African Journal of Laboratory Haematology and Transfusion Science

Vol 2, Issue 3, page 214 - 221 | September 2023 | www.ajlhtsonline.org
Article DOI : 10.59708/ajlhts.v2i3.2327
DOI URL: doi.org/10.59708/ajlhts.v2i3.2327

ORIGINAL ARTICLE

Frequency of Rh-e Antigen and reference values of Erythrocyte Sedimentation Rate, Red Cell Indices in an Undergraduate Students' population in Port Harcourt, Nigeria.

Ransom Baribefii Jacob[1*], Chizarum Boms[1], Orokwu Eziaku Chukuigwe-Igbere[1] and Nwika, Goodnews Nkabari[2]

[1] Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

[2] Faculty of Medical Laboratory Science, Federal University Otuoke, Bayelsa State, Nigeria

Corresponding Author:
ransom.jacob@ust.edu.ng
08069356274

Submitted:26-08-2023
Accepted:15-09-2023
Published 30-09-2023

Abstract

Introduction:
Red cell antigens alongside red cell indices provide an essential support to the diagnosis and monitoring of haematological diseases while the erythrocyte sedimentation rate (ESR) indicates and monitor an increase in inflammatory activity within the body. This study aims to determine the frequency of Rh-e antigen and reference values of Erythrocyte Sedimentation Rate, Red cell indices in an undergraduate student's population in the Rivers State, Port Harcourt, Nigeria.

Material and Methods: One hundred and fifty (150) undergraduate students aged between 17-28years were enrolled in the study and standard venipuncture technique used to collect 5ml of blood. Determination of the Rh-e antigen was carried out using anti-e monoclonal antibodies (Lorne Diagnostics UK), Red cell indices obtained using BC 5000 Mindray Haematology Analyser and ESR by Westergren method.

Results: Among the 150 subjects, 130 (86.6%) were positive while 20 (13.3%) were negative for Rh-e antigen. The mean±SD of the mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width co-efficient of variation (RDW-CV), red cell distribution width-standard deviation (RDW-SD) and erythrocyte sedimentation rate (ESR) were 83.12 ± 10.74, 31.13 ± 3.25, 34.00 ± 3.32, 13.32 ± 1.61, 39.70 ± 2.26 and 30.36 ± 2.15 in the same order, while the reference values were 61.64-104.6 for MCV, 24.63-37.63 for MCH, 27.36–40.64 for MCHC, 10.10–16.54 for RDW (CV), 35.18–44.22 for RDW (SD) and 26.06-34.66 for ESR. Gender had no effect on MCV ($p=0.3007$) and MCHC ($p=0.1436$) but significant effect on MCH ($p=0.030$), RDW-CV ($p<0.001$), RDW-SD ($p=0.0005$) and ESR ($p=0.036$) with a perfect positive correlation ($r=1$) between all the studied parameters.

Conclusion: This study revealed a high (86.6%) prevalence for Rh-e antigen, established normal reference ranges for red cell indices and erythrocyte sedimentation rates and showed that gender has significant effects on MCH, RDW-CV, RDW-SD and ESR among

healthy undergraduate students of Rivers State University. These findings are of clinical and research relevance.

Keywords: Rh-e Antigen, Erythrocyte Sedimentation Rate, Red Cell Indices, Agglutination.

Introduction

Rh antigens, red cell indices and Erythrocytes Sedimentation Rate are useful parameters in the study of the immunogenicity of blood cell, classification of anaemia and monitoring/ evaluation of inflammatory activities in humans. Blood as a tissue albeit in fluid form (plasma) and composed of formed, cellular and structural components such as the white blood cell, red blood cell and platelets circulated in the vascular system of the body performing specific physiologic functions (1). Healthy human adults produce about 200 billion red blood cells (RBCs) daily to replace those lost by senescence through a process termed erythropoiesis (2). Erythropoiesis is exquisitely regulated by an oxygen-sensing mechanism that has evolved to maintain RBC numbers within a narrow physiological range (3) as red blood cell stimulated production emanates from haemopoietic stem cells in the bone marrow and survive in circulation for about 120 days (4).

The Rhesus blood group system is one of the most polymorphic and antigenic blood group systems. It is made up of 49 blood group antigens of which five are most important which are D, C, c, E and e (5-7). The Rh blood group locus is composed of two related structural genes, RhD and RhCE which encode the membrane proteins that carry the D, Cc and Ee antigens (6-8). The antigens C, D, E, c and e (except d) are antigenic and capable of stimulating production of antibodies if introduced into the body of an individual whose red cells lack them. However, the Rhesus antigens vary in their degree of antigenicity (6-8). The Rh antigens are thought to play a role in maintaining the integrity of the RBC membrane as RBCs which lack Rh antigens have been found to possess an abnormal shape (5-8).

Red cell indices are haematological values derived from complete blood count analysis using automated analyzers or calculated from values of packed red cell (PCV), haemoglobin concentration (Hb) and red blood cell (RBC) count valuable in the morphological classification of anaemias and thus have clinical value in the management of patients with anaemia. Red cell indices consist of mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) and together with parameters such as red blood cell count and packed cell volume are used in characterizing and diagnosing anaemia as well as establishing blood disorders (9-12). Anaemias are classified, according to the size of the red cell, as being normocytic (normal MCV), macrocytic (increased MCV), or microcytic (decreased MCV) (9,13).

Erythrocyte sedimentation rate (ESR) is one of the most common test used for assessment of inflammation or tissue injury and thus of great diagnostic value in clinical practice (14). It is measured as the distance of fall of erythrocytes in the plasma expressed as millimeters in 1 hour (14). High values of ESR suggest infection, non-infectious inflammatory disorders, neoplasms or non-inflammatory conditions such as pregnancy and drug use (14,15). Research have shown that erythrocyte-related parameters such as size and number and non-erythrocyte-related parameters such as fibrinogen and immunoglobulins are two

principal factors that may affect ESR values (16).

Although many inflammatory illnesses can increase the ESR, other conditions exist that can lower the ESR and these factors can exist either as isolated disease processes or in conjunction with other pathologic conditions that raise the ESR, thus giving a lower-than-expected ESR results in light of a serious underlying inflammatory process (17). Polycythemia will increase blood viscosity and can cause a reduced ESR (reduces the rate at which RBC rouleaux will settle to the bottom of the Westergren tube); haemoglobinopathies such as sickle cell disease lower ESR due to the abnormal shape of red blood cells that impairs rouleaux formation (17). Established prevalence and reference ranges for Rh-e antigen, red cell indices and erythrocytes sedimentation rate are lacking in facilities within the university and its environs; this study aims to determine Rh-e antigen, Erythrocyte Sedimentation Rate and Red cell indices values in apparently healthy undergraduate students of the Rivers State University, Port Harcourt Nigeria.

Materials and Methods

Study Design and Population

A cross sectional study design involving one hundred and fifty (150) apparently healthy undergraduate students of Rivers State University, Port Harcourt Nigeria and comprising of seventy-six (76) male and seventy-four (74) female within the age range of 17-28 years, with an average age of 20.73 ± 2.72 years from different departments in the university were enrolled in the study. A well-structured questionnaire was used to obtain the demographic information of the participants.

Sample Collection

Five millilitres (5ml) of venous blood was collected from each student into ethylene diamine tetracetic acid (EDTA) anticoagulant tube and mixed properly. The samples were analyzed within twenty-four hours of blood collection.

Procedures

Determination of Rh-e Blood Group by Microwell Agglutination Technique using Monoclonal antibodies Lorne Diagnostics UK. A 5% suspension of red blood cells was prepared using normal saline. 0.2ml of Rh-e antibody was added into a micro-titre plate. 0.2ml of the washed red cell was added into the micro-titre plate containing the Rh-e antibody. The sample was incubated for 2 hours while rocking intermittently and observing for agglutination every 30 minutes. If there was no agglutination after 2 hours, 0.2ml of LISS antibody was added and observed for agglutination for 30 minutes. If no visible agglutination, the sample was placed on a slide and observed microscopically for agglutination. Absence of agglutination indicates a negative result while presence of agglutination indicates a positive result.

Determination of Red Cell Indices and Erythrocyte Sedimentation Rate (ESR): Red cell values were obtained from complete blood count (CBC) results generated using BC 5000 Mindray Haematology Analyser while Erythrocyte Sedimentation Rate (ESR) values was assayed for using the Westergren method with strict compliance to standard.

Statistical Analysis

The data obtained was analyzed using Graphpad prism software version 6.0 produced by Graphpad software Inc. USA and results presented in tables.

Results

A total of 150 apparently healthy participants were enrolled for the study, comprising of 74 female and 76 male students of the Rivers State University, within the age of 17-28 years with an average age of 20.73 ± 2.72 years. The demographic details of the participants are shown in Table 1.

Among the 150 subjects who participated in the study, 130 (86.6%) [68 (45.3%) males and 62 (41.3%) females] were positive for Anti-e while 20 (13.3%) [8 (5.3%) males and 12 (8.0%) females] were negative for anti-e as shown in table 2.

Table 3 shows that the mean ± SD of the mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width-ccoefficient of variation (RDW-CV), red cell distribution width- standard deviation (RDW-SD) and erythrocyte sedimentation rate (ESR) were 83.12 ± 10.74, 31.13 ± 3.25, 34.00 ± 3.32, 13.32 ± 1.61, 39.70 ± 2.26 and 30.36 ± 2.15 in the same order, while the reference values were 61.64 - 104.6 for MCV, 24.63 - 37.63 for MCH, 27.36 – 40.64 for MCHC, 10.10 – 16.54 for RDW (CV), 35.18 – 44.22 for RDW (SD) and 26.06-34.66 for ESR.

The MCV, MCHC values among females and males in the study revealed a mean ± SD of 84.04 ± 10.98 and 82.22 ± 10.49 (p=0.3007); 33.59 ± 3.12 and 34.39 ± 3.49 (p=0.1436) respectively resulting in a statistically insignificant difference. The mean ± SD for MCH revealed 34.35 ± 4.19 for the females and 27.99 ± 2.58 for the males (p=0.030); 13.86 ± 1.96 and 12.78 ± 0.89 (p<0.0001) for RDW-CV and 41.19 ± 2.62 and 38.25 ± 2.47 (p=0.0005) for RDW-SD and 36.12 ± 2.99 for females and 24.75 ± 2.95 (p=0.036) for ESR respectively resulting in a statistically significant difference as shown in table 4.

The Correlation analysis in study population shows a perfect positive correlation (r=1) between all the studied parameters as shown in Table 5.

Table 1: Demographic Data of Study Population

Parameters	Number or Range
Age of Participants (Years)	17-28
Number of Male Subjects	76
Number of Female Subjects	74
Total Number of Subjects	150

Table 2: Percentage Expression of Rh-e antigen in study population

Rh-e Antigen	Male (n=76)	Female (n=74)	Frequency (%)
Rh-e Positive	68	62	130 (86.6)
Rh-e Negative	8	12	20 (13.3)

Key: Rh-e= Rh-e antigens, n= number of subjects

Table 3: Red cell indices and Erythrocytes Reference values in study population

Parameter	Mean±SD	Reference Range
MCV (g/dl)	83.12 ± 10.74	61.64 – 104.6
MCH (g/dl)	31.13 ± 3.25	24.63 – 37.63
MCHC (g/dl)	34.00 ± 3.32	27.36 – 40.64
RDW(CV)	13.32 ± 1.61	10.10 – 16.54
RDW(SD)	39.70 ± 2.26	35.18 – 44.22
ESR (mm/hr)	30.36 ± 2.15	26.06 – 34.66

Key: ESR= Erythrocytes Sedimentation Rate, MCV=Mean cell volume, MCH=Mean cell haemoglobin, MCHC=Mean cell haemoglobin concentration, RDW-CV=Red cell distribution width coefficient of variation, RDW-SD= Red cell distribution width standard deviation.

Table 4: Effect of Gender on red cell indices and erythrocyte sedimentation rate in study population.

Parameter	Female (n=74)	Male (n=76)	t-test	Remark
MCV (g/dl)	84.04 ± 10.98	82.22 ± 10.49	0.3007	NS
MCH (g/dl)	34.35 ± 4.19	27.99 ± 2.58	0.030	S
MCHC(g/dl)	33.59 ± 3.12	34.39 ± 3.49	0.1436	NS
RDW(CV)	13.86 ± 1.96	12.78 ± 0.89	<0.0001	S
RDW(SD)	41.19 ± 2.62	38.25 ± 2.47	0.0005	S
ESR (mm/hr)	36.12 ± 2.99	24.75 ± 2.95	0.036	S

Key: ESR= Erythrocytes Sedimentation Rate, MCV=Mean cell volume, MCH=Mean cell haemoglobin, MCHC=Mean cell haemoglobin concentration, RDW-CV=Red cell distribution width coefficient of variation, RDW-SD= Red cell distribution width standard deviation, S= Significant at p<0.05, NS= Not Significant at p>0.05.

Table 5: Correlation Analysis in study population

Correlation	AGE	ESR	MCV	MCH	MCHC	RDW(CV)	RDW(SD)
AGE(YEARS)	1						
ESR(mm/hr)	-0.03	1					
MCV (g/dl)	0.11	0.042	1				
MCH (g/dl)	-0.055	-0.028	0.115	1			
MCHC (g/dl)	0.115	-0.008	-0.430	0.046	1		
RDW (CV)	-0.103	0.001	-0.011	0.130	-0.238	1	
RDW (SD)	-0.008	0.050	0.204	0.374	-0.032	0.374	1

Key: ESR= Erythrocytes Sedimentation Rate, MCV=Mean cell volume, MCH=Mean cell haemoglobin, MCHC=Mean cell haemoglobin concentration, RDW-CV=Red cell distribution width coefficient of variation, RDW-SD= Red cell distribution width standard deviation

Discussion

In this study, 86.6% of the participants were positive for Rh-e antigen while while 13.4% were negative . This finding is slightly at variance with other reportedworks in terms of lower percentage but not in high prevalence, Results obtained from this study is lower than the 94.2% percentage expression reported earlier in 2022 among apparently healthy descent of Bonny kingdom, Nigeria (5); 90.8% percentage expression reported earlier in 2023 among multiparous women attending antenatal clinic in tertiary hospital, Port Harcourt, Nigeria (8). This study is not also in agreement with the findings of Jeremiah and Buseri (18) who carried out similar research among 4 main ethnic groups in Port Harcourt, Nigeria and reported a prevalence of Rh-e to be 98.7%. These percentages are higher than those seen in the present study and these differences may be reflective of an ethnic variation in the distribution of e antigens

in different population as well as increase awareness and screening for Rh-e antigens.

Findings from this study revealed that the average MCV, MCH, MCHC and RDW were within the normal established reference values for MCV (80-100fL), MCH (27-31pg) (12,19), MCHC (32-36g/dL) (12,20), RDW-CV (11-16%) (21) and RDW-SD (36-47fL) (22), but the average of the ESR was higher in our study population (30.36 ± 2.15) when compared to the established range for healthy adults less than 50 years (≤15mm/hr for men and ≤20mm/hr for women) (23).

Consequently, findings in this study is not in agreement with the reports of Emelike et al (24) who in their study among healthy Nigerians in 2010, showed a mean ESR value of 11.99 ± 9.48 for males and 13.69 ± 8.59 for females. Although, the average values for all parameters except ESR in our study were within the established Western ranges, the reference ranges established in our study showed a decreased lower limit and an increased upper limit. For ESR, both the lower and upper limit were higher than the established Western reference values. The ESR also revealed a statistically significant difference as the females showed a higher ESR value than the males. This is in line with previous studies by Emelike et al (24), however, the higher limits of the reference values for the ESR in men and women 50 years or younger are 15 and 20 mm/hr, respectively and this is not in line with findings in the present study.

This study revealed a statistically significant difference in the MCH, MCHC and RDW between the male and female student population. It was observed that the females had higher MCH, MCHC, RDW and ESR values when compared to the males. Other studies carried out in Nigeria and other African countries to establish reference ranges for haematological parameters have shown that values for red cell parameters such as haematocrit, haemoglobin and red cell indices like MCV, MCH and MCHC are higher in males than females (10,-12, 25,- 29). Such gender differences have been attributed to menstrual blood loss in females and androgenic hormonal influences in males (24,27). However,the findings of this study is not in agreement with this as the values were higher in the female population than in the males. The higher values in red cell indices for females in this study could be attributed to high awareness among female student on maintenance of a hygienic and healthy lifestyle before and after menstruation demonstrated, intake of iron supplements and routines drugs that boost blood production as a way of compensating for blood due to menstrual flow.

Red cell indices constitute important parameters used as screening tools for anaemia (30). In order to interpret and determine the clinical implication of the full blood count including the red cell indices of any individual in the state of health or disease, knowledge of the normal reference values for that locality is needed. Like many other African countries, most Nigerian health facilities and organizations often rely on laboratory reference values derived from Western populations for results interpretation. It is worthy to note that there is an inter-regional, inter-country and inter-racial differences in haematological reference values and these are well documented (25).

Conclusion

This study revealed a high (86.6%) prevalence for Rh-e antigen with higher percentage expression among females, established normal reference values for red cell indices and erythrocyte sedimentation rates and showed that gender has significant effects on MCH, RDW-CV, RDW-SD and ESR among healthy undergraduate student's population in Rivers State. These findings are unique, and relevant for clinical and research decision making in health facilities within the University and environs.

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How to cite this article:
Jacob RB, Boms C, Chukwuigwe-Igbere OE, Nwika GN. Frequency of Rh-e Antigen and reference ranges of Erythrocyte Sedimentation Rate and Red Cell Indices in an Undergraduate Students' population in Port Harcourt, Nigeria. Afr J Lab Haem Transf Science 2023; 2(3): 214 - 221.
DOI: https://doi.org/10.59708/ajlhts.v2i3.2327