anti-HIV agents are still needed. An alternative approaches particularly anti-infective or immunomodulating medicinal herbs have been attempted. A thirty Asymptomatic HIV-1 infected patients were selected based on certain criteria and a prepared phytochemical drug therapy was given as in capsule form for a three year period to assess the efficacy over the CD4, CD8 cells and CD4/CD8 ratio of the patients. The CD4, CD8 cells and CD4/CD8 ratio were monitored before, during and after the treatment. The CD4, CD8 cells and CD4/CD8 ratio were incresed during and at the end of the treatment. The study suggests that the combination of herbs used in this study is effective against CD4, CD8 cells and CD4/CD8 ratio.

Keywords: HIV, Herbal Therapy, CD4 Cells, CD8 Cells.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) and HIV-2 are closely related lentiviruses with different biological and epidemiological characteristics. Like HIV-1 infection, HIV-2 infection leads to immune suppression and AIDS, but with slower $CD4^+$ T-cell decline, lower plasma viral load levels, and hence slower progression of the disease[1-3]. In addition, HIV-2 shows lower transmission rates than HIV-1[4, 5]. While HIV-1 has spread worldwide, HIV-2 has remained mainly confined to West Africa, with most countries now reporting a decrease in HIV-2 prevalence[6]. HIV-1- and HIV-2-infected patients matched for plasma viral load levels showed equal rates of $CD4^+$ T-cell decline[7]. At the time of AIDS diagnosis, the mortality rate was found to be more influenced by the $CD4^+$ T-cell count than by the HIV type[8]. HIV-1 and HIV-2 also show comparable levels of cytopathicity in vitro[9].

Progressive depletion in numbers of circulating CD4+ T cells occurs in almost all cases of untreated HIV infection. The number of circulating CD4+ T cells is widely used as a measure of global "immune competence" and provides a predictor of the immediate risk for opportunistic illnesses[10]. Earlier in the course of infection, many HIV-infected persons have a syndrome of generalized lymphadenopathy characterized by accumulation of lymphocytes within inflammatory lymph nodes and upregulation of adhesion molecule expression. Early in the course of infection, memory CD4+ T cells are selectively depleted from circulation; as disease advances, CD4+ T cells of both the naive and memory phenotype are lost from circulation[11]. In advanced disease, all CD4

cell populations are depleted from circulation and from lymphoid tissue sites. Functional abnormalities of CD4+ T cells are also characteristic of progressive HIV infection. Failure of CD4+ lymphocytes to undergo cell division, for example, has been demonstrated following stimulation of T cells from infected individuals with antigens or mitogens in vitro. A sequential loss of immune responsiveness to recall antigens, followed by alloantigens and then mitogens was also described[12].

In early HIV infection, CD8+ T-cell numbers tend to increase, reflecting expansion of memory CD8+ T cells, particularly HIV-reactive cells. CD8 cell expansions persist until far advanced stages of HIV disease, when all T-cell numbers tend to fall[13]. In contrast to memory CD8 cell expansions, proportions of naive CD8 cells tend to fall in early infection but absolute numbers of these cells do not fall until HIV disease progresses. For example, in earlier disease CD8+ T cells that recognize cytomegalovirus are present in large numbers but in advanced disease the cytolytic function of CD8+ T cells directed against opportunistic pathogens is demonstrably impaired[14]. It is not entirely clear whether the CD8+ cells present in early disease are functionally "normal," as the maturation phenotype of CD8+ T cells recognizing pathogen-derived peptides was found to be variably perturbed[15]. Whether this is the cause or consequence (or the interaction of both) of greater exposure to opportunistic pathogen-derived antigens in HIV-infected immunosuppressed persons is difficult to sort out.

AIDS remains an enormous health threat, although chemotherapeutic agents have increased in number and effectiveness. Both nucleoside (AZT, DDI, DDC, D4T, 3TC) and nonnucleoside (nevirapine, delavirdine) HIV reverse transcriptase (RT) inhibitors and HIV protease (saquinavir, indinavir, ritonavir, nelfinavir) inhibitors have been licensed by the US FDA. Also, combination therapy of inhibitors of both groups results in undetectable levels of HIV in the blood of infected patients. However, despite this success, toxicity and, especially, drug resistance still present severe problems.

The US Food and Drug Administration (FDA) has approved a number of anti-HIV drugs for clinical use. However, these medications have limitations such as high cost, decreased sensitivity due to the rapid emergence of drug-resistant mutants, and adverse effects like peripheral neuropathy, bone marrow suppression, and anemia[16]. Thus, more effective and less toxic anti-HIV agents are still needed. In addition, alternative approaches, including herbal therapies after long-term screening of plant extracts, particularly anti-infective or immunomodulating medicinal herbs and the structural modification of lead compounds, have been attempted[17]. Recently, the author has reported that the medicinal plants treatment to a single asymptomatic female individual has performed well up to the elimination of proviral DNA, as the outcome of that the present study was undertaken[18].

The CD4, CD8 and CD4/CD8 ratio were taken as the parameters to assess the efficacy of the phytochemical therapy in this present study.

MATERIALS AND METHODS

Patient selection

For the present study, altogether 14 patients on their own total willingness were selected from India and 16 patients from Madagascar. Upon invitation, treatment of asymptomatic AIDS patients was taken up at Madagascar. The study was carried out by following the ethical guidelines as per Helsinki declaration. Informed consent were obtained from all patients. The initial diagnosis of AIDS was done by the own Medical Physicians either accidentally or voluntarily by the patients. They were subjected to confirmatory test before they were subjected for this study. All the patients investigated were asymptomatic having CD4-T Cells more than 500 per cubic millimeter and the viral load was less than one lakh.

Herbal Treatment

The asymptomatic AIDS patients thus selected were subjected to herbal therapy as outlined below. Six herbs were selected, of which five possessed antiviral molecules and the last one (No.6) contained immunostimulant property.

1.*Curcuma longa* - antiviral property[19,20]
2.*Ocimum sanctum* - IFN-γ inducing property
3.*Phylanthus emblica* - IFN-γ inducing property
4.*Phylanthus niruri* - IFN-γ inducing property
5.*Withania Somnifera* has IFN-γ inducing property
6. *Piper longum* - IFN-γ inducing property[21]

Preparation of herbal regimen

The rhizome of *Curcuma longa* and *Withania somnifera* , the fruits of *Phylanthus emblica* and *Piper longum*, the leaves of *Phylanthus niruri* and *Ocimum sanctum* were collected, shade dried and powdered and one gram from each of the above sample was mixed together and filled into capsules (1g/capsule). The capsules were administered six at a time before food and likewise three times a day in asymptomatic HIV patients. Before administering the drug, the patient's blood was taken and subjected to various clinical parameters.

CD4 Enumeration

CD4 Cells were isolated from 10ml of patients' EDTA whole blood samples by an immunomagnetic

method using anti-CD4 coated beads (Dynabeads M450 CD4, Dynal AS, Oslo, Norway) following the protocol (Benoit kadamba et al., 2005) as outlined in Becton Dickinson Fascan Flowcytometer technology.

CD8 Enumeration

CD8 Cells were isolated from 10ml of patient EDTA whole blood samples by an immunomagnetic method using anti-CD8 coated beads (Dynabeads , Dynal AS, Oslo, Norway) following the protocol as outlined in Becton Dickinson Fascan Flowcytometer technology.

CD4/CD8 Ratio

The ratio between CD4 and CD8 cells was calculated and recorded in all the patients.

Statistical analysis

The one way ANOVA was performed using SPSS 17.0 Software.

RESULTS

Phytochemical therapy

Phytochemical therapy was employed on patients throughout the treatment, as this kind of therapy is yet to take strong routes in developing countries. The following herbs were employed in the treatment.

Plant name	Parts used	Antiviral compounds	BioActivity
1.Phyllanthus niruri	Leaves	Limonene	antiviral
2.Withania somnifera	Roots	Beta-sitosterol	antiviral
3.Ocimum sanctum	Leaves	Eugenol	antiviral
4.Curcuma longa	Rhizome	Curcumin	antiviral
5.Phyllanthus emblica	Fruits	Ascorbic acid	antiviral
6.Piper longum	Fruits	-	Immunostimulant and P-glycoprotein inhibitor

The following are the justifications for selection of the aforesaid medicinal herbs.

1. Antiviral
2. Immunostimulant

(The selection of medicinal herbs is based on Duke's phytochemical database).The selection of herbal plants as phytochemical therapy is also based on Siddha and Ayurvedic literature, namely Kunappadam and Saraha samgithai. The medicinal herbs were employed either in single or in combination with other herbs. When the herbs were employed independently they hardly produced any effect on the patients. The data on the single treatment of herbs on the patients are not presented, as they did not produce any alleviatory effect.

Mode of treatment

All the parts of the medicinal herbs, as specified in the aforesaid table were filled in capsules (1gram per capsule) and administered on the patients six in number prior to each meal totalling 18 capsules per day. The alternative form of employing alcoholic extract and further purification was not acceptable and palatable to the patients and therefore, the present method of capsular administration was followed. The patients were thoroughly examined clinically before and after administration of herbal drugs (Phytochemical therapy) for a period over 36 months. As the parameter CD4 and CD8 cells were monitored before and after treatment in both Indian and Madagascar patients. As mentioned in Tables 1,2 and 3,4 upon treatment there was a progressive increase in CD4 cell population up to the period of treatment (36 months). On the contrary, CD8 cells did not show any appreciable increase/ decrease during the period of treatment (Tables 3 and 4).In CD4/CD8 cell ratio was also determined before and after the treatment (Tables 5 and 6). In both India and Madagascar, the patients recorded higher ratio of CD4/CD8 cells indicating progressive development of CD4 cells.

In statistical analysis, the one way ANOVA reveals the F value for the result is 8.976 ($P<0.0001$), this is statistically highly significant.

DISCUSSION

In the present investigation, patients were selected on their own willingness for treatment from both India and Madagascar. Because of the popularity of herbal treatment in India, part of the present programme was AIDS remains as an enormous health threat in spite of different kinds of treatments. The present work relates to selected a few asymptomatic AIDS patients from India (Tamilnadu and Karnadaka states) and Madagascar. The NAVAL AIDS RESEARCH CENTER, an Unit of INDIAN SOCIAL SERVICES located at Namakkal, Tamilnadu, India was solely involved in the treatment of patients in India. In view of Madagascar being an underdeveloped Island and the possibility of exploring the same kind of treatment as it was being followed at Namakkal, another transient center was planned at Madagascar.

Mode of selection of patients

The following parameters were employed in the identification of asymptomatic AIDS patients.

1. The patients without any external visual symptoms
2. The CD4 cell count was not lower than 500 cells per cubic milliliter of serum

also carried out in Madagascar upon invitation by a charitable nongovernmental organization (Ste'Oriental Madagascar, Antananarivo). Two major types of HIV have been identified so far i.e. HIV-1 and HIV-2. HIV-1 is the cause for worldwide epidemic. There are many different strains of HIV-1, which can be classified into groups and subtypes. HIV-2 is much less pathogenic and occurs rarely (West Africa)

The particular phase of the study, the effects of the herbal therapy on immune cells such as CD4 and CD8 and their ratio were studied. It is well known CD4 cell acts as the receptor for HIV adhesion. Therefore, the level of CD4 was monitored. As given in Tables 1 and 2, the CD4 cell count progressively increased during the course of the treatment by two-fold, compared to the cell count prior to treatment. The CD4 cells have proven importance in restricting viral replication (22, 23) in humans and SIV- infected macaques (24). AIDS, which, results from immunosuppression, is characterized by steady decline in CD4 cell count (25).An analogy can be drawn from the present observation that anti retroviral therapy employed on 30 patients proved that CD4 cells increased and the number remained the same even after two years of treatment (26, 27). These results and that of the present observation point out to the fact that there is possibly an immunological recovery. It is also well known that CD8 cells play a crucial role in the control of Immunodeficiency virus (HIV) and Simian Immunodeficiency Virus (SIV) infections. The CD8 cell number remained almost the same throughout the period of study. Therefore, it is presumed that CD8 cells carried out such functions normally as assigned to them i. e .ability to produce different cytokines and cytotoxic cells and as well as induction of apoptosis (25). Possibly there was no impairment in the functions of CD4 and CD8 cells under the influence of herbal therapy (Tables 1,2,3, and 4). HIV is known to down-regulate virus specific CD4 and CD8 T cells that are not restored even with Highly Active Anti Retroviral Therapy (HAART). Restoration of CD4 cells and steady maintenance of CD8 cells indicate the possible success of the phytochemical therapy in the progression of health of asymptomatic AIDS patients. At this juncture, it may be confided that patients with no herbal therapy could be treated as control. As human subjects were involved, their cooperation in this regard was essential. Coercion could not be exercised. In continuation, the ratio of CD4/CD8 was also monitored in the patients and the results are presented in Tables 5 and 6. It was observed that in view of steady progression in CD4 cell count, CD4/CD8 cell count ratio increased by 1.5 to 2-fold over the treatment period. The increased ratio might indicate progression of the HIV patients towards restoration of HIV- induced impairment of health. In general HIV infection is characterized by the decreased CD4/CD8 cell ratio. The CD4/CD8 ratio may also help to identify people who are likely to become progressors/non progressors/slow progressors of the disease (13, 28).

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Table 1: Cd4 cell counts before and after treatment with medicinal herbs over a period of 36 months in asymptomatic aids (HIV-1) patients in india.
(cells/µl of blood). The data are the mean of three different assays.

PATIENT IDENTIFICATION NUMBER (PIN)	BEFORE TREATMENT	AFTER TREATMENT (MONTHS)				
		6TH MONTH	12TH MONTH	18TH MONTH	24TH MONTH	36TH MONTH
1	450	570	610	760	850	1010
2	510	590	640	800	840	1190
3	500	570	670	870	910	1250
4	540	610	690	750	830	1190
5	505	600	680	750	850	1000
6	475	570	650	700	790	1310
7	540	600	650	770	860	1100
8	525	670	710	800	910	1080
9	505	780	850	910	1100	1250
10	540	690	780	870	910	1290
11	500	610	770	870	890	1150
12	560	790	850	910	940	1100
13	500	690	760	870	960	1090
14	525	600	710	790	850	1290

Table 2: Cd4 cell counts before and after treatment with medicinal herbs over a period of 36 months in asymptomatic aids (HIV-1) patients in madagascar. (cells/µl of blood). the data are the mean of three different assays

PATIENT IDENTIFICATION NUMBER (PIN)	BEFORE TREATMENT	AFTER TREATMENT (MONTHS)				
		6^{TH} MONTH	12^{TH} MONTH	18^{TH} MONTH	24^{TH} MONTH	36^{TH} MONTH
1	540	670	780	870	980	1090
2	510	590	640	800	840	1190
3	500	570	670	870	910	1250
4	540	610	690	750	830	1190
5	505	600	680	750	850	1000
6	475	570	650	700	790	1310
7	540	600	650	770	860	1100
8	525	670	710	800	910	1080
9	510	750	870	890	910	1050
10	500	650	690	710	750	1090
11	505	590	640	690	760	1070
12	550	600	670	750	870	1070
13	545	670	690	750	850	1000
14	500	650	700	750	800	1050
15	540	640	740	840	940	1040
16	530	640	730	820	910	1000

Table 3: Cd8 cell counts before and after treatment with medicinal herbs over a period of 36 months in asymptomatic aids (HIV-1) patients in india.(cells/..µl of blood). the data are the mean of three different assays.

PATIENT IDENTIFICATION NUMBER (PIN)	BEFORE TREATMENT	AFTER TREATMENT (MONTHS)				
		6^{TH} MONTH	12^{TH} MONTH	18^{TH} MONTH	24^{TH} MONTH	36^{TH} MONTH
1	990	970	990	980	1000	1010
2	1100	1100	1080	950	1000	1050
3	950	940	950	930	960	980
4	1000	1100	1100	950	1000	1090
5	980	990	990	980	999	1000
6	1000	1010	1000	1020	1000	1100
7	990	980	950	800	800	800
8	750	760	760	770	750	750
9	780	790	750	750	760	750
10	780	800	770	770	760	750
11	1000	900	900	950	910	910
12	750	760	770	760	780	760
13	760	750	780	760	760	760
14	800	800	810	800	820	800

Table 4: Cd8 cell counts before and after treatment with medicinal herbs over a period of 36 months in asymptomatic aids (HIV-1) patients in madagascar..(cells/µl of blood). The data are the mean of three different assays.

PATIENT IDENTIFICATION NUMBER (PIN)	BEFORE TREATMENT	AFTER TREATMENT (MONTHS)				
		6^{TH} MONTH	12^{TH} MONTH	18^{TH} MONTH	24^{TH} MONTH	36^{TH} MONTH
1	760	770	800	790	750	760
2	750	770	780	770	760	760
3	800	810	750	750	760	770
4	760	770	760	770	760	760
5	759	767	768	768	787	789
6	800	810	820	810	815	815
7	769	750	760	750	760	760
8	770	760	780	760	780	780
9	760	760	800	810	790	780
10	770	760	800	780	750	750

11	740	770	750	760	770	770
12	750	760	770	760	770	760
13	760	800	860	850	860	850
14	750	770	770	780	770	770

Table 5: Cd4/cd8 cell ratio before and after treatment with medicinal herbs over a period of 36 months in asymptomatic aids (HIV-1) patients in india.(cells/µl of blood). The data are the mean of three different assays.

PATIENT IDENTIFICATION NUMBER (PIN)	BEFORE TREATMENT	AFTER TREATMENT (MONTHS)				
		6^{TH} MONTH	12^{TH} MONTH	18^{TH} MONTH	24^{TH} MONTH	36^{TH} MONTH
1	0.45	0.58	0.61	0.77	0.85	1.00
2	0.46	0.53	0.59	0.84	0.84	1.13
3	0.52	0.60	0.70	0.93	0.94	1.27
4	0.54	0.55	0.62	0.78	0.83	1.09
5	0.51	0.60	0.68	0.76	0.85	1.00
6	0.47	0.56	0.65	0.68	0.79	1.19
7	0.54	0.61	0.68	0.96	1.07	1.37
8	0.70	0.88	0.93	1.03	1.21	1.72
9	0.64	0.98	1.13	1.21	1.44	1.66
10	0.69	0.86	1.01	1.12	1.19	1.72
11	0.50	0.67	0.85	0.91	0.97	1.26
12	0.74	1.03	1.10	1.19	1.20	1.44
13	0.65	0.92	0.97	1.14	1.26	1.43
14	0.65	0.75	0.81	0.98	1.03	1.61

Table 6: Cd4/cd8 cell ratio before and after treatment with medicinal herbs over a period of 36 months in asymptomatic aids (HIV-1) patients in madagascar. (cells/µl of blood). The data are the mean of three different assays.

PATIENT IDENTIFICATION NUMBER (PIN)	BEFORE TREATMENT	AFTER TREATMENT (MONTHS)				
		6^{TH} MONTH	12^{TH} MONTH	18^{TH} MONTH	24^{TH} MONTH	36^{TH} MONTH
1	0.71	0.87	0.97	1.10	1.30	1.43
2	0.73	0.88	1.01	1.14	1.27	1.44
3	0.62	0.82	1.00	1.16	1.19	1.43
4	0.65	0.84	0.98	1.12	1.18	1.43
5	0.69	0.87	0.97	1.01	1.10	1.26
6	0.62	0.82	0.90	0.96	1.04	1.46
7	0.72	0.93	1.06	1.29	1.57	1.71
8	0.70	0.97	1.08	1.18	1.19	1.39
9	0.67	0.98	1.08	1.09	1.15	1.34
10	0;64	0.85	0.86	0.91	1.00	1.45
11	0.68	0.76	0.85	0.90	0.98	1.38
12	0.73	0.78	0.87	0.98	1.12	1.40
13	0.71	0.83	0.86	0.88	0.98	1.17
14	0.66	0.84	0.90	0.96	1.03	1.36
15	0.71	0.84	0.96	1.07	1.22	1.33
16	0.66	0.81	0.93	1.05	1.15	1.27

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