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The Reference Ranges for Lymphocytes Subsets of Healthy Adults individuals by Immunophenotyping.

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ABSTRACT

***Introduction and aims*:** Immunophenotyping by flow cytometry is the most advance and accurate technique for differentiation between lymphocyte sub populations of B and T cells. This descriptive cross-sectional study was performed to establish the normal reference ranges for several lymphocyte sub-populations of healthy adult Sudanese: (CD3, CD4, CD8 and CD19). ***Materials and Methods*:** Peripheral blood samples (n = 100) were collected from healthy adult Sudanese from Khartoum State, Sudan. Their mean age was (31± 13) years ranged from (18 to 80) years, and their sex ratio male to females was (1:1). The hematological parameters of them were analyzed by hematology analyzer (Sysmex). The flow cytometry was used to determine percentages and absolute count (cell/µl) of CD3, CD4, CD8 T cells and CD19 B cell**.** SPSS version 16 was used for statistical analysis. ***Results*:** The percentages and mean absolute count (cell/µl) of lymphocytes and lymphocyte sub-populations were obtained as follows: lymphocytes (35.8% ± 7.3 ; 2120.1 ± 652), CD3 (58.9% ± 14.7 ; 1242 ± 481), CD4 (35.9 % ± 20.1% ; 761 ± 315), CD8 (18.1% ± 11.1% ; 425 ± 195), CD19 (9.8% ± 5.4%; 214 ± 141). The CD4/CD8 ratio was found to be (1.98 ± 0.85). ***Conclusion*:** there was no significant difference according to sex and age in different CD values obtained.

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INTRODUCTION

The normal reference ranges or the absolute count for certain T and B subsets designated by their cluster differentiation are used to differentiate between different diseases. Higher or lower absolute count for these values for example low absolute count for CD4 indicates infection by HIV, also higher absolute count or values for other cells indicated certain or specific type of leukemia. Accordingly, set up or determination of the normal reference range is very important for each country to differentiate between healthy individuals and other leukemic patients (Bibhu *et al.*, 2008).

The normal reference ranges for different parts of the world were cited below. It showed variation between different countries due to various factors not ethnic only. However, variable reagents, techniques and equipments (Flow cytometers models) were used by different researchers in different countries. With regard to lymphocytes percentages, a study in India (Sexana, *et al*. 2003) showed that the percentages of CD3, CD4, CD8, CD4/CD8 and CD19 were 68.65%, 37.10%, 34.04%, 1.2% and 14.67% respectively, while in Oman (Al Jabri, *et al.* 2005) revealed 68.53%, 40.40 %, 25.8% , 1.6% and 13.7% respectively. Relatively low percentages for CD3 (54.9%), CD8 (11.5%) and CD19 (4.7%) were reported by (Bisset *et al.*2004) in Switzerland.

As far as the absolute count (mean± SD) is concerned, Al Qouzi *et al.* (2002) in Saudi Arabia reported 1618 ± 547, 869 ± 310, 615± 276, 1.6 ± 0.7, 230± 130 and 262± 178 for CD3, CD4, CD8, CD4/CD8 for T cell ,CD19 B cell and CD16 NK cells respectively. In Oman Al Jabri, *et al.(* 2005) reported 1701± 489 for CD3, 1006 ± 319 for CD4 , 638± 225 for CD8, 1.6± 0.8 for CD4/CD8, 349± 158 for CD19 and 221±115 for CD16. While in Turkey, Yaman *et al.* (2005) reported 1680±528, 1095±391, 669±239, 1.68±0.43 and 254±122 counts respectively. In Ethiopia an absolute count was made for all lymphocytes except T cell CD3 by Tsegaye *et al.* (1999) their study showed counts of 753±227, 777± 362, 1.1±0.4 and 184±96 for CD4, CD8, CD4/CD8 and CD19 B cells respectively. A similar study was performed in Saudi Arabia by Shahabuddin *et al.* (1996). The

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study illustrated counts of 880 ±270, 890±290, 1.1±0.3 and 290±90 respectively. In Dutch land Tsegaye *et al.* (1999) results revealed 993±319, 506±220, 2.2±1.0 and 313±147 respectively.

Flow cytometry is an important tool for diagnosis of hematological and immunological disorders. Flow cytometric immunophenotyping forms the basis for modern classification of acute and chronic leukemias. The reference values for different lymphocytes subpopulations in peripheral blood of healthy individuals, has to be establish for each country with regard to age, sex and race. This invaluable diagnostic information will greatly contribute for therapy selection and for determining prognosis and can help in the detection of relapse or of secondary leukemia. The importance of this study, that it will establish for the first time the normal reference values of lymphocytes sub set populations for Sudanese healthy individuals in comparison to WHO values. This also will help for diagnosis, treatment and follow up of other diseases causing lymphocytosis or lymphocytopenia.

The normal reference value for T and B subset populations (CD3, CD4, CD8 and CD19) now a day in use for diagnosis of leukemia in Sudan based on WHO Western or European standard, which might be different for ours (genetic make up, race, age, sex, geography, and environmental conditions). Even with the advent of large multicenter therapeutic trials for the determination of chemotherapeutic efficacy, individual variability in tumor characteristics often leads to a poor therapeutic outcome.

So the main objective of this study is to establish the normal reference values and ranges for T and B lymphocytes subset populations, for Sudanese healthy individuals (n, 100). After that we can compare the normal reference range of Sudanese healthy individuals with those of other countries. Moreover the study aimed to determine the normal range in association with age and sex.

MATERIALS AND METHODS

This descriptive cross sectional community based study was carried at Khartoum state from May to September 2010. The practical flow cytometry was done at Radiation and Isotopes Center of Khartoum (RICK) that located at the south west part of Khartoum teaching hospital. RICK is main hospital which provides diagnosis, chemotherapy and radiotherapy for cancer patients. The study aims were explained to all participants and their consent was obtained and a questionnaire was filled.

Blood samples were randomly collected from adult healthy individuals from Khartoum state, their ages ranged from 18 to 80 years. Their sex ratio male to female was (1:1) Venous peripheral blood samples (n = 100) about 2.5 ml of blood were collected from each adult individual in EDTA vacontainer (3ml) and were mixed gently. Complete Blood Count (CBC) was done for all samples by Hematology analyzer (Sysmex corporation), which performs blood cell count by DC detection method. After that sample preparation for immunophenotyping by the flow cytometry was done for all blood samples as follow: 100 µl of anticoagulated (EDTA) blood was transferred to 12 X 75 mm polystyrene test tube (10^4 cells). Then 20 µl of antibody was added and well mixed with vortex mixer. Then the sample was incubated in the dark place at room temperature at (20-25C) for 15 minutes. 1.5 ml of lysing Solution was added to each sample and well mixed with a vortex mixer. After that was incubated for 10 minutes at room temperature in the dark place. Then centrifugation at 1500 RPM for 5 minutes was performed. Then the supernatant was aspirated and discarded leaving approximately 50 µl of fluid. Which resuspend in 2 ml (0.01 mol/l Phosphate buffer saline (PBS)) by using vortex mixer. Centrifugation at 1500 RPM for 5 minutes was performed. Then the supernatant was aspirated and discarded leaving approximately 50 µl of fluid. Finally the pellet was resuspend in an appropriate fluid for flow cytometry to analyze on a flow cytometer.

Data of all samples were acquired on Beckman Coulter EPICS XL Flow cytometer using system II software (Beckman Coulter, Miami, FL, USA). Alignment was check daily using flow-check (Miami, Fl, USA).Flow–Set (Miami, Fl, USA) was used weekly to monitor the standardization. Compensation was checked continually with normal lymphocytes and adjusted if required. Moreover negative control **(**Code No. ISOCONTFITCIGG2a) antibody was used. All parameters under measurement were calculated within the gated lymphocyte region from forward scatter / side scatter (FS/SS) histogram.

Results:

Table 1: Absolute counts of lymphocytes and lymphocyte subsets (CD3, CD4, CD8 and CD19) of healthy males (n = 50) and females (n= 50)

Cells	Absolute counts cells/µl			
	Gender	Mean	± SD	p- value
Lymphocyte	Female	2211.56	621.68	*0.164*
	Male	2028.64	682.39	
	Both	2120	652.02	
	Female	1327.55	543.26	*0.76*

CD3 +ve cell (T cells)	Male	1156.68	397.15	
	Both	1242	470.20	
CD4 +ve cell (T helper cells)	Female	804.94	347.57	*0.164*
	Male	716.92	276.27	
	Both	761	312	
CD8 +ve cells (T cytotoxic cells)	Female	467.68	216.25	*0.027*
	Male	381.72	162.08	
	Both	424.7	189.2	
CD19 +ve cells (B cells)	Female	233.55	135.61	*0.168*
	Male	194.42	146.15	
	Both	214	140.9	

Table 2: Percentages of lymphocytes subsets (CD3, CD4, CD8, and CD19) in 100 healthy males and females.

Gender	Cells	Percentages (%)			
		Mini	Max	Mean	± SD
Female (n=50)	Lymphocytes	19	47	35.3	7.2
	CD3 +ve cells	18.5	84.5	59.4	16.1
	CD4 +ve cells	10.7	65.7	38.0	11.8
	CD8 +ve cells	4.86	32.4	17.4	5.6
	CD19 +ve cells	2.86	20.8	10.2	4.1
Male (n=50)	lymphocytes	19	47	36.3	7.5
	CD3 +ve cells	17.6	76.7	58.3	13.2
	CD4 +ve cells	9.43	55.4	33.7	9.4
	CD8 +ve cells	5.64	36.6	19.9	6.4
	CD19 +ve cells	2.19	45	9.4	6.6

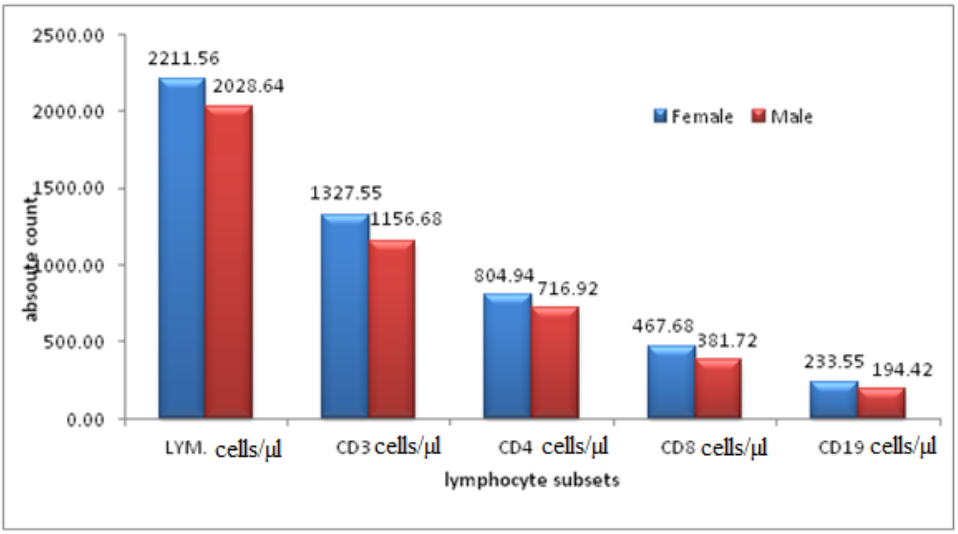

Fig. 1: Absolute counts for lymphocytes and lymphocyte subsets (CD3, CD4, CD8 and CD19) with regard to gender.

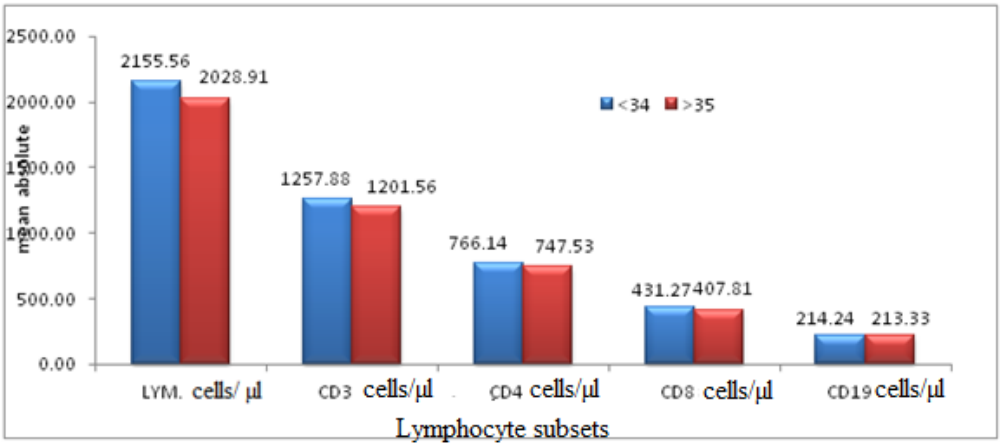

Fig. 2: Absolute counts for lymphocytes and lymphocyte subset (CD3,CD4,CD8 and CD19) with regard to age group.

Percentages and absolute count of CD3+/CD4-/CD8-

The percentage and absolute count for CD3+/CD4-/CD8- was found to be (3.19% ± 1.97; 64.7 ± 40.2).

Percentages and absolute count of CD3+/CD4+CD8+

The percentage and absolute count for CD3+/CD4+/CD8+ was found to be (0.7% ± 0.59; 15.2 ± 14.4).

Discussion:

Lymphocytes:

Information on lymphocyte subsets populations in Sudan is lacking. Hence, this study aimed to provide the normal ranges of percentage and absolute counts of lymphocyte subsets; T cells (CD3, CD4, CD8 and CD4/CD8) and B cells (CD19) of healthy Sudanese adults. The major finding of this study that it established and defined the normal reference ranges for the most important lymphocyte sub-populations of healthy adult Sudanese using Immunophenotyping (Flow cytometry), that will be applied for the first time for diagnosis, treatment and follow up of patients with AIDS and leukemia in Sudan. The percentages (%) and mean absolute count of lymphocytes sub-populations (cells/µl) were obtained as follows**:** lymphocytes (35.8% ± 7.3 ; 2120.1 ± 652), CD3 (58.9% ± 14.7 ; 1242 ± 481), CD4 (35.9 % ± 20.1% ; 761 ± 315), CD8 (18.1% ± 11.1% ; 425 ± 195), CD19 (9.8% ± 5.4% ; 214 ± 141) and the CD4/CD8 ratio (1.98 ± 0.85). Moreover, this research did not reveal any significant influence for age and or sex.

CD3:

Great variation for CD3 mean absolute count was reported even in the same cotenant such as Asia (see Table 1.3). The CD3 absolute count of Sudanese (1242) was less than those reported in India (1881), Oman (1701), Turkey (1680), and Saudia Arabia (1618), Malaysia (1599), Singapore (1590), China (1547) and Hong Kong (1370) (Chng *et al.*, 2004; Al Jabri *et al.*,2005; Yaman *et al.*, 2005 and Al Quzi *et al.*, 2002).

The Sudanese CD3 count was near to those reported for Senegal (Mair *et al.*, 2007). However, other data from Africa and Europe were not available. That African may have lower CD3 count, however ethnic, genetic composition, and geographic differences might be the reason.

CD4:

The present study revealed great variation in CD4 mean absolute count in the world specially Asian and African countries (see Table 1.3). The CD4 count was high in Asian than in African and not low as reported by Menarad *et al.*, 2003. The highest CD4 mean absolute count was reported in Turkey (1095) and the lowest in Senegal (711) which was almost similar to **Sudanese (760).** The Sudanese CD4 absolute count was similar to those reported from other African countries but less than those of Arabic countries (Saudi and Oman). The Sudanese CD4 values were intermediate between Asian and African countries. The CD4 absolute count were arranged from the highest to the lowest as follows; Turkey (1095), Oman (1006), Dutch Land (993), India (958), Central African Republic (934), Saudia (869), Malaysia (856), Singapore (838), China (812), **Sudan (760)**, Botswana (759), Ethiopia (753), Tanzania (746), Hong Kong (725), and Senegal (711) (see Table 1.3 for values) (Yaman *et al.*, 2005; Al Jabri *et al.*,2005; Tsegaye *et al.*, 1999; chng *et al.*, 2004; Menarad *et al.*, 2003; Al Quzi *et al.*, 2002; Bussmann *et al.*, 2004; Ngowi *et al.*, 2009 and Mair *et al.*, 2007).

The different CD4 values indicated the heterogeneity of the African populations, racial and genetic makeup also might contribute, in addition to the variable methods applied (Bofill *et al.*, 1992). Moreover Smoking increase CD4 count but height and underweight may decrease it (Mair *et al.*, 2007).

This obtained the normal range for CD4 subset mean absolute count value among healthy Sudanese adults was (760 ± 315) cells/µl. This was significantly lower than what has been reported for Caucasians (844 ± 247 cells/µl) (Lebranchu *et al.*, 1991) and is consistent with previous findings from Saudi Arabia and Singapore. This finding is important because CD4 lymphocyte counts are used for clinical classification, to determine prognosis, and to decide whether to prescribe prophylaxis for opportunistic infections.

CD8:

Concerning the mean absolute count for CD8, the highest value was reported from Central African Republic (807) and the lowest value was from Tanzania (504) (Menarad *et al.*, 2003 and Ngowi *et al.*, 2009). It is very difficult to draw a clear conclusion or idea about this matter in Asia and Africa. The mean Absolute count for CD8 in the **Sudan (424)** was the lowest value in the World. It was similar to other reports from the some African countries and China. The CD8 mean absolute counts for some countries were listed below starting from the highest value; Central African Republic (807), Ethiopia (777), India (707), Turkey (669), Malaysia (661), Singapore (642), Oman (638), China (629), Saudia (615), Hong Kong (589), China (540), Senegal (520), Botswana (509), Dutch Land (506), Tanzania (504) and **Sudan (424)** (see Table 1.3) (Menarad *et al.*, 2003; Tsegaye *et al.*, 1999; chng *et al.*, 2004; Yaman *et al.*, 2005; Al Jabri *et al.*,2005; Al Quzi *et al.*, 2002; Jiang *et al.*, 2004; Mair *et al.*, 2007; Bussmann *et al.*, 2004 and Ngowi *et al.*, 2009). Different flow cytometers versions and reagents were used in addition to other factors listed before. This indicated the

importance of this unique study, that it established the normal reference CD8 mean absolute count for CD8 in Sudan. Due to the great variation for these values in different countries, each country must establish its own normal reference mean absolute count for lymphocytes subsets.

The present study found that the normal range for CD8 in Sudanese was lower than all those reported before in Africa and the World (See Table 1.3). Variation in the mean CD8 absolute count may be attributed to acute or chronic viral infection (hepatitis), persistent chronic antigenic stimulation, endemic infectious diseases (tuberculosis intestinal and parasitic infections. as helminthes). Also other factors such as genetic heterogeneity, ethnic composition (racial and interracial differences), altitude, poor nutrition and physical exercise could not be ruled out. Recently, Clerici *et al.*, 2000 demonstrated that immune activation in Africans is environmentally driven and not genetically predetermined**.**

CD19:

Concerning the mean absolute count for CD19, the highest value was reported from India (514) and the lowest value was from Ethiopia (184) (Chng *et al.*, 2004, Tesgaye *et al.* 1999). It is very difficult to draw a clear conclusion or idea about this matter in Asia and Africa. The mean Absolute count for CD19 in the **Sudan (214)** was close to the lowest value in the World. The CD19 mean absolute counts for some countries were listed below starting from the highest value; India (514), Malaysia (422), Singapore (353), Oman (343), China (330), Deutch land (313), Turkey (254), Saudi (230), Hong Kong (221), Sudan (214) and Ethiopia(184) (see Table 1.3) (Chng *et al.*, 2004; Al Jabri *et al.* 2005; Tesgaye *et al.* 1999*;* Yaman *et al.* 2005 and Al Quzi *et al.* 2002). Different flow cytometers versions and reagents were used in addition to other factors listed before. This indicated the importance of this unique study, that it established the normal reference CD19 mean absolute count for CD19 in Sudan. Due to the great variation for these values in different countries, each country must establish its own normal reference mean absolute count for lymphocytes subsets.

CD4/CD8:

The highest CD4/CD8 ratio was reported in Dutch Land (2.2) and the lowest one in Ethiopia (1.1) (Tesgaye *et al.*, 1999). The CD4/CD8 ratio for Sudan was very high (1.98) and immediately after Dutch Land. These ratios were quite different from country to country or cotenant. However, this study obtained very high CD4/CD8 ratio (1.98). The CD4/CD8 ratios for different parts of the world were listed below starting from the highest one; Dutch Land (2.2), **Sudan (1.98)**, Senegal (1.7), Oman (1.68), Botswana (1.63), Tanzania (1.6), Saudia (1.6), China, (1.49), Central Africa republic (1.35), India (1.2) and Ethiopia(1.1) (See Table 1.3) (Tesgaye *et al.*, 1999; Mair *et al.*, 2007; Al Jabri *et al.*,2005; Bussmann *et al.*, 2004; Ngowi *et al.*, 2009; Al Quzi *et al.*, 2002; Jiang *et al.*, 2004; Menarad *et al.*, 2003 and Saxena *et al.*, 2003).

Sex:

In this study the mean absolute counts for all investigated lymphocytes and the lymphocyte subsets CD3, CD4, CD8, CD19 and CD4/CD8 were higher in females in comparison to males, though the difference is not statistically significant (Table (1) and Figure (1)). These findings were in consistent with those reported before in Africa; Senegal, Central African Republic, Tanzania, and Botswana (Mair *et al.*, 2007; Menard *et al.*, 2003; Ngowi *et al.*, 2009 and Bussmann *et al.*, 2004) (Table 1.3). The variation from male to female may be due to the effects of hormones, modulation of thymic involution by sex hormones.

The demographic and genetic factors, infections and behavioral factors have been reported to be associated with variations in CD4 cell counts of healthy individuals (Clerici *et al.*, 2000). Healthy African and Asian populations typically have lower CD4 lymphocyte counts than their western European and Caucasian counterparts.

Paradoxically, cigarette smoking has been associated with higher CD4 counts in several studies. Underlying infectious diseases, such as pneumonia and tuberculosis (TB), have been associated with decreased CD4 levels. In western populations, black race, low body mass index (BMI) and injection drug use have also been associated with lower CD4 lymphocyte counts and women tend to have CD4 levels 1–200 cells/µl higher than men with comparable demographic and behavioral patterns (Reichert *et al.*, 1991).

Age:

The present study did not revealed significant differences, between subjects aged $\leq$ 34 and $\geq$ 35, and concerning the absolute counts for all investigated lymphocyte subsets CD3, CD4, CD8, CD19 and CD4/CD8 (Table 4.2a and Figure 4.5). Similar findings were reported by many authors, that age did not significantly influence the lymphocyte sub-population absolute count (Chng *et al.*, 2004; Jiang et al., 2004). In this study lymphocytes and specially T lymphocytes subsets increased slightly in younger ($\leq$34years).

Conclusion:

This study established for the first time the normal reference ranges (percentages (%) and absolute counts cell/µl) for healthy Sudanese individuals. The percentages and absolute counts for lymphocytes and lymphocyte subsets were as follows **lymphocyte** (35.8% ± 7.3 ; 2120.1 ± 652) **CD3** (58.9% ± 14.7, 1242 ± 481), **CD4** (35.9 % ± 20.1% , 761 ± 315), **CD8** (18.1% ± 11.1%, 425 ± 195), **CD19** (9.8% ± 5.4%, 214 ± 141). The C**D4/CD8** ratio was found to be (1.98 ± 0.85). As reported before this study didn't reveal any significant differences due to age and gender.

Recommendations:

- To apply the findings for the normal reference values for diagnosis, treatment and follow up of patients with AIDS and leukaemia.
- New study with larger sample size that will consider age, ethnic, and sex of Sudanese individuals from different parts of Sudan should be conducted.
- Special study for normal range is needed for children due to the increasing numbers of those patients.
- Further research to study the different types of leukaemia in comparison to the normal range must be conducted.

REFERENCES

Al Jabri A.A., A.K. Al-Shukaili, Z.T. Al-Rashdi and S.S. Ganguly, 2008. Reference ranges for lymphocyte subsets in healthy adult male Omanis. *Saudi Med J.* (3): 409-412.

Al Qouzi, A.A., R. Al Salamah, A. Al Rasheed, K. Al Musalam, O. Al Khairy, S. Kheir, A. Al Ajaji and H. Hajeer, 2002. Immunophenotyping of peripheral blood lymphocytes in Saudi men. Clin. Diagn. Lab. Immunol., (9): 279-281.

Benett, J.S., 2005. Structure and function of the platelet integrin alphaII beta 3. J. Clini. Investigation., (115): 3363-3369.

Bibhu R. Das., A. Aparna, R. Bhanushali, K.D. Khadapkar, Jeswani, Mita Bhavsar, A. Dasgupta, 2008. Reference ranges for lymphocyte subsets in adults from western India: Influence of sex, age and method of enumeration India. J. of med. Sci., (62): 397-406.

Bisset, L.R., T.L. Lung, M. Kaelin, E. Ludwig and R.W. Dubs, 2004. Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. Eur. J. Haematol., (72): 203-212.

Bofill, M., G. Janossy, C.A. Lee, D. MacDonald-Burns, A.N. Phillips and C. Sabin, *et al*., 1992. Laboratory control values for CD4 and CD8 T lymphocytes. Implications for HIV-1 diagnosis. Clin. Exp. Immunol., *(*88): 243–252.

Brown, M. and C. Wittwer, 2000. Flow Cytometry: Principles and Clinical Applications in Hematology. *Clini. Chem.*, (46): 1221-1229.

Bussmann, H., C.W. Wester, K.V. Masupu, *et al.*, 2004. Low CD4+ T-lymphocyte values in human immunodeficiency virus-negative adults in Botswana. Clin. Diagn. Lab. Immunol., 11: 905-930.

Chisari, F.V., I.F. Ruggeri, *et al*., 2005. Platelets mediate cytotoxic T lymphocyte–induced liver damage. *Nature Medicine*., (11): 1167-1169.

Chng , W.J., G.B. Tan, P. Kuperan, 2004. Establishment of adult peripheral blood lymphocyte subset reference range for an Asian population by single-platform flow cytometry: influence of age, sex, and race and comparison with other published studies. Clin. Diagn. Lab. Immunol., 11: 163-173.

Clerici, M.S., and M. Lukwiya, *et al.*, 2000. Immune activation in Africa is environmentally-driven and is associated with upregulation of CCR5. Itali.–Ugand. AIDS Proj., (14): 2083-2092.

Fujimoto, M., Y. Fujimoto, J.C. Poe, P.J. Jansen, C.A. Lowell, A.L. De Franco *et al.*, 2000. CD19 regulates *src* family protein tyrosine kinase activation in B lymphocytes through processive amplification. *Immuni.* (13): 47.

Ishikawa, H., N. Tsuyama, M.S. Mahmoud, *et al.*, 2003. "CD19 expression and growth inhibition of tumours in human multiple myeloma.".Leuk. Lymphoma., (43): 606-613.

Jiang, W., L. Kang, *et al.*, 2004. Normal values for CD4 and CD8 lymphocyte subsets in healthy Chinese adults from Shanghai. Clin. Diagn. Lab. Immunol., (11): 811-813.

Kaleem, Z., E. Crawford, H. Pathan, L. Jasper, M.A. Covinsky, L.R. Johnson and G. White, 2003. Flow cytometric analysis of acute leukemias. Archi. Pathol. Lab. Med., (42): 127-142.

Kassa, A.T., T.T. Messele, E.T. Hailu, R.D. Sahlu, A.L. Fontanet and T.F. Rinke, 1999. Immunohematological reference ranges for adult Ethiopians. Clin. Diagn. Lab. Immunol., (6): 410-414.

Lebranchu, Y.G., D. Thibault and P. Bardos, 1991. Abnormalities in CD4+ T lymphocyte subsets in patients with common variable immunodeficiency. Clin. Immunol. Immunopathol., (61): 83-92.

Mair, S.E., H.D. Hawes, P.S. Agne, I.N. Sow, L.E. doye, P.L. Manhart, F.G.S. Gottlieb and N.B. Kiviat, 2007. Factors associated with CD4 lymphocytes count s in HIV –ve Senegalese inidividuals. Clin. Exp., Immunol.,151(3): 432-440.

Menard, D., M.J. Mandeng, M.B. Tothy, E.K. Kelembho, G. Gresenguet and A. Talarmin, 2003. Immunohematological reference ranges for adults from the Central African Republic. Clin. Diagn. Lab. Immunol., (10): 405-443.

Ngowi, B.J., S.G. Mfinanga, J.N. Bruun, O. Morkve, 2009. Immunohaematological reference values in human immunodeficiency virus-negative adolescent and adults in rural northern Tanzania. B.M.C. Infect. Dis., (13): 9-11.

Nowicki, M.J., R. Karim, W.J. Mack, *et al*., 2007. Correlates of CD4+ and CD8+ lymphocyte counts in high-risk immunodeficiency virus (HIV)-seronegative women enrolled in the women's interagency HIV study (WIHS). Hum. Immunol., (68): 309-342.

Reichert , T., M. De Bruyere, V. Deneys, T. Totterman, P. Lydyard and F. Yuksel, *et al*., 1991. Lymphocyte subset reference ranges in adult Caucasians. Clin. Immunol. Immunopathol., 60: 190-208.

Saxena, R.K., V. Choudhry, I. Nath, S.N. Das, R.S. Paranjape, G. Babu, S. Ramlingam, D. Mohanty, H. Vohra, M. Thomas, Q.B. Saxena and N.K. Ganguly, 2004. Normal ranges of some select lymphocyte sub-populations in peripheral blood of normal healthy Indians.*Res. Communi.*

Shahabuddin, S., I.H. Al Ayed, M.O. El Rad and M.I. Qureshi, 1998. Lymphocyte subset reference ranges in healthy Saudi Arabian children. Pediatr. Allerg. Immunol., 9: 44-48.

Sullivan, J.G. and T.B Wiggers, 2000. Immunophenotyping leukemias: The new force in hematology.Clini. Lab. Sci., (2): 117-122.

Tsegaye, A.T., T.T. Messele, E.T. Hailu, R.D. Sahlu, A. Fontanet and T. Rinke de Wit., 1999. Immunohematological reference ranges for adult Ethiopians. Clin. Diagn. Lab. Immunol., (6): 410-414.

Uppal, S.S., S. Verma, P.S. Dhot, 2003. Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity, and smoking. Cytometry B. Clin. Cytom., (52): 32-36.

Yaman, A., S. Cetiner, F. Kibar, Y. Taşova, G. Seydaoğlu and I.H Dündar, 2005. Reference ranges of lymphocyte subsets of healthy adults in Turkey. Med Princ Pract., (3): 189-192.

Zloza, A., Y.B. Sullivan, E. Connick, A.L. Landay and L. Al-Harthi, 2003. CD8+ T cells that express CD4 on their surface ($CD4^{dim}CD8^{bright}$ T cells) recognize an antigen-specific target, are detected in vivo, and can be productively infected by T-tropic HIV *Blood.*, (102): 2156-2164.

ELECTRONIC REFRENCES AND INTERNET LINKS:

ER1 (http://www.hcdm.org/MoleculeInformation/tabid/54/Default.aspx).

ER 2 (http://en.wikipedia.org/wiki/List_of_human_clusters_of_differentiation).

ER3 (http://www.ncbi.nlm.nih.gov/pubmed/16020511).

ER4 (http://doctor-jones.co.uk/Immunology/Tutorial/MHC-I.jpg).