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HIV INFECTION AND IMMUNOSENESCENCE: A COMPARISON OF IMMUNE STATUS AND T CELL HOMING MARKERS BETWEEN HIV INFECTED AND UNINFECTED ELDERLY INDIVIDUALS IN KISII, KENYA

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ABSTRACT

Objectives: **To examine if differences exist among people aged ≥50 years in T-cell phenotypes frequencies and homing capacity between human immunodeficiency virus (HIV) infected and HIV uninfected controls that impacts Immunosenescence.**

Design: **Laboratory-based cross-sectional study.**

Setting: **Immunology laboratory at Kisii Teaching and Referral Hospital (KTRH) in Kisii, Kenya.**

Subjects: **Asymptomatic elderly HIV infected patients attending HIV clinic and HIV uninfected controls all aged ≥ 50 years. Age used is according to the criteria of the American Centres for Disease Control and Prevention (CDC) which describes HIV/AIDS infected individuals aged ≥ 50 years as "elderly".**

Procedure: **T-cell subset counts were resolved utilising FACScan flow cytometer and outcome processed employing FlowJo computer programme. Differences in cell count and percentages of T lymphocytes were analysed utilising Student's *t*-test and linear regression based on HIV status and age**

Main outcome measures: **Cell count and percentage of T lymphocytes, CD4/CD8 ratio and age**

Results: **Significant difference were found in $CD45^+$ (*P = 0.045 and 0. 003*), $CD3^+$ (*P=0.001*), $CD4^+$ (*p<0.001*), $CD8^+$ (*p<0.001*), $CD8^+CCR7^-$ (*p<0.001*) and $CD4^+CCR7^-$ (*p<0.001* and *P=0.002*) cells between HIV infected and uninfected people.**

Conclusion: **T-cell phenotypes frequencies and homing capacity are significantly altered among elderly HIV infected compared to the HIV uninfected controls leading to greater impairment of the T cell apportionment among older HIV infected and consequently HIV accelerates Immunosenescence.**

INTRODUCTION

With the utilisation of highly active antiretroviral therapy (HAART), persons infected with HIV are surviving longer [1]. Elderly HIV infected people (EHIP) encounter many new challenges which may include inevitable hastened ageing and increased occurrence of co-morbidities including cardiovascular disease and geriatric syndromes at an early age than HIV uninfected people (EHUP) [2]. Most geriatricians and the World Health Organisation (WHO) describe "elderly" as an individual who is 60 years or elder. Notwithstanding, because of the effect of HIV/AIDS infection and ART on aging, a person aged 50 years old is well thought to be elderly by CDC [3]. Growing old is accompanied by Immunosenescence which results in an increased occurrence of infectious and non-infectious comorbidities and eventual death [4]. Opposing opinions exist about whether HIV accelerates immune ageing [5]. Many immunologic changes that are found in EHIP are strikingly comparable to those found in the EHUP [6]. The Limited research on mechanisms of Immunosenescence among EHIP that has been done is chiefly localised to industrialized countries [7]. Different geographical regions may influence immunological status because of different existing predisposing risk factors that vary from country to country [8].

The hallmark of age and HIV related Immunosenescence is changes in frequencies of circulating blood lymphocyte subsets ($CD45^+$ expressing cells) and homing of T-cells. [9]. Typically there is a depletion in $CD4^+$ and $CD8^+$ T cells repertoire, a reduction in the CD4/CD8 ratio and elevation memory T cells.; central memory T cells ($CCR7^+$ cells) and effector memory T cells ($CCR7^-$) with advancing age [10]. Homing of T cells to peripheral lymphoid organs is determined by the expression of CCR7 which links to complementary ligands CCL19 and CCL2 [11]. The present study aimed to find if there are differences in profiles of T cell biomarkers of immune status and homing capacity ($CD45^+$, $CD3^+$, $CD4^+$, $CD8^+$, CD4/CD8 ratio, $CD4^+CCR7^+$, $CD4^+CCR7^-$ $CD8^+CCR7^+$, and $CD8^+CCR7^-$) among asymptomatic EHIP and EHUP attending HIV/AIDS clinic at KTRH, Kenya.

MATERIALS AND METHODS

Study site

The inquiry was performed at KTRH immunology laboratory. This is a regional referral hospital serving South Nyanza and parts of South Rift valley regions of Kenya. The hospital has a well-established HIV/AIDS Clinic. The overall HIV prevalence among adults and children in Kisii County is 8.9% [12].

Study design and population

The inquiry was a laboratory-based cross-sectional study design involving elderly HIV infected patients attending HIV/AIDS clinic and HIV uninfected individuals. Asymptomatic HIV infected patients of either gender aged ≥50 years who gave written consent and had normal range of CD4 cells count (≥ 500 cells/mm^3 per of blood) were included as the study group. The control group included HIV uninfected individuals aged ≥50 years who gave written consent. All patients with medical conditions that can affect lymphocyte counts e.g. diabetes, tuberculosis, pregnancy and malnutrition were excluded for the study. A total of 31 EHIP and an equal number of EHUP were recruited into the study between March 2019 and December 2019. This sample size was settled at guided by a proportionate sample size determination formula [13].

Data collection

Recruitment of study participants: Elderly individuals who fitted the inclusion criteria (persons not manifesting other conditions

that mighty affect the frequency of lymphocytes) were recruited into the study by the principal researcher and one trained assistant by purposive sampling procedure until the desired sample size was achieved. The entry point was the HIV/AIDS clinic reception office where all patients attending HIV/AIDS clinic report on arrival.

Sample Collection: Blood sample was used for this study. The puncture area was identified, swabbed with alcohol and 5ml of blood was drawn by venipuncture from the median cupita vein, on the interior forearm using a 10ml syringe and 21g needle which was well sterilised before use. Specimens of whole blood were gathered in 5 ml K3E K3EDTA vacutainer tubes and mixed adequately with the EDTA by gently inverting up and down for at least ten times to avoid blood clotting. The specimens of blood were kept at 4°Ċ for assaying later.

Graithing of Blood Specimens for Flow Cytometry Analysis

The manufacturer's guidelines were followed in graithing blood specimens, briefly, 20µL each of appropriate fluorochrome-conjugated monoclonal antibody was added to 100 µL of whole blood in a 12x75mm tube. The mixture was vortexed mildly then incubated up to 30 minutes in darkness at room temperature (20°C-25°C). 2ml of 1x BD FACS lysing mixture was then added succeeded by mild vortexing. The mixture was incubated for 10 minutes in darkness at a temperature of 22°C and succeeded by centrifuging for 5 minutes at 300g. The upper portion of the mixture was removed by decanting. 3ml of BD CellWASH solution (or wash buffer) was added followed by centrifuging at 200g for 5 minutes. The upper portion of the mixture was removed by decanting. 0.5ml of BD CellFIX solution (or 1% paraformaldehyde solution) was added and mixed thoroughly. The preparation was stored at 4°C until analysis which did not exceed 24 hours as recommended by the manufacturer.

Flow Cytometry Analysis

Blood specimens from this people were analysed for CD45, CD3, CD4, CD8, CCR7 and CD4: CD8 ratio to establish if there are differences in the profiles of T cell biomarkers of immune status and homing capacity. This study used monoclonal antibody isotypes (MAb) (all from BD Biosciences) in panels for flow cytometry. This included: - Anti-CD3-FITC, anti-CD4-PE, anti-CD45-PerCP, and anti-CD8-APC; Anti-CD3-FITC, anti-CD4-PE, anti-CD45-PerCP, and anti-CCR7-APC; Anti-CD3-FITC, anti-CD8-PE, anti-CD45-PerCP, and anti-CCR7-APC. Four-colour phenotypic characterisations of circulating blood lymphocytes were determined as per established flow cytometry protocols (FACSCalibur, BD Biosciences, USA) [14]. The specimen were analysed before the end of 18 hours from the time of blood collection. The analysis was performed using CellQuest software (BD Biosciences). For quality control, suitable antibody controls (IgG1-FITC, IgG1-PE, IgG1-APC, IgG2a-PE) (BD Biosciences) were utilised in ensuring the accuracy of the study results. . Data for each lymphocyte subset specimen was procured for 10,000 events in line with FSC/SSC dispersion. For equipment setup, CaliBRITE beads and FACSComp software (BD Biosciences) was used for mounting the photomultiplier tube (PMT) voltages, the fluorescence balancing and for curbing the sensitivity of the flow cytometry machine on a daily basis. Data obtained from flow cytometry was further evaluated and dissected using FlowJo software (Tree Star Inc., San Carlos, CA, USA).

Data analysis

Statistical Package for the Social Sciences, *lection* 25 was utilised for statistical computation. The noticeable divergences in mean cell count and proportions of lymphocyte subsets were computed utilising Student's *t*-test for parametric values. To find out if a linear relationship existed

betwixt age in years and the proportion of T lymphocytes, linear regression analysis was performed.

Ethical Considerations

This study was commenced after obtaining ethical approval from the University of Eastern Africa, Baraton research ethics committee and permitted by National Commission for Science, Technology, and Innovation, Ministry of Education and Ministry of Health, Kenya. Scripted informed consent was obtained from potential study participants before data collection. Unique letters and numbers were used to de-identify study participants for identity confidentiality.

RESULTS

Among the 62 individuals recruited into the study, 31 (50%) were elderly HIV infected people and 31 (50%) were elderly HIV uninfected people. Blood specimens from this people were compared for CD45, CD3, CD4, CD8, CCR7 and CD4: CD8 ratio to establish if there are differences in the profiles of T cell biomarkers of immune status and homing capacity as indicators of immune status and Immunosenescence among asymptomatic EHIP and EHUP.

Comparison of lymphocyte phenotypes between EHIP and EHUP

Markers of Immune Status

Cell count and percentage of $CD45^+$ lymphocytes was found to be significantly higher in EHIP (4872 cells; 48.68%) when compared to EHUP (3967 cells; 36.87%; $p = 0.045$ *and* 0.003 respectively). The cell count and proportion (in %) of total T lymphocyte subpopulations was different between EHIP and EHUP. Enumeration of circulating T lymphocytes ($CD3^+$ lymphocytes) was significantly higher in EHUP (1275.23cells) compared with EHIP (886.50cells; $p = 0.001$). There was a substantially raised number and proportion of $CD8^+$ cells in EHUP (485.77 cells; 19.81%) as compared to EHIP (284.27cells; 14.65%; both was $p < 0.001$). Similarly, substantially raised number and proportion of $CD4^+$ cells in EHUP (830.22 cells; 29.21%) was observed as compared to EHIP (574.11 cells; 22.12%; both was $p < 0.001$).

Markers of Homing Status

Cell count and percentage of $CD8^+CCR7^-$ Cells was significantly higher in EHIP (126.19 cells; 17.83%) compared to EHUP (60.17 cells; 13.18%; both was $p = < 0.001$). There was a significantly higher cell count and percentage of $CD4^+CCR7^-$ Cells in EHUP (141.91 cells; 19.60%) as compared to EHIP (99.33 cells; $p < 0.001$ *and* 0.002 respectively). These outcomes are displayed in Table 1.

Table 1

Lymphocyte phenotype distribution (cell count and %) for EHIP and EHUP

Cell subset	Mean Cell count ±SD	HIV Status		Difference Δ	*p-value*
		HIV Infected (*N=31*)	**HIV Uninfected (*N=31*)**		
CD45$^+$	Cell counts ±SD	4872±1872	3967±1599	905	**0.045**
	% ±SD	48.68±14.07	36.87±15.86	11.81	**0.003**
CD3$^+$	Cell counts ±SD	886 ± 223	1275 ± 567	389	0.001
	% ±SD	53.58±14.36	49.62±14.49	3.96	0.283
CD4$^+$	Cell counts ±SD	574 ± 226	830 ± 155	256	<0.001
	% ±SD	22.12±9.51	29.21±8.44	7.09	<0.001
CD8$^+$	Cell counts ±SD	284 ± 223	485 ± 164	201	<0.001
	% ±SD	14.65±5.77	19.81±3.91	5.16	<0.001
CD8$^+$CCR7$^-$	Cell counts ±SD	126 ± 30	60 ± 29	66	<0.001
	% ±SD	17.83±3.52	13.18±5.19	4.65	<0.001
CD8$^+$CCR7$^+$	Cell counts ±SD	1 ± 1	0 ± 0	1	0.138
	% ±SD	0.04±0.098	0.45±1.23	0.41	0.066
CD4$^+$CCR7$^-$	Cell counts ±SD	99 ± 31	142 ± 39	43	<0.001
	% ±SD	14.80±6.41	19.60±5.64	4.8	0.002
CD4$^+$CCR7$^+$	Cell counts ±SD	1 ± 3	6 ± 17	5	0.202
	% ±SD	0.12±0 .24	0.41±0.956	0.29	0.111
CD4/CD8 Ratio	Ratio ±SD	1.42 ± 0.77	1.47 ± 0.84	0.05	0.807

Mean Cell count is expressed as cells/μL of blood.
% is the percentage of total lymphocytes.

Linear relationship of proportions of T cell subsets with age in EHIP and EHUP

Linear regression computation outcome for CD45+, CD3$^+$, CD8$^+$, CD4$^+$, CD8$^+$CCR7$^+$, CD8$^+$CCR7$^-$, CD4$^+$CCR7$^+$, CD4$^+$CCR7$^-$, and CD4CD8 ratio percentages compared to age are shown in Figure 1. There were no substantial disparities betwixt per centum of lymphocyte subset considered and age.

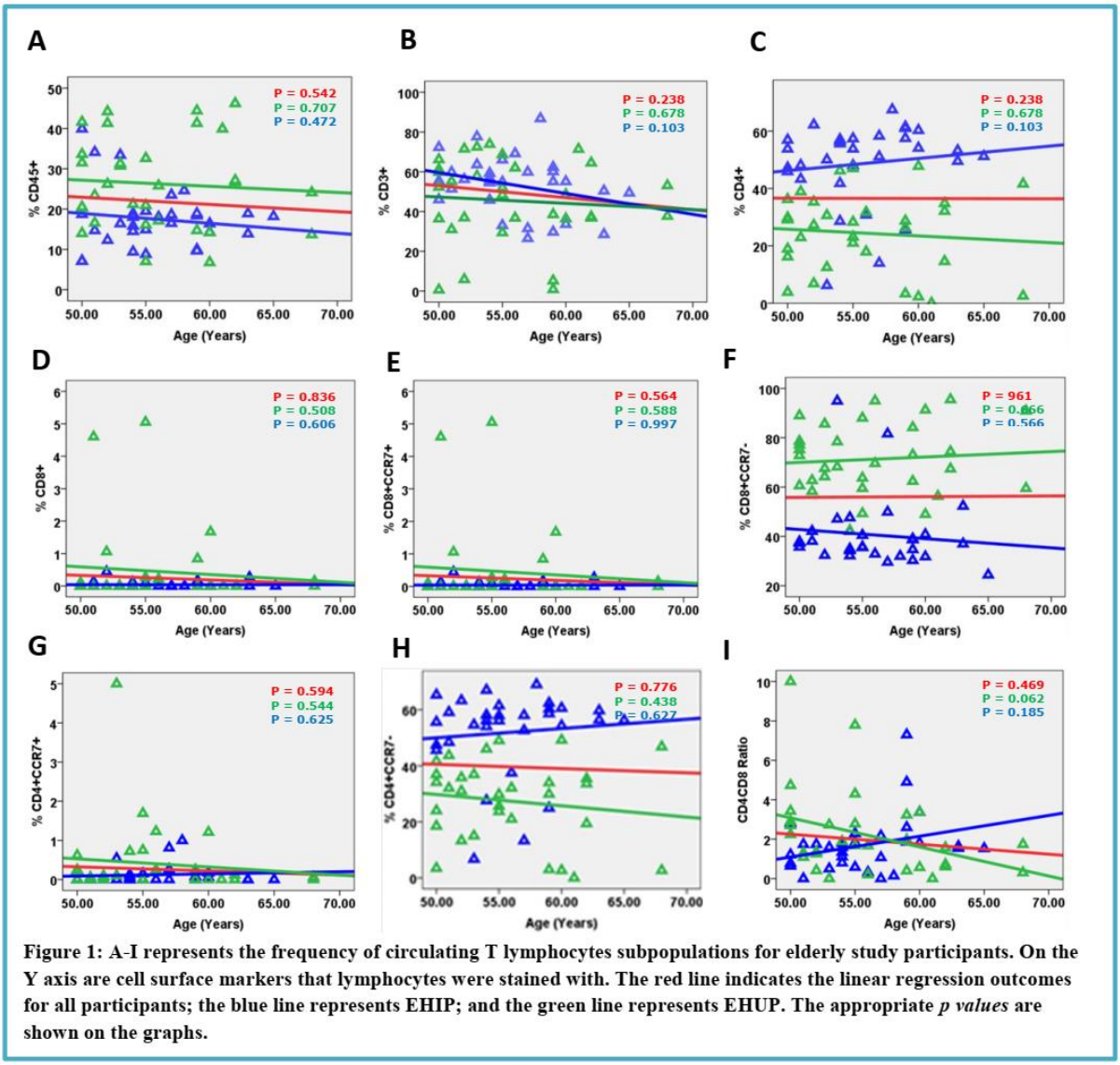

Figure 1: A-I represents the frequency of circulating T lymphocytes subpopulations for elderly study participants. On the Y axis are cell surface markers that lymphocytes were stained with. The red line indicates the linear regression outcomes for all participants; the blue line represents EHIP; and the green line represents EHUP. The appropriate *p values* are shown on the graphs.

DISCUSSION

The current study sought to find if HIV infections contribute to Immunosenescence among elderly asymptomatic HIV infected population attending KTRH. The study found significant variations in frequencies of $CD45^+$ lymphocytes and T lymphocytes subpopulations when a comparison was done between EHIP and EHUP. Firstly, population of $CD45^+$ lymphocytes was found to be higher in EHIP when compared to EHUP. Secondly, enumerations of circulating CD3+, CD8+ and CD4+cells were significantly higher in EHUP compared with EHIP. Thirdly, substantially elevated cell count of $CD4^+CCR7^-$ was discovered among EHUP when compared to EHIP. On the other hand, cell counts of CD8+CCR7- Cells were substantially increased among EHIP when compared to EHUP.

The elevated enumeration of $CD45^+$ leukocytes could be emanating from other lymphocytes not included in the current study. The observed lower counts of T cells in EHIP can be explained by consistent results of other studies; firstly, T cell-mediated immunity declines with advancing age because of involution of the thymus, a primary lymphoid organ generating and maturing T lymphocytes [15]. Secondly, despite successful treatment by HAART,

there remains a continuous undetectable immune activation and inflammation which results in an immune accelerated ageing pattern [16]. Thirdly, the difference in the number and composition of lymphocytes may result directly from HIV disease or its treatment [17].

Optimum operation of the immune control depends on the continuous aptness of T cells to transmigrate to and inside tissues (homing) which are controlled by the expression of receptor CCR7 [18]. In the present inquiry, we have compared the possession of CCR7 in EHIP and EHUP to determine differences in homing capacity found in T lymphocytes. The elevated cell count of $CD4^+CCR7^-$ subpopulation of $CD4^+$ T cells among EHUP implies a possession of enhanced effector control and potential of $CD4^+$ cells to transmigrate into inflamed tissues than in EHIP. This observation is justified by other studies that report substantial $CD4^+$ T-cell repertoire perturbations during HIV infection [19]. On the other hand, the increased Cell count of $CD8^+CCR7^-$ subpopulation of CD8 T cells among EHIP suggests that CD8+ T cells of EHIP possess raised functional agility and the potential to transmigrate to and inside inflamed tissues as compared to EHUP an observation that agrees with results from other studies attesting that despite viral depletion by HAART, there remains a continuous low immune activation and inflammation [16]. The results referred to here, however, are based on small sample size and the comparisons are dependent on a few isolated not fully comparable studies, and hence more research along ageing in HIV infected people is justifiable.

No substantial divergences were found in the frequencies of T lymphocytes when compared with age both in EHIP and EHUP. This result is not consistent with previous studies that looked at the distribution of lymphocytes from individuals of different ages. The studies have however mainly compared young and old, male and female without referring to HIV status and people aged ≥50 years which we have done in the current study. Yet in another study in Kenya working with a population aged between 16 and 55 years [20], there were no significant differences in lymphocyte distribution with age groups and thus consistent with our findings. The lack of significance when lymphocyte frequencies were compared with increasing age could be explained by the low sample size utilised in the current study. We suggest further studies to look at frequencies of T lymphocytes with advancing age amongst EHIP and EHUP utilising larger sample sizes.

This study included comparatively small sample size and limited group grandness; most inclinations reported in the current investigation can result in statistical importance if the groups are enlarged which we suggest should be explored. We also were unable to test for the effect of duration ART treatment on number and composition lymphocytes in the treated population. The current study also did not answer how the frequencies of T lymphocytes are affected with advancing age amongst EHIP which we also suggest as an area to be looked into in further studies.

CONCLUSIONS

The quantitative and qualitative disparities between EHIP and EHUP in the distribution of T cell subpopulations discussed above involving $CD45^+$, $CD3^+$, $CD4^+$ and $CD8^+$ cells and their differential expression of cysteine chemokine receptor (CCR7) suggest greater impairment of the T cell compartment of EHIP as compared to EHUP and consequently there is accelerated Immunosenescence among EHIP which is a result of HIV infection. Also the effector function and ability of migration of CD8 T cells of HIV infected people is raised suggesting higher inflammation while at the

same time the effector function and ability of migration of CD4 T cells is diminished suggesting that the function of this cells is compromised and therefore indicating greater immune senescence as compared to HIV uninfected controls.

Low CD4/CD8 ratio directly impacts on the distribution of T subpopulations negatively suggesting greater impairment of the immune system among people with inverted CD4/CD8 ratio and therefore accelerated immune aging. The alterations in the T cell immune compartment among EHIP in the foregoing discussion implying accelerated Immunosenescence should be considered in future management of elderly HIV patients to develop a unique HIV treatment regimen targeting persons living with HIV aged ≥50 years so as to improve their end of life quality of health.

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