Impact of age and gender on the $CD4^+$ t-lymphocyte count among apparently healthy under-5 children as seen in Sokoto, Nigeria

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Abstract

Background: $CD4^+$ T-lymphocyte is a subset of T-lymphocyte that plays a pivotal role in immune response. It is a major target of viruses like HIV. As a result, its monitoring forms an important part of the management of HIV infected patients.

Objectives: To determine the effect(s) of the age and gender on the $CD4^+$ T-lymphocyte count and percentage among apparently healthy under-5 Nigerian children.

Methodology: It was a prospective cross-sectional study conducted among apparently healthy well-nourished HIV-negative under-5 children aged 6 months to 59 months from January 1st to June 30th 2011. The age, gender and weight of these children were documented. The $CD4^+$ T-lymphocyte counts and percentages were determined using Partec cytoflow machine. Data was analyzed using SPSS statistical package version 20.0. A p-value ≤ 0.05 was regarded as statistically significant.

Results: One-hundred children were studied over the period. The mean age (±SD) was 19.1±9.7 months. There were 63 males and 37 females. The mean $CD4^+$ T-lymphocyte count was highest among those aged 6.0 – 11.9 months (2675.7±464.5cells/µL) and lowest among 48 –59.9months of age (1770.0±70.7cells/µL) showing a negative correlation (r= - 0.52, *p*=0.0001). There was no statistically significant difference in the mean $CD4^+$ T-lymphocyte count of the females (2144±553cells/µL) and the males (2120±483cells/µL) (t= 0.22, *p*= 0.83). There were no significant differences in the mean $CD4^+$ T-cell percent across the age group (F=0.28,*p*=0.89) and between the gender (t=0.03,*p*=0.98).

Conclusion: The age has depletive effect on the $CD4^+$ T-lymphocyte count; however, gender seems to have no significant effect on the $CD4^+$ T-lymphocyte count among under-5 children.

Key Words: Impact, Age, Gender, Under-5, $CD4^+$ T-Lymphocyte

Introduction

Lymphocytes are mononuclear white blood cells (WBC) with scanty agranular cytoplasm and are key elements in the production of immunity.[1] The naive T cell expresses surface molecules and proteins called cluster cells of differentiation (CD).[2,3]

The interaction of these T cell receptors with peptide/MHC prompts the T cells to make a commitment to either $CD4^+$ or $CD8^+$ linage, hence, the two broad categories of T cells into $CD4^+$ expressing cells ($CD4^+$ T/helper cell) and $CD8^+$ expressing cells ($CD8^+$ T/suppressor/cytotoxic cells). The $CD4^+$ T-lymphocyte plays a pivotal role in cellular mediated immunity and it also stimulates humoral immunity. The CD4 binds to constant regions of MHC II, while CD8 binds to constant regions of MHC I.[1,4] Based on the cytokines released by these cells when in contact with pathogens, the $CD4^+$ T cells can become inflammatory T_H1 cells or helper T_H2 cells. The inflammatory T cells activate macrophages and leads to cell-mediated immunity while the helper T cells activate B lymphocytes and stimulate humoral immunity. The inflammatory T cells play important roles in cell-mediated and delayed hypersensitivity reactions produced against antigens of intracellular parasites such as viruses, bacteria (mycobacteria), fungi and some protozoa. T helper cells play a central role in regulating humoral immunity.[1-4]

An adult individual has about 3000 lymphocytes per mm^3 in the peripheral blood with about 70%–80% being T-lymphocytes and 65% of these T-lymphocytes bear $CD4^+$ antigens.[5] The absolute $CD4^+$ cell count measures the number of functional $CD4^+$ cells circulating in the blood, while the $CD4^+$ percentage is the percentage of the total lymphocytes that are $CD4^+$ cells. Unlike $CD4^+$ count, $CD4^+$ percentage is relatively stable and more useful in young children. Most health care systems recommend use of $CD4^+$ percentage to guide treatment decisions and monitoring HIV treatment in children.[5]Both the $CD4^+$ count and percentage are higher in children than in adult; and higher in infants compared to older children. In children, the number of circulating T-cells increases from mid-gestation until the infant is about 6 months. This peak is followed by a gradual decline throughout childhood until adult levels are reached at 6 years of age.[7-9]

Several studies have documented inverse relationship between the age and $CD4^+$ T-lymphocyte count among children and higher values in younger children compared to older children and adults.[7-9] Reports on gender variation in $CD4^+$ T-lymphocyte are among adults cohorts and are mostly from the developed countries.[10-12] They have been inconsistent as some reported higher values among females while some reported no

significant difference between the genders. There is paucity of data on the effect of age and gender or even reference values for $CD4^+$ T-lymphocyte count in our community. This study was conducted to determine the effect(s) of the age and gender on the $CD4^+$ T-lymphocyte count and percentage among apparently healthy under-5 children seen in Sokoto, Nigeria.

Methodology

This was a prospective cross-sectional study conducted among apparently healthy well-nourished HIV-negative children aged between 6 months and 59.9 months who were seen at the immunization clinic and Paediatric Outpatient Clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto from January 1st to June 30th 2011. The hospital is a tertiary health facility in Sokoto, Northwestern Nigeria. It serves as a referral centre for other neighbouring states Zamfara, Kebbi and Niger.

The subjects were recruited consecutively. The age, gender and weight of these children were obtained. Each child was assessed for signs of acute febrile illness and active infection and malnutrition using World Health Organization's classification for protein-energy malnutrition.[13] The HIV infection was ruled with HIV-DNA polymerase chain reaction for those aged less than 18months and by Rapid Test kits (Unigold, Determine and Stat pak) for those aged 18 months and above. A child is HIV negative when the 3 testing kits were non-reactive. Those with febrile illness, HIV infection and malnutrition were excluded from the study and were referred to the appropriate units for treatment. The haematocrit, total leucocyte count and absolute lymphocyte count were obtained using Swelab Alfa 3-part haematology analyser (Boule Medical, Stockholm, Sweden, 2006), an Automated Coulter method. The $CD4^+$ T-lymphocyte counts and percentages were determined using Partec cytoflow machine. An informed consent was obtained from the parents or care-givers of the children and an approval was obtained from the Health Research Ethics Committee of the hospital. Data was analysed using SPSS statistical package version 20.0. A p-value ≤ 0.05 at 95% confidence interval was regarded as statistically significant.

Results

One-hundred children were studied over the 6 month period. The mean age (±SD) was 19.1±9.7months. There were 63 males and 37 females. The absolute lymphocyte count

among the studied population ranged from 1.2 – 3.8 × 10^9cells/L with a mean 2.1(±0.5) × 10^9cells/L.The mean absolute lymphocyte count was highest (4.8(±1.1) × 10^9cells/L) in children aged 6.0 – 11.9 months and lowest (3.2(±0.6) × 10^9cells/L) in the 24.1 – 35.9 months age group. The difference was statistically significant (F= 7.8, p=0.0001) as depicted in Table 1.

Table 1: The mean (±SD) value of some haematological parameters of the apparently healthy under-5 children in Sokoto.

Age Group (months)	n	Haematocrit (%)#	TLC(×10^9cell/L)*	ALC(×10^9cell/L)+
6.0 – 11.9	32	33.6±2.1	7.8±1.7	4.8±1.1
12.0 – 23.9	56	34.0±1.6	7.0±1.4	4.0±0.8
24.0 – 35.9	8	35.4±0.7	6.3±1.3	3.2±0.6
36.0 – 47.9	2	38.4±0.1	5.2±0.6	3.3±0.4
48.0 – 59.9	2	35.2±0.3	6.2±0.4	4.3±0.1

NB: n=Number of children, TLC= Total Leucocyte Count, ALC= Absolute lymphocyte Count. #means p=0.01;*means p=0.02 and +means p=0.0001

The mean $CD4^+$ T-lymphocyte count was highest (2675.7±464.5cells/µL) among those aged 6 – 11.9 months and lowest (1770.0±70.7cells/µL) among 48 –59.9 months of age group showing a negative correlation (r= - 0.52, *p*=0.0001) as shown in figure 1.

Table 2: Comparison of the Mean $CD4^+$ T-Lymphocyte Count in Apparently Healthy Under-5 Children seen in Sokoto based on Gender.

Age Group	Male (n)	Female (n)	t-test	p-value
6.0 – 11.9	2420.6±446.1(20)	2671.7±478.5 (12)	1.5	0.14
12.0 – 23.9	2031.3±453.2 (36)	1970.3±391.3 (20)	0.5	0.61
24.0 – 35.9	1746.6±282.3 (5)	1572.7±105.8 (3)	0.9	0.36
36.0 – 47.9	1720.0 (1)	1634.0 (1)	-	-
48.0 – 59.9	1620.0 (1)	1520.0 (1)	-	-
Total	2120.3±483 (63)	2144.8±553 (37)	0.2	0.83

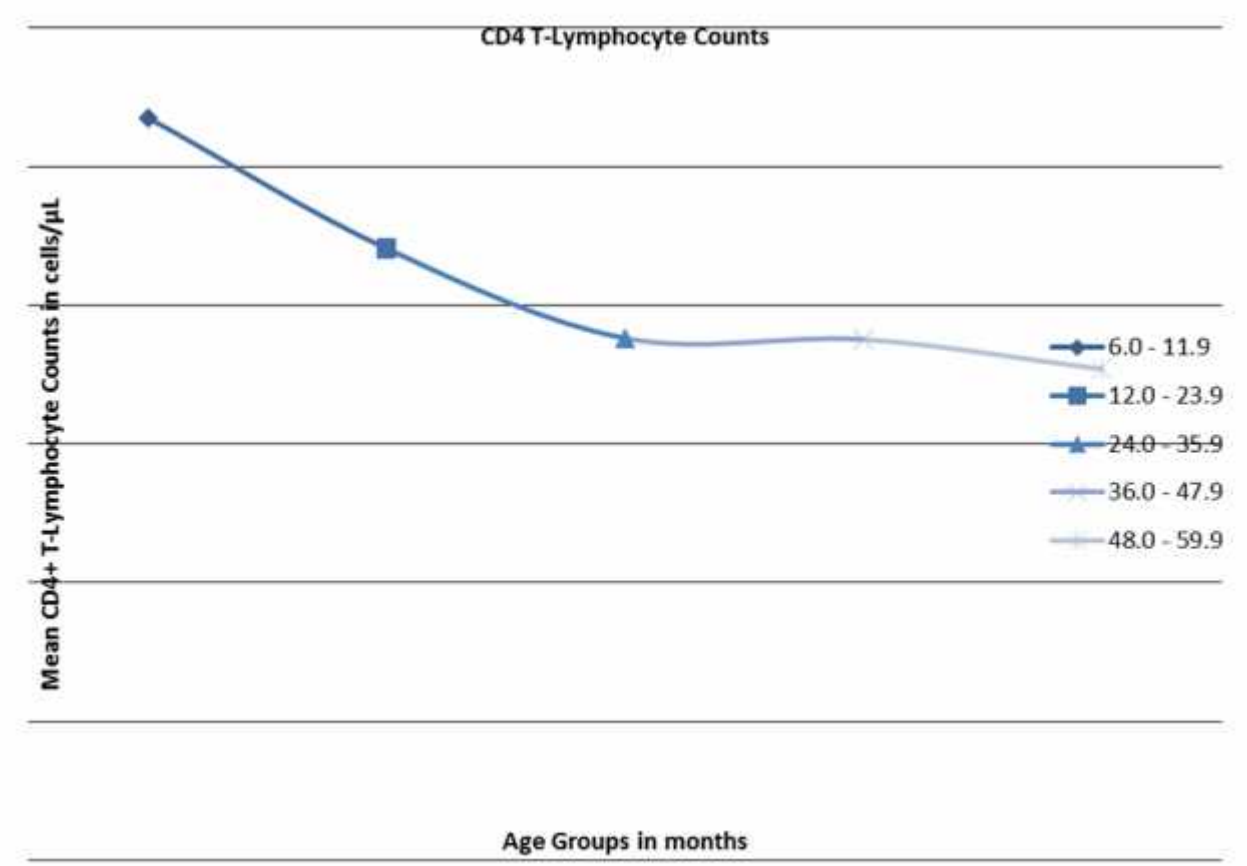

Figure 1: The mean $CD4^+$ T-Lymphocyte Counts in Apparently Healthy Under-5 Children in Sokoto.

There was no statistically significant difference in the mean $CD4^+$ T-lymphocyte count of the males (2120.3±483cells/µL) and the females (2144.8±553cells/µL) (t= 0.22, *p*= 0.83) as shown in the Table 2. The mean $CD4^+$ T-lymphocyte percentage ranges between 49.1% and 51.4% across the age groups; and it was 50.3% in both genders. There were no significant differences in the mean $CD4^+$ T-cell percentage across the age group (F= 0.28, *p*= 0.89) and between the gender (t=0.03,*p*=0.98).

Discussion

The findings of this study have demonstrated the effects of demographic factors such as age and sex on the $CD4^+$ T-lymphocyte count in apparently healthy

well-nourished under five children in our community using cytoflowmetric technique.

The $CD4^+$ T-lymphocyte count has been shown to be variable in this age group. In this study the mean $CD4^+$ T-lymphocyte count was shown to be decreasing with increasing age with significant negative correlation between the age and $CD4^+$ T-lymphocyte count. This inverse relationship is more evident in children aged 3yeras and below in this study probably because of the few subjects aged above 3years were enrolled for the study. This finding is similar to the pattern observed among the healthy young children as reported in previous studies both in and outside Nigeria.[7-9]The absolute $CD4^+$ T-lymphocyte counts for age in this series was within normal reference values reported among American[7] and Saudi Arabian children[8] but were higher compared to the values reported by Emmanuel *et al*[9] among healthy Nigerian children in 2009 in Lagos. The difference may be related to the machine (FACScount machine) used in enumerating $CD4^+$ T-cells count in their study. The machine could only determine count ≤2000 cells/µL and perhaps the larger sample size in Lagos series. The higher value of $CD4^+$ T-lymphocyte counts among infants compared to young children could be related to the higher absolute lymphocyte count among this age group as a positive correlation has been established between absolute lymphocyte count and the $CD4^+$ T-lymphocyte counts.[5] This observation implies that interpretation of the $CD4^+$ T-lymphocyte counts in under-5 children has to be age-adjusted and indirectly not reliable for monitoring of disease conditions such HIV infection.

In this series, the mean absolute $CD4^+$ T-lymphocyte was similar in both males and females. A similar finding was reported by Foca M and colleagues[14] in USA, but in contrast to that reported by Mandala and coworkers[15] who reported higher $CD4^+$ T-lymphocyte count among females compared to the male Malawian children. This shows that gender has no significant effect on the $CD4^+$ T-lymphocyte count in children in our community. Therefore, there is no need for different reference values for different gender in interpreting the CD4 T-lymphocyte count in both genders in children.

The $CD4^+$ T-cell percentage was comparable in all age groups below 5 years and between the genders as shown in this study. This is similar to earlier findings among under-5 children both in and outside Nigeria.[5-7] This implies that the $CD4^+$ T-cell

percent is relatively stable with no significant change in children below 5 years; hence, it is very useful and reliable as a guide in treatment decisions and monitoring of under-5 children with HIV infection. A limitation of this study was that the majority of the subjects recruited were aged less than 3 years with very few above 3 years.

Conclusion

In conclusion, age has depletive effect on the $CD4^+$ T-lymphocyte count; however, gender seems to have no significant effect on the $CD4^+$ T-lymphocyte count among under-5 children. The $CD4^+$ T-lymphocyte percentage is relatively similar among under-5 irrespective of the age or gender, and therefore, can be useful in treatment decisions and monitoring under-5 children with HIV infection. A large cohort multicentre study would be needed to establish normal reference values for $CD4^+$ T-lymphocyte subsets in our community.

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