Monitoring of the CD4+ T cell subsets among Sudanese HIV sero-positive individuals

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Abstract:

Purpose: HIV\AIDS is developing major health problem in Sudan. This study investigate the CD4+ level among Sudanese HIV sero-positive subjects with and without AIDS symptoms in comparison to the HIV sero-negative control to establish base line data about CD4+ count in HIV infection in Sudan.

Methods: Eighty six individuals were selected for this study, their age ranged between (13 –53) years, (60) subjects were males and 26 were females. The HIV seropositive were 60, of whom 54 were AIDS patients. The presence of HIV specific antibodies was detected and confirmed by ELISA and immunoblotting techniques according to WHO criteria. CD4 and CD8 were counting by Dynabeads T4- T8 Quantitative method.

Results: $CD4^+$ and $CD8^+$ levels were counted together Differential of TWBCs. Significant lower counts of CD4+ were detected among AIDS/HIV patients compared with AIDS/HIV sero-negative individuals. Increased of CD4+ counts were observed among treated AIDS/HIV patients. No significant differences detected in either $CD8^+$ count or the total WBCs in HIV seropositive and seronegative subjects.

Conclusion: The counting of CD4+ T lymphocytes is important

in the staging of AIDS/HIV disease for various purposes such as deciding when to initiate prophylactic or therapeutic intervention, and in monitoring the response following antiretroviral treatment.

مستخلص:

الهدف يتسبب فيروس نقص المناعه البشريه بمشكله صحيه كبيره في السودان. في هذه الدراسه نبحث مستوي الخلايا التائيه (CD4+) بين المصابين بفيروس نقص المناعه البشريه مع ظهور اعراض ومع المصابين بغير اعراض مقارنه باشخاص اصحاء حتي نتمكن من وضع قاعده بيانات لمعرفه عدد الخلايا التائيه (CD4+) بين مرضي فيروس نقص المناعه البشريه. الطريقه تم اختيار ست وسبعون شخصا لهذه الدراسه تراوحت اعمارهم بين ثلاثة عشره سنه وثلاث وخمسون سنه. بينهم ستون من الذكور وست وعشرون من الاناث. بلغ عددالمصابين بفيروس نقص المناعه البشريه ستون شخصا منهم اربعه وخمسون شخصا مصابين مع ظهور اعراض نقص المناعه المكتسبه. تم الكشف عن وجود الاجسام المضاده لفيروس نقص المناعه البشريه بواسطة تقنيه تفاعلات الانزيم المناعي الملتصق وتم تاكيد الفحص بواسطة تقنيات التجلط المناعي وفقا لمعايير منظمة الصحه العالميه. خلايا الدم التائيه (CD4+) (CD8) تم تعدادها بواسطة طريقة الحبيبات الممغنطه (Dynabeads T4- T8 Quantitative method).النتائج تم حساب الخلايا التائيه (CD4+) (CD8) مع كريات الدم البيضاء . وجد ان هناك انخفاض ملحوظ لخلايا الدم التائيه (CD4+) بين مصابي فيروس نقص المناعه البشريه مقارنة بغير المصابين. كما لوحظ انه ليس هناك اي اختلاف بين المصابين وغير المصابين في خلايا الدم البيضاء والخلايا التائيه نوع (CD8) . الخلاصه يعد حساب الخلايا التائيه (CD4+) (CD8) مهم لتحديد مراحل مرض فيروس نقص المناعه المكتسبه وذلك لتحديد وقت التدخل العلاجي والوقائي كما انه مهم فى مراقبه الاستجابه للعلاج بواسطة المضادات الفيروسيه .

Introduction:

Acquired immunodeficiency syndrome (AIDS):

AIDS is a group of clinical syndrome caused by Human Immunodeficiency Virus (HIV), characterized by profound immune suppression with diverse clinical features, including opportunistic infection, malignancies and central nervous system infections (Osmond *et al.,*1991)

In Sudan the first identified HIV patient was a hemophiliac boy in November 1987(March, 1987-1989). Screening of 52,000 healthy male volunteer blood donors showed an HIV-seroprevalence rate of 0.05%. HIV seroprevalence of 1.2% was detected among 1118 children admitted to Khartoum Teaching Hospital

during the period 1985-1995. (Hashim *et al*. 1997). In 1988 the first two HIV-positive individuals from southern Sudan were reported, including one with clinical AIDS. (Woodruff *et al,* 1988). From 1987 - 2002 the total number of HIV positive were 8669, 4428 were asymptomatic and the rest were AIDS cases. While 652 AIDS were reported in 2000, and 678 in the year 2001.The male to female ratio of seropositive was 2:1. The age distribution of AIDS commonly ranged from 20 up to 39 years and included the high sexual active age. (SNAP, 2002).

CD4+ cells:

The decline in CD4+ in HIV infection cells reflects the progression of HIV infection. CD4+ counts were used for monitoring of HIV infection to indicate the progress of HIV\AIDS symptoms. However, CD4+ count alone does not always reflect the clinical status of HIV-infected individuals; untreated individuals with similar CD4+ counts may have very different functional status, frequency of opportunistic infections, and constitutional symptoms and signs.

Treatment with antiretroviral therapy often results in increase CD4+ counts to levels that probably confer normal immune protection against opportunistic infection. Counting of CD4+ T lymphocytes is important in the staging of HIV disease for various purposes such as deciding when to initiate prophylactic or therapeutic intervention (CDC. 1992a; National institute of Health. 1990). CD4+ counts it is also important for defining the risk for mother-to-infant transmission of HIV (Newell and Peckham. 1993) and in monitoring the response following antiretroviral treatment (Stein *et al*., 1992). It helps to determine how advanced HIV disease (staging) and to predict the risk of complications (prognosis). Medical conditions, such as oral thrush, Pneumocystis carinii pneumonia (PCP), and Mycobacterium avium complex (MAC) disease, occur at particular stages of HIV disease (Turner *et al.,* 1994; Fei *et al.,* 1993).

Objective:

The aim of this study to monitor the CD4+ level among Sudanese HIV sero-positive subjects with and without AIDS symptoms in comparison with the HIV sero-negative control and to establish base line data about CD4+ count among HIV infected subjects.

Materials and Methods:

This is a cross sectional study was done to determine the CD4+ and CD8+ T cell subsets counts. This study was done in the Department of virology, National Health laboratory, Khartoum, Sudan and Institute of Endemic Diseases, University of Khartoum.

Two groups were included in this study, the first group included AIDS/HIV patients in Khartoum teaching Hospital. While the second group included subjects who attended at Department of virology as voluntary for AIDS/HIV test.

The study was ethically approved by the institute of Endemic Diseases ethically committee and Federal Ministry of Health. The participants of this study were selected after filling formal consent; male and female with deferent ages were included. The study group included (76) subjects, (60) were HIV seropositive and (16) were HIV seronegative controls. The (60) HIV seropositive were clinically examined and classified into symptomatic and asymptomatic according to CDC AIDS classification.

Data of age, gender, history of illness, socioeconomic status were collected via questionnaire designed for this study and base line data was collected by completion of this questionnaire.

Sample collection:

Five ml of venous blood was collected from each consented individual in ethylene diamine tetra acetic (EDTA) vacutainer tubes (BD vacutainer) and submitted to the laboratory. The obtained blood samples were tested for the presence of HIV specific antibodies using ELISA and immunoblotting techniques.

Enzyme linked Immunosorbent Assay (ELISA):

All individuals were tested for the presence of HIV specific antibodies in plasma. Plasma was separated by centrifugation of

the EDTA blood at (1000) r.p.m for 5 min. Two different ELISA techniques Human ELISA test, Dong-A AIDSDIA ½ test and immunochromatographic as simple test were used for the diagnosis and confirmation of HIV infection.

Human ELISA was used for screening of HIV antibodies against HIV-1 and HIV-2 and subtype, on microtiter strips wells. The microtiter strips wells were coated with synthetic peptide (Pept), and recombinant antigen (rAg), gp 41, gp 36, and the p 24 gag.

The positive and equivocal samples in screening test (Human ELISA) were confirmed by the Dong-A AIDSDIA 1/2 as a second ELISA. The immunochromatographic technique was used as confirmatory tests for positive and equivocal samples.

Dynabeads T4- T8 Quantitative method:
(Dynal A.S OSLo Norway):

4.5 ml of whole blood collected in EDTA tubes were rotated-for 2 minutes at room temperature within one hour of collection.

225µl-washing/phosphate buffer saline (PBS) solution and 2 X 125µl blood. (Total 250µl) were added to dynal test tubes.

Monocytes depletion:

25µl of magnetic beads coated with anti CD14 antibodies (Dynabeads ® M-450 CD14) diluted ½ in (PBS) were added to the test tubes, mixed and incubated with a tilt-and rotatedfor 10 minutes at room temperature on Dynal rotator (Dynal Sample Mixer MXI) (tilt-and-rotate action). Magnetic particle concentrator (Dynal MPC ®-M) separated the Monocytes within 2 minutes. 200µl monocyte-depleted blood was transferred to each of 2 test dynal tubes.

CD4+ - CD8+ Lymphocyte isolation:

200µl washing/ PBS solution was added to monocyte depleted blood tubes.

25µl of beads coated with anti CD4 (Dynabeads ® M450 CD4) monoclonal antibodies were added to dynal tube. 25µl of beads coated with anti CD8 (Dynabeads ® M450 CD8)monoclonal

antibodies were added to the second tube. The tubes were mixed carefully, and incubated with a tilt-and rotated for 10 minutes at room temperature on a dynal mechanical rotator. The beads were separated by the magnetic MPCQ placed for two minutes. 500 µl of PBS was used to wash the isolated cells 2Xtimes 2 minutes each.

CD4+ and CD8+counting:

The isolated CD4+ and CD8+ T lymphocyte were re-suspended in 50µl lysis solution for 5 minutes at room temperature. The nucleius of CD4+ and CD8+ were stained by Gentian violet and counted by light microscope using haemocytometer. The results were expressed as cells per microliter whole blood.

Results:

A total of (76) subjects were included in this study, 55 (72.36%) of them were male, and 21 (27.64%) were female (Table 1). The age of the studied subjects ranged between 13 and 53 years, more than 70%, were of age between 20 and 40 years (Table 2). There were 26 (34 %), married patients and 50 (66 %)were single. Spouses, solders and, drivers were 19.7% , 14.5%, 5.3% respectively (Table 3).

(Table 1): Distribution of gender of HIV positive and negative subjects

	HIV +ve	AIDS	HIV -ve	Total
Male	5	35	10	50
Female	1	19	6	26
	6	54	16	76

Note: No significant differences were detected between males and females.

(Table 2): The age groups of studied individuals

Age group	HIV positive	HIV negative	AIDS
20 – 10	0	0	3
30 – 21	9	1	15
40 – 31	6	4	18
50– 41	1	1	15
60 – 51	0	0	3

(Table 3): Occupational of included subjects

Occupational	Frequency
Spouses	(% 19.7) 15
Solders	(% 14.5)11
Drivers	(% 5.3)4
Other	(% 60.5)46

(Table 4): AIDS among HIV positive individuals

HIV serology negative	HIV serology positive and AIDS	
(% 21.1) 16	(% 78.9) 60	
	AIDS positive	AIDS negative
	(% 10) 6	(% 90)54

60 (78.9%) of studied individuals were positive for HIV, of whom 90% were AIDS patients.

(Table 6): Mode of transmission among HIV serology positive cases

Sexual contact	Infected parents	Blood transfusion	HIV positive
(% 90.7) 49	(% 1.9) 1	(% 7.4) 4	With symptoms
6	0	0	Without symptoms

Sexual contact was the significant mode of transmission

(Table 7): The marital status of HIV positive individuals

	Married	Single
HIV positive	(% 28.3) 17	(% 71.7) 43
HIV negative	(% 56.3) 9	(% 43.8) 7

Significant difference in marital status was noticed.

(Table 8): Common clinical symptoms of AIDS patients

Symptoms	Frequency	
	symptoms	No symptoms
Weight loss	51	3
Diarrhea	44	10
Fever	32	22
Cough	24	30
Dermatitis	15	39
Candidasis	38	16
LAD	16	38
KS	4	50

Among AIDS patients no significant values of different symptoms although 51 of 54 were presented with weight loss.

(Table 9): Total white blood cells count of the studied individuals

	3x10 ^{9}\L>	to 7)x10 ^{9}\L 3)	7x10^{9}\L<
HIV positive	19	38	3
HIV negative	2	12	2

No significant difference were detected

(Table 10): CD4 counts of studied individuals

CD4 cells\µl	Frequency
Less than 100	21
100-199	15
200-299	9
300-399	8
400-499	2
More than 500	21

AIDS patints had significant less CD4+ counts

(Table 11): CD4 counts among different age groups.

CD4 cells\µl	Age groups					
	10-20	21-30	31-40	41-50	51-60	Total
Less than 100	1	6	6	6	2	21
100-199	2	3	7	2	1	15
200-299	0	4	2	3	0	9
300-399	0	2	3	3	0	8
400-499	0	0	0	2	0	2
More than 500	0	10	10	1	0	21

No significant difference among different age group

HIV serology negative individuals have significantly higher CD4+ count than HIV serology positive

No significant reduction in CD4 counts among HIV serology positive patients without symptoms.

(Table 12): CD4 counts in AIDS patients with different clinical symptoms

CD4 cells\µl	W.L	Diarrhea	Fever	Cough	Der-matitis	Candi-dasis	KS
Less than 100	21	19	14	12	10	21	1
100-199	15	14	8	5	5	11	2
200-299	9	8	7	4	0	4	0
300-399	5	3	3	3	0	2	1
400-499	1	0	0	0	0	0	0
More than 500	0	0	0	0	0	0	0

There is significant frequency association between CD4 count less than 400cells\µl and diarrhea, fever, cough, candidiasis and Kaposi Sarcoma.

Significant CD4 count less than 200 cells\µl with dermatitis (15 infected patients)

Significant weight loss with CD4 counts less than 200 cells\µl (Table 13): CD8 counts among HIV positive and negative individuals.

CD8 cells\µl	Less than 250	800 – 250	More than 800
HIV Positive	14	20	26
HIV negative	0	12	3

No significant differences in CD8 count among each HIV serology negative and positive patients.

Fig. 1. CD4 count of the study individuals

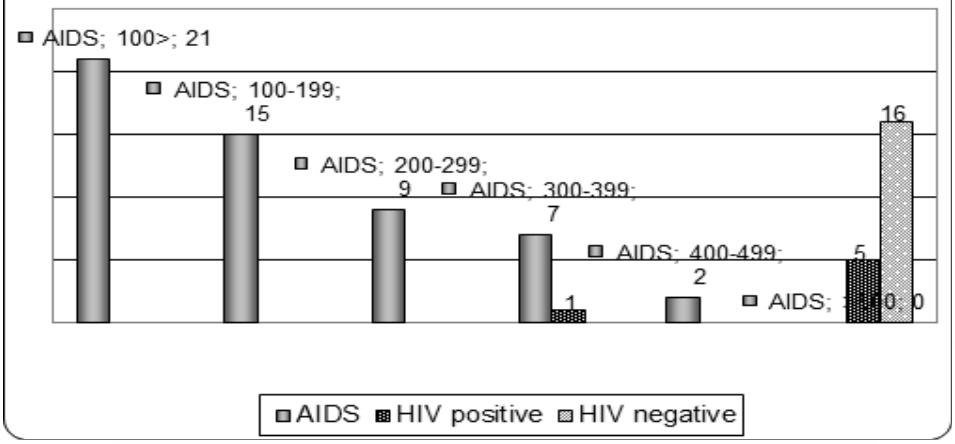
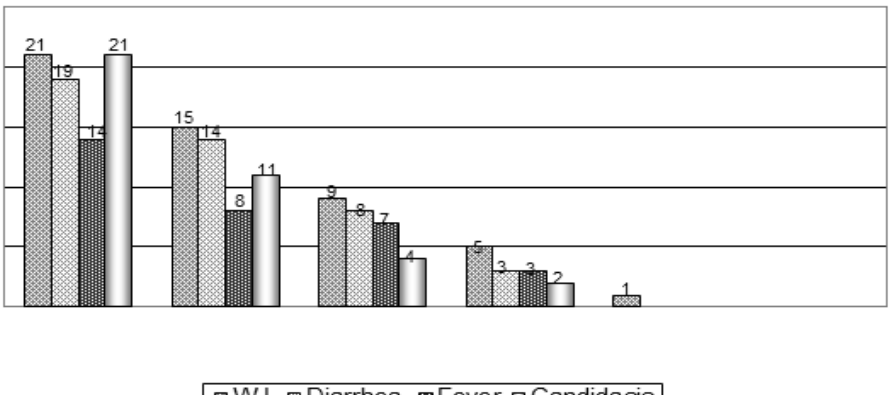


Fig. 2. CD4 count and symptoms in AIDS patients



Discussion

Although significant data on CD4+ counts of HIV infected individual is globally available, no data is available on the immune status of HIV infection in Sudan. Total of (76) consent individuals were included in this study. All individuals were reporting voluntary for HIV serology testing either for travelling requirement or for diagnosis of suspected HIV infection. In this study (50/76) studied individuals were males, twice the number of females recruited in this study. The high number of male in this study could be due to the fact that most of individuals who seek HIV testing for travel requirement are males, furthermore, males presented themselves to clinics more than females due to socio-economic cultural reasons.

It was noticeable that most of HIV positive individuals were within the age rang of (20-40) years. This age group is the sexually active group and can explain this finding by the fact that (90%) of studied HIV positive individuals in this study were infected by sexual contact. Similar previous studies in Sudan and sub-Saharan Africa indicated that sexual contact is the common mode of transmission of HIV (McCarthy *et al*., 1989).

Soldiers and drivers were more affected than other occupation and both occupations were associated with traveling to eastern and southern regions of Sudan this results is similar to s study done by McCarthy (1989) which reported high risk of HIV infection in solders.

There was no significant difference in the total white blood cell counts among HIV serology positive compared with HIV serology negatives however, all AIDS patients had significant lower CD4 count compared with clinical signs of AIDS regard less to the presence of HIV specific antibodies. All patients with clinical signs of AIDS had CD4 count less than 500 cell\mm^3. It appeared that development of AIDS symptoms is associated with decreased CD4 to <500 cell\mm^3. Similar findings were reported in studies from other counties (Barker *et al* 1998). The finding indicates that

an increase in CD4 count cells to >500 cell\mm^3 lowers the possibility of opportunistic infections in AIDS patients.

In this study the decrease of the CD4 count to less than <400 cell\mm^3 was significantly associated with diarrhea, fever, cough, candidiasis and Kaposi Sarcoma Similar results were reported by Pistone at Fann University Hospital in Dakar, Senegal and by Supanaranond (Pistone *et al.,* 2002). (Supanaranond *et al.,* 2001).

70% of the studied AIDS patients were infected with *candidiasis* as first clinical sign, similar results were reported by Van meter (Van Meter *et al*., 1994) and Spinillo (Spinillo *et al*, 1994).

The CD4 levels <200cells\µl were significant associated with weight loss and Dermatitis, similar to the results reported by Forrester (Forrester *et al.*, 2001).

All HIV seronegative individuals had CD4 count >500cell\mm^3 and non had opportunistic infection. Among normal Sudanese CD4≥500cell\mm^3 seem to be protective from opportunistic infection. Similar to the results reported in other countries (Gruber *et al*., 1991).

The antiviral drugs increased the number of CD4 count. 4 of 5 treated patients had elevated CD4 count after treatment for one month and clinical symptoms decreased gradually, as previously reported by Giorgi in USA (Giorgi *et al*., 1998).

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