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ORIGINAL ARTICLE

Assessment of Serum Ferritin and Haemoglobin Concentration in Multi-Transfused Patients in Owo, South Western Nigeria

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Abstract

Introduction: Blood transfusion is a therapeutic, life-saving intervention. It has been widely used and overused in medical practice since the early 20th century to treat anaemia and haemorrhage. Blood transfusion may cause some adverse effects in serum ferritin levels and haemoglobin concentrations, as may be occasioned by overload. This study was carried out to determine the effects of multiple transfusions on patients' iron storage and haemoglobin concentration in Owo, southwest Nigeria.

Methodology: Ethical approval was obtained as required. A total of 87 participants were recruited for this study, comprising 67 recipients of multiple transfusions and 20 subjects who had never received a blood transfusion. Haemoglobin was estimated using a standard ELISA technique, and serum ferritin was estimated using a colourimetric method. Data were analysed with SPSS version 25.0. Values were considered significantly different at $P < 0.05$.

Result: The mean ± standard error of the mean (SEM) of haemoglobin concentration revealed a significant decrease when multiple transfused subjects with 5 pints and above (12.57± 1.17) were compared to recipients of 2-4 pints (12.16± 1.25) with p=0.001. The mean ± standard error of the mean (SEM) of serum ferritin level was significantly elevated in multiple transfused subjects with 5 units and above (308.38±114.09); as compared with recipients of 2-4 units (279.95± 95.36) and those never transfused (100.92±20.29) with a p-value of 0.001. Gender has no significant effect on serum ferritin levels (p=0.227). The mean standard error of the mean of haemoglobin concentration revealed a significant difference in males (13.19±1.25) as compared to females (12.19±1.55) with p =0.001.

Conclusion: The significantly elevated serum ferritin in multi-transfused patients suggests iron overload, which is reflected in the

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haemoglobin concentration, which also increased with the increase in the number of units transfused. Thus, assessment of serum ferritin and haemoglobin concentration should be considered as post-transfusion monitors in anaemic patients.

Key words: Multiple transfusions, anaemia, serum ferritin, haemoglobin.

Introduction

Blood transfusion is a therapeutic life-saving intervention whose importance cannot be overemphasized. A pint of blood (450 ml) contains approximately 250 mg of iron, representing about 30% of the average body iron stores (BIS) in males and nearly 80% in females (1). Iron deficiency is probably one of the most common micronutrient deficiencies in developing nations with low socio-economic status. It is responsible for a higher incidence of morbidity due to the lack of proper investigation, prophylactic and therapeutic measures (1, 2). Recent reports have shown that the body's iron reserves are generally small, and iron depletion is more frequent in blood recipients than in non-recipients (3). There is an inverse correlation existing between iron storage and absorption. The continued iron loss of an individual either reaches equilibrium at a lower concentration of iron stores or becomes iron-depleted, eventually leading to iron-deficient erythropoiesis and, consequently, anaemia (4).

Iron is a dietary nutrient necessary for synthesising several haem and non-haem proteins involved in oxygen transport, storage, and energy production (5). The major function of haemoglobin is transporting oxygen from the lungs to all body tissues. The oxygen binding capacity of haemoglobin is 1.34 mL O2 per gram. Each globin subunit of the haemoglobin molecule can bind with one Fe2 ion. The affinity of haemoglobin towards oxygen is gained by the Fe2+ ion. Each Fe2+ can bind with one oxygen molecule. The binding of oxygen oxidizes Fe2+ into Fe3+. One atom of the oxygen molecule, which binds to Fe2+, becomes a superoxide, where the other oxygen atom protrudes at an angle (6). The oxygen-bound haemoglobin is referred to as oxyhaemoglobin. When blood reaches an oxygen-deficient tissue, oxygen is dissociated from haemoglobin and diffused into the tissue. The O2 is the terminal electron acceptor in the oxidative phosphorylation process in ATP production. The removal of O2 turns the iron into its reduced form. The oxygen-unbound haemoglobin is referred to as deoxyhaemoglobin. Oxidation of Fe2+ into Fe3+ creates methaemoglobin, which cannot bind to O2 (7).

Haemoproteins are haemoglobin and myoglobin (that are responsible for oxygen binding and transportation), catalase and peroxidase enzymes (which are associated with oxygen metabolism), and cytochromes (which are associated with electron transport and mitochondrial respiration). Non-haem iron-containing proteins are involved in DNA synthesis, cell proliferation and differentiation, gene regulation, drug metabolism, and steroid synthesis (8). Ferritin has a spherical protein shell that contains 24 subunits of a different proportion of two subunit types, H and L, which differ in structure and immunochemical properties (9). The iron uptake and release are thought to occur in three passages through the protein channels and the deposition or mobilization on the surface of the interior microcrystals (10). Iron uptake is associated with the oxidation of Fe2+ to Fe3+ by ferritin itself, and mobilization is associated with reduction by reduced flavins (11). Ferritin is mainly localized intracellularly in all tissues, but

significant amounts are also present in serum and body fluids. These extracellular ferritins have been recognised with immunological methods using antibodies specific for the L-subunit-rich, basic ferritin from the liver or spleen and by antibodies for H-subunit-rich, acidic ferritin from heart or Hella cells(12). The immunochemical properties of ferritin in the various biological fluids vary greatly; for instance, serum ferritin in iron-overloaded patients is essentially recognized only by basic ferritin antibodies. Most of the ferritin in mature milk is recognized by acidic antibodies (13). Serum ferritin appears to be partially glycosylated and iron poor, and composed of two peptides named L and H subunits. The biochemical studies carried out in sera from iron-overloaded patients did not show the presence of detectable proportions of H-chain in serum ferritin. Therefore, the direct evidence that this peptide can be actively secreted is still lacking. This may be an important consideration since several studies have shown that circulating acidic isoferritin (rich in H-subunit) may originate from malignant cells (14).

About 200–1000 mg of iron is stored in hepatocytes (liver cells) as ferritin, while 150 mg of iron is found in the bone marrow (15). The duodenum plays a very significant role in dietary iron absorption. The absorbed iron can be stored in the erythrocytes or enter the circulatory system and bound to the liver-derived plasma protein transferrin. It is then taken up by tissues and utilized for many processes, such as erythropoiesis in the bone marrow, myoglobin synthesis in muscle, and oxidative metabolism in all respiring cells. Splenic, hepatic, and bone marrow macrophages, which belong to the reticuloendothelial system (RES,) recycle iron from senescent erythrocytes (16). The liver has an important storing and regulatory function. The production of the hormone hepcidin controls the release of iron from enterocytes and macrophages into the circulation (17). Approximately 1–2 mg of iron is lost daily from the body through enterocyte and skin desquamation haemorrhages and parasitic infestations. No active mechanism of iron excretion exists. Consequently, 1–2 mg of intestinal iron absorption daily is required for iron homeostasis. This demand is increased in physiological conditions such as growth, pregnancy, and menstruation (18). Meanwhile, the RES recycled about 25 mg of iron daily as senescent erythrocytes are phagocytosed. This means most of human iron homeostasis depends on iron recycling (19).

Erythrophagocytosis predominates conducted by splenic macrophages but also occurs in the liver and bone marrow (20). In this process, senescent red blood cells are engulfed by a phagolysosome, where they undergo proteolysis to release the haem subunit. The haem subunit is then transported from the lumen of the phagolysosome into the macrophage cytosol by the haem-responsive protein gene-1(21). Thereafter, haem-oxygenase lyses iron from the haem subunit in the ribosome of the endoplasmic reticulum. Iron liberated from haem binds to apoferritin and is stored in the cytosol as ferritin, before being exported from the macrophage into the blood via ferroportin and finally being delivered to erythroid precursor cells (22).

Iron deficiency is when the body's iron content is less than normal. The earliest stage of iron deficiency is the depletion of iron stores, in which the serum iron, transferrin saturation and haemoglobin levels will be normal, but the storage of iron is decreased or absent. The further advanced stage is iron deficiency without anaemia, characterised by depleted iron stores, low serum iron and transferrin saturation but without anaemia. Iron deficiency anaemia is most prevalent in women and children in regions where meat intake is low, food is not fortified with iron, and where malaria, intestinal infections and parasitic worms are common (23). The study aimed to assess the serum ferritin levels and haemoglobin concentrations of multi-

transfused patients in Ondo State.

Materials And Methods

Study Design

This was a cross-sectional study of multi-transfused patients at the Federal Medical Centre (FMC) Owo, Ondo state.

Study Population

The study population consisted of patients admitted to Federal Medical Center, Owo; who received more than one pint of whole blood or packed red cells.

Sample size determination

The sample size was calculated using the formula

$$N = \frac{Z2(p \times q)}{d2}$$

Where;

N= required sample size ,

Z = confidence level at 95% (Standard value = 1.96),

p = estimated prevalence

q = (1-p)

d = desired level of significance or precision chosen for the study. It is 0.05 in this study, prevalence of iron deficiency was reported as 6%(24),

The sample calculated was 87.

Study Area

Owo, Ondo State, Nigeria, is located at 7° 11′ 0″ North and 5° 35′ 0″ East. It is situated in southwestern Nigeria, at the southern edge of the Yoruba Hills, at the intersection of roads from Akure, Kabba, Benin City, and Siluko. Owo is halfway between the towns of Ile Ife and Benin City.

Sample Collection

Each participant's blood sample was collected in a volume of 6mls. Three ml of the blood sample was dispensed into a plain bottle, and the remaining into an Ethylene K3EDTA bottle for analysis within four hours of collection. The blood samples in the plain bottles were centrifuged at 4000g for five minutes, and the serum was dispensed into plain bottles and stored at -100C until used for analysis.

Laboratory Procedures

Haemoglobin concentration was estimated spectrophotometrically using the cyanmethaemoglobin method. Serum ferritin was estimated using the standard ELISA technique in which polystyrene microwells pre-coated with monoclonal antibodies specific to Serum Ferritin were employed. The participant's serum sample was added to the microwell together with a second antibody-conjugated enzyme and directed against the serum ferritin. During incubation, the specific complex formed was captured on the solid phase. After washing to remove sample serum proteins and unbound HRP-conjugate antibody, chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide were added to the wells. In the presence of the antibody-antigen-antibody "sandwich" immuno-complex, the colourless TMB Solution was hydrolysed by the bound HRP-conjugate antibody to a blue-coloured product. The blue colour turns yellow after stopping the reaction with acid. The amount of colour intensity was measured spectrophotometrically with an ELISA plate

reader, and it is proportional to the amount of serum ferritin captured in the wells and to its amount in the sample, respectively.

Data Analysis: Data was analyzed using SPSS 25.0 version, and ANOVA was used as the tool for comparison. P-value of < 0.05 was considered as evidence of significant statistical difference.

Results

Age has no significant effect on the haemoglobin concentration of the participants (p>0.05), whereas the serum ferritin level increases with age, from age 20 upward, and the trend reveals a statistically significant difference (p< 0.05). Also, the serum ferritin levels were significantly elevated as the units of blood transfused increased (p< 0.05). There is also a significant difference (p< 0.05) in haemoglobin concentration, with the normal control group having the highest (14.42±0.89g/dL).

The haemoglobin concentration of participants varied with gender significantly (P<0.05), but the serum ferritin level did not (p>0.05). Details of these results are depicted in tables 1 to 3, as below.

Table 1: The haemoglobin concentration and serum ferritin levels of different age groups

Age Group(year)	Ferritin(mg/L)	Hb(g/dL)
≤20	291.20±19.65	13.23±0.68
21-30	215.32±104.23	13.15±1.37
31-40	267.63±126.97	12.39±1.66
41-50	341.35±136.99	12.34±0.89
Above 50	350.90	11.50±0.71
F –value	2.867	1.937
p-value	0.028	0.211

P is considered significant at 0.05 and below.

Table 2: The Haemoglobin concentration and serum ferritin levels of participants in respect to units of blood received

Units of blood received	Ferritin(mg/L)	Haemoglobin(g/dL)
5 and above	308.38±114.09	12.57±1.17
2-4	279.95± 95.36	12.16±1.25
None	100.92±20.29	14.42±0.89
F-value	34.203	26.127
P-value	0.001	0.001

P significant at p < 0.05

Table 3: Haemoglobin concentration and serum ferritin levels of participants with gender

Gender	Ferritin	Hb
Female	227.92±113.96	12.19±1.55
Male	260.72±126.51	13.19±1.25
F -value	1.482	10.888
p-value	0.227	0.001

P significant at $P < 0.05$

Discussion

This study shows that Red Blood Cell transfusion is still the most reliable therapeutic intervention in moderate to severe anaemic conditions, which agrees with outcomes of studies conducted elsewhere (25, 26). In this study, haemoglobin levels significantly decreased in multiple transfused subjects compared with individuals who needed no transfusion (P=0.001), confirming that haemoglobin is a diagnostic parameter for anaemia. In this study, the mean haemoglobin level observed in the females was significantly lower compared to the male subjects. This suggests physiological iron losses incidental to menstrual losses and pregnancy, which are peculiar to females.

This study showed that serum ferritin level was significantly increased in multiple transfused subjects compared with individuals who have not been transfused, and this is proof of propensity towards iron overload. A similar pattern was reported by Okoh and Nwabuko in sickle cell patients who were previously transfused and had elevated ferritin levels when compared with the non-transfused patients (27). Patients with inflammatory conditions may, however, have restricted availability of iron for erythropoiesis and other cell functions due to increased hepcidin expression, despite normal or high levels of serum ferritin (28). This explained the reason of decreased haemoglobin values seen in multi-transfused subjects despite normal or high levels of serum ferritin in this study.

Conclusion

This study showed that serum ferritin among multi-transfused patients suggests an increased iron level, tending towards haemosiderosis, while haemoglobin concentration may remain in the lower limit of the normal range. Thus, serum ferritin and haemoglobin concentration should be assessed for recipients of multiple transfusions to rule out and prevent iron overload. This will optimize the goals of blood transfusion in anaemic cases.

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