Some hematological parameters and the prognostic value of CD4, CD8 and total lymphocyte counts and CD4/CD8 cell count ratio in healthy HIV sero-negative, healthy HIV sero-positive and AIDS subjects in Port Harcourt, Nigeria

Nijerya'da (Port Harcourt) sağlıklı HIV seronegatif, sağlıklı HIV seropozitif ve AIDS hastası olgularda CD4, CD8 ve toplam lenfosit sayımı ve CD4/CD8 hücre sayımı oranının prognostik değeri ve bazı hematolojik parametreler

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Abstract

Objective: The present study attempts to determine normal values of CD4, CD8, CD4/CD8 ratio, total WBC and differential counts, hematocrit and total lymphocyte count (TLC) in healthy HIV sero-negative and sero-positive subjects, and to assess the prognostic significance of these parameters in these subjects as compared to AIDS subjects.

Material and Methods: A total of 300 subjects (147 M, 153 F) aged between 17 and 71 years were recruited into the study. Subjects were separated according to sex and divided into three groups: Group A: healthy HIV sero-negative subjects; Group B: healthy HIV sero-positive newly diagnosed ART-naïve subjects; and Group C: AIDS subjects. CD4 and CD8 counts were determined by flow cytometry; hematocrit was determined using Hawksley micro-capillary tubes; total WBC and differential counts were determined manually with the improved Neubauer counting chamber; and TLC was obtained by multiplying the percentage of lymphocytes by the total WBC count.

Results: For male subjects, significant differences were found in CD4 count, CD4/CD8 count ratio, hematocrit, total WBC and TLC, whereas for female subjects, significant differences were found only in CD4 and CD4/CD8 count ratio in the three groups of subjects. In both sexes, however, these parameters were found to be highest in healthy HIV sero-negative subjects and lowest in AIDS subjects, with HIV sero-positive subjects having intermediate values.

Conclusion: The results confirm previous reports that the CD4 count and CD4/CD8 count ratio are fairly reliable indicators of the progression of HIV infection. In addition, the results also apparently suggest that the prognostic value of CD8 count is limited and that of TLC possibly sex-dependent. The results could be of importance in our environment since previous reports have been relatively scarce. *(Turk J Hematol 2008; 25: 181-6)*

Key words: CD4 counts, CD8 counts, CD4/CD8 ratio, total lymphocyte count, human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS).

Received: May 21, 2008 *Accepted: September 17, 2008*

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Özet

Amaç: Bu çalışmanın amacı sağlıklı HIV seronegatif ve seropozitif olgularda normal CD4, CD8 değerleri, CD4/CD8 oranı, total lökosit ve periferik yaymadaki kan hücrelerinin sayımları hematokrit ve total lenfosit sayımının (TLS) tespit edilmesi ve AIDS olgularına kıyasla bu kişilerde söz konusu parametrelerin prognostik öneminin değerlendirilmesidir.
Yöntem ve Gereçler: Onyedi ile 71 yaş aralığındaki toplam 300 olgu (147 E, 153 K) çalışmaya alınmıştır. Olgular cinsiyete göre ayrılmış ve üç gruba bölünmüştür: Grup A: Sağlıklı HIV seronegatif olgular; Grup B: sağlıklı HIV seropozitif (yeni teşhis edilmiş ART-naïve) olgular; ve Grup C: AIDS olguları. CD4 ve CD8 sayımları akış sitometrisi ile; hematokrit Hawksley mikrokapiler tüpler ile; total lökosit ve periferik yaymadaki kan hücrelerinin sayımları geliştirilmiş Neubauer sayım kabı kullanılarak manuel olarak tespit edilmiş ve TLS de lenfosit yüzdesinin toplam lökosit sayımı ile çarpılmasıyla elde edilmiştir.
Bulgular: Üç çalışma grubunda erkek olgularla ilgili olarak CD4 sayımı, CD4/CD8 sayımının oranı, hematokrit, toplam lokösit ve toplam lenfosit sayımına yönelik anlamlı farklılıklar gözlemlenirken kadın olgularda yalnızca CD4 sayımı ve CD4/CD8 oranında anlamlı farklılıklar bulunmuştur. Ancak her iki cinsiyete yönelik olarak bu parametrelerin en yüksek değerleri HIV seronegatif olgularda ve en düşük değerleri de AIDS olgularında gözlenmiştir. HIV seropozitif olgular ise ara değerlere sahiptir.
Sonuç: Elde edilen sonuçlar ile CD4 sayımı ve CD4/CD8 oranının, HIV enfeksiyonunun gelişimine yönelik oldukça güvenilir göstergeler olduğuna dair önceki raporlar onaylanmaktadır. Ayrıca elde edilen sonuçlar ile, CD8 sayımı prognostik değerinin sınırlı olduğu ve toplam lenfosit sayımının ilgili değerinin ise cinsiyete bağlı olduğu açıkça ileri sürülmektedir. Önceki raporlar nispeten sınırlı olduğundan elde edilen sonuçlar son derece önem taşımaktadır.
(*Turk J Hematol 2008; 25: 181-6*)
Anahtar kelimeler: CD4 sayımı, CD8 sayımı, CD4/CD8 oranı, toplam lenfosit sayımı, insan bağışıklık yetmezlik virüsü (HIV), edinilmiş bağışıklık yetmezlik belirtisi (AIDS).

Geliş tarihi: 21 Mayıs 2008 Kabul tarihi: 17 Eylül 2008

Introduction

Since its identification in 1981, human immunodeficiency virus (HIV) infection and the associated acquired immunodeficiency syndrome (AIDS) remain a major health burden globally. Recent estimates indicate that over 35 million people are affected worldwide, with mortality counts of over 20 million [1]. About 70% of these deaths have occurred in sub-Saharan Africa [1], where the burden of disease is high and poverty an important accomplice. In Nigeria, the current national average HIV seroprevalence rate is estimated at about 4.4%, with Rivers State predictably having a higher rate of 5.4% [2]. This is possibly so following the recent rapid urbanization of Port Harcourt, the capital of Rivers State, due to influx of multinational concerns involved with petroleum exploitation and exploration [3].

In individuals with HIV infection, assessment of CD_4 and CD_8 cell counts is fairly common and they are routine indices for the evaluation of immune status and decision to initiate anti-retroviral drug therapy (ART) [4]. The CD_4 cell counts of healthy HIV sero-negative Caucasians have been reported to be between 500 and 1500 cells/µl, while the CD_8 cell count ranges lower, between 300 and 1000 cells/µl [5]. However, significant geographical and racial differences have been reported in CD_4 count between Asians and Caucasians [6] and even amongst healthy Africans of different countries [7-9]. Racial differences have also been established in white blood cell (WBC) counts between Africans and Caucasians, with a leuko-neutropenia seen in the Africans [10].

With recent up-scaling of ART in developing countries, including Nigeria, and the resultant anticipated increase in the number of individuals accessing ART, determination of CD_4 cell count would expectedly become more frequent in Nigeria. However, with the relatively high cost of CD_4 cell count determination, total lymphocyte count (TLC) has been suggested as an alternative in situations where facilities for CD_4 cell count are not readily available or resources are limited. This is because TLC is easily obtained from routine complete blood cell counts by multiplying the percentage of lymphocytes by the WBC count [11,12]. However, a number of reports have suggested an inconsistency in the correlations between total lymphocyte and CD4 cell counts [13].

Given that, in our environment, reports on this subject are relatively scarce, the present study attempted to determine values of CD_4, CD_8, total lymphocyte, total WBC and differential cell counts in healthy HIV sero-negative, healthy HIV sero-positive and in persons with AIDS. The study also determined the CD_4/CD_8 cell count ratio and attempted to assess the possible prognostic value of these parameters using these three groups of subjects. In addition, the study attempts to establish normative values of these parameters, in our environment, for healthy HIV sero-negative and healthy HIV sero-positive subjects who have yet to commence ART. This could possibly assist Nigerian physicians with the assessment and management of HIV infection in affected individuals.

Materials and Methods

Subjects: A total of 300 subjects (147 M, 153 F; age range: 17-71 years) were recruited into the study. Subjects were separated according to sex and were further divided into three groups: Group A [controls] consisted of healthy HIV sero-negative subjects, Group B consisted of healthy HIV sero-positive

subjects, and Group C consisted of AIDS subjects. Groups B subjects were newly diagnosed subjects yet to commence ART. Groups B and C were attending the HIV clinic of a tertiary health care facility in Port Harcourt, southeastern Nigeria; Group A [control] subjects were apparently healthy staff and students of the University of Port Harcourt, Nigeria. Each control subject was examined and no evidence of acute or chronic infections or any hematologic, cardiovascular or metabolic disease likely to influence any of the hematological parameters under investigation was found. All subjects gave informed consent before recruitment into the study; ethical clearance was obtained from our institutional ethics committee. All pregnant female subjects were excluded from the study.

Methods: Five milliliters of venous blood was collected from each subject from an antecubital vein with the subject comfortably seated and with minimum stasis. The blood was immediately transferred into EDTA specimen bottles and carefully mixed. All blood specimens were collected between 9 a.m. and 12 noon each day and analyzed within 2 hours of collection.

The HIV status of each subject was determined routinely using the Chembio HIV 1/2 Stat-pak assay kit (Chembio Diagnostic Systems Incorporated, USA). The CD_4 cell and the CD_8 cell counts were both determined by flow cytometry using the Partec Cytoflow counter FMC system (Partec GmbH, 2006).

CD_4/CD_8 count ratio for each subject was obtained from the product of dividing the CD_4 cell count by the CD_8 cell count. Hematocrit was determined using Hawksley micro-capillary tubes centrifuged at 3000 rpm for 10 minutes; the mean of two separate readings was taken as the hematocrit value. Total WBC and differential WBC counts were determined manually using the improved Neubauer counting chamber [14]. TLC was obtained by multiplying the percentage of lymphocytes by the total WBC count [11,12].

Statistics: The results obtained are expressed as means ±standard errors of means (SEM); ranges are in parenthesis. Statistical significance was determined using the analysis of variance (ANOVA) or the Student's t-test as appropriate. A p value less than 0.05 ($p<0.05$) was considered statistically significant.

Results

The results obtained from the present study for each Group are as shown in Tables 1 and 2 for male and female subjects, respectively.

Table 1 presents the ages, CD_4 and CD_8 counts, CD_4/CD_8 count ratio, hematocrit, total WBC count, percentage neutrophil, lymphocyte, monocyte, eosinophil and basophil, and TLC for the male subjects involved in the present study. ANOVA showed

Table 1. Hematological parameters, CD_4 and CD_8 counts and ratio in male HIV sero-negative and sero-positive subjects and AIDS subjects.

Parameter	Healthy HIV- negative subjects [Group A] [n= 58]	Healthy HIV- positive subjects [Group B] [n=37]	AIDS subjects [Group C] [n= 52]	Significant differences
Age (years)	36.98±0.98 [18-71]	35.68±0.87 [17-63]	41.23±0.99 [19.0-71.0]	No [p>0.05]
CD_4 count (Cells/µl)	1019.0±23.74 [468.0-1609.0]	545.76±29.48 [58.0-2285.0]	99.04±4.36 [12.0-195.0]	Yes [p<0.05]
CD_8 count (Cells/µl)	701.76±22.99 [137.0-1420.0]	896.30±36.5 [144.0-2381.0]	781.65±20.47 [296.0-1385.0]	No [p> 0.05]
CD_4/CD_8 count ratio	1.71±0.07 [0.44-5.22]	0.79±0.07 [0.08-4.07]	0.13±0.01 [0.02-0.29]	Yes [p<0.05]
Hematocrit (%)	40.29±0.46 [30-58]	31.97±0.41 [22.0-42.0]	31.02±0.36 [21.0-38.0]	Yes [p<0.05]
Total WBC count (Cells/µl)	5.56±0.07 [3.90-7.30]	4.74±0.09 [2.80-8.30]	4.45±0.08 [2.60-7.10]	Yes [p<0.05]
Neutrophil count (%)	59.95±0.56 [45.0-73.0]	57.51±0.65 [34.0-72.0]	61.75±0.53 [50.0-78.0]	No [p>0.05]
Lymphocyte count (%)	39.43±0.55 [26.0-54.0]	40.73±0.60 [21.0-65.0]	37.35±0.56 [21.0-49.0]	No [p>0.05]
Monocyte count (%)	0.21±0.04 [0.0-2.0]	0.22±0.04 [0.0-2.0]	0.31±0.05 [0.0-2.0]	No [p>0.05]
Eosinophil count (%)	0.34±0.05 [0.0-3.0]	0.32±0.05 [0.0-2.0]	0.40±0.05 [0.0-2.0]	No [p>0.05]
Basophil count (%)	0.05±0.02 [0.0-1.0]	-	0.04±0.02 [0.0-2.0]	No [p>0.05]
Total lymphocyte count (%Cells/µl)	219.1±4.09 [114.8-340.8]	193.15±4.62 [86.1-332.0]	168.01±4.40 [72.6-302.4]	Yes [p<0.05]

Values=mean ± SEM, range in parenthesis

that significant differences existed in CD_4 cell count, CD_4/CD_8 cell count ratio, hematocrit, total WBC count and TLC for male subjects between the three groups under consideration: healthy HIV sero-negative subjects (Group A), healthy HIV sero-positive subjects (Group B) and AIDS subjects (Group C) ($p<0.05$). Each of these parameters was generally the highest in the healthy HIV sero-negative subjects (Group A) and lowest in the AIDS subjects (Group C), with healthy HIV sero-positive subjects (Group B) having intermediate values.

Similarly, Table 2 presents the values of the investigated parameters in all the female subjects involved in the present study. ANOVA showed that significant differences existed only in CD_4 cell count and CD_4/CD_8 cell count ratio for the three female groups under consideration ($p<0.001$). Unlike for male subjects, in female subjects, no significant differences were found in hematocrit, total WBC count and TLC between the three groups under consideration ($p>0.001$). However, as in male subjects, both CD_4 cell count and CD_4/CD_8 cell count ratio were highest in the healthy HIV sero-negative subjects (Group A) and lowest in the AIDS subjects (Group C), with healthy HIV sero-positive subjects (Group B) having intermediate values.

Amongst the HIV sero-positive (Group B) subjects, 8 (21.6%) males and 26 (41.3%) females had CD4 cell counts less than 350 cells/µl. All the AIDS (Group C) subjects were found to have CD_4 cell counts less than 350 cells/µl. None of the healthy HIV sero-negative (Group A) subjects had CD_4 cell counts less than 350 cells/µl.

Discussion

The present study presents normative values for CD_4 cell counts, CD_8 cell counts, TLC and CD_4/CD_8 cell count ratio in healthy HIV sero-negative and healthy HIV sero-positive male and female subjects in Port Harcourt, Nigeria. Previous studies in this regard have been relatively scarce and have focused on the effects of highly active anti-retroviral therapy (HAART) on CD_4 cell count [15]; on use of absolute lymphocyte count as a marker of CD_4 cell count and criteria for initiating ART [16]; and on hematological parameters in HIV-infected Nigerians in Port Harcourt [17].

The CD_4 cell counts obtained in the present study are in the same range as in a recent report in HIV sero-negative Nigerians [18] and are fairly similar to values reported in Caucasians [5,8], Kuwaitis [19], Indians [20], and Tanzanians [21]. However, the CD_8 cell counts obtained in the present study are marginally higher than values reported for Caucasians5. The non-significant differences in the CD_8 cell counts between the three groups is at variance with a recent report from Zaria, northern Nigeria, in

Table 2. Hematological parameters, CD_4 and CD_8 counts and ratio in female HIV sero-negative and sero-positive subjects and AIDS subjects.

Parameter	Healthy HIV- negative subjects [Group A] [n= 42]	Healthy HIV- positive subjects [Group B] [n=63]	AIDS subjects [Group C] [n=48]	Significant differences
Age (years)	38.93±1.13 [17-71]	33.86±0.88 [19-71]	31.73±0.73 [18-65]	No [p>0.05]
CD_4 count (Cells/µl)	920.52±24.10 [528.00-1671.00]	451.46±20.23 [89.00-1377]	94.46±4.43 [14.00-196.00]	Yes [p<0.05]
CD_8 count (Cells/µl)	834.69±24.54 [326.00 -1452.00]	804.27±41.86 [269.00-3943.00]	800.10±54.88 [58.00-5055.00]	No [p>0.05]
CD_4/CD_8 count ratio	1.23±0.04 [0.46-2.41]	0.67±0.04 [0.15-2.51]	0.19±0.02 [0.02-1.76]	Yes [p<0.05]
Hematocrit (%)	33.45±0.31 [24.00-41.00]	30.49±0.44 [17.00-44.00]	30.38±0.41 [20.00-44.00]	No [p>0.05]
Total WBC count (Cells/µl)	5.07±0.10 [3.30-7.80]	4.85±0.11 [2.80-9.80]	4.82±0.11 [2.10-8.50]	No [p>0.05]
Neutrophil count (%)	64.74±0.54 [50.00-78.00]	61.48±0.71 [37.00-76.00]	61.77±0.62 [43.00-80.00]	No [p>0.05]
Lymphocyte count (%)	34.33±0.56 [21.00-48.00]	37.94±0.75 [22.00-73.00]	37.46±0.62 [20.00-57.00]	No [p>0.05]
Monocyte count (%)	0.24±0.04 [0.00-2.00]	0.17±0.04 [0.00-2.00]	0.21±0.03 [0.00-1.00]	No [p>0.05]
Eosinophil count (%)	0.62±0.07 [0.00-4.00]	0.57±0.07 [0.00-6.00]	0.58±0.06 [0.00-2.00]	No [p>0.05]
Basophil count (%)	0.12±0.03 [0.00-2.00]	0.02±0.01 [0.00-1.00]	-	No [p>0.05]
Total lymphocyte count (%Cells/µl)	178.04±5.33 [84.0-336.0]	186.7±6.14 [66-489.1]	177.10±3.84 [60.9-277.5]	No [p>0.05]

Values=mean ± SEM, range in parenthesis

which both CD_4 and CD_8 cell counts were significantly lower in patients compared to controls [22]. However, the significant differences in the CD_4 cell counts seen in the present study are consistent with that report, although our values are lower than the CD_8 cell counts reported in healthy controls [22].

The results of the present study suggest that sex variations apparently do exist in both the pattern of differences and possibly in the prognostic value of the parameters under investigation. For instance, although in both sexes CD_4 cell count and CD_4/CD_8 cell count ratio consistently showed significant differences in the three groups of subjects, TLC followed a similar pattern only in males. These sex differences in both the TLC and total WBC count seen in the present study are perhaps expected based on the reported sex variations in WBC and neutrophil counts [23] and the reported cyclic variation in WBC population during the normal menstrual cycle [24]. Apparently, menstrual cyclic variations in the WBC count could possibly contribute to obscuring the pattern in females likely leading to a sex distinction. This finding would, however, require further investigation. Perhaps these cyclic variations in females could indeed account for the absence of significant differences in the hematocrit scores in females as opposed to the pattern seen in males. Cyclic changes in the hematocrit scores during the normal menstrual cycle have also been reported by the present authors in Nigerians [25] and have been similarly described in Caucasians [26].

Amongst all the parameters studied, the results of the present study apparently suggest that in our environment, the CD_4 cell count and CD_4/CD_8 cell count ratio are fairly reliable indicators of the progression of HIV infection in both males and females. This is of possible prognostic value and confirms the findings of previous studies in this regard [27]. The results, however, also suggest that the prognostic value of the CD_8 cell count is limited and that of TLC is possibly sex-dependent.

From the results of the present study, we suggest that Nigerian physicians consider both the CD_4 cell count and the CD_4/CD_8 cell count ratio more critically in determining the immune status of persons infected with HIV. In addition, the results suggest that the usefulness of TLC in females is limited and therefore can only be used with some caution in place of both the CD_4 cell count and the CD_4/CD_8 cell count ratio.

In conclusion, the present study attempsted to report normative values for CD_4, CD_8, TLC, and CD_4/CD_8 cell count ratio in healthy HIV sero-negative and HIV sero-positive (ART naïve) individuals in Port Harcourt, southeastern Nigeria. In addition, the study reports significantly higher CD_4 cell and CD_4/CD_8 cell count ratio in healthy HIV sero-negative subjects compared to HIV sero-positive (ART naïve) subjects and AIDS subjects. Our results could be of possible prognostic importance and likely assist in the management of individuals infected with HIV in our environment.

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