






Research Article

# Neutrophil/Lymphocytes Ratio and Haemoglobin Electrophoretic Pattern in an Undergraduate Student's Population Rivers State University, Port Harcourt, Nigeria

Ransom Baribefii Jacob<sup>1\*</sup> , Chioma Favour Ndamati<sup>1</sup> , Serekara Gideon Christian<sup>1</sup> , Evelyn Mgbeoma Eze<sup>1</sup> , Teddy Charles Adias<sup>2</sup> 

<sup>1</sup> Department of Haematology and Transfusion Science, Rivers State University, Port Harcourt, Nigeria

<sup>2</sup> Faculty of Medical Laboratory Science, Federal University Otueke, Bayelsa State, Nigeria.

\* Correspondence: ransom.jacob@ust.edu.ng

<https://doi.org/ciki/10.59652/aim.v2i1.127>

**Abstract:** Neutrophil/lymphocyte ratio (NLR) is a very cheap and accurate method of accessing inflammation and is fast emerging as a prognostic biomarker in many diseases. This study determines the Neutrophil/lymphocyte ratio and haemoglobin electrophoretic patterns in an undergraduate student's population at Rivers State University, Port Harcourt, Nigeria. One hundred and fifty (150) undergraduate students aged between 17 and 30 years old were recruited for the study. Five millimeters (5ml) of venous blood was collected from each participants into ethylene diamine tetraacetic acid (EDTA) vacutainer bottle for the determination of haemoglobin genotype using cellulose acetate electrophoresis method while the neutrophil/lymphocyte ratio was calculated from neutrophil and lymphocyte values obtained from BC 5000 Mindray Hematology Auto-Analyzer. The results obtained showed that the mean $\pm$ SD value of Neutrophil/Lymphocyte ratio was  $1.21 \pm 0.07$  for male and  $1.14 \pm 0.06$  for female participants with both within normal reference ranges and with no significant difference ( $p=0.4692$ ). 100 (66.7%) subjects had haemoglobin genotype AA (HbAA) out of which 48 (32%) male, 52 (34.7%) female while 50 (33.3%) participants had haemoglobin genotype AS (HbAS) of which 24 (16%) male, 26 (17.3%) female. No haemoglobin genotype SS/SC (HbSS/HbSC) traits were seen in the study population. Furthermore, results also showed that haemoglobin genotype and sex had no effects on the neutrophil/lymphocyte ratio ( $p=0.05$ ). This study shows a 66.7%, 33.3%, and 0% expression for HbAA, HbAS, and HbSS/HbSC, respectively, and that the Neutrophil/Lymphocyte ratio is within the normal reference range. Further studies to include other haemoglobin variants such as haemoglobin SS (HbSS) and haemoglobin SC (HbSC) is recommended.

Received: 09 Jan. 2024

Accepted: 24 Jan. 2023

Published: 25 Jan. 2023



**Copyright:** © 2023 by the authors.

Submitted for open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license

(<https://creativecommons.org/licenses/by/4.0/>).

**Keywords:** Neutrophil/Lymphocytes Ratio, Haemoglobin Electrophoretic patterns, Cellulose Acetate Electrophoresis Method.

## 1. INTRODUCTION

Inflammatory activities occurring *in vivo* constitutes normal immune defense mechanism in virtually all apparently healthy individuals. The neutrophil-to-lymphocyte ratio (NLR) in peripheral blood is an assay that strikes a balance between systemic inflammation and immunity and is thus an emerging prognostic biomarker in many diseases (1). The neutrophil-to-lymphocyte ratio (NLR) also serve as a biomarker connecting two part of the immune system; the innate and adaptive immunity, both mediated by the neutrophil and lymphocytes respectively. It is calculated as a simple ratio between the neutrophil and lymphocyte counts measured in peripheral blood (1).

Neutrophils are granulated cells that function as the first line of host immune response against invading pathogens through different mechanisms, which includes; chemotaxis, phagocytosis, release of reactive oxygen species (ROS), granular proteins, and the

production/liberation of cytokines (2). They also actively partake in adaptive immunity by playing an important regulatory role as main effector cells during the systemic inflammatory response (SIRS). Neutrophil as a major regulator of innate immunity, recruits, activates, and programs other immune cells and secretes an array of proinflammatory and immunomodulatory cytokines/chemokines capable of enhancing and advancing the effector function of other immune cells such as B cells, NK (Natural killer) cells, dendritic cells, CD4/CD8 cells (3).

Lymphocytes exist in different forms such as B cells; T cells, CD4-positive, CD4/CD8-negative or CD8-positive; natural killer T cells and are mainly responsible for adaptive immunity, providing an antigen-specific response regulated by the major histocompatibility complex (MHC) class I (4). Lymphocyte activity is involved in the host's response to viruses, tumor cells, atopy, and in the systemic inflammation response (4).

Haemoglobin electrophoretic patterns are the genotypic expression of haemoglobin that include both normal homozygous forms (HbAA) and abnormal heterozygous forms (HbSS, HbSC, HbCC, etc). The variant HbSC is formed by the replacement of glutamic acid with lysine at the 6th position of the  $\beta$ -globin chain while HbSS is formed by the replacement of glutamic acid with valine at the 6th position of the  $\beta$ -globin chain of the molecule (5,6,7). There are six major haemoglobin electrophoretic patterns inherited in the homozygous state (HbAA, HbCC, and HbSS) or heterozygous state (HbAS, HbAC, and HbSC) (8,9,10). The inheritance of HbS from both parents results in a homozygous state (HbSS) known as sickle cell anaemia/disease. The inheritance of HbS from one parent and HbA from the other leads to a heterozygous state (HbAS) which is known as sickle cell trait (SCT) (8,11). Subjects with HbSS gene trait are easily prone to anaemia due to destruction of red blood cell by various mechanism that decrease red cell production, increase red cell destruction and ineffective red cell production (12). Management of the disease associated with heterozygous state of haemoglobin S (HbSS) are usually expensive and often not affordable by the poor as most drugs used in its management are insufficiently ineffective, expensive and toxic to cell (13).

Researches have shown that there is an isolated rise in neutrophil count and consequently an elevated neutrophil/lymphocyte ratio in several conditions including; bacteria or fungal infection (14,15,16) atherosclerosis (17) all of which are common features with haemoglobin SS genotype. This is because the early hyperdynamic phase of infection is characterized by a proinflammatory state, mediated by neutrophils and other inflammatory cells (14). Thus, NLR is often characterized by an increase in neutrophils and a decline in lymphocytes. Lower NLR is usually associated with favorable prognostic factors in every field of application, mirroring a preserved immune balance (18). This study is aimed at determining Neutrophil/Lymphocytes ratio and electrophoretic pattern in apparently healthy undergraduate student of Rivers State University, Port Harcourt Nigeria.

## 2. MATERIALS AND METHOD

### 2.1 Study Design/population

This cross-sectional study was aimed at determining the Neutrophil/Lymphocyte ratio and Haemoglobin Electrophoretic patterns in apparently healthy students in Rivers State University. The study was carried out in August-November 2022 with a total of one hundred and fifty (150) apparently healthy male and female participants aged between 16 to 45 years recruited through a well-structured questionnaire.

### 2.2 Sample Collection, Transportation, Processing and Preservation

Five (5) millilitres (ml) of venous blood sample were collected from each participants through venepuncture techniques into ethylene diamine tetraacetic acid (EDTA) vacutainer bottle and transported under recommended condition to the laboratory for analysis.

### 2.3 Methodology

Estimation of Neutrophil, Lymphocyte count was carried using BC 5000 Mindray Hematology Auto-Analyzer and neutrophil/lymphocyte ratio calculated. Haemoglobin

electrophoretic pattern was carried out using cellulose acetate electrophoresis method under an electric current and at an alkaline pH (8.4 - 8.6).

#### 2.4 Calculation of Neutrophil/Lymphocyte Ratio

Neutrophil/lymphocyte ratio was calculated by dividing absolute values of neutrophil and lymphocyte count estimation from full blood count haematology autoanalysers. Although, NLR can also be calculated from full blood count autoanalysers when values of neutrophil and lymphocyte are presented in percentage. This was done by respective calculation and multiplying neutrophil and lymphocyte values by white blood cell count and dividing by 100 and then dividing the results of neutrophil by lymphocytes.

#### 2.5 Data Analysis

Data was statistically analyzed using Statistical package for Social Sciences (SPSS) version 23 and results presented in tables.

### 3. RESULTS

#### 3.1 Demographic Data of Studied Subjects

Table 3.1 shows a total of one hundred and fifty (150) subjects were enrolled for the study, comprising of seventy-six (76) males and seventy-four (74) females participants aged between 17-30years.

**Table 3.1:** Demographic Data of Studied Participants

<i>Parameters</i>	<i>Frequency (%)</i>
<i>Age range in years</i>	17-30
<i>Male</i>	76(50.7%)
<i>Female</i>	74(49.3%)
<i>Total</i>	150

#### 3.2 Percentage Distribution of Haemoglobin Genotype in Studied Participants

Result in the study shows that out of the total study population of 150 subjects, 100 (66.7%) participants were haemoglobin genotype A (homozygous A (HbAA) of which 48 (32%) were male and 52 (34.7%) were females. 50 (33.3%) of the studied participants were haemoglobin genotype AS (heterozygous AS (HbAS) of which 24 (16%) were male and 26 (17.3%) females as shown in table 3.2.

**Table 3.2:** Percentage Distribution of Haemoglobin Genotype in Studied Participants

<i>Genotype</i>	<i>Frequency (%)</i>
<i>Haemoglobin A (HbAA)</i>	
<i>Male</i>	48 (32%)
<i>Female</i>	52 (34.7%)
<i>Total</i>	100 (66.7%)
<i>Haemoglobin AS (HbAS)</i>	

Male	24 (16%)
Female	26 (17.3%)
Total	50 (33.3%)

### 3.3 Result of Neutrophil, Lymphocytes and Neutrophil/Lymphocyte Ratio of Studied Participants

Table 3.3 shows the mean±SD values of Neutrophil, Lymphocyte and Neutrophil/Lymphocyte Ratio in female participants as 46.22 ± 4.91(%) ( $p=0.6373$ ), 44.09 ± 4.56 (%) ( $p=0.5745$ ) and 1.14 ± 0.06 ( $p=0.4692$ ) respectively. The mean±SD values of neutrophil, lymphocyte and neutrophil/lymphocyte ratio in male participants were 47.02 ± 5.85(%) ( $p=0.6373$ ), 43.22 ± 4.37(%) ( $p=0.5745$ ) and 1.21 ± 0.07 ( $p=0.4692$ ).

**Table 3.3:** Result of Neutrophil, Lymphocytes and Neutrophil/Lymphocyte Ratio of Studied Participants

Parameter	Male (n=76)	Female (n=74)	P value	Remark
Neutrophil (%)	46.22 ± 4.91	47.02 ± 5.85	0.6373	NS
Lymphocyte (%)	44.09 ± 4.56	43.22 ± 4.37	0.5745	NS
NLR	1.21 ± 0.07	1.14 ± 0.06	0.4692	NS

**KEY:** n= number of participants, NLR=Neutrophil/Lymphocyte Ratio, NS= Not Significant when compared at  $p<0.05$

### 3.4 Effect of Haemoglobin Genotype on Neutrophil, Lymphocyte and Neutrophil/Lymphocyte Ratio

Table 3.4 shows the mean±SD values Neutrophil, Lymphocyte and Neutrophil/lymphocyte ratio in participants with haemoglobin AA (HbAA) as 46.65 ± 5.27(%) ( $p=0.9585$ ), 44.05 ± 4.10 (%) ( $p=0.4690$ ), and 1.16 ± 0.05 ( $p=0.6057$ ) respectively. Participants with haemoglobin AS (HbAS) had mean±SD values of neutrophil, lymphocyte and neutrophil/lymphocyte ratio as 46.56 ± 5.66(%) ( $p=0.9585$ ), 42.86 ± 5.13 (%) ( $p=0.4690$ ), 1.21 ± 0.09 ( $p=0.6057$ ) respectively.

**Table 3.4** Effects of Haemoglobin Genotype on Neutrophil, Lymphocyte, Neutrophil/Lymphocyte Ratio

Parameters	HbAA (n=100)	HbAS (n=50)	P value	Remark
Neutrophil (%)	46.65 ± 5.27	46.56 ± 5.66	0.9585	NS
Lymphocyte (%)	44.05 ± 4.10	42.86 ± 5.13	0.4690	NS
NLR	1.16 ± 0.05	1.21 ± 0.09	0.6057	NS

**KEY:** NLR=Neutrophil/Lymphocyte Ratio, n= number of participants, HbAA=haemoglobin genotype AA, HbAS=haemoglobin genotype AS, NS= Not Significant when compared at  $p<0.05$

## 4. DISCUSSION

The Neutrophil/lymphocyte ratio is an important haematological parameter derived by dividing absolute values of neutrophils by lymphocyte. It establishes the balance between systemic inflammation and immunity and is a rising prognostic biomarker in many diseases (19). This study was carried out to determine the Neutrophil/Lymphocyte ratio and

haemoglobin electrophoretic patterns in the apparently healthy undergraduate student population of Rivers State University.

Results for neutrophil/lymphocyte ratio showed that both male and female participants had NLR values all within established normal reference ranges. The findings in this study are deviant from the study of Alagbe and Olaniyi (20), who recorded significantly higher NLR values in subjects with HbSS compared to HbAA, and Beatrice *et al.* (21), who recorded lower values for NLR while assessing the effect of cART on neutrophil/lymphocyte ratio in HIV positive patients initiating antiretroviral therapy.

The finding in this study, however, is not surprising since there was no sickle cell haemoglobin genotype discovered among the participants in the study, which could have been a predisposing factor to inflammation and thus triggered elevated or decreased NLR values in the study population. It is known that sickle cell anaemia has always focused on the primary genetic defect, the abnormal sickle haemoglobin that polymerizes when deoxygenated. This polymerization within the red cell is capable of causing deformability of the cell and consequently resulting in the cell becoming rigid, obstructing blood flow. Obstruction of blood flow will trickle down to acute and/or chronic tissue damage due to poor perfusion of the cell.

Furthermore, the Neutrophil/lymphocyte ratio as a cheap assessment method of inflammation is somewhat more stable and not easily influenced by physiological, pathological, or stressful events known to influence neutrophil and platelet counts and activities. Therefore, since all participants in this study were apparently healthy, the triggers of inflammation that could have caused alterations in neutrophil/lymphocytes ratio are not present, and thus, the results observed in the study.

In this study, 100 (66.7%) participants comprising of 52(34.7%) male and 48(32%) females expressed the homozygous haemoglobin genotype AA (HbAA) while 50 (33.3%) comprising of 24 (16%) male and 26 (17.3%) females expressed the heterozygous haemoglobin genotype form AS (HbAS). No HbSS, HbAC and HbSC traits was seen in the study population. This finding slightly agrees with the findings of Erhabor *et al.* (22) who reported a 69.1% expression, Abdulrahaman *et al.* (7) who reported 70% expression of HbAA, Moses *et al.* (23) with 73.9% HbA and 26.1% HbAS in their study of pregnant women attending the antenatal clinic in Plateau State Specialist Hospital; Umoh *et al.* (24) who found out a 78.7% HbAA, 19.6% HbAS, 1.5% HbSS, 0.2% HbAC and 0.04% HbSC in their five (5) year retrospective study on haemoglobin genotypes as an implication for reproductive health in Uyo, Nigeria. The finding in this research is also within the normal reference range reported in blacks and in tandem with the reports of Abdulrahaman *et al.* (7)

From the findings in this research, it could be inferred that the HbAA is the most prevalent haemoglobin genotype among students of Rivers State University and this high percentage expression of HbAA, average prevalence of HbAS and no HbSS or HbSC can be attributed to the genes expressed in the population, high level of awareness and sensitization on the part of parents of the students on the negative social economic implications of haemoglobin genotype especially the HbSS on the health and wellbeing of their children. Also, the zero frequencies of HbSS observed in this study is a possible indication that the sickle cell gene trait in Port Harcourt is gradually reducing due to increased awareness, pre-marital counselling, increased awareness and knowledge of the devastating socio-economic implications and complications of the disease associated with HbSS gene. The absence and decrease prevalence in the HbSS and HbAS traits might also be attributed to the improved and active program for prenatal screening and diagnosis among pregnant women in Port Harcourt, Nigeria.

There was no statistically significant difference in the Neutrophil/Lymphocytes values in haemoglobin genotype AA (HbAA) compared to haemoglobin genotype AS (HbAS) ( $p=0.6057$ ). This indicates that haemoglobin genotype have no significant effects on neutrophil/lymphocytes ratio. This could be because there was no haemoglobin genotype SS and other abnormal variants which are highly connected with inflammatory processes. It is known that subjects with HbSS gene trait are easily prone to anaemia due to the destruction of red



blood cells by various mechanism that decrease red cell production, increase red cell destruction and ineffective red cell production. Fragment of red cell has ability to stimulate immune response capable of increasing neutrophil and lymphocyte count in peripheral blood.

Although Neutrophil/Lymphocytes values were slightly elevated in males compared to female participants, it shows no statistically significant difference ( $p=0.4692$ ) indicating that sex/gender have no significant effects on neutrophil/lymphocytes ratio. This could be because both the male and female participants had no condition or disease that affected or was capable of triggering the production of neutrophil/lymphocyte above normal reference values.

## 5. CONCLUSION

This study has revealed that haemoglobin genotype AA is the most prevalent genotype among the study population. Neutrophil/lymphocyte ratio of participants is within the normal reference range, and the haemoglobin genotype and sex have no effect on the neutrophil/lymphocyte ratio. Further studies to include other haemoglobin variants, such as haemoglobin SS (HbSS) and haemoglobin SC (HbSC), is recommended.

## REFERENCES

1. Minkyo, S., Barry, I. G., Charles, S., Rabkin A. & Eric, A. E. (2021). Neutrophil-to-lymphocyte ratio and mortality in the United States general population. *Nature Research* 19, 5-6 <https://doi.org/10.1038/s41598-020-79431-7>.
2. Esmacil, M., Shamila, D. A., Ian, M.A., Sharon, M. & Leo, K. (2018). Update on Neutrophil Function in Severe Inflammation. *Frontiers in Immunology*, 9(2171), 1-2. <https://doi.org/10.3389/fimmu.2018.02171>.
3. Li, Y., Wang, W., Yang, F., Xu, Y., Feng, C. & Zhao, Y. (2019). The regulatory roles of neutrophils in adaptive immunity. *Cell Communication and Signaling*, 17, 147 - 152. <https://doi.org/10.1186/s12964-019-0471-y>.
4. LaRosa, D. F. & Orange, J. (2008). Lymphocytes. *Journal of Allergy and Clinical Immunology*, 121, 364–369. doi: 10.1016/j.jaci.2007.06.016.
5. Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries Part 2. *Cambridge University press*, 2, 280-370.
6. Weatherall, D.J. (2010). The inherited diseases of hemoglobin are an emerging global health burden. *Blood*, 115, 4331-4336. doi: 10.1182/blood-2010-01-251348. .
7. Abdulrahman, Y., Isaac, Z.I., Erhabor, O., Sanusi, B., Udomah, F.P., Ezimah, A., Mainasara, Y., Wase, A., Uko, E.K., Adias, T.C., Iwueke, I., Ikhuenbor, D., Aghedo, F., Igbineweka, O. & Balarabe, I.A. (2013). Haemoglobin electrophoretic pattern among resident in Sokoto, Nigeria. *Journal of Medical Disorders*, 1, 2. <http://dx.doi.org/10.7243/2053-3659-1-2>
8. Akhigbe, R. E., Ige, S. F., Afolabi, A. O., Azeez, O. M., Adegunlola, G. J. & Bamidele, J. O. (2009). Prevalence of Haemoglobin Variants, ABO and Rhesus blood groups in Ladoko Akintola University of Technology, Ogbomosho, Nigeria. *Trends in Medical Research*, 4, 24-29. <https://scialert.net/abstract/?doi=tmr.2009.24.29>
9. Christian, S.G., Jacob, R.B., Adedeji, M.R. (2020). Evaluation of haemo-rheological variability of haemoglobin genotypes in descent of rumuche community, emohua local government area, rivers state Nigeria. *Nigerian Biomedical Science Journal*, 17(2), 6–11.
10. Jacob, R.B., Ken-Ezihuo, S.U., Bartimaus, E.S. & Eze, E.M. (2023). P-selectin level in haemoglobin-S variants steady-state subjects in Port Harcourt Nigeria. *Hematology and Transfusion International*, 11(4),110–113. DOI: 10.15406/htij.2023.11.00316.
11. Medugu, J. T., Abjah, U., Nasir, I. A., Adegoke, S. & Asuquo, E. E. (2016). Distribution of ABO, Rh D blood groups and hemoglobin phenotypes among pregnant women attending a Tertiary Hospital in Yola, Nigeria. *Journal of Medicine in the Tropics*, 18(1), 38-42. <https://doi.org/10.4103/2276-7096.177829>
12. Adias, T.C., Uko, E & Erhabor, O. (2006). Anaemia in Immunodeficiency virus: A review. *Nigeria journal of Medicine*, 15(3), 203-206. doi: 10.4314/njm.v15i3.37203.
13. Olayanju, A.O., Kyesman, N.I., Jacob, R.B., Adeniyi, T.D., Enitan, S.S. & Olayanju, A.J. (2021). Effects of Anti-Sickling Polyherbal Mixture on Haematological Indices in Normal and Anaemia-Induced Rabbits. *Asian Journal of Medicine and Health*, 19(12), 15-26. DOI: [10.9734/ajmah/2021/v19i1230413](https://doi.org/10.9734/ajmah/2021/v19i1230413).
14. Lowsby, R., Gomes, C., Jarman, I., Lisboa, P., Nee, P. A., Vardhan, M., Eckersley, T., Saleh, R. & Mills, H. (2015). Neutrophil to lymphocyte count ratio as an early indicator of blood stream infection in the emergency department. *Emergency Medical Journal*, 32, 531–534. doi: 10.1136/emmermed-2014-204071.
15. Jiang, J., Liu, R., Yu, X., Yang, R., Xu, H., Mao, Z. & Wang, Y. (2019). The neutrophil-lymphocyte count ratio as a diagnostic marker for bacteraemia: A systematic review and meta-analysis. *American Journal of Emergency Medicine* 37, 1482–1489. doi: 10.1016/j.ajem.2018.10.057.
16. Niu, D., Huang, Q., Yang, F., Tian, W., Li, C., Ding, L., Fang, H. C. & Zhao, Y. (2021). Serum biomarkers to differentiate Gram-negative, Gram-positive and fungal infection in febrile patients. *Journal of Medical Microbiology*, 70, 1360. doi: 10.1099/jmm.0.001360.
17. Adanstein, N. H., MacFadyen, J. G., Rose, L. M., Glynn, R. J., Dey, A. K., Libby, P., Tabas, I. A., Mehta, N. N. & Ridker, P. M. (2021). The neutrophil–lymphocyte ratio and incident atherosclerotic events: Analyses from five contemporary randomized trials. *European Heart Journal*, 42, 896–903. doi: [10.1093/eurheartj/ehaa1034](https://doi.org/10.1093/eurheartj/ehaa1034).

18. Agata, B., Benedetta, S., Michele, C. & Lorenzo, M. (2022). Neutrophil/lymphocyte ratio: An emerging marker of the relationships between the immune system and diseases. *International Journal of Molecular Sciences*, 23, 3636. doi: [10.3390/ijms23073636](https://doi.org/10.3390/ijms23073636)
19. Paquissi F.C. (2016). The predictive role of inflammatory biomarkers in atrial fibrillation as seen through neutrophil-lymphocyte ratio mirror. *Journal of Biomarkers*, 10, 1155-1165. DOI: [10.1155/2016/8160393](https://doi.org/10.1155/2016/8160393)
20. Alagbe, A. E. & Olaniyi, J. A. (2019). Pattern of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in sickle cell anemia patients at steady state and vaso-occlusive crisis. *Journal of Applied Haematology*, 10, 45-50. *Gale Academic OneFile*, [link.gale.com/apps/doc/A592874924/AONE?](https://link.gale.com/apps/doc/A592874924/AONE?link.gale.com/apps/doc/A592874924/AONE?)
21. Beatrice, W. M. I., Jacob, R.B., Christian, S.G. & Eze, E.M, (2022). The effect of cART on neutrophil: Lymphocyte ratio in HIV+ patients initiating combined antiretroviral therapy. *Biomedical Sciences*, 8(2), 57-62. DOI: 10.11648/j.bs.20220802.12.
22. Erhabor, O., Adias, T.C., Jeremiah, Z.A. & Hart, M.L. (2010). Abnormal hemoglobin variants, ABO, and Rhesus blood group distribution among students in the Niger Delta of Nigeria. *Pathology and Laboratory Medicine International*, 2, 41–46. DOI <https://doi.org/10.2147/PLMI.S9488>
23. Moses, D. L., Umanka, Y. P., Ogbonnaya, U. N., Naancin, I. V., James, G. D. (2018). Distribution of Haemoglobin Genotype, ABO and Rhesus (D) Blood Groups among Pregnant Women in North Central, Nigeria. *World Journal of Pharmaceutical and Medical Research*, 4(6), 54-58.
24. Umoh, A. V., Abah, G. M., Ekanem, T. I. & Essien, E. M. (2010). Haemoglobin genotypes: A prevalence study and implications for reproductive health in Uyo, Nigeria. *Nigerian Journal of Medicine: Journal of the National Association of Resident Doctors of Nigeria*, 19(1), 36–41. DOI: [10.4314/njm.v19i1.52473](https://doi.org/10.4314/njm.v19i1.52473).