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Determination of CD4+ T- Lymphocytes in Healthy Children of Kathmandu

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ABSTRACT

Background: The cluster differentiation (CD) of T-cell is the good marker for the immunological competence study. Nepal does not have a reference value for CD4+ T cell count and percentage for children, which severely limits the prospect of pediatric prognosis.

Methods: This cross-sectional study was conducted in Kathmandu valley where total 207 children of age 0-14 year age group were recruited in this study. We analyzed 50 cord blood and 157 peripheral blood samples in order to calculate the absolute count of CD4+ T lymphocyte using Fluorescence-activated cell sorting methodology.

Results: The reference range for absolute CD4+ T cell count was found to be 634-4040 cells/μL(mean1470; median: 1335 and 95% CI [1322-1617]) for male children and 491-2922 cells/μL (mean: 1443 median: 1326 and95% CI [1298-1588]) for the female children. We also observed elevated CD4 to the CD3 ratio in younger children (0.67 from cord blood Vs 0.53 from 10-14yr) compared to older ones.

Conclusions: The observed CD4+ T cell counts among healthy children of Kathmandu highlights the gender differences skewed for male as well the need of defining specific reference values for other lymphocyte subsets as well in a country like Nepal which has a population with diverse genetic and socio-cultural parameters.

Keywords: CD4+ T lymphocyte; children; HIV, immunophenotyping, Kathmandu; Nepal.

INTRODUCTION

Relevant clinical lymphocyte reference range is important for prognosis of HIV infection among children who are using ART. As there has been increased dependency of immune status for the treatment of these patients, reference data of lymphocytes subset population have been generated and recorded demographically around the world thus concluding the impact of climate, genetic predisposition and cultural and social environment.[1-4] Unfortunately, Nepal does not have any previous data to compare the CD4 counts of HIV infected children population of same stratified age group. This study was designed to establish the value of CD4+ T cells counts among healthy children (0-14yr). Nepal being a multi-ethnic country consisting of diverse socio-cultural, environmental and nutritional habits, has particular importance for establishing reference T-lymphocyte count for healthy Nepalese children CD4+ T cells population.

METHODS

This cross-sectional study was conducted in Kathmandu. Total 207 children of age 0-14-year group were recruited for this study. Blood was collected in EDTA (50 cord blood from Paropakar Maternity and Woman's Hospital, Thapathali, Kathmandu and 157 peripheral blood samples from different schools of Kathmandu). Samples were collected in between 10.00 AM to 1.00 PM from the collection site and transported to the National Public Health Laboratory (NPHL), Teku, Kathmandu. The CD4 count was performed on the same day using whole blood. After that the plasma was separated and stored at -20^{0}C for the further serological assay. Ethical approval was obtained from the Nepal Health Research Council (NHRC) to conduct this study. The study participants were informed by their guardian and oral/

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written consent was also obtained from the guardian on behalf of them.

The T cells subpopulations were analyzed by using a FACSCaliburflowcytometer (Becton, Dickinson and Company, California, USA). In brief, 20 µL of Multitest (CD3/CD4/CD45-TriTEST 3-color, Dickinson, and Company, California, USA) monoclonal antibodies were dispensed to Truecounttubes containing reference beads (Becton Dickinson Immunocytometry Systems). Whole blood (50 µL) was mixed and incubated at room temperature for 15 min in dark. Red blood cells were then lysed by adding 450 µL of fluorescence-activated cell sorter lysing solution (ACK buffer). The tubes were incubated at ambient temperature for 15 min followed by acquisition for the absolute count of subset profile ($CD3^+$, $CD4^+$, and $CD45^+$). The determination was done in four tubes with TriTEST and by calculating the ratio of regional events for each subset to bead events using the BD Biosciences-developed software, Becton Dickinson Immunocytometry Systems.The screening for HIV, HCV, HBV and Syphilis was done by using by ELISA (Wantai Co., China) and Latex agglutination method and if found positive were excluded from the study.

The health status and demographic data (income, nutritional status, family smoking environment, andethnicity) of the participants was assessed by a pre-decided questionnaire (not shown). Data generated in the BD Flow Cytometry (BD-Bioscience) was analyzed using Graph Pad Prism 6.0. The study participants were stratified into respective age group before the analysis. The mean, median, standard deviation and percentile (2.5^{th} and 97.5^{th}) was calculated for absolute CD4+ T cell count and percentages. Frequency distribution of absolute CD4+ T cell count was analyzed on the basis of gender. The Wilks-Shapiro test was used to analyze the normality of population and data were considered to have followed the non –Gaussian distribution. The reference range was defined as the 95% of the area under distribution curve were thus from 2.5 to 97.5%. The *p*-values less than 0.05 were considered significant unless stated otherwise.

The children with history of recent morbid conditions, any sickness within past one month, major surgery and blood transfusion within the last 6 months, and with known chronic illnesses as well as those with a history of fever, cold or cough in previous 2 weeks, using medication and who tested positive for HIV, HCV, HBV and Syphilis antibodies were not included in the study.

RESULTS

Total of 207 healthy children (0-14yr) was prospectively enrolled in the study with 1:0.99 ratio of male (n=103) to female (n=104). The total mean absolute CD4+ T cell count was 1446 ±750 cells/µL (median 1326, range: 415-4387 cells/µL). Ninety-one percent of the study population showed absolute CD4 counts between 400-2500 cells/µL (Table 1).The absolute CD4+ T cell count in male (mean: 1487 ±841, median: 1215 cells/µL) were significantly higher than CD4+ T cell count in female (mean: 1406 ±645, median: 1355cells/µL). The age wise absolute CD4+ T cell count from male and female participants are given in table 1. The values were significantly higher in male than female participants intheage group 0-2 years ($p<0.001$) but other age groups did not differ significantly (Table 1). The reference range for healthy Nepali child (0-14yr) for male population was 634-4040 cells/µL and for the female population 491-2922cells/µL. CD4+ cell count in the cord blood of both sexes though did not show much difference, however, the CD4+ cell count in male children were found gradually decreasing from young individuals (cord blood, median value 2313) to older ones (14 years of age, median value 891). In girl population, the count remains almost similar till the age of 10 however, it decreased significantly (<0.001) from the age 10 till 14.

The total mean CD4+ T cell percentage was 40±9 % (median: 39%, range: 23-58%). We observed significantly higher CD4+ T cell percentage in male participants (mean: 41±8%, median: 40%) compared to female participants (mean: 39±10%, median: 37%). The CD4+ T cell percentage was significant among males of 6-10-year age group as compared to female from the same age group. The reference range for CD4% for Nepali male children population was 23-56% and for female children, population was 24-60% (Table 2).

The overall mean absolute CD3+ T cell count was 2420±1166 cells/µL (median 2255, range: 705-7498 cells/µL). The absolute CD3 + T cell counts in male participants were significantly higher than CD3+ T cell counts inafemale in age group 0-2yr (mean absolute CD3 counts: 4032±1894 in male and 2241±1742 inthefemale, $p < 0.001$) (Table 3). The CD3 counts of the participants from 0-2yr age group(mean: 3162±2012, median: 2817 cells/µL) were significantly higher than the CD3 counts of the participants from the other regions age group with the lowest CD3+ T cell counts in the participants from the 10-14yr age group (mean: 1879±422, median: 1784 cells/µL). The reference range for absolute CD3+ T cell count for Nepali male population was 1033-6176 cells/µL and for the female population, it was 852-4440 cells/µL. The ratio of absolute CD4 count to absolute CD3 count in cord, 0-2yr, 2-6yr, 6-10yr and 10-15yr blood was 0.67, 0.63, 0.56, 0.53 and 0.53 respectively (Figure 1).

Table 1.The absolute CD4 count in the Nepali children (0-14yr) population.

	Male			Female			Total		
Age	No. Of Samples	Mean±SD	2.5th-97.5th percentile	No. Of Samples	Mean±SD	2.5th-97.5th percentile	No. Of Samples	Mean±SD	2.5th-97.5th percentile
Cord blood	24	1532±814	598-3182	26	1686±698	695-2991	50	1612±752	641- 3455
0-2 years	18	2542±1062	950-4387	17	1420±1036	415-3597	35	1997±1180	415-4130
2-6 years	20	1377±479	824-2474	21	1437±359	930-2077	41	1408±418	827-2343
6-10 years	20	1106±408	717-1988	19	1404±503	675-2256	39	1251±475	712-2082
10-14 years	21	997±312	565-1593	21	1019±302	610-1505	42	1008±303	588-1549
Total	103	1487±841	634-4040*	104	1406±645	491-2922*	207	1446±750	546-3716

**significant at p<0.05*

Table 2. The CD4 percentage in the Nepali children (0-14yr) population.

	Male			Female			Total		
Age	No. of Samples	Mean±SD	2.5th - 97.5th percentile	No. of Samples	Mean±SD	2.5th - 97.5th percentile	No. of Samples	Mean±SD	2.5th - 97.5th percentile
Cord blood	24	45±10	31-65	26	44±11	31-65	50	45±10	24-67
0-2 years	18	41±10	17-56	17	43±12	23-63	35	42±11	20-58
2-6 years	20	40±5	31-65	21	37±5	31-66	41	39±5	31-65
6-10 years	20	38±6	27-49	19	32±8	23-45	39	35±7	23-49
10-14 years	21	37±5	23-58	21	36±5	28-44	42	36±5	29-47
Total	103	41±8	23-56*	104	39±10	24-60*	207	40±9	23-58

**significant at p<0.05*

Table 3.The absolute CD3 count in the Nepali children (0-14yr) population.

Male	Female			Total					
Rage	N	Mean±SD	2.5th-97.5th percentile	N	Mean±SD	2.5th - 97.5th percentile	N	Mean±SD	2.5th- 97.5th percentile
Cord blood	24	2299±1072	997-4669	26	2451±1222	1137-4881	50	2374±1143	1026-5319
0-2 years	18	4032±1894	1149-6922	17	2241±1742	705-6408	35	3162±2012	705-7008
2-6 years	20	2556±840	1548-4334	21	2426±575	1516-3450	41	2489±710	1524-3804
6-10 years	20	2178±554	1490-3303	19	2470±816	1299-3896	39	2320±701	1302-3564
10-14 years	21	1931±462	1208-2794	21	1827±382	1236-2454	42	1879±422	1222-2535
Total	103	2553±1263	1033-6176*	104	2289±1050	852-4440*	207	2420±1166	916-5719

**significant at p<0.05*

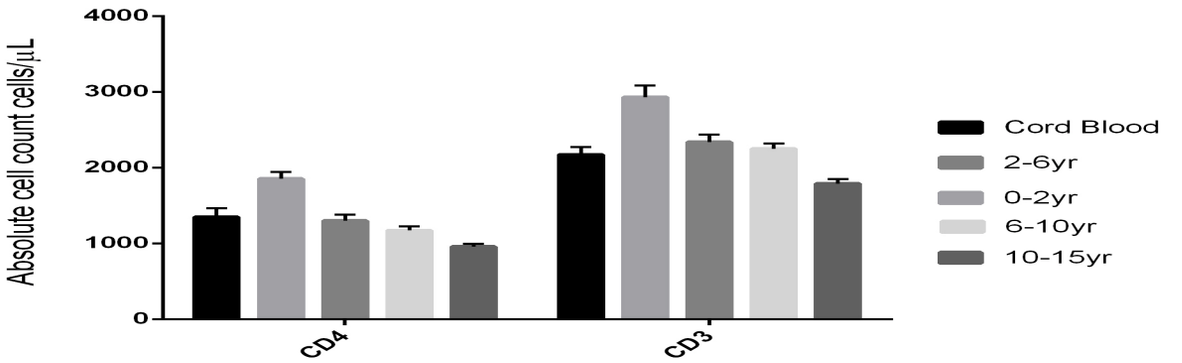

Figure 1. Absolute cell count comparison between CD4+ and CD3 subtypes (error bars represent standard deviation of the mean).

DISCUSSION

Reliable lymphocyte range data in normal infants has become indispensable for taking care of childhood diseases[6]. This study has determined the CD4 count of healthy children (aged 0-14 years) of Kathmandu valley of Nepal. The result shows variation among CD4 mean absolute count in different age group children and the children growing in diverse behavioral and socio-economic surroundings. Cord blood mean CD4 count was observed higher than that of Brazil,[7] but lower to Cameroon.[1] The mean CD4 count in male and female children (2-6yr) in this study is slightly higher than that of United States, Hong Kong, Cameroon, and Italy.[1,6,8,9]

The observed differences in CD4 values in different age group in our study might be due to the heterogeneity of population as a result of ethnic-racial variations (Table 4) and probably due to the differences in the instruments used in different laboratory to measure CD4 count in Nepal.[10] The difference between the results obtained from our population with other studies indicates that it is necessary for each geographical boundary to establish an internal range for these markers. From neonatal period to childhood, the percentage of CD4 showed less variation than that of CD4 count (Table 2). This shows that percentage of CD4 count can be preferred as the best surrogate marker for monitoring HIV-infected children of 0-14-year age. In this study, the mean CD4 value was found to be higher in male compared to female; while the CD4 % was found to be higher in female than male. Also, the CD4 to CD3 ratio was decreasing as per increase in age. The CD4+ T cell population count in male children was found gradually decreasing from neonatal up to the 14 years age group, however, consistency was observed in female population till the age of 10 years. The variation in this cell population from male to female may be due to the effects of hormones, modulation of thymic involution by sex hormones.[11,12]

Also, in view of the statistical difference, in both genders during childhood, it is imperative to establish a reference range separately for male and female population. The demographic and genetic factors, infections and behavioral factors have been reported to be associated with variations in CD4 cell counts of healthy individuals.[8] The data obtained in this work clearly shows that the nutritional status, economic status of family and smoking behavior of parents haveadirect significant impact on the children's' CD4+ T cell counts (Table 4). However, the ethnicity was found to be very least influential to these immune cells. Immunophenotypic changes of lymphocytes from birth throughout adulthood have been examined in a small number of studies. One of the limitations of our study is the small sample size. However, the significant difference obtained in the comparison between the populations indicates that it had enough power to detect variations in the studied variables. Hence, the observed differences for reference ranges in CD4+ T lymphocytes populations demonstrate the need of defining specific reference values of other lymphocytes as well. In our study, asymptomatic infectious diseases if any like tuberculosis and pneumonia were not ruled out, which needs to be targeted in future studies in Nepalese population.[13, 14]

CONCLUSIONS

Nepal being a small country with diversity in population composition has probably diverse immune functions and alterations foraspecific set of infection conditions. Especially in HIV PMTCT, the observed reference value of CD4 T cell in healthy children would be useful for diagnosing the progress of ART in children with HIV. This baseline will assist in monitoring and reschedule the effectiveness of ART in coming future.

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