Evaluation of Haematological Profile of Geriatric Subjects in Port Harcourt Metropolis of Niger Delta of Nigeria

Azuonwu Obioma*, Nnenna I and Uwuma OE

Department of Medical Laboratory Science, Faculty of Sciences, Rivers State University of Science and Technology, Nkpolu, Port Harcourt, Nigeria

*Corresponding author: Dr Azuonwu O, Department of Medical Laboratory Science, Rivers State University of Science and Technology, Nkpolu, Port Harcourt, Nigeria, **E-mail:** bimajacobs@yahoo.co.uk

Abstract

The haemopoietic process of the geriatrics involves inactive yellow marrow, replacing the red marrow (active form) thus; it could be persuasive to assume that blood counts may be low in the elderly, because of the establishment of fatty molecules cum variation in the availability of marrow type with respect to age. However, this cross-sectional observational study was designed and had the purpose of evaluating the haematological profile of geriatric subjects in Port Harcourt Metropolis of Nigeria. Thus, this would further uncover the relevance of ascertaining the geriatrics haematological profile, detecting the possible alterations in the geriatrics with respect to sex, and determining the effect of these non-modifiable biological factors (age and sex) on haematological parameters, such as Haemoglobin concentration, packed cell volume, total white blood cell count, platelet count and erythrocyte sedimentation rate. These parameters were estimated using Mythic 18 haematology auto-analyzer (Switzerland) and Westergren method for ESR. The study recruited 140 participants randomly selected from those who met the inclusion criteria, comprising of 100 apparently healthy geriatrics aged 70 ± 4years, 50 males and 50 females. Also, a control group included a total of 40 young adults age 34 ± 6years; 20 males and 20 females respectively. SPSS version 21 was used to analyze the data for mean, standard deviation, and t-test at 0.05 alpha level. The result showed a significant difference (p<0.05) in the mean values of the haematological parameters between the geriatrics and control (young adults). However, no disparity was observed among geriatrics males and females. An inverse relationship exists between Hb, PCV, WBC, PLT and age whereas, a direct relationship was observed between age and ESR. Nevertheless, it is therefore strongly recommended that, preventive haematological testing remains an essential component of health care delivery approach for the geriatrics, as anaemia could be a strong pointer for a a disease condition, even in asymptomatic subjects that may not show any signs and symptoms of an illness.

Keywords: Haematological; Anaemia; Parameter; Geriatrics; Port Harcourt Metropolis; Niger Delta

Introduction

Geriatrics, also known as the older stage of life which entails the later part of life is the period after youth and middle age, usually with reference to deterioration in organs and cells functioning capacity. The United Nations (UN) has agreed that sixty years and above may usually be recognize as old age. The Geriatrics stage of life is accompanied with distinguishing variations from physical, mental and haematological characteristics. These transitional marks do not occur at the same chronological age for every individual. They occur at different rates and order for different people because each individual is unique in nature and gene makeup, thus marks of old age varies between people, even in those of same chronological age.

Haematological parameters are quantifiable constituents of blood like erythrocytes and its indices, leukocytes and platelets [1]. These blood components originate from the haemopoietic stem cell, these occupy the entire capacity of the bones at birth but it is been replaced with fatty marrow with increase in age, thereby affecting these blood parameters based on these, even as different reference ranges could be seen for different age groups[1]. The assessment of haematological parameters is very necessary because, they are important proxy indicators useful in the assessment of immune status, therapeutic purposes and monitoring of disease progression and treatment outcome for proper patient management. The developmental stages of life vary directly with basic biological variables of age and sex independently. In pursuant of effective health care through accurate diagnosis, haematological parameters are routinely assessed in different phases of life. Notably, several factors including modifiable (life style- dietary patterns, attitude) and non-modifiable (genetics- age, sex) affect haematological parameters according to Hoffman RG [2] though, the variations in these factors depend hugely on the population, nutrition, geographic locations of the study populace thus, changes in haematological parameters in geriatrics will not be surprising if radical variations become visible from various studies across the globe.

Nonetheless, review of available literatures revealed that there is an inverse relationship between most haematological parameters and age, for instance, increase in age causes a decline in Hb, PCV, WBC and PLT; this could be an indication of a diminished reserved capacity of the bone marrow. On the other hand, parameter like ESR has been reported to have a direct relationship with age; which means ESR increases with respect to increasing age, it is suggestive of age related inflammatory response, this causes the plasma viscosity to increase as a result of an increase in plasma proteins like fibrinogen and other acute phase proteins [1,3-12].

Ageing is related to the progressive decline in functional reserve of multiple organ systems increasing the probability of dysfunction and disease. Progressive reduction in stem cell due to exhaustion of pluripotent cytokines and production of haemopoietic growth factors cause an imbalance of the haemopoietic modulation as reported by Mariza DS et al. [13]. Due to the universality of these age-related changes, it is generally and strongly suggested that separate reference ranges should be established for each age group as well as sex. Thus, there is need to establish these, particularly in developing countries for stratification. In

addition, in the area of this present study, haematological reports are centered on paediatrics and young adults while little or nothing is known about geriatrics. Nevertheless, it is generally assumed that haematological parameters decrease with increasing age without any empirical evidence from this given population (Nigeria). This shows that basically, the geriatrics have no reference range thus, no actual value is considered physiologically normal for this age group, rather comparison is made with the available (young adults) or that from the Caucasians which is not suitable for this population often times. Thus, there is a need for the establishment of a physiological range for the geriatrics haematological parameters for proper assessment and evaluation. Hence, this cross-sectional observational study was designed to evaluate the haematological profile of geriatric subjects in Port Harcourt Metropolis, with the aim of ascertaining the relevance of geriatrics haematological assessment, detecting the possible alterations in the geriatrics with respect to sex and determining the effect of these non-modifiable biological factors (age and sex) on some haematological parameters such as Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Total White Blood Cell (WBC) count, Platelet Count (PLT) and Erythrocyte Sedimentation Rate (ESR) respectively. It is strongly believed that data generated from this present empirical evidence based study, would be helpful in prompt diagnosis, treatment and management of the diseases of the elderly in the region, given the scenario of increasing challenge of death of Health infrastructure and manpower, coupled with weak government Health policies to underpin the gains of robust Health care delivery system in the region [14].

Study Location

Port Harcourt is one of the greatest cities in South-South part of Nigeria, the city potentially lies along the Bonny River and its location remains very strategic as the capital of all Niger Delta States cum capital of Oil rich River State [14]. Port Harcourt is also regarded as the center nerve of oil and gas activities in Nigeria, given her role in engendering development through the oil and gas economy, both in the past and in the present Nigeria. The population of Port Harcourt is about 1,382.592 and 2,700/km² (7,100/sqmi) density as reported by 2006 Nigerian Population census.

Nonetheless, history recorded it that, Port Harcourt exported the first shipload of oil in 1958 after the huge oil deposit was discovered in Oloibiri in 1956, thereby fulfilling one of its core mandates of creation. Port Harcourt as at today remains the home of huge oil and gas activities in Nigeria, thus playing host to a myriad of oil and gas multinationals and other allied industries. The city has witnessed tremendous exponential growth in development, urbanization, and a population that is though, not in consonance with the level and quality of social infrastructure on the ground [14]. However, as a result of the massive oil and gas activities within the region, the populace have suffered different categories of environmental pollution of the air, water, and land, even as the agricultural activities have remained unproductive with a large amount of hunger and poverty in the region [14]. Presently, there is increasing trend of black sooth in the air, due to probably the activities of oil and gas operations, which have increasingly domiciled in Port Harcourt. It is strongly believed that such condition might promote the spread of respiratory diseases and cancer of the lungs and skin as well as blood disorders, if urgent steps that are scientific and research based, are not taking in good time by the relevant authorities. Port Harcourt play host to two active refineries that produce about 210, 000 barrels of crude oil a day. The city is inclined with a tropical monsoon climate which features heavy and lengthy rain fall and short dry season, thus only December and January partially witness dry season. The harmattan season that is always pronounced in most West African cities remains hardly visible in Port Harcourt, with little or no influence. September is the month of heaviest precipitation in Port Harcourt with an average rainfall of 20 mm and the average temperatures are literally between 25°C-28°C in the city. 4°49′27″N 7°2′1″E Coordinates area of 369 km² (142 sqmi) metropolis, 360 km² (140 sqmi) land, 9 km² (3 sqmi) water, 158 km² (61sqmi) urban, 1,900 km² (700 sqmi). The Port Harcourt metropolis covers two local government areas- Port Harcourt and Obio-Akpor literally.

Materials and Methods

The study recruited 140 participants randomly selected, sample sized estimated according to Cochran WG [15]. An unbiased simple random selection (sampling technique) was employed in this study, each study participants had equal chances of being selected to curb bias from the selection procedure till the required sample size was achieved. The entire process of sampling was done in a simple way with each subject selected independently of the other members of the study participants and balloting without replacement was adopted in selecting the participants in each facility. The selection involved each subject been assigned a unique number. All numbers were placed in a ballot bag and mixed thoroughly then stipulated numbers were picked to work with per day based on their availability. In this study, both the researcher and the study participants were not aware of the particular number to be picked per time (double blinded), any number picked became the assigned number.

Outpatient department/family medicine clinics of Braithwaite Memorial Specialist Hospital and Civil servants Clinic, Rivers State Secretariat were used for this study. The eligible subjects were selected through medical records of the facilities used. A standard self structured questionnaire was formed specifically designed for the study to extract data on socio-demographic details (sex, age, education, occupation, and religion), weight, height, basal metabolic index (BMI), blood pressure, family history etc.

A combination of primary and secondary data were used. All paediatrics, menstruating/pregnant women and adults who did not consent by endorsing a written/informed consent, were excluded from the study while those young adults and geriatrics who gave their consent participated in the study. A comprehensive history and laboratory evaluation were performed on the study participants, those with extreme low or high values were excluded from the study to rule out (confounders) known cases of abnormalities (anaemia, polycythaemia, leukaemia, etc.) among the study population were not included as well. Apparently healthy individuals were considered as those in good health condition with no observable signs and symptom of blood disorders or any known illness activity restrictions, chronic diseases- cardiac problems which may affect cardiac output etc. In addition, iron deficiency, folate deficiency, Vitamin B_{12} deficiency though were not evaluated but those on medications (supplementation/fortification); oral mineral supplements (used in mineral deficiencies)-vitamin B_{12} supplementation, B complex, folic acid etc. Also, those taking medications like erythropoiesis-stimulating agents and colony-stimulating factors (erythropoiesis enhancer) were excluded from the study. Furthermore, physical examination was performed. Typical symptoms of anaemia, such as fatigue, weakness and dyspnea, seemed unspecific especially in the elderly patients which could be attributed to advancing age; however, conjunctival pallor was a helpful diagnostic clue used.

Mythic 18 haematology auto-analyzer (Switzerland) and 4 ml of venous blood collected into K2EDTA (BD, USA) was used for the haematology indices whereas, Westergren method for ESR involved 2 ml of venous blood collected into a tube containing 0 .5 ml of sodium citrate.

Statistical analysis

Normal distribution test for parametric data was checked with the use of Shapiro-Wilk (S-W) test which matched up the data to a normal distribution resulting to no indication of statistical significance ($p>0.05$)

which suggested that the samples were normally distributed. Mean, standard deviation and Independent sample t-test were used to measure the distribution at 0.05 level of significance using SPSS version 21 statistical package. Also, data were further analysed using SPSS Version 21. Descriptive statistics for continuous variables included means and standard deviations were also explored. Whereas, test of significance involved the use of independent t- test at 0.05 alpha level.

Ethical Consideration

Ethical authorization for this study was sought from the ethics committee of the Rivers State University of Science and Technology, University of Port Harcourt Teaching Hospital, Hospital Management Board, School of Health Technology Maternity Centre Board and the Braithwaite Memorial Specialist Hospital, all in Port Harcourt Metropolis. Moreover, permission from the family medicine/outpatient clinic was also obtained. Furthermore, informed written consent was obtained from their parents after detailed information and procedure of the study was explained to them and those who gave their consent endorsed on the consent form to participate.

Results

One hundred and forty participants were recruited in this study, comprising of 100 apparently healthy geriatrics of 50 males and 50 females. Also, a control group included a total of 40 young adults; 20 males and 20 females. Age distribution from this study revealed a mean age of 70 ± 4 years and 69 ± 4 years for geriatrics males and females respectively whereas; the control group had mean age of 30 ± 5 years and 37 ± 8 years for males and females respectively.

Table 1 shows the distribution of the haematological parameters for geriatrics male and female. Mean haemoglobin concentrations of 12.3 ± 1.9g/dl and 12.0 ± 0.5g/dl for geriatric males and females accordingly, with no evidence of statistical significance {t(98)=1.079; p=0.283}.Similarly, the white blood cell count, platelet count and erythrocyte sedimentation rate showed no indication of statistical significance t_{98}=1.118, p=0.266; t_{98}=1.483, p=0.141; t_{98}=0.985, p=0.985 for white blood cell count, platelet count and erythrocyte sedimentation rate for the geriatrics males and females respectively. However, comparison of the packed cell volumes for geriatrics male and females revealed an evidence of statistical significance (t_{98}=4.008, p=0.000) with a mean of 37.7 ± 2.1 and 36.3 ± 1.3 respectively.

In addition, an independent-samples t-test was conducted to compare haematological parameters in geriatrics males and control males (young adults), almost all parameters showed that there were indications of statistical significant difference, apart from white blood cell count with means of 70 ± 4.0years, 12.3 ± 1.9g/dl, 37.7 ± 2.1%, 5.9 ± 0.6x10^9/L, 182 ± 23 × 10^9/L and 42.4 ± 3.7mm/hr for geriatrics male and control males 30 ± 5.0 years, 14.0 ± 1.0g/dl, 40.5 ± 3.1%, 6.2 ± 0.6 × 10^9/L, 206 ± 32 × 10^9/L and 16.1 ± 6.1mm/hr; with a t-values of t(68)=35.136, p=0.000; t(68)=3.786, p=0.000; t(68)=4.371, p=0.000; t(68)=1.889, p=0.063; t(68)=18.143, p=0.038 and t(68)=22.084, p=0.000 for age, haemoglobin concentration, packed cell volume, white blood cell count, platelet count and erythrocyte sedimentation rate (Table 2).

Further comparison of Mean, SD and t test distribution of haematological parameters in geriatrics females and control females showed an indication of statistical significant as revealed in this study (Table 3).

Table 1: Mean, SD and t test Distribution of Haematological Parameters in Geriatrics Males and Female

Parameters	Male N=50 Mean ± SD	Female N=50 Mean ± SD	t-value	DF	95% CI	P-value
Age (Years)	70 ± 4	69 ± 4	1.250	98	-0.59–2.59	0.214
Hb (g/dl)	12.3 ± 1.9	12.0 ± 0.5	1.079	98	-0.251–0.851	0.283
PCV (%)	37.7 ± 2.1	36.3 ± 1.3	4.008	98	0.707–2.093	0.000
WBC (10^9/L)	5.9 ± 0.6	6.0 ± 0.2	1.118	98	-0.277–0.077	0.266
PLT (10^9/L)	182 ± 23	188 ± 17	1.483	98	-14.03–2.03	0.141
ESR (mm/hr)	42.4 ± 3.7	41.5 ± 5.3	0.985	98	-0.914–2.714	0.985

Table 2: Mean, SD and t test Distribution of Haematological Parameters in Geriatrics Males and Control Males

Parameters	Male N=50 Mean ± SD	Male N=20 Mean ± SD	t-value	DF	95% CI	P-value
Age (Years)	70 ± 4.0	30 ± 5.0	35.136	68	37.73–42.27	0.000
Hb (g/dl)	12.3 ± 1.9	14.0 ± 1.0	3.786	68	-2.59– -0.84	0.000
PCV (%)	37.7 ± 2.1	40.5 ± 3.1	4.371	68	-4.08– -1.52	0.000
WBC (10^9/L)	5.9 ± 0.6	6.2 ± 0.6	1.889	68	-0.62–0.02	0.063
PLT (10^9/L)	182 ± 23	206 ± 32	18.143	68	-137.64– -110.36	0.000
ESR (mm/hr)	42.4 ± 3.7	16.1 ± 6.1	22.084	68	23.92–28.68	0.000

Table 3: Mean, SD and t test Distribution of Haematological Parameters in Geriatrics Females and Control Females

Parameters	Female N=50 Mean ± SD	Female N=20 Mean ± SD	t-value	DF	95% CI	P-value
Age (Years)	69 ± 4	37 ± 8	1.250	68	29.14–34.86	0.000
Hb (g/dl)	12.0 ± 0.5	12.5 ± 0.4	1.079	68	-0.750– -0.814	0.000
PCV (%)	36.3 ± 1.3	37.8 ± 1.3	4.008	68	-4.078– -1.522	0.000
WBC (10^9/L)	6.0 ± 0.2	6.2 ± 0.5	1.118	68	-0.617– -0.017	0.019
PLT (10^9/L)	188 ± 17	198 ± 20	1.483	68	-137.64– -110.36	0.038
ESR (mm/hr)	41.5 ± 5.3	22.3 ± 10	0.985	68	23.924–28.676	0.000

Discussion

This study clearly demonstrated the effects of ageing on some haematological parameters which are evident in the significant differences in the haematological parameters between the geriatrics and the control (young adults). The fact that a variety of parameters changed with age could be attributed to the effect of increasing age on various cells, organs and systems as reported by Brigden ML et al. [11].

Report has it that, at birth, there is an initial increase in haematological parameters but immediately after the post natal period, there tend to be a fair steeply decrease from few days to about three months of life, and gradually rise to almost adult levels at puberty and then adulthood with a subsequent decline seen in the old adults [1].

There were statistical differences in the haemoglobin concentration and packed cell volume between the geriatrics and young adults, the haemoglobin level and packed cell volume were lower in the geriatrics when compared to the control (young adults) with an indication of statistical significance. This observation is similar with Nilsson-Ehle H et al., Smith JS et al. [4,5]. Also, in almost all the literatures reviewed in relation to ageing and haemoglobin level/ packed cell volume of geriatrics had decreased haemoglobin concentration/ packed cell volume when compared to young adults [1,4,7-12]. The lower haemoglobin level that was observed in the elderly people could probably suggest a physiologic reduction, rather than pathologic condition according to a Japan based longitudinal study by Yamada M et al. [6]. This physiologic anaemia seen in the elderly, as reported in this study is as well in conformity with the work of Olivares M et al. [16] which involved two hundred and seventy-five apparently healthy older adults as participants and a prevalence rate of four percent was observed in their study. Also, other scholars have been able to establish the fact that physiologic anaemia could be seen in apparently healthy elders [17-19]. The reason behind this geriatric physiologic anaemia is unclear however; some scholars have attributed it to the replacement of the red bone marrow with the fatty yellow marrow or cellularitis of the bone marrow in geriatrics. On the other hand, previous health issues and lifestyle choices create a diverse constellation of diseases and symptoms in different individual. Hence, the appearance of symptoms depends on the remaining healthy reserves in the cells, tissues, organs and systems [20]. In addition, the decline in physiological reserve makes the elderly develop some kinds of diseases, and have more complications from mild to severe problems however; asymptomatic individuals are at risk as well thus, there is need for preventive haematologic testing in order to avert presumed morbidity and mortality that could result from anaemia. Furthermore, this study revealed a significant sex disparity in haemoglobin concentration and packed cell volume between male geriatrics and female geriatrics with the aged males having higher values compared to the females. This is in agreement with the work of Mangwendeza MP et al. [8] in Harare Zimbabwe, which reported a higher mean values in the elderly males compared to the elderly females.

The total white blood cell count was also higher in the young adults when compared to the aged group. This finding is comparable with those of Odunukwe NN et al. and Sharland DE [7,12]. This decrease in total leukocyte count could be attributed to a quantitative decrease in the suppressor T-cells [21] on the other hand; there was no proof of statistical significant sex difference in the total leukocyte count when matched between sexes for both groups as obtained in this study, this is related to the work of Odunukwe NN et al. [7]. Also, platelet count showed a marked difference between the two groups with the younger adults (control) having a higher platelet count when compared to the geriatrics. Moreover, several factors account for varied platelet counts like age, drugs, consumption rate, bone marrow suppression, genetic etc. [22]. Genetic factors responsible for variation in platelet count in healthy individuals are being investigated and a few have been identified [23,24] but they account for only a little proportion of the phenotypic variance.

From the foregoing evidence based proof, as obtained in this study, erythrocyte sedimentation rates showed a direct relationship with age; the higher the age, the higher the erythrocyte sedimentation rate as the geriatric group showed a very significant increase in the erythrocyte sedimentation rate when compared to the control group. Similarly Odunukwe NN et al. and Brigden ML et al. [7,11] revealed this as well. Additionally, significance difference was reported between sexes for both groups. Females had higher erythrocyte sedimentation rate than males for both groups which is consistent with the reports of Odunukwe NN et al. and Brigden ML et al. [7,11]. Furthermore, this geriatrics triggered elevated erythrocyte sedimentation rate could be associated with some of the common ageing processes like increased plasma and acute phase proteins, due to inflammatory response to tissue injury during ageing process causing the promotion of rouleux formation and thereby increasing the rate of sedimentation as earlier stated. Erythrocyte sedimentation rate however, does not provide definite diagnosis and thus, adequate care must be taken to avoid misleading diagnosis and management of an illness in elderly subjects.

Conclusion

This present study reported that some haematological parameters exhibited considerable variations at different stages of life, in a population. Since age and sex are non-modifiable biological factors, it is imperative for reference values to be age and sex specific for use in clinical evaluations. The emphatic haematological parameters estimated in this study on the geriatrics showed a marked variation when compared to the control (young adults) group.

Besides, the evidence based results above have strongly demonstrated the importance of age and sex as a specific haematological parameter reference indices, which cannot be overemphasized as a necessary tool for the assessment, monitoring and management of patients, especially during the interpretation and comparisons of results with respect to reference values. Therefore, the geriatric haematology reference values established in this study will in no small measure help in proper diagnosis and improve the management of the geriatrics. Absence or inappropriate reference ranges could increase the chances of misdiagnosis, unnecessary additional investigations and failure to detect the underlying disease in question. Thus, the values established in this present study could serve as baseline for further research investigations for the geriatric populaces and this study have actually filled the gap of lack of information concerning the reference haematological parameters for the geriatrics within the region of this present study.

Conflict of interest

Non observed among authors

References

1. Dacie JV, Lewis SM (2010) Reference ranges and normal values. In: Practical Haematology, 10th Editionion, Churchill Livingstone, Edinburgh 559-574.

2. Hoffman RG (1971) Establishing quality control and normal ranges in the clinical laboratory. Ex-position Press, New York, New York, 30.

3. Nilsson-Ehle H, Landahl S, Lindstedt G, Netterblad L, Stockbruegger R, et al. (1989) Low serum cobalamin levels in a population study of 70- and 75-year-old subjects. Gastrointestinal causes and hematological effects. Dig Dis Sci 34: 716-723.

4. Nilsson-Ehle H, Jagenburg R, Landahl S, Svanborg A, Westin J (1988) Haematological abnormalities and reference intervals in the elderly. A cross sectional comparative study of three Swedish population samples aged 70,75 and 81 years. Acta Med Scand 224: 595-604.

5. Smith JS, Whitelaw DW (1971) Haemoglobin values in aged men. Can Med Assoc J 105: 816-818.

6. Yamada M, Wong FL, Suzuki G (2003) Longitudinal trends of hemoglobin levels in a Japanese population-RERF's Adult Health Study subjects. Eur J Haematol. 70: 129-135.

7. Odunukwe NN, Imonugo IO, Akanmu AS, Nnodu OE, Okany C et al. (2004) Ferritin and Haematological values in healthy elderly Nigerians. Turk J Haematol 21: 71-77.

8. Mangwendeza MP, Mandisodza A, Siziya S (2000) Haematology reference values for healthy elderly blacks residing in Harare, Zimbabwe. Cent Afr J Med 46: 120-123.

9. Ross DW, Ayscue LH, Waston J, Bently SA (1988) Stability of haematologic parameters in healthy subjects. Intra-individual versus Inter-individual variations. Am J Clin Pathol 90: 262-267.

10. Hamblin TJ (2007) Haematological problems in the elderly. Clinical Haematology 1: 271-593.

11. Brigden ML, Healthcare JC (2000) Problems in Interpreting Laboratory tests: What do unexpected results mean? Postgrad Med 107: 145-146.

12. Sharland DE (1980) Erythrocyte Sedimentation Rate: The normal range in the elderly. J Am Geriatr Soc 28: 346-348.

13. Mariza DS, Schivanke AHC, Bauer M, Clarice L, Irana MC (2007) Haematological and nutritional parameters in apparently healthy elderly individuals. RevistaBrasileiraHematologia e Hemoterapia 29: 10.

14. Azuonwu O, Nnenna I, Douglass AS, Ntaa NB (2016) Consequences of Haemolytic Disease of the Fetus and New-born (HDFN) and the Clinical Significance of Antibody Screening in Prenatal Diagnosis: A Study of Multigravidal and Primigravidal Women in Port Harcourt, Niger Delta. J Clin Lab Med 1: 106.

15. Cochran WG (1977) Sampling techniques (3rd ed.) New York: John Wiley & Sons.

16. Olivares M, Hertrampf E, Wegner D, Capurro MT, Letelier A, et al. (1994) Normal values of the hemogram and other hematological variables in subjects over 60 years old. Rev Med Chil122:1289-1293.

17. Blain H, Lerouge S, Blain A, Lacomski D, Virion JM, et al. (2001) Determination by flow cytometry of reference values of erythrocyte parameters in aged subjects. Presse Med 30: 779-784.

18. Weggemans RM, de Groot LC, Haller J (1997) Factors related to plasma folate and vitamin B_{12}. The Seneca study. Int J Food Sci Nutr 48:141-150.

19. de Groot CP, van Staveren WA (2002) Undernutrition in the European SENECA studies. Clin Geriatr Med 18: 699-708.

20. Fletcher C, Peto R (1977) The natural history of chronic airflow obstruction. Br Med J 1: 1645-1648.

21. Fox RA (2005) Immunology of ageing. In: Textbook of Geriatric Medicine and Gerontology, Churchill Livingstone, Edinburgh 82-104.

22. Drachman JG (2004) Inherited thrombocytopenia: when a low platelet count does not mean ITP. Blood 103: 390-398.

23. Gieger C, Radhakrishnan A, Cvejic A, Tang W, Porcu E, et al. (2011) New gene functions in megakaryopoiesis and platelet formation. Nature 480: 201-218.

24. Girotto G, Pirastu N, Sorice R, Biino G, Campbell H, et al. (2011). Hearing function and thresholds: a genome-wide association study in European isolated populations identifies new loci and pathways. J Med Genet 48: 369-374.