CD3 T Lymphocytes Count of Apparently Healthy Adult Individuals in Ekpoma, Edo State, Nigeria

Babatope, I.O.[1*], Esumeh, F.I.[2], Orhue, P.O.[1], Amaechi, R.A.[1], Inojie, V.[1]

[1]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria and Department of Haematology, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria

Abstract

The CD3 protein complex is an important T cell marker. This study aimed at determining the CD3 T lymphocytes count of apparently healthy adult subjects in Ekpoma, Nigeria. A total of two hundred and sixty (260) apparently healthy subjects were randomly recruited for the study. The subjects were of the both sexes and belonged to 17 to 50 years of age range. Five millitres (5ml) of whole blood was collected from the antecubital vein and dispensed into Ethylene Diamine Tetraacetic Acid (EDTA) bottles. Flow cytometry was used to determine the absolute counts (cells/µl) of CD3+ T lymphocytes. The mean CD3 absolute count of the male subjects is 1445.88 cells/µl (Median 1460.00 cells/µl, Range 783.38-2222.70 cells/µl) compared to a mean CD3 absolute count of the female subjects – 1720.27 cells/µl (Median 1741.00 cells/µl, Range 686.20 – 2145 cells/µl). There is a significant higher difference ($P<0.05$) in the mean CD3 count of the female subjects compared to males. The overall (total) mean CD3 absolute count of the subjects studied was 1483.08 ± 381.55 cells/µl (Median 160.00 cells/µl, Range 788.73 – 2159.00 cells/µl). The age groups of 17-24 years, 25-32 years and 33-40 years showed a statistical significant difference ($P<0.05$) when the mean CD3 absolute counts of the female subjects were compared to males. However, the age group of 40 and above did not reveal any significant difference when both means were compared. In conclusion, the study has revealed that the mean CD3+ T lymphocyte count obtained in our study population is unique to the study and different from those of other populations and this will guide the management of immune-related disorders.

Keywords: CD3 count, Reference ranges, Apparently healthy adults, Ekpoma.

1. Introduction

The Cluster of Differentiation (CD) used in lymphocyte nomenclature, is a protein expressed on the cells of haemopoietic system. Cells with different functions express different CD molecules. For instance, CD3 cells are total T lymphocytes, while CD4 cells are T-helper cells and over 300 molecules have so far been reported (WHO, 2007). Cluster of differentiation 3 (CD3) is a multimeric protein complex, known historically as the T3 complex, and is composed of four distinct polypeptide chains that assemble and function as three pairs of dimers. The CD3 complex serves as a T cell co-receptor that associates non-covalently with the T cell receptor (TCR) (Smith-Garvin et al., 2009). The CD3 protein complex is a defining feature of the T cell lineage, therefore anti-CD3 antibodies can be used effectively as T cell markers (Chetty & Gatter, 1994). The CD3 protein complex is an important T cell marker for the classification of malignant lymphomas and leukaemias (T cell neoplasms).

The effector, memory and immunoregulatory roles of CD3 T lymphocytes (CD3 T cells) have been variously described especially in this era of unfortunate HIV/AIDS challenge (Klose et al., 2007). It has also been established for years that CD3 T cells play critical roles in the functioning of B cells and effective responses of CD8 T cells. Moreover, the role of CD3 T cells in cancer immunotherapy and in the control of auto-immunity is gaining increased attention (Idigbe et al., 2010). CD3 can also be used for the identification of T cells in coeliac disease (Leon, 2011), lymphocytic colitis and collagen colitis (Mosnier et al., 1996; Sapp et al., 2002).

Full understanding of the values of CD3 T cells in African populations are not fully understood with both relatively high and low values being reported in various countries (Kalinkovich et al., 1998; Bussman et al., 2004., Klose et al., 2007). There is paucity of published data on CD3 T cells of Nigerians and even in Ekpoma inhabitants. The only data available are on CD4 T cells in Nigeria, and most of the studies were on immunocompromised populations (Nwokedi et al., 2007; Forbi & Agwale, 2009; Forbi et al., 2010). Therefore, there might be variation in the CD3 T lymphocytes count from one population to another and even in the instruments used for the enumeration (WHO, 2007). The aim of the present study is to determine the values of CD3 T cells among apparently healthy individuals residing in the semi-urban population of Ekpoma, Nigeria.

2. Materials and Methods

This study was carried out in Ekpoma, the administrative headquarters of Esan West Local Government Area of Edo State, Nigeria. Ekpoma is the fourth largest town in Edo State and lies between latitude $6^0$43'N and $6^0$45'N of the Equator and longitude $6^0$6'E to $6^0$8'E of the Greenwich Meridian with a population of 170,123 people

(National Population Commission (NPC),2012). The town, Ekpoma has a land area of 923 square kilometers and has the state-owned Ambrose Alli University (A.A.U.). Majority of the people residing in Ekpoma are civil servants, traders, businessmen/women, transporters, farmers, teachers, lecturers and students by occupation.

2.1. Study Population

A total of two hundred and sixty (260) apparently healthy subjects were randomly recruited into this study. The subjects were of both sexes and belonged to 17-50 years of age.

2.2. Ethical Approval

Approval was obtained from the Research and Ethics Committee (HREC NO:ISTH/HREC/2015/NOVEMBER/014) of Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria in accordance with the code of ethics for biomedical research involving human subjects. Informed consent of each participant was also obtained before sample collection.

2.3. Inclusion and Exclusion Criteria

Only subjects that were apparently healthy adults, seronegative for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Venereal Disease Research Laboratory (VDRL) were recruited for the study while ill subjects and those seropositive for HIV, HBV, HCV and VDRL were excluded from the study.

2.4. Specimen Collection

Five (5) millitres of whole blood was aseptically collected from each participant by venepuncture of the antecubital vein. Samples were dispensed in EDTA anticoagulated bottles and inverted between eight (8) to fifteen (15) times before it was set in a rack. All the field samples were placed in cold transport boxes with a temperature range of 2^0C-8^0C before they were transported to the laboratory for analysis. All samples were collected between 9.00am-12.00noon each day. Samples were analysed between 1-2 hours after collection and not longer than 6 hours.

2.5. Sample Analysis

CD3 cell counts were determined by flow cytometry using the Partec cyflow counter (Partec GmbH, 2006) adapted to single platform technology. Forward scatter and side scatter signals were measured using a linear scale. To ensure the optical alignment of the equipment and fluorescence compensation settings, countcheck bead green were run everyday and the count was compared with the manufacturer's range.

2.6. Statistical Analysis

The data obtained were expressed as means and standard errors of meams (S.E.M.). The medians were calculated and the reference ranges of absolute counts were determined at 2.5^{th} and 95^{th} percentiles. Statistical significance was determined using the analysis of variance (ANOVA) or the Student's t-test where appropriate. $P < 0.05$ was considered significant. All statistical analyses were done using SPSS version 21.0.

3. Results

Table 1 reveals the socio-demographic characteristics of the subjects studied. Of a total of 260 apparently healthy adults recruited into the study, 130 (50%) of the participants were males and 130 subjects (50%) were female subjects. Based on occupational status, half (50.0%) of the study subjects were students, followed by teachers/civil servants (23.08%) while others recorded the least frequency distribution of 0.38%. The educational status of the subjects revealed that most of them (90.77%) have formal education with just a fraction of them (9.23%) having no formal education. Of the studied population, 53.85% were single, 38.46% married individuals while 1.92% were divorced. With regards to religion, Christianity,muslims and others accounted for 96.15%, 3.08% and0.77% respectively.

The mean CD3 count ranges of the subjects studied are shown in table 2. The male subjects (n=130) recorded a mean CD3 absolute count of 1445.88 ± 409.44 cells/µl (Median 1460.00cells/µl, Range 783.38 – 2222.70cells/µl) against a mean CD3 absolute count of 1720.27 ± 294.49 cells/µl (Median 1741.00cells/µl, Range 686.20 – 2145 cells/µl) for the female subjects. There was a significant higher difference ($P < 0.05$) in the mean CD3 count of the female subjects compared to males. The overall (total) (n=260) mean CD3 absolute count of the subjects studied was 1483.08 ±381.55 cells/µl (Median 1603.00 cells/µl, Range 788.73 – 2159.00 cells/µl).

Table 3 shows the normal CD3 count ranges of the studied subjects according to age groups with the mean, median and ranges. Age groups 17 – 24 years, 25-32 years and 33-40 years showed a statistically significant difference ($P < 0.05$) while the mean CD3 absolute counts of female subjects were compared to males. The age group of 40 and above did not reveal any significant difference ($P>0.05$) when both means were compared.

Table 1: Socio-demographic characteristics of the study subjects

Socio-demographic characteristics	Number of participants	Percent (%)
Gender		
Male	130	50
Female	130	50
Occupational status		
Student	130	50.0
Farmer	10	3.85
Daily Labour	10	3.85
Merchant	15	5.77
Housewife	30	11.54
Teacher/Civil Servant	60	23.08
Driver	4	1.54
Others	1	0.38
Educational Status		
No Formal Education	24	9.23
Formal Education	236	90.77
Marital Status		
Single	140	53.85
Married	100	38.46
Divorced	5	1.92
Widowed	15	5.77
Religion		
Christian	250	96.15
Muslim	08	3.08
Others	02	0.77

Table 2: Normal CD3 count ranges of the subjects studied

	Males n=130			Females n=130			
Parameter	**Mean±SD**	**Median**	**2.5TH-95TH Percentile**	**Mean±SD**	**Median**	**2.5TH-95TH Percentile**	**P-value**
CD3	1445.88±409.44	1460.00	783.38-2222.70	1720.27±294.49	1741.00	686.20-2145.15	0.000

Table 3: Normal CD3 count ranges of subjects studied according to age groups

	Males				Females				
AGE (Yrs)	**N**	**Mean±SD**	**Median**	**2.5TH-95TH Percentile**	**N**	**Mean±SD**	**Median**	**2.5TH-95TH Percentile**	**P -value**
17-24	22	1635.23±176.79	1637.00	1420.00-2048.25	64	1844.60±227.44	1855	1515.00-2133.00	0.000
25-32	54	1435.30±393.20	1419.00	903.38-2354.00	22	1737.05±178.26	1673.00	1485.00-2019.85	0.001
33-40	29	1479.00±472.72	1600.00	766.00-2226.00	21	1621.91±295.47	1544.00	1278.00-2285.00	0.002
>40	25	1263.72±450.20	1065.00	783.00-2084.00	23	1431.48±356.40	1391.00	835.00-2211.00	0.211

4. Discussion

In this study, the mean CD3 absolute count of the subjects studied is 1483.08±381.55 cell/µl (Median 1603.00 cells/µl Range 788.73-2159.00 c of Kalinkovich et al (1998) in some African populations and Mair et al. (2007) in Senegal who both found mean CD3 absolute counts of 141 adult subjects they studied respectively. In contrast, our mean values was less than those reported in Botswana (1555 cells/µl), Ethiopia (1636 Burkina Faso (1801 cells/µl) by Bussmann et al. (2004), Gize et al. (2014), Bosire et al. (2013), Zekeng et al. (1997), Adoga et al. (2012) and CD3 mean absolute counts were also reported in Asia. For example, in countries like India (1881 cells/µl), Oman (1701 cells/µl) Turkey (168 (1599 cells/µl), Singapore (1590 cells/µl)and China (1547 cells/µl), different mean CD3 absolute counts were reported by Chng et al. (2004), A et al. (2002) respectively. The mean CD3 absolute count obtained in Ekpoma was higher than those reported for Sweden (1075 cells/µl), Su Kong (1370 cells/µl) by Bisset et al (2003), Abdelltef et al. (2014), Feki et al. (1998) and Al-Quozi et al. (2002) respectively. The plausible r composition and geographic differences.

Also in this study, the female subjects had higher mean CD3 absolute counts compared to their male counterparts (1720 cells/µl versus 1445 reports of Adoga et al. (2012), Gize et al. (2014) and Abdelltef et al. (2014). Prins et al (1999) attributed a sex hormone effect as one of the po count between genders. Other authors have speculated that gender and age-related variations within the immune system system parameters ma age-related diseases such as autoimmune disorders in female patients (Santagostino et al., 1999; Mair et al., 2007; Ngowi et al., 2009). In ad high levels of the hormone dehydroepiandrosterone (DHEA), which is a critical up-regulator of Th 1 immune response.

We observed that the mean CD3 absolute count based on the different age groups showed a statistical significant difference in only one age group (17-24 years) compared to other age groups. This observation corroborates the report of previous authors in Uganda and Nigeria respectively (Lugada et al., 2004; Adoga et al., 2012). The reason for this difference is not clear but Bosire et al (2013) suggested that a more comprehensive study design might be required to give a clear picture of the lymphocyte changes with advancing age. It is however well known that the pattern of T lymphocyte generation with age originates from dynamic changes in thymic as well as extra thymic functions, along with sequential developmental steps from stem cell to ultimately mature cells (Globerson, 1997).

In conclusion, our study has revealed that the mean $CD3^+$ T lymphocyte count values obtained in our study population are different from those of other populations. It is our opinion that the values established in this study will be of assistance in the management of immune-related disorders.

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