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Original Research Article

Ferritin and transferrin levels of the elderly population at Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

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ABSTRACT

Background: The elderly have limited regenerative abilities thus predisposing them to various diseases. Measuring both serum ferritin and transferrin serve as biomarkers of iron deficiency. This study, using enzyme-linked immunosorbent assay (ELISA) determined the serum levels of ferritin and transferrin in the elderly. Full blood count was also done and correlated with serum ferritin and transferrin levels of every participant.
Methods: This was a cross-sectional study at the Geriatric Clinic of Lagos State University Teaching Hospital (LASUTH). Following receipt of written consents from ninety (90) elderly participants, venous samples were drawn for full blood count (FBC) and samples for serum ferritin and transferrin ELISA assay collected and stored at -40^{o}C until the required sample size was obtained. Data were analyzed using SPSS version 23.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill). The Pearson chi-square test was used for statistical analysis. P value was considered to be statistically significant when <0.05.
Results: Participants consisted of 50 (55.6%) females and 40 (44.4%) males. The mean age of all participants was 71.31±7.38 years. The Majority, 90% (36 of 40) of the males had haemoglobin values lower than 13 gm/dl, while 66% (33 of 50) of females had haemoglobin values lower than 12 gm/dl. The mean ferritin concentration of all participants was 196.19 ±121.21ng/ml. The overall mean serum transferrin was 0.187±0.157 ng/ml with a minimum of 0.03 ng/ml and a maximum of 1.18 ng/ml.
Conclusions: Anaemia in the elderly is very common using the World Health Organization (WHO) haemoglobin cut-off values, however, iron deficiency anaemia prevalence is low.

Keywords: Ferritin, Transferrin, Elderly

INTRODUCTION

Ageing comes with numerous health challenges such as anorexia because of reduced metabolism, poor food absorption due to inflammatory bowel diseases, dental caries, dementia, inadequate and unbalanced food intake, and limited regenerative ability. These impacts negatively on the status of iron in the body. Iron is needed for haemoglobin formation, deficiency of which results in anaemia. Iron is transported by transferrin and stored majorly as ferritin.

The World Health Organization (WHO) defines the elderly as a person of 65 years and above.[1] However, Jacobs et al differ with the 65 years of WHO suggesting 70 years as the cut-off age of the elderly based on

improvement in health, cognition, and functional ability particularly in the developed economy.[2]

Biological, compared with chronological age has been suggested as a better determinant of the elderly's age.[3,4] Biological age is the combination of chronological age, genetics, lifestyle, and diseases present.

Haemoglobin stores 65% of body iron, 30% is stored in ferritin while about 5% is stored in myoglobin and iron-containing enzymes.[5]

Iron deficiency accounts for half of the causes of anaemia in the elderly in developing countries.[6] In 1972, serum ferritin was assayed in a normal population, individuals with iron deficiency and overload. Low serum ferritin was for the first time associated with iron deficiency while high serum ferritin was associated with overload.[7] Normal reference ferritin level is 15-300 µg/l in men, however, this value is lower in premenopausal women and children.[8]

Plasma iron is transported by transferrin.[9] Iron reversibly binds transferrin, the latter is up-regulated in iron deficiency and down-regulated in iron overload.[10,11] Via the transferrin receptor pathway, transferrin transports four molecules of iron to the cells.[12] To meet the erythropoiesis daily needs, transferrin has a daily turnover rate of ten times.[13] Transferrin's normal reference range is between 250-300mg/dl.[14] Its half-life is 8-10 days.[15] Higher than the normal value of transferrin has been reported in those with nutritional supplementation.[16] This study aimed at determining the serum levels of ferritin and transferrin in the elderly to evaluate their iron status.

METHODS

Study location

The Geriatric Clinic of Lagos State University Teaching Hospital (LASUTH) was used. The hospital was established as a cottage hospital in 1955, transformed into a secondary health centre in 1970, and later metamorphosed into a teaching hospital in July 2001 following the establishment of Lagos State University College of Medicine on 9th February 1999.

Study population

The participants of the study were recruited from the geriatric clinic of LASUTH located in the General out-patient department and run by family physicians. The clinic runs every Wednesday.

Study design

This study was a descriptive and cross-sectional study.

Inclusion criteria

Consenting elderly people of the age range of 65 years and above.

Exclusion criteria

Adults below 65 years of age. Those who refused to give informed consent. Critically ill elderly people. Those on folic acid and Vitamin B12 supplements.

Sampling technique

Participants were recruited consecutively as they consented to participate in the study.

Questionnaire administration and history taking

With the use of an interviewer-administered questionnaire, each participant was interviewed to obtain relevant socio-demographic and clinical data like age at last birthday, history of blood transfusion, and drug history particularly use of an iron tablet.

Specimen collection

Following receipt of written consents from the participants, the researcher, and a trained research assistant collected blood samples from the participants from intravenous access, under aseptic conditions using a vacutainer needle, 4.5 ml of blood was collected into an EDTA bottle to obtain blood for haemoglobin, packed cell volume, red cell count, red cell indices, total white blood cell count with differentials, and platelet counts another 3 ml was collected into plain bottle and content centrifuged to obtain the serum for the ferritin and transferrin using enzyme-linked immunosorbent assay (ELISA).

Sample analysis

The EDTA samples were run on Sysmex KN-21N (manufactured by Sysmex Corporation, Kobe Japan with a serial number B6991). It is a three-part auto-analyzer able to run 19 parameters per sample which include white blood cell count, haematocrit, red blood cell count, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration among others. All FBC samples were run on the day of collection. While sera from plain bottles were stored at -40^{o}C for ELISA of ferritin and transferrin. Ferritin ELISA kit was manufactured by Calbiotech, Catalogue number FR248T. The optical density obtained was used to determine the ferritin concentration in ng/ml for each sample. The concentration was extrapolated from a standard curve obtained by plotting the optical densities versus known concentrations of six standards provided in the kit. The transferrin ELISA kit was manufactured by Elabscience with a catalogue number E-TSEL-H0001. The transferrin kit has a sensitivity of 0.13 ng/ml and a detection range of

0.14 to 100 ng/ml, a coefficient of variation of <10%. The optical density obtained was used to determine the transferrin concentration in ng/ml for each sample. The concentration was extrapolated from a standard curve obtained by plotting the optical densities versus known concentrations of eight standards provided in the kit. All assays were done in the Haematology Laboratory in LASUTH.

Sample size determination

Sample size was determined using the Daniel's formula.[17]

$$N = \frac{z^2pq}{d^2}$$

where N= sample size, z= statistics for confidence level of 95% set at 1.96, p= Reported prevalence, q= 1-p, d= degree of precision =0.05.

A reported prevalence of anaemia in the elderly is 3.7%.[18]

$$N = \frac{1.962 \times 0.037 \times 0.963}{0.052}$$

$$= \frac{3.8416 \times 0.035631}{0.0025}$$

=54.75

10% Attrition = 5.475; 54.75 + 5.475 = 60.2 ≈ 60

The calculated sample size of 60 was increased to 90 to allow for a margin of error.

Therefore, total subjects recruited =90.

Confidentiality

Participants were not identified by their names and electronic data were pass-worded in the storage facility.

Ethical considerations

Ethics committee approval for the study was obtained from the Health Research Ethics Committee of LASUTH, Ikeja

Statistical analysis

Data were analyzed using SPSS version 23.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill). The continuous variables were given as means±standard deviation (SD). The Pearson Chi-square test was used to test for association between discrete variables. P value was considered to be statistically significant when <0.05.

RESULTS

A total of ninety (90) participants were recruited consisting of 50 (55.6%) females and 40 (44.4%) males. The mean age of all participants was 71.31±7.38 years. The mean age of male participants was 71.24±7.51 years and of females were 71.02±7.45 years. The majority (68.9%) were Christians while only 31.15% were Muslims. A total of 33.3%, 30.0%, 22.2%, and 14.4% had tertiary, secondary, primary and no formal education respectively. Almost all (98.9% and 94.4%) neither smoked nor on alcohol respectively.

The full blood count parameters of participants are presented in Table 1.

A total of 55 of 90 (61.1%) of all participants had haemoglobin values lower than 12 gm/dl while 35 of 90 (38.9%) had haemoglobin values between 12 to 17 gm/dl. The highest value of haemoglobin was 16.6 gm/dl. The majority 90% (36 of 40) of the males had haemoglobin values lower than 13 gm/dl, while 66% (33 of 50) of females had haemoglobin values lower than 12 gm/dl.

Similarly, 22 of 90 (24.4%) had MCH lower than 27 pg, majority of them 58 of 90 (64.4%) had MCH within the reference range of the laboratory (27 to 32 pg) while 10 of 90 (11.1%) had MCH greater than 32pg.

The mean ferritin concentration of all participants was 196.19±121.21 ng/ml while the male and female participants had mean ferritin values of 207.76±131.04 ng/ml and 186.93±113.22 ng/ml respectively. The minimum ferritin for males was 33.33 ng/ml and the maximum was 500 ng/ml while the minimum for females was 15 ng/ml and the maximum 500 ng/ml.

Table 2 shows categorization of serum ferritin into <15 ng/ml, 15-300 ng/ml, and >300 ng/ml.

Using chi-square between age and ferritin p value of 0.90 was obtained but the two parameters correlated positively with each other r=0.52, while p values were obtained using independent t-tests between age and serum ferritin / mean corpuscular haemoglobin (MCH) and serum ferritin of participants were 0.17 and 0.60 respectively.

The overall mean serum transferrin obtained in this study was 0.187±0.157 ng/ml with a minimum of 0.03 ng/ml and a maximum of 1.18 ng/ml. The gender-specific serum transferrin concentrations are presented in Table 3. The minimum transferrin for males was 0.07 ng/ml and the maximum was 1.18 ng/ml while the minimum for females was 0.03 ng/ml and the maximum was 0.51 ng/ml.

Table 1: Full blood count parameters of all participants.

Parameters	Range	Minimum	Maximum	Mean	Std deviation
WBC*10^9/l	12.87	3.20	16.07	5.94	1.95
HbG/dl	11.5	5.1	16.6	11.38	1.77
PCV%	34.8	16.3	51.1	34.83	5.13
Platelet count*10^9/l	641	102	743	230.76	84.45
Lymphocyte%	64.9	11.8	76.7	40.10	12.18
Neutrophil%	68.2	17.5	85.7	51.60	12.83
RBC concentration	4.88	2.0	6.88	4.01	0.71
MCH pg	15.4	19.7	35.1	28.59	2.85
MCV fl	32.9	70.5	103.4	86.96	6.78
MCHC g/dl	8.7	26.9	35.6	32.84	1.18

Keys: WBC-white blood cells, HbG-haemoglobin, PCV-packed cell volume, RBC-red blood cells, MCH-mean corpuscular haemoglobin, MCV-mean corpuscular volume, MCHC-mean corpuscular haemoglobin concentration.

Table 2: Categorization of serum ferritin in groups.

Group (ng/ml)	Frequency	Percentage (%)
<15	1	1.1
15-300	73	81.1
>300	16	17.8
Total	90	100

Table 3: Gender-specific serum transferrin concentration.

Transferrin Concentrations	Mean (ng/ml)	Frequency	P value
Males	0.21±0.20	40	
Females	0.51±0.16	50	0.001
Total	0.187±0.157	90	

DISCUSSION

Anaemia's definition is influenced by age, ethnicity, and gender.[19,20] It is commoner in the elderly than in the younger population because of a progressive decrease in haematopoiesis due to ageing, in addition to various comorbidities associated with ageing.[21] Anaemia in the elderly is associated with poor quality of life, and linked with unplanned re-admission of patients and increased hospital length of stay.[22-27] This study reported that more than half of the elderly participants were anaemic using haemoglobin values of less than 13 gm/dl in men and 12 gm/dl in women as the recommended haemoglobin cut-off values of anaemia in males and females respectively by the World Health Organisation (WHO).[28] Utilizing the WHO cut-offs, the majority of this study's participants, 90% and 66% of male and female respectively were anaemic. These values are much higher than United Kingdom (UK) values which are 20.1% and 13.7% of elderly men and women respectively. UK is a developed economy and belongs to a high-income country where the elderly enjoy numerous benefits including free health care services unlike in Nigeria, the prevalence of anaemia in the elderly is bound to be much lower. The WHO cut-offs published over 50 years ago was however challenged by Beutler and Waalen in 2006, asserting that the WHO study was based on small study participants who were healthy and young.[19] They suggested that haemoglobin cut-offs should be based on age, ethnicity, and gender. If lower cut-offs were used in this study, the prevalence of anaemia in the elderly will be much lower than reported. Izaks et al reported increased mortality in the elderly of 85 years and above who are anaemic using the WHO haemoglobin cut-off values.[29]

Causes of anaemia in the elderly are multifactorial. Apart from iron deficiency anaemia, others include anaemia of chronic kidney disease, nutritional anaemias, anaemias due to chronic inflammation, and anaemia of undetermined origin.[30] In a study of the elderly 65 years and above involving 475 participants and using Hb of 12 gm/dl as the cut-off value of anaemia, iron deficiency anaemia accounted for 45% of all causes of anaemia and 10% of non-anaemic participants also had evidence of iron deficiency.[31] However, the prevalence of iron deficiency anaemia of 1.1% obtained in this study is similar to values obtained in the elderly in the US, 4% and in the UK 3.5 -5.3% using Hb value of 12 gm/dl.[32,33]

The level of serum ferritin was used in this study to determine the prevalence of iron deficiency anaemia because it is the best non-invasive diagnostic parameter for determining iron deficiency anaemia in patients of all ages.[34] However, caution must be taken in diagnosing iron deficiency in the elderly using serum ferritin because

it is an acute-phase protein whose level rises with ageing.[35] Hence, normal or increased serum ferritin may be associated with iron deficiency anaemia in the elderly in those with concurrent chronic inflammation, liver diseases, and cancers which are common morbidities in the elderly.[36] The WHO cut-off of serum ferritin of 15 µg/l was used in this study to define iron deficiency anaemia, however, it was reported that lower limit of ferritin cut-off in elderly patients should be as high as 75 µg/l and iron deficiency anaemia is likely in the elderly who has serum ferritin up to 45 µg/l but unlikely in the elderly if the serum ferritin is greater than 100 µg/l.[8,34,37]

The mean serum ferritin obtained in this study is lower in females than males, this is similar to a previous study.[38] Hormonal gender differences may account for higher ferritin in elderly men than women. Testosterone has been reported to inhibit hepcidin in males thus increasing ferritin levels.[39] Through hepcidin inhibition, 17β estradiol a product of oestrogen has also been reported to increase iron absorption to compensate for menstrual iron loss in pre-menopausal women, however, this response is blunted in postmenopausal women.[40]

A quarter of the population studied (24.4%) had reduced MCH indicating microcytosis, apart from iron deficiency anaemia, sideroblastic anaemia, anaemia of chronic disease, lead poisoning, and thalassaemia could account for the wide gap between the 1.1% of participants with reduced ferritin concentration and the 24.4% of participants with reduced MCH.

The mean serum transferrin is however higher in females than males, the level of serum transferrin is a reflection of body iron, the higher the transferrin level, the lower the body iron. The result is in keeping with a lower iron level in elderly females compared with males. A major function of transferrin is the transportation of iron from the sites of absorption in the duodenum to sites of utilization and storage.[41] Transferrin is very active in areas where there is rapid erythropoiesis and active cell division.[42]

Major study limitations were other causes of anaemia not determined which include, folate and vitamin B12 deficiencies, though, outside the study objectives, they could account for the other causes of anaemia reported in the elderly. Secondly, C reactive protein assay would have been useful in categorizing causes of anaemia between anaemia of chronic diseases like inflammatory bowel diseases, malignancies associated with ageing, and iron deficiency anaemia, given that both conditions could present with microcytosis. However, serum ferritin would be normal and usually greater than 100 ng/ml in anaemia of chronic disease unlike in iron deficiency anaemia. Other iron profiles like serum iron, transferrin receptor, hepcidin, and transferrin saturation assays would have made the picture even clearer.

CONCLUSION

Anaemia in the elderly is very common using the WHO haemoglobin cut-off values, however, iron deficiency anaemia prevalence is low and comparable to values obtained in developed countries. Iron deficiency anaemia is commoner in females than males.

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