Immunohaematological Reference Values of Apparently Healthy Adult Nigerians in Benin City, Edo State

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ABSTRACT

Background: *Full blood analysis is the most frequently ordered blood investigation panel by medical practitioners.*
Aim*: This study was set out to determine a locally based immunohaematological reference values for apparently healthy adult Nigerians in Benin City, Edo state.*
Methods*: The research was carried out in Benin City within the three local government areas which comprise Oredo, Ego and Ikpoba-Okha local council areas of the state, with a total of three hundred (300) subjects aged between 20 to 50 years comprising 150 males and 150 females who were randomly enrolled. Reference ranges were calculated using nonparametric methods.*
Result: *The immunohaemmatological reference values of this study presents as follows: The total white blood cell count show a reference range of 4.3 – 10.0 (10^9/l); lymphocytes 22.1 - 42.0 (%); neutrophils 50.1 - 73.0 (%); red blood cells 3.8 - 5.4 (10^{12}/l); haemoglobin 11.0 - 16.0 (g/dl); haematocrit 33.9 - 49.8 (%); mean corpuscular volume 73.0 - 94.0 (%); mean corpuscular haemoglobin 4.0 – 36.0 (%); mean corpuscular haemoglobin concentration 24.0 - 38.6 (%); platelets 150 – 305 (10^9/l); and cluster of differentiation (CD4+) cell count of 419 – 1807 cells/ul.*
Discussion*: The result obtained showed a slight significant differences with a higher mean red blood cells, haemoglobin, haematocrit, mean cell volume, and mean corpuscular haemoglobin values in the males than the females ($p < 0.05$). There is however, no significant difference in the CD4+ values ($p > 0.05$).The study also shows a distribution of immunohaematological indices along age boundaries as there are slight significant differences in red blood cell, white blood cell, and platelet distribution between the age groups, with the highest mean value in age group 40 to 49, and lowest mean value in age group 20 to 29 ($p < 0.05$).*
Conclusion: *It is expedient to provide region-specific reference values which could be used to guide patient management plans, interpretation of clinical research findings, and ultimately improve the quality of healthcare delivery.*

Keywords: *Immunohaematological, Blood, Reference, values*

INTRODUCTION

Immunohaematological reference values for Apparently Healthy adult Nigerians in Benin City have never been established. Although similar efforts have been made to develop both haematologic and immunologic indices in other regions (Bolaji *et al.*, 2015).Platelet count values as well as reference haematological values for infants, children, and adolescents, have also been documented in some populations (Azikiwe, 1984; Buseri et al., 2010). It is noteworthy that reference ranges being used in most places were mere appendages from manufacturer's kits and textbooks which refer mainly to Caucasian subjects (Hoffbrand and Pettit, 1993; Wintrobe, 1999). It has been observed however, that Immunohaematologic survey carried out among black races have shown a wide disparity with those of their white counterparts in many studies (Hoffman, 1977; Inwood, et al., 1983; Lewis, 2006; Nduka et al., 1988). Significant differences have been observed in various haematological indices with special reference to haemoglobin and neutrophil absolute values which are often used as toxicity index with wide implication in clinical trials, and health management (Etienne et al., 2009).The adoptions of Caucasian immunohaematologic reference values for other races have established a serious negative implication in the enrolment of their African counterparts in vaccine trial participatory role. This is so because the result outcome from the critical steps in the exercise from screening to monitoring were usually tied to these adopted reference values, and with the disparity among the races, there is an implied high exclusion figures for the Africans, thereby making them to be side lined (Leigh et al., 2008). Immunohaematologic indices are greatly influenced by diversities of factors such as age, sex, geographical location, physiological disposition, life style, genetic composition and many other factors including disease distribution as well as agents of diseases (Bain, 1996; Choong et al., 1995; Evans et al., 1999). Genetic influences however, have been reported to show the highest disparity of immunohaematologic indices (Dal Colletto et al., 1993; Sharper and Lewis, 1971).It is now a common understanding based on research findings that immunohaematologic reference values varies between different populations as well as geographical settings. This understanding therefore, calls for the establishment of a peculiar region based values that would be the true reflection of the inhabitants and would be applied in more appropriate research ventures and for general healthcare management.Immunohaematologic reference values are veritable tools in the day to day health evaluation and monitoring practise in every economy. Critical haematological conditions as well as routine health checks and responses to treatment regimens such as in persons living with immunodeficiency virus have found usefulness in these values (Pomerantz, 2001; Stein et al., 1992).

Clinical reference ranges being used in most places in Nigeria were mere appendages from manufacturer's kits and textbooks which refer mainly to Caucasian subjects (Hoffbrand and Pettit, 1993; Wintrobe, 1999). Study has shown however, that Immunohaematologic survey carried out among black races has a wide disparity with those of their white counterpart due to overbearing influence of many factors (Hoffman, 1977; Inwood et al., 1983; Lewis., 2006; Nduka et al., 1988). It is therefore very imperative to establish a locally based immunohaematological reference values that would be the true reflection of the inhabitants to guide proper healthcare management and research ventures. The study was aimed at establishing a locally based immunohaematologic reference values in Benin City, Edo state.

MATERIALS AND METHODS

Research Area
This research was carried out in the metropolitan city of Edo state within the three local government areas which comprises Oredo, Ego and Ikpoba-Okha local council areas of the state.

Study population
A cross sectional study of the haematological and immunologic parameters of apparently healthy adults was carried out in this study over a period of five months.A total of three hundred (300) subjects aged between 20 to 50 years comprising 150 males and 150 females were randomly enrolled into the study, with a total of one hundred participants from each local Council area.

Ethical approval/consent
After obtaining an ethical approval from the Edo state Hospital management board, oral informed consent was granted by each participant.

Inclusion criteria
Apparently healthy volunteers who have not been on medication for the last one month with age range of 20 - 50 years were enrolled in the study after filling out a questionnaire.

Exclusion criteria
Subjects with genotype HbSS, hypertension, diabetes mellitus, human immunodeficiency virus (HIV), cancer, hepatitis, and regular smokers, those who had vaccination during the past six months were excluded from this study. Subjects with bleeding disorder, or regular blood donors, and those recently transfused with blood within twelve (12) months period, were also excluded from the research study. Both alcoholics and recently hospitalized subjects were also excluded in the study. Additionally, women on oral contraceptives, menstruating, gravid and breastfeeding mothers did not take part in the exercise relying on the information generated from the questionnaires.

Sample collection
Using EDTA vacutainer tubes, five (5) millilitres of whole blood was drawn from the cubital vein of subjects sterilizing the site and with minimal stasis for CD4 and complete blood count between 9.00 am to 12.00 noon. Samples were properly mixed and labelled using marker. The collected blood samples were immediately sent to the laboratory in cold chain boxes for CD4 counts as well as haematological analysis, after six hours.

Haematological Analysis (F B C)
Using the Automated HaematologicalAnalyser, Sysmex, KXN21(Japan), red blood cells, haemoglobin, haematocrit, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), total white blood cells, platelets, lymphocytes and neutrophils were analysed in this study.

Principle
The automated Haematological analyser utilises the principle of fluorescence flow cytometry to quantitate standard differentials based on the migration of blood cells directed against a laser beam. The florescent labeled cell components in a stream are then excited to produce

light at different wavelengths specific to each cell. The characterization of various cell components are expressed in a computer read out.

CD4 (+) Analysis

The CD4 counts in this study were assayed using the cy-flow counter; model SL – 3 which uses the principle of Flow cytometry by which cells or micro particles in suspension is differentiated and counted according to size and internal structure (Calman et al., 2007).

Principle

Monoclonal antibodies binds to the CD4+ antigen on the mononuclear cell (T –lymphocyte and monocyte), and in a buffer suspension, the complex is passed through the flow cuvette in a single stream of flow. The complex is excited by solid state laser light at a wavelength of 532 nm, causing the complex to emit light which is captured by photomultiplier tube and transmitted into digital read out as count.

Statistical Analysis

Both parametric, non-parametric, and analysis of variance (ANOVA), where the statistical approach used in analysing the haematological and immunologic profiles generated in this research. The mean, medians, standard deviation and reference ranges were calculated non - parametrically. Statistical differences between genders in the study population were computed by means of parametric student's t – test regime. Finally, the analysis of variance (ANOVA) was the choice statistical method used to establish relationship between different age groups in the immunohaematological profiles. SPSS statistical software version 21.0 were used in the computations (Ogbeibu,2005).

RESULTS

Table 1: **Mean standard deviation, reference Range, and median of Haematological indices and CD4 + cells count for male and female of the study population.**

Subjects	Indices	WBC (10^9/L)	RBC (10^9/L)	HGB (G/DL)	HCT (%)	MCV (FL)	MCH (PG)	MCHC (G/DL)	PLTS (10^9/L	LYM (%)	NEUT (%)	CD4 (CELLS/UL)
Male (N= 150)	Mean± SD	5.98±1.39	4.72±0.41	14.90±1.00	44.24±3.12	87.53±3.86	31.13±2.95	34.42±2.36	226.85±39	31.97±5.67	63.35±5.38	918.31±219.67
	Ref. Range	4.5-10	4.2-5.4	12.3-16	38-49.8	83-94	26-36	25-38	150-300	22-42	51-72	421-1760
	Median	5.6	4.6	15.1	44	86.9	31.60	34.8	216	31.8	63.85	906.5
Female (N = 150)	Mean± SD	5.69±1.05	4.29±0.41	12.3±0.86	37.79±2.85	82.94±3.76	28.37±1.63	31.73±2.20	233.7±38.27	32.17±5.67	63.74±5.56	935.09±235.19
	Ref.Range	4.3-9.7	3.8-5.1	11-15.4	33.9-45.2	73-89	24-31	24-33	150-305	23-42	50.1-73	419-1807
	Median	5.5	4.1	12.2	36.8	82.6	28.3	33	229.5	31.5	64.75	900
	p-values	0.04*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.126	0.758	0.539	0.523
	t-values	2.06	9.74	23.97	18.73	10.43	10.02	10.22	-1.53	-0.31	-0.62	-0.64
OverallValues (N = 300)	Mean ±SD	5.84±1.24	4.49±0.47	13.60±1.59	41.01±4.39	85.23±4.44	29.75±2.75	33.07±2.65	230.28±38.75	32.1±5.67	63.54±5.47	926.7±227.34
	Median	5.5	4.4	13.5	40.9	84.6	29.6	33	220	31.6	64.2	905
	Ref. Range	4.30-10	3.80-5.4	11-16	33.9-49.8	73-94	24-36	24-38.6	150-305	22.1-42	50.1-73	419-1807

Significance ($p<0.05$)

Table 1 above shows the distribution of the haematological and CD4 (+) cell profiles among males and females in the study population. The result expressed significant differences in total white blood cells, red blood cell, haemoglobin, haematocrits, mean cell haemoglobin, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration ($p < 0.05$). There was no significant difference in platelets, lymphocytes, neutrophils, and CD4 (+) cell counts along gender lines ($p > 0.05$).

Table 2: **Immunohaematological Reference Range of the study population**

Indices	WBC (10^9/L)	RBC (10^9/L)	HGB (G/DL)	HCT (%)	MCV (FL)	MCH (PG)	MCHC (G/DL)	PLTS (10^9/L	LYM (%)	NEUT (%)	CD4 (CELLS/UL)
Male	4.5-10.0	4.2-5.4	12.3-16.0	38-49.8	83-94	26-36	25-38	150-300	22-42	51-72	421-1760
Female	4.3-9.7	3.8-5.1	11.0-15.4	33.9-45.2	73-89	24-31	24-33	150-305	23-42	50.1-73	419-1807
Absolute	4.30-10.0	3.8-5.4	11.0-16.0	33.9-49.8	73-94	24-36	24-38.6	150-305	22.1-42	50.1-73	419-1807

Significance ($p<0.05$)

Table 2above shows the distribution of haematological and CD4 (+) reference values among males and females in the study population. Obvious differences occur in red blood cell, haemoglobin, haematocrits, mean cell volume, mean cell concentration and mean cell haemoglobin concentration. There were however, little or no difference in platelets, total white blood cell, lymphocytes, neutrophils, and CD4 (+) cell counts.

Table 3: **Comparism of Hematological indices and CD4 + cell counts among different age groups in the study population.**

AGE GROUP (YRS)	INDICES	WBC (10^9/L)	RBC (10^9/L)	HGB (G/DL)	HCT (%)	MCV (FL)	MCH (PG)	MCHC (G/DL)	PLTS (10^9/L	LYM (%)	NEUT (%)	CD4 (CELLS
20 – 29 (N= 105)	Mean±SD	4.98±0.95	4.37±0.46	13.17±13.17	40.26±3.97	82.75±3.29	28.52±2.07	33.48±2.79	216.66±34.50	33.76±5.89	62.59±5.27	932.82
30 – 39 (N = 137)	Mean±SD	5.82±1.41	4.47±0.44	13.60±13.56	40.70±4.65	83.01±3.42	28.82±2.02	33.40±2.73	237.15±39.68	30.80±5.38	64.25±5.70	792.05
40 – 49 (N = 58)	Mean±SD	5.85±1.24	4.77±0.48	14.07±14.07	42.66±3.96	86.48±4.61	29.21±1.89	34.03±2.76	224.33±39.83	32.00±5.18	63.74±5.14	1224.3
	P – value	0.000*	0.000*	0.001*	0.002*	0.000*	0.106	0.329	0.000*	0.000*	0.062	0.000*
	F-value	16.203	14.824	6.835	6.158	22.906	2.265	1.117	8.900	8.474	2.814	141.15

Significance ($p<0.05$)

Table 3 above compares the haematological indices and CD4 (+) cell counts among different age groups in the study population. Significant differences occur in total white cell, red blood cell counts, haemoglobin haematocrits, mean cell volume, platelets, lymphocytes as well as CD4 cell counts ($p < .05$). All other parameters showed no significant difference ($p > 0.05$).

DISCUSSION

Immunohematological indices comprising of white blood cell (WBC) count, red blood cell (RBC), hemoglobin (HB), haematocrit (HCT), mean corpuscular volume (MCV),mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLTS), lymphocytes (LYM), neutrophils, (NEUT), and CD4+ cell counts were analysed for males and females, for the purpose of establishing reference values in this study.

The result obtained showed a slight significant differences with a higher mean red blood cells (RBCs), haemoglobin (HB), haematocrit (HCT), mean cell volume (MCV), and mean corpuscular haemoglobin (MCH) values in the males than the females of the study population ($p < 0.05$). The differences between the male and female in these parameters confirms a previous study that gender could cause variations in these profiles as a result of variation of hormone types and the effect of hormonal changes in females (Usman and Rao, 2007).It is this physiological phenomena in females that explains the low mean values of red cells and red cell subsets in association with low mean red cell indices when compared with the males.

There is no significant difference in the platelet values recorded in this study ($p > 0.05$), however, the higher mean difference in female population of the study may be associated with hormonal response to periodic low haemoglobin and packed cell volume due to the physiological changes in menstrual cycle (Usman and Rao, 2007). This trend also play out in the similar study carried out within regional, sub regional and continental sphere, thus supporting gender differences as a factor for establishing immunohaematological reference values.

The low haemoglobin and packed cell volume levels noted in this study with a similar low values in other African regions compared with higher values among the white population have been cited in previous studies carried among African population (Badenhorst *et al.*, 1995). These dissimilarities are likely as a result of low iron diets among Africans, which could result in iron deficiency anaemia as a consequence of ineffective haematopoiesis. Plasmodiasis, and protozoan infestation as it is common with African population however, could also lead to low haemoglobin and packed cell volume respectively (Coetzee *et al.*, 1994).

An overview of previous study has indicated marked differences in red cell values among African countries. The higher red blood cell values among Kenyan population, a mountainous East African nation, compared with lower values in this study, agrees with other studies that altitude or geographical location might be an essential variables that could influence haematological parameters (Bain, 1996; Choong *et al.*, 1995). Reference platelet values expressed in the study shows degree of differences between regions in African, ethnic groups and with the Caucasians.

These dissimilarities conforms with other studies that haematological parameters shares both ethnic, regional and continental differences (Hoffman., 1977).The lower platelet values obtained in this study and among other African countries compared with those of the Caucasians are nearly the same with studies with African population (Gill and Marshal 1979). While the direct cause or causes of the low platelet counts among Africans are unknown, however, environmental factors, genetic factors, and unidentified illnesses, have been implicated (Bain, 1996).

Although there is a slight significant difference in the total white blood cell (WBC) values ($p < 0.05$), however, there is no significant difference in both lymphocytes and neutrophils of male and female in the study population ($p > 0.05$). The cause of gender variation in total white blood cell values cannot be vividly explained, however, study has shown that stress and habits may influence white blood cell parameters (McBride and Shapley, 1968).

Disequilibrium of health status and insincere divulging of vital information may be another reason. Since there was no clinical screening carried out on the participants, there is therefore the likelihood of some underlying clinical features amongst them. Exclusion criteria used in this study such as cigarette smoking and chemotherapy may not have been sincerely observed since participants were not quarantined. The absolute white blood cell (WBC), neutrophil and lymphoccyte counts and reference ranges in this study population have something in common with those reported in previous studies carried with other African populations, and are lower than values of the white population (Ezeilo,1972).

The causes of these differences may be unknown, however, genetics factors, dietary, and environmental factors could be a possibility (Shaper and Lewis, 1971).The study does not show a significant difference in CD4+ cell values among gender spread ($p > 0.05$), however, the lower mean value of male compared with the female may be associated with stress factors that may be found in men, while higher mean CD4+ values in the females might be as a result of haemopoietic feedback stimulus following monthly blood loss due to regular menstruation. This similar observation is reflected in the study of Miri-Dashe et al., (2014).

The CD4+ reference values in this study reflect the values obtained in some other regions in Nigeria, especially the neighbouring state of Ondo in (Bolaji and Adedeji, 2015). Studies of other African population have shown that several factors especially infection can influence CD4+ cell count values.

The study also shows a distribution of immunohaematological indices along age boundaries. There were slight significant differences in red blood cells and red blood cell subsets of age groups in this study ($p < 0.05$), with a highest mean value in age group 40 to 49, and lowest mean value in age group 20 to 29. This characterization can be explained in terms of social class syndrome. Apparently healthy subjects in age group 40 to 49 were likely better placed socially than other groups. Adequate dieting however implied an increase in erythropoietic activity which resulted in the upward classification of red blood cells and its fractions as expressed in this study. The study showed similar significant differences of total white blood cell distribution among the age groups. The highest mean value in age group 40 to 49 may be a predisposing factor of predictive systemic conditions requiring a further laboratory screening as ageing is synonymous with illness. This same observation is reflected in the works of Weijenberg*et al*(1996), who noted raised White blood cell counts as the risk factor of coronary heart disease in elderly men. Significant differences of platelets distribution were observed among the age groups in the study ($p < 0.05$). The falling mean values among age group 20 to 29; 40 to 49, is suggestive of age related decline of platelets in health. This physiological variable could be an associated decline of thrombopoietin production correlating the works of Segal and Moliterno,(2006) that platelets is evidently reduced in elderly. There were significant differences of CD4 (+) cell counts among the age groups in the study ($p < 0.05$), with a fall and rise in mean values. The falling mean values from age group 20 to 29 to age group 30 to 39 could be an associated stress factor and emotions common in a deprived economy when individuals strives to make both ends meet. It is likely that the higher mean value expressed in age group 40 to 49, could be an attainment of expected social status with stress factor abrogation. Decline in CD4 (+) cell counts have been

implicated in stress and emotional states in previous scientific studies (Mills*et al.*, 1996; Naliboff*et al.*, 1991; Oladepo*et al.*, 2009).

CONCLUSION

The variability of immunohaematological indices have been a source of worry in interpretation of clinical laboratory results for proper healthcare practise in Nigeria. The sources of these wide differences have been identified in this study as expressed in previous scientific studies. This work therefore, has provided solution to the problems associated with improper laboratory result application in medical healthcare due to adopted reference values, and would therefore be used in a local setting.

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