The Effect of Iron Deficiency Anemia Treatment on Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio

Burak Uz[1,*], Ilhan Dolasik[1], Hasan Atli[2] and Deccane Duzenci[2]

[1]*Fırat University Faculty of Medicine, Department of Internal Medicine, Division of Hematology*

[2]*Fırat University Faculty of Medicine, Department of Internal Medicine*

Abstract: *Background:* Both neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are cost-effective and readily available biomarkers. An increment in either NLR or PLR is an indicative of a prolonged chronic inflammatory condition and increased host inflammatory response. Iron deficiency anemia (IDA) is frequently associated in chronic disorders.

Aims: We decided to investigate whether the efficient treatment of IDA should affect NLR and/or PLR values in an adult population with IDA.

Methods: This was a retrospective (case-series) observational study conducted at an adult Hematology clinic in Turkey. Patients were ≥ 18 years-old, with IDA defined according to the World Health Organisation criteria. The hematological parameters, NLR, and PLR levels were noted before and after oral iron (Fe^{+2}) repletion treatment.

Results: A total of 200 patients with IDA (median age 44 years, IQR 32-52 years, women 91%) were included. NLR values did not differ significantly in terms of IDA treatment (2.07 vs. 2.01, *p*= .558). PLR levels were significantly decreased after IDA treatment (170.63 vs. 140.32, *p*< .001). The NLR and PLR were positively correlated (p= .01). A low-unremarkable inverse correlation between NLR, and serum iron levels (*p*= .024) and Tfsat (*p*= .038) was observed; a similar negative correlation was also observed between PLR, and serum iron (*p*= .002) and Tfsat (*p*= .013) levels.

Conclusion: The treatment of IDA did not affect NLR, whereas it was associated with significant decrease in PLR. The NLR and PLR were positively correlated. However, both the NLR and PLR were inversely correlated with serum iron and Tfsat levels.

Keywords: Iron deficiency, Anemia, Neutrophils, Lymphocytes, Platelets.

INTRODUCTION

Anemia is a widespread public health problem and is defined as a low hemoglobin concentration with respect to normal cut-off values. Global anemia prevalance in 2010 was 32.9% [1]. Iron deficiency affects more than 2 billion people worlwide, and iron deficiency anemia (IDA) remains to be the top cause of anemia [2].

Neutrophil to lymphocyte ratio (NLR), calculated from complete blood count with differential, is a readily available and inexpensive marker of inflammation in ischaemic heart diseases [3], and even in different types of solid tumors [4]. Similarly, platelet to lymphocyte ratio (PLR) is a cost-effective and inexpensive inflammatory biomarker with independent prognostic value in solid tumors [5].

To the best of our knowledge, there is no published study about the effects of IDA treatment on NLR or PLR. Therefore, the aim of the current study was to define the alterations in NLR and/or PLR before and after the treatment of IDA in a Turkish adult population.

MATERIALS AND METHODS

Between January 2014 and July 2014, a total of 200 patients with IDA, 182 female (91%) and 18 male, were enrolled to this study. The study protocol was approved by the local ethics commitee (Date: 24.03.2015, Desicion Number: 04).

Hemoglobin levels of <13 g/dL in men, and <12 g/dL in women have been used to define anemia [6]. IDA was defined as: ferritin level <15 ng/mL (normal range: 20-300 ng/mL), transferrin saturation <15%, and mean corpuscular volume <80 fL. Patients with the following conditions were excluded from the study: age of <18 years, other nutritional anemias (vitamin B_{12}, folate), inherited or acquired red cell disorders (haemoglobinopathies), acute or chronic infections (malaria, tuberculosis, HIV), known malignancies or chronic diseases (chronic renal or liver disease, severe cardiac or respiratory disease), pregnancy, alcoholism, and gastric surgery.

Treatment protocole, contained 2 x 100 mg/day ferrous (Fe^{+2}) glycine sulfate complex perorally until the

*Address correspondence to this author at the Göksu Mah, Erzurum Kongre Cad., Metrokent Sitesi, A-3 Blok, No= 26, Eryaman / Ankara, Turkey;
Tel: +90 (312) 202 55 79; Fax: +90 (312) 223 67 14;
E-mail: burakuz78@gmail.com

E-ISSN: 2408-9877/16

hematological parameters normalised and serum ferritin ≥ 40 ng/mL. Blood samples for complete blood count and biochemical analyses were collected before and after the treatment protocole for all patients. All blood samples were drawn in the fasting state. Data on blood cell counts and iron status were extracted in a retrospective fashion from the electronic medical records database.

The NLR was defined as the ratio of absolute neutrophil count divided by the absolute lymphocyte count, and PLR was defined as the ratio of platelet count divided by the absolute lymphocyte count.

STATISTICAL ANALYSIS

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 20. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. Descriptive analyses were presented using medians and interquartile range (IQR) for the non-normally distributed variables. The Wilcoxon test was used to compare the non-normally distributed numeric variables before and after the treatment of IDA. While investigating the associations between non-normally distributed variables, the correlation coefficients and their significance were calculated using the Spearman test. A 5% type-I error level was used to infer statistical significance. A value of less than .05 was considered to show a statistically significant result.

RESULTS

1. Clinical Characteristics

Ninety-one percent (n= 182) of the patients were female. Median age was 44 (min-max: 19-93) years. Median follow-up of the patients was 63 [Interquartile Range (IQR): 36-137] days.

Table 1: The Comparison of Hematologic Parameters, Neutrophil/Lymphocyte Ratio (NLR), and Platelet/Lymphocyte Ratio (PLR) in Patients with Iron Deficiency Anemia (IDA) before and after Oral Iron Repletion Treatment

Hematologic Parameters	Reference Range	Before Treatment [Median (IQR)]	After Treatment [Median (IQR)]	*p*
Hemoglobin (g/dL)	11.1-17.1	10.50 (9.10-11.43)	12.70 (11.90-13.40)	< .001
Hematocrit (%)	33-57	33.20 (29.50-35.00)	38.70 (36.75-40.70)	< .001
MCV (fL)	78-100	76.00 (68.15-80.00)	84.00 (78.00-88.00)	< .001
RDW (%)	12-15	16.75 (14.60-19.30)	17.55 (15.20-22.60)	< .001
MCH (pg)	24-31	23.60 (20.55-26.35)	27.25 (24.90-29.30)	< .001
MCHC (g/dL)	28-34	31.30 (30.02-32.40)	32.51 (31.76-33.38)	< .001
WBC (x 10^9/L)	3.8-8.6	6.40 (5.31-8.02)	6.35 (5.40-7.93)	.792
ANC (x 10^9/L)	2.1-6.1	3.81 (2.94-5.07)	3.81 (2.96-5.17)	.437
Neutrophil (%)	40-77	59.80 (53.45-65.68)	59.60 (54.65-65.35)	.975
ALC (x 10^9/L)	1.3-3.5	1.92 (1.51-2.32)	1.87 (1.57-2.25)	.722
Lymphocyte (%)	16-44	29.20 (23.75-36.05)	30.10 (24.80-34.95)	.279
NLR		2.07 (1.50-2.75)	2.01 (1.62-2.70)	.558
Platelet (x 10^9/L)	140-360	320.00 (261.50-378.00)	272.00 (231.50-331.50)	< .001
MPV (fL)	7-9	8.40 (7.75-9.20)	8.80 (7.98-9.63)	< .001
PDW (%)		37.30 (18.20-51.15)	18.30 (15.65-39.00)	< .001
PCT (%)		0.27 (0.22-0.32)	0.24 (0.20-0.29)	< .001
PLR		170.63 (134.58-222.89)	140.32 (116.42-182.42)	< .001
Iron (µg/dL)	60-170	23.00 (14.00-42.25)	65.00 (42.00-86.50)	< .001
TIBC (µmol/L)	250-450	393.00 (346.50-437.00)	349.00 (304.75-377.75)	< .001
Tfsat (%)		6.00 (3.40-10.60)	18.85 (12.93-26.70)	< .001
Ferritin (ng/mL)	20-300	5.75 (2.70-10.63)	31.50 (26.30-45.05)	< .001

Abbreviations: IQR: interquartile range; MCV: mean corpuscular volume; RDW: red cell distribution width; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; WBC: white blood cell; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; NLR: neutophil/lymphocyte ratio; MPV: mean platelet volume; PDW: platelet distribution width; PCT: platelet crit; PLR: platelet/lymphocyte ratio; TIBC: total iron binding capacity; Tfsat: transferrin saturation.

2. Complete Blood Count & Iron Parameters

White blood cell (WBC) count, absolute neutrophil count, neutrophil percent, absolute lymphocyte count, lymphocyte percent, and NLR did not significantly differ before and after IDA treatment. However, there was a statistically difference between all other hematologic parameters including iron metabolism and platelets before and after the treatment of IDA (Table **1**).

NLR

NLR values did not differ significantly in terms of IDA treatment (2.07 vs. 2.01, *p*= .558; Figure **1**).

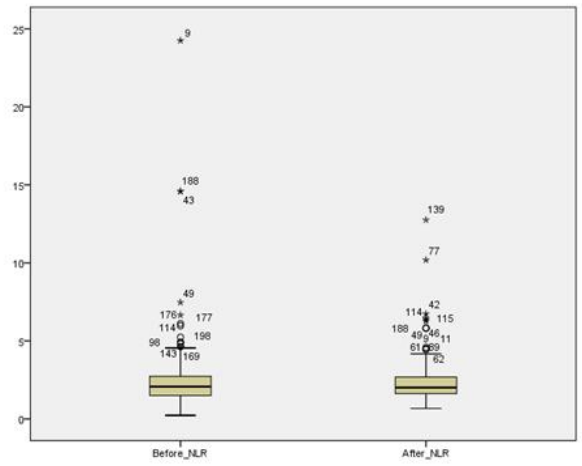

Figure 1: Neutrophil to lymphocyte ratio (NLR) before and after the treatment of iron deficiency anemia (IDA) is shown by box plots. NLR did not differ significantly in terms of IDA treatment (p= .558).

PLR

PLR levels were significantly decreased after IDA treatment (170.63 vs. 140.32, *p*< .001; Figure **2**).

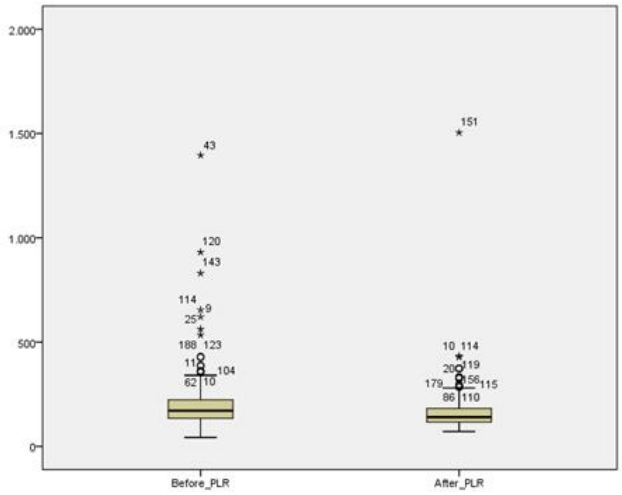

Figure 2: Platelet to lymphocyte ratio (PLR) before and after the treatment of iron deficiency anemia (IDA) is shown by box plots. PLR decreased significantly after IDA treatment (p< .001).

Iron Metabolism Parameters

Serum iron, Tfsat, and ferritin levels were significantly increased; whereas serum TIBC levels were significantly decreased after the treatment of IDA (Table **1**).

Platelets and Related Parameters

Platelet counts, PDW, and PCT were significantly decreased; whereas MPV was significantly increased after IDA treatment (Table **1**).

3. Correlation Analyses

NLR

Correlation analyses between pre-treatment NLR and pre-treatment iron parameters were calculated. A low-unremarkable inverse correlation between NLR, and serum iron (*p*= .024) and Tfsat (*p*= .038) was observed; whereas there was no correlation between NLR, and TIBC or ferritin levels (Table **2**). The NLR and PLR were positively correlated either before (r= .547; p= .01) or after (r= .460; p= .01) the treatment of IDA.

Table 2: Spearman's Coefficients (*r*) between NLR and Iron Parameters

Parameters	Spearman Coefficient	*p*-Value
Iron (µg/dL)	-0.163	0.024
TIBC (µmol/L)	-0.082	0.258
Tfsat (%)	-0.151	0.038
Ferritin (ng/mL)	0.140	0.052

Abbreviations= TIBC: total transferrin binding capacity; Tfsat: transferrin saturation

PLR

Correlation analyses between pre-treatment PLR and pre-treatment iron parameters were calculated. A low-unremarkable inverse correlation between PLR, and serum iron (*p*= .002) and Tfsat (*p*= .013) was observed; whereas there was no correlation between PLR, and TIBC or ferritin levels (Table **3**).

Table 3: Spearman's Coefficients (*r*) between PLR and Iron Parameters

Parameters	Spearman Coefficient	*p*-Value
Iron (µg/dL)	-0.226	0.002
TIBC (µmol/L)	-0.113	0.121
Tfsat (%)	-0.180	0.013
Ferritin (ng/mL)	-0.094	0.199

Abbreviations= TIBC: total transferrin binding capacity; Tfsat: transferrin saturation

DISCUSSION

Since anemia is associated with hypoxia and ischemia, it may affect WBCs via increasing vascular reactivity to catecholamine through glucocorticoids [7]. Singh *et al*. has presented that in a very small cohort (n= 20) with nutritional anemia, total leukocyte count was found to be insignificantly higher in anemic patients compared to those of controls. On differential count of leukocytes, there was increment in neutrophils without alteration in lymphocytes. Additionally, NLR was significantly (p< .001) higher in anemic vs healthy subjects, and platelet count was decreased in anemic patients [8]. In a Turkish pediatric study, focused on iron-deficient children with a mean age of 13.8, the percentage of neutrophils with stimulated oxidative burst activity was found to be significantly lower than those of healthy controls. In addition, although the difference is borderline and not significant, the ratio of neutrophils with phagocytic activity was also lower in the anemic group [9]. In iron deficient pediatric patients T lymphocytes, CD4+ T-cells, and the CD4:CD8 ratio were all significantly lower than those noted in healthy children. Iron replation was associated with significantly improved CD4+ cell counts [10]. Therefore, not only the quantitative properties of neutrophils, but also qualitative aspects of neutrophils may be associated with inflammatory response in iron deficiency. The present study was carried out in 200 patients with IDA. Either total or differential leukocyte counts (neutrophils, lymphocytes) were in normal ranges, and we also did not observe any significant alterations in NLR after the treatment of IDA. Pre-treatment platelet parameters were all in normal ranges except PDW, and PDW was found to be higher in patients with IDA according to normal reference values. Platelet counts, PLR, PDW, and PCT were significantly decreased; whereas MPV was significantly increased after the treatment of IDA (Table **1**).

A high NLR is related with an adverse outcome in many solid tumors. Most recently, in a systematic meta-analysis including 100 studies with a total of 40,559 patients, it has been suggested that a high NLR may reflect either greater tumor burden or a more prolonged chronic inflammatory process [4]. Although the exact mechanism about the prognostic role of NLR in subjects with cancer remains unclear, most studies have pointed out a potential and/or possible association between a high NLR and inflammation [4]. Several inflammatory cytokines (especially IL-6) and chemokines released by both the cancer cells and associated host cells (*i.e*. neutrophis) [11, 12] may trigger a “cytokine storm” like process and eventually contribute to a systemic inflammatory response. Additionally, it has also been suggested that NLR may also correlate with non-cancer conditions including, myocardial ischemic events [13], acute pancreatitis [3], or inflammatory bowel diseases [14]. As well as NLR, an increased PLR is also indicative of an increased host inflammatory response [15]. In the present paper, PLR decreased significantly with the improvement of IDA; but NLR did not differ significantly after IDA treatment.

Platelets are known to be active players in the induction of inflammatory response, in addition to their participation in hemostasis [16, 17]. In contrast to acute infections with viruses or bacteria, chronic inflammation is often related with increased megakaryopoiesis and reactive thrombocytosis [17]. Iron deficiency (ID) is a recognized cause of reactive thrombocytosis even outside the setting of inflammation [18, 19]. However, the exact mechanism of thrombocytosis in ID is not yet clear. Interleukin (IL) 6 plays a key role in the systemic inflammatory response and thrombocytosis by several mechanisms including; increasing the synthesis of acute phase proteins such as C-reactive protein (CRP), serum amyloid A protein, and/or fibrinogen [20], decreasing albumin biosynthesis in the liver [20], stimulating human megakaryocytic proliferation and differentiation synergistically with IL-3 [21], and stimulating thrombopoietin production [17]. Contrastly, in an animal iron-deficieny model, Evstatiev *et al*. have recently showed that ID did not alter the production of hematopoeitic growth factors including thrombopoietin, IL-6 or IL-11 [22]. In the present analysis, pre-treatment platelet counts were significantly higher than the post-treatment platelet counts (even both of them were within normal reference ranges) which can, at least partly, be explained by a “relative” reactive thrombocytosis induced by IDA. The improvement of this reactive thrombocytosis after iron replacement without an alteration in lymphocytes, gave rise to a significant decrease in PLR.

A small peptide called “hepcidin” has a crucial role in the control of iron availability to tissues. Hepcidin expression is upregulated by inflammation. IDA is frequently associated with anemia of chronic diseases, including chronic heart failure, cancer, chronic kidney disease, rheumatoid arthritis, obesity, and inflammatory bowel diseases [23]. It has also been shown that IL-6 enhances the synthesis of hepcidin in the liver which regulates iron recycling, resulting in anemia due to hypofferemia [20, 24].

This study had some limitations. Firstly, other markers of systemic inflammation such as CRP, or hypoalbuminemia should be obtained in order to

compare them with NLR and PLR by multivariate analyses. Second, we were not able to determine the aforementioned hematopoieitic growth factors or the master regulator of iron homeostasis, hepcidin. Despite these factors, our study may help to better understand the inflammatory aspects of iron deficiency anemia by clinicians. Further prospectively designed studies with more patients are needed in order to elucidate the association of inflammation and IDA.

REFERENCES

[1] Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, *et al*. A systematic analysis of global anemia burden from 1990 to 2010. Blood 2014; 123: 615-24. http://dx.doi.org/10.1182/blood-2013-06-508325

[2] Camaschella C. Iron-deficiency anemia. New Engl J Med 2015; 372: 1832-43. http://dx.doi.org/10.1056/NEJMra1401038

[3] Park JJ, Jang HJ, Oh IY, Yoon CH, Suh JW, Cho YS, *et al*. Prognostic value of neutrophil to lymphocyte ratio in patients presenting with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention. Am J Cardiol 2013; 111: 636-42. http://dx.doi.org/10.1016/j.amjcard.2012.11.012

[4] Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaňa A, *et al*. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst 2014; 106: dju124. http://dx.doi.org/10.1093/jnci/dju124

[5] Templeton AJ, Ace O, McNamara MG, Al-Mubarak M, Vera-Badillo FE, Hermanns T, *et al*. Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 2014; 23: 1204-12. http://dx.doi.org/10.1158/1055-9965.EPI-14-0146

[6] World Health Organisation. Nutritional anemias: report of a WHO scientific group. World Health Organ Tech Rep Ser 1968; 405: 5-37.

[7] Ernst E, Hammerschmidt DE, Bagge U, Matrai A and Dormandy JA. Leukocytes and the risk of ischaemic diseases. JAMA 1987; 257: 2318-24. http://dx.doi.org/10.1001/jama.1987.03390170074031

[8] Singh K. Leukocyte counts in anaemia. Indian J Physiol Pharmacol 2010; 54: 85-8.

[9] Ekiz C, Agaoglu L, Karakas Z, Gurel N and Yalcin I. The effect of iron deficiency anemia on the function of the immune system. Hematol J 2005; 5: 579-83. http://dx.doi.org/10.1038/sj.thj.6200574

[10] Mullick S, Rusia U, Sikka M and Faridi MA. Impact of iron deficiency anemia on T lymphocytes & their subsets in children. Indian J Med Res 2006; 124: 647-54.

[11] Kantola T, Klintrup K, Väyrynen JP, Vornanen J, Bloiqu R, Karhu T, *et al*. Stage-dependent alterations of the serum cytokine pattern in colorectal carcinoma. Br J Cancer 2012; 107: 1729-36. http://dx.doi.org/10.1038/bjc.2012.456

[12] Jablońska E, Kiluk M, Markiewicz W, Piotrowski L, Grabowska Z and Jabloński J. TNF-alpha, IL-6 and their soluble receptor serum levels and secretion by neutrophils in cancer patients. Arch Immunol Ther Exp (Warsz) 2001; 49: 63-9.

[13] Azap B, Jaglall N, Atallah JP, Lamet A, Raja-Surya V, Farah B, *et al*. Neutrophil-lymphocyte ratio as a predictor of adverse outcomes of acute pancreatitis. Pancreatology 2011; 11: 445-52. http://dx.doi.org/10.1159/000331494

[14] Acarturk G, Acay A, Demir K, Ulu MH, Ahsen A and Yuksel S. Neutrophil-to-lymphocyte ratio in inflammatory bowel disease – as a new predictor of disease severity. Bratisl Lek Listy 2015; 116: 213-7. http://dx.doi.org/10.4149/bll_2015_041

[15] Kwon HC, Kim SH, Oh SY, Lee S, Lee JH, Choi HJ, *et al*. Clinical significance of preoperative neutrophil-lymphocyte versus platelet-lymphocyte ratio in patients with operable colorectal cancer. Biomarkers 2012; 17: 216-22. http://dx.doi.org/10.3109/1354750X.2012.656705

[16] Wagner DD. New links between inflammation and thrombosis. Arterioscler Thromb Vasc Biol 2005; 25: 1321-4. http://dx.doi.org/10.1161/01.ATV.0000166521.90532.44

[17] Klinger MH, Jelkman W. Role of blood platelets in infection and inflammation. J Interferon Cytokine Res 2002; 22: 913-22. http://dx.doi.org/10.1089/10799900260286623

[18] Dan K. Thrombocytosis in iron deficiency anemia. Intern Med 2005; 44: 1025-26. http://dx.doi.org/10.2169/internalmedicine.44.1025

[19] Kulnigg Dabsch S, Schmid W, Howaldt S, Stein J, Mickisch O, Waldhör T, *et al*. Iron deficiency generates secondary thrombocytosis and platelet activation in IBD: The randomised, controlled thrombo VIT trial. Inflamm Bowel Dis 2013; 19: 1609-16. http://dx.doi.org/10.1097/mib.0b013e318281f4db

[20] Ohsugi Y. Recent advances in immunopathophysiology of interleukin-6: an innovative therapeutic drug, tocilizumab (recombinant humanized anti-human interleukin-6 receptor antibody), unveils the myterious etiology of immune-mediated inflammatory diseases. Biol Pharm Bull 2007; 30: 2001-6. http://dx.doi.org/10.1248/bpb.30.2001

[21] Imai T, Koike K, Kubo T, Kikuchi T, Amano Y, Takagi M, *et al*. Interleukin-6 supports human megakaryocytic proliferation and differentiation in vitro. Blood 1991; 78: 1969-74.

[22] Evstatiev R, Bukaty A, Jimenez K, Kulnigg-Dabsch S, Surman L, Schmid W, *et al*. Iron deficiency alters mekacaryopoiesis and platelet phenotype independent of thrombopoietin. Am J Hematol 2014; 89: 524-9. http://dx.doi.org/10.1002/ajh.23682

[23] Lopez A, Cacoub B, Macdougall IC and Peyrin-Biroulet L. Iron deficiency anemia. Lancet 2015 Aug 24. pii: S0140-6736(15)60865-0. [Epub ahead of print]

[24] Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, *et al*. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004; 113: 1271-6. http://dx.doi.org/10.1172/JCI200420945

Received on 07-12-2015 Accepted on 13-01-2016 Published on 20-01-2016

http://dx.doi.org/10.15379/2408-9877.2016.03.01.01