ORIGINAL ARTICLE

Assessment of Serum Hepcidin Level in First Time and Repeat Blood Donors at the University of Maiduguri Teaching Hospital, Nigeria.

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ABSTRACT

Background: Iron deficiency remains one of the frequent complications encountered by regular blood donors, with each unit (450mls) of donated blood containing about 250mg of iron. Although most centers utilize capillary haemoglobin in determining the eligibility to serve as a donor, haemoglobin level is a poor indicator of body iron status since depletion of iron store can be present in the absence of overt anaemia. Serum hepcidin has been found to be low in people with iron deficiency, and may serve as a surrogate for depleted iron stores.

Aim: To study the levels of serum hepcidin as a marker of iron deficiency in first time blood donors and subjects with history of multiple blood donation at a tertiary hospital in North-East Nigeria.

Methods: Eligible and consenting voluntary first time and repeat blood donors were consecutively recruited at the transfusion unit of the University of Maiduguri Teaching Hospital. Serum hepcidin level was assayed in all participants using quantitative ELISA technique, and classified into low, normal and high based on recommendation of the manufacturer of assay kits.

Results: One hundred and eighty apparently healthy donors comprising 90 first time donors (control) and 90 repeat donors (subjects) were studied. Their ages ranged from 18 years to 60 years, with a mean of 30+6.20 years and 27.43+5.28 years for subjects and controls respectively (p=0.45). The median serum hepcidin of subjects and controls was 1.43(2.47) and 1.23 (2.43) respectively (p=0.375). Twenty one (23.3%) of subjects had low serum hepcidin compared to 22 (24.4%) of the controls. Serum hepcidin level did not significantly differ between male subjects and controls (p=0.079) or female subjects and controls (p=0.77) No correlation was observed between the frequency of donation and hepcidin (p=0.323).

Conclusion: There is a high prevalence of low hepcidin levels among individuals deemed eligible for blood donation in this environment. This implies that screening for donor eligibility using haemoglobin could result in use of iron deficient individuals as donors. Detection of low hepcidin in blood donors will help identify donors with low iron store, which may be important in preventing them from becoming anaemic following further donation.

KEYWORDS: Hepcidin, Blood Donors, Iron Deficiency, Anaemia

Introduction

Iron deficiency anaemia (IDA) remains one of the frequent complications encountered by regular blood donors, with each unit of blood containing about 250mg/450mls of iron[1,2]. Repeated donation leads to increased erythropoiesis requiring high iron supply from stores and gut absorption. Most blood transfusion centers utilize capillary haemoglobin (Hb) in determining donor eligibility. However, haemoglobin level is a poor indicator of body iron status because depletion of iron store can be present in the absence of overt anaemia[3,4]. Utilizing haemoglobin alone, individuals with

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depleted iron level may have haemoglobin values above the arbitrarily defined limit for anaemia, thus increasing the risk of developing IDA following further donation. Laboratory method of measuring iron status includes screening and definitive methods. The screening measurements are non-specific and include review of peripheral blood film, haemoglobin, transferrin saturation, red cell indices and zinc protoporphyrin (ZPP). Definitive method includes measurements of storage (ferritin and marrow haemosiderin) and tissue iron (sTfR)[4]. The peptide hormone hepcidin, synthesized mainly by hepatocytes, is the main regulator of systemic iron homeostasis. It inhibits release of iron by macrophages and attenuates iron uptake in the gut. Levels are raised in chronic inflammation and iron overload, and low in hypoxia and iron deficiency[5,6,7]. A number of studies have validated the diagnostic usefulness of hepcidin in determining IDA. Pasricha and coworkers demonstrated that hepcidin showed a combined excellent sensitivity and specificity as diagnostic tool for IDA in healthy non anaemic blood donors when compared to other parameters of iron status, and they suggested that hepcidin may be a useful indicator of deficient iron store[8]. Similarly, Lofti et al tested a broad spectrum of parameters including ferritin, transferrin, transferrin receptor, erythropoietin and reticulocytes, in order to predict spontaneous recovery from iron loss following donation, and showed that serum hepcidin had the most significant result starting one day post donation and remained consistently so throughout the period of study when compared with the other parameters[9]. Blood donors are particularly at risk of developing iron deficiency, and early diagnosis remains challenging. Subclinical iron deficiency (SID) occurs in about 10% of blood donors who meet the eligible Hb cutoff for donation, defined as ferritin level of < 10ng/ml[10]. Using serum hepcidin to determine SID, Baart et al found a prevalence of 27.4% and 24.7% in males and females respectively [11]. Individuals with SID are asymptomatic, and prone to developing clinical features following anaemia upon further donation. Although not routinely tested, measurement of serum hepcidin may help identify IDA in high risk individuals. The aim of this study was to assess the level of serum hepcidin as a marker of iron deficiency in first time blood donors and subjects with history of multiple blood donation at a tertiary hospital in North-East Nigeria. One hundred and eighty eligible blood donors were consecutively enrolled from January 2014 to September 2014. The participants consisted of 90 repeat donors (subjects) and 90 first time donors (controls). Use of iron supplements, presence of active inflammatory conditions, pregnancy, obstructive airway diseases and refusal of consent served as exclusion criteria.

Methodology

Ten milliliters of venous blood was obtained from the subjects via the antecubital vein, and 5mls was added each into EDTA and plain specimen bottles, and samples forhepcidin analysis were allowed to clot and centrifuged for 15min at 100g, and stored at -80^{0}C. The samples for haemtological parameters were analysed using Advia 120 hema system analyser (Bayer). The quantitative sandwich enzyme immunoassay technique was employed for hepcidin assay. Human Hepc (Hepcidin) ELISA Kit (catalogue No E-EL-HOO77 from Elabscience Biotechnology Co. Ltd,) was used. Instructions provided with the test kit were strictly adhered to. The concentration of hepcidin was obtained from a graph of the optical density and standard concentrations using the Curve expert software version 1.4 (Daniel Hyams, Microsoft corporation copyright© 1995-2009). Iron deficiency was defined as serum hepcidin levels of < 0.4ng/ml for females and < 0.6ng/ml for males[12].

The data was analyzed using the statistical

package for social sciences version 20.0 (SPSS Chicago III USA.). Normality of data was tested using Kolmogorov-Smirnov test, and continuous variables expressed using means (SD) and median (interquartile range) as appropriate. Continuous variables were compared using Student's t-test and Mann-Whitney test as appropriate. ANOVA was used in comparing level of hepcidin on the basis of frequency of donation. A p value of <0.05 was considered significant for all statistical analysis.

Results

One hundred and eighty apparently healthy donors comprising 90 first time donors (control) and 90 repeat donors (subjects) were studied. The subjects were made up of 72(80%) males and 18(20%) females, while the controls were made up of 66(73.3%) males and 24(26.7%) females respectively. Their ages ranged from 18 years to 60 years, with a mean of 30±6.20 years and 27.43±5.28 years for subjects and controls respectively (p=0.45). Age, haematological parameters and hepcidin of the subjects and controls are illustrated in Tables 1-3. The median serum hepcidin for subjects and controls was 1.43(2.47) and 1.23 (2.43) respectively (p=0.375). Serum hepcidin level did no significantly differ between male subjects and controls (p=0.079) or female subjects and controls (p=0.77). Twenty one (23.3%) of the subjects had low serum hepcidin compared to twenty two (24.4%) of the controls. Thirty four (34.4%) of the subjects had donated twice, sixteen (8.9%) three times, twenty six (28.9%) four times, and seventeen (18.8%) have donated more than five times (Table 4). No correlation was observed between the frequency of donation and hepcidin (p=0.323).

Table 1: Shows the mean values of the laboratory profile amongst subjects and controls

Parameters	Subjects	Controls	p-value
Age	30 ± 6.2*	27.43 ± 5.28*	0.045
Haematocrit	0.41 ± 0.03*	0.41 ± 0.03*	0.494
WBC	5.03 ± 1.4*	4.7 ± 1.34*	0.106
Platelets	237.01 ± 86.6*	237.80 ± 76.2*	0.953
Hepcidin	1.43 (2.47)**	1.23 (2.43)**	0.375

* Mean ± Standard deviation ** Median (Interquartile range)

Table 2: Shows the mean value of laboratory profile amongst male subjects and controls

Parameters	Male Subjects	Male controls	p -value
Age	30.07 ± 6.3*	28.30± 6.2*	0.079
Haematocrit	0.42 ± 0.03*	0.42± 0.02*	0.178
WBC	4.92 ± 1.41*	4.8 ± 1.40*	0.308
Platelets	228.05 ± 82.8*	236 ± 79.7*	0.524
Hepcidin	1.47 (3.06)**	1.09 (2.40)**	0.423

* Mean ± Standard deviation ** Median (Interquartile range)

Table 3: The mean laboratory profile of female subjects and controls

Parameters	Female subjects	Females control	p-value
Age	29.74 ± 6.21*	24.91 ± 5.14*	0.009
Haematocrit	0.39 ± 0.016*	0.39 ± 0.018*	0.672
WBC	5.4 ± 1.3*	4.75 ± 1.18*	0.816
Platelets	270.7 ± 94.5*	240.34 ± 66.6*	0.852
Hepcidin	1.28 (1.85)**	1.62 (2.27)**	0.77

* Mean ± Standard deviation ** Median (Interquartile range)

Table 4: Shows the frequency of blood donation amongst the subjects

Donor types	Frequency of donation	Number of donors	Percentage of donors
Subjects	2	31	34.4
	3	16	17.8
	4	26	28.9
	5	2	2.2
	6	5	5.6
	7	3	3.3
	8	3	3.3
	9	2	2.2
	10	2	2.2
	TOTAL	**90**	**100**

Discussion

This was an observational case-control study carried out at the University of Maiduguri teaching hospital in North east Nigeria amongst voluntary blood donors. Serum levels of hepcidin in repeat blood donors were compared with those of first time blood donors to determine the prevalence of low hepcidin. Iron deficiency and erythropoiesis are both associated with suppression of hepatic hepcidin release, which in turn allow for absorption of iron from the gut and release from stored macrophages[13]. Compared with other parameters of iron metabolism such as ferritin (marker of iron store) and serum transferrin receptor (marker of erythropoeitc marrow iron depletion), serum hepcidin represents a signal that indicates iron is needed. Therefore, a low hepcidin serve as an essential physiological response in iron deficit state[14]. In the current study, the prevalence of low hepcidin was similar among the subjects and controls at 23.3% and 24.4% respectively. A study conducted in Pakistan by Baart et al to determine the prevalence of subclinical iron deficiency in whole blood donors using hepcidin concentration, also demonstrated a high prevalence of 27.4% and 24.7% for males and females respectively [11]. In addition, we found no significant difference in the median serum hepcidin level of subjects and control. This finding reflects a low iron stores in both subjects and controls, which may be attributed to the high prevalence of IDA in this region, attributable to multiple factors

including infections like malaria and poor nutritional intake[15]. A study conducted amongst blood donors in southern Nigeria found the prevalence of iron deficiency (serum ferritin <12 ng/mL) as 20.6% and iron-deficiency anaemia (haemoglobin<11.0 g/dL + serum ferritin <12.0 ng/mL) as 12.0%[16]. In addition, our finding demonstrate that despite being eligible for donation based on the cutoff haematocrit, these individual have depleted iron store and risk further depletion following donation, which may potentially affects their health.

We found no correlation between the serum level of hepcidin and the frequency of donation. Studies have shown that serum hepcidin levels tend to recover to normal levels in blood donors with sufficient iron available to support new red blood cell synthesis and to maintain pre-donation haemoglobin values [17,18]; the inter donation interval required to replenish iron store may differ amongst individual. In their study, Alan et al, showed that serum level of hepcidin declined with repeated donation, however, levels tend to correlate significantly with the period since last donation and not the total number of donation; with levels recovering to normal in between donation in some individuals[17]. The current study is limited because only the total number of donations was considered and not the inter donation interval. Decrease in serum hepcidin level following consecutive donation may results in compensatory increase in gut absorption of iron. However, because of its erythropoietic effect, even in the presence of low iron store, hepcidin is able to drive erythropoiesis with restoration of acceptable pre-donation haemoglobin level at the risk of further depletion of existing store [18]. This may explain why such donors are able to maintain a pre donation haematocrit or Hb that meets the eligible level for donation.

Hepcidin is not yet part of the repertoire of iron parameters testing; the high prevalence of low hepcidin in current study raises the possibility of developing a point of care testing which can help detect SID/IDA in asymptomatic donors with otherwise eligible pre-donation haemoglobin value. This will allow for early detection of individuals who may benefit from intervention with iron supplementation and improved nutritional intake, given that iron is important in many cellular and immune processes of the body such as DNA synthesis, transfer of electron during oxidative process[19]. Detection of low hepcidin in blood donors will help identify donors with depleted iron store, which may be important in preventing them from becoming anaemic following further donation.

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