Assessment of Efficiency of Serum Transferrin Receptor (sTfR) and sTfR/ Ferritin Index Comparable to Stainable Iron in predicting iron presence in bone marrow of anemic patients

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ABSTRACT

Objective: The current study is planned to distinguish between true iron deficiency anemia (IDA), anemia of chronic diseases (ACD) and coexistence iron deficiency anemia in patients with chronic inflammatory disorders (ACD/ IDA) by using new noninvasive and sensitive parameters as serum transferrin receptors and transferrin receptors – ferritin index (sTfR / Log ferritin) and assessment the efficiency of these measurements in comparison to histochemical examination of stainable iron in bone marrow aspiration which remains the most definitive method to distinguish between IDA and ACD. **Subjects and Methods:** 64 anemic patients and 10 control healthy subjects were used in this study. There were insignificant differences between the studied groups as regard sex and age. The anemic patients were classified into three groups according to the presence or absence of stainable iron in bone marrow and clinical data. I- IDA group**:** patients had no stainable iron in the bone marrow. II- ACD group**:** patients had stainable iron in the bone marrow with chronic disorders. III-COMBI group (ACD/ IDA): patients had no stainable iron in bone marrow together with an infectious diseases, chronic inflammatory diseases or non-hematological malignancy, in addition to patients who had a C-reactive protein (CPR) above 20 mg/L. **Samples and analysis:1)** Bone marrow aspiration was collected from the sternal bone or iliac crest, and many thin smears were made immediately and stained by Aldrich Perl's Prussian for examination of stainable iron, **2)** Blood analysis and complete blood count with the assessment of RBCs indices were done, **3)** Blood smears stained with Leishman's stain were examined by LM , **4)** Examination and count of reticulocytes in peripheral blood smears stained by Brilliant Cresyl blue stain were illustrated, **5)** Serum iron profile included serum iron concentration and % Saturation were calculated. Moreover, **6)** Determination of serum ferritin level, **7)** Determination of serum transferrin receptors level (sTfR) and **8)** Calculation of serum sTfR / Log ferritin ratio were done. **Results**: Microscopic examination of **stained bone marrow smears** demonstrated the absence of stainable iron in bone marrow of patients of IDA and ACD/IDA groups, presence of stainable iron in bone marrow of patients of ACD group as large granules in small clumps, and also appearance of stainable iron in bone marrow of healthy normal control subjects group as a normal positive small sparse of iron particles, **MCH&MCV values** indicated significant decrease between IDA, ACD/ IDA groups with normal group, but there was insignificant difference between ACD and normal groups.The microscopic examination of stained **peripheral blood films** showed normal biconcave disc-shaped red cells with normal central pallor and lacks intra-cytoplasmic inclusions in normal control and ACD groups, while anisipoikilocytosis and microcytosis appeared with large central pallor cytoplasm in IDA ACD/ IDA patient groups. **MCHC value** recorded significant difference between all groups; there was significant decrease difference between IDA, ACD/IDA patient groups and control one and with ACD group, but there was insignificant difference between normal and ACD groups, and between IDA and ACD/IDA groups. **The reticulocytes** were stained with brilliant cresyl blue and displayed a black-blue network or dots. There was significant increase difference between the three patient groups in comparable to normal group, and also between IDA and ACD/IDA groups with ACD group while there was insignificant difference between IDA with ACD/IDA groups. **% Saturation value** recorded significant difference between the three patient groups and normal one, but there was insignificant difference between group IDA and ACD/IDA groups. **Ferritin levels** illustrated significant decrease between IDA and normal groups, and significant increase between ACD and ACD/IDA groups with normal one. **Serum transferrin receptors level** recorded significant increase of IDA and ACD/IDA groups comparable with normal one, but insignificant differences between ACD and normal groups, and between IDA with ACD/IDA groups. **sTFR / Log Ferritin ratio** showed significant difference between all groups; there was significant increase difference between IDA, ACD/IDA and normal groups, but there was no

significant difference between ACD and normal groups. **In conclusion:** the present data suggested that the sTfR and transferrin receptors – ferritin index (sTfR / Log ferritin) could be used as reliable parameters in differentiation between IDA, ACD and ACD/IDA.

Keywords: True iron deficiency anemia (IDA), anemia of chronic diseases (ACD) , coexistence iron deficiency anemia in patients with chronic inflammatory disorders (ACD/IDA), iron, ferritin, %Saturation ,transferrin receptors (sTfR) and transferrin receptors – ferritin index (sTfR / Log ferritin).

INTRODUCTION

Iron deficiency is one of the most frequent clinical disorders in humans, caused by dietary deficiency, chronic blood loss or pregnancy .Iron-deficiency anemia (IDA) occurs when this condition is severe enough to reduce erythropoiesis. The diagnosis of IDA can be very difficult in patients with coexistent pathologies, such as inflammatory and autoimmune diseases, malignancies, or infections (acute or chronic) because anemia of chronic disease (ACD), frequently occurring in these conditions, can be a confounding factor **(1).** Differentiating between IDA and ACD, and identifying the coexistence of mixed IDA/ACD is, therefore, a serious diagnostic challenge, mainly because these conditions of anemia need different treatments and in ACD patients an inappropriate supplementation of iron could aggravate the underlying disease. The gold standard for diagnosing iron deficiency is the bone marrow examination (BME), which establishes the absence of stainable iron **(2)**. BME is, however, invasive, expensive and operator-dependent so that it cannot be routinely performed in clinical practice (**3&4).**

Traditionally, the distinction between different causes of anemia is based on a hematological algorithm starting with the interpretation of the mean corpuscular volume (MCV). Accordingly, micro-, macro- or normocytic erythrocyte conditions may hint at different causes for anemia. Besides MCV, basic laboratory parameters should include reticulocyte count, differential blood cell count, red cell distribution width, and serum ferritin, TfS and C-reactive protein (CRP) concentrations **(5).**

Consequently, the measurement of serum ferritin concentration is the first level test for diagnosing IDA. Blood ferritin concentrations are reported to reflect the amount and the changes of intracellular ferritin, the main iron storage protein, as confirmed by robust data. However, ferritin failed to recognize true iron deficiency in chronic disorders cases due to the fact that ferritin is considered as an acute phase protein **(6)**.

The availability of further biomarkers, not affected by concurrent chronic disease and inflammation and therefore able to identify mixed IDA/ACD, could have a great clinical value. The soluble transferrin receptor (sTfR) may play this role, as its serum concentration is expected to rise in IDA, but not in ACD **(7)**. This is a single polypeptide chain of 85 kDa, produced by the proteolytic cleavage of the 190 kDa transmembrane transferrin receptor, a glycoprotein primarily expressed in cells requiring iron. Plasma sTfR concentrations reflect the receptor density on cells and the number of cells expressing the receptor, therefore it is closely related to cellular iron demands and erythroid proliferation rate **(8)**. Some authors also reported the use of the sTfR/Log ferritin ratio, i.e. the sTfR index, theoretically taking advantage of the reciprocal relationship between the two variables influenced by iron deficiency (increase of sTfR and decrease of ferritin concentrations) **(9&10).** However, a recent meta-analysis demonstrated the greater clinical value of sTfR rather than the sTfR index **(11)**.

The present investigation focused on the differentiation between IDA, ACD and ACD / IDA by using histological, histochemical and biochemical studies.

SUBJECTS & METHODS

I. Study design:

The current study was conducted on 64 anemic patients, in addition to, 10 healthy subjects serving as healthy control group admitted to Al-Fadaly hospital, Tanta, Egypt. The anemic patients were categorized into three groups based on the presence or absence of stainable iron in bone marrow and according to the clinical data as:-

IDA group: Twenty-seven patients (15women and 12 men): who fulfilled the morphologic criteria of iron deficiency and had no stainable iron in the bone marrow**,** were known as IDA patients.

ACD group: Twenty-seven anemic patients (10 women and 17 men) were classified as ACD patients, and these patients had stainable iron in their bone marrow. These patients had recurrent or chronic infections and chronic diseases (non-hematologic malignancies or inflammatory diseases).

COMBI group (ACD/IDA): Ten anemic patients who had no stainable iron in the bone marrow together with an infectious disease, or a chronic inflammatory disorder (such as rheumatoid arthritis or colitis ulcerosa) or a non-hematological malignancy. In addition, patients who had a C-reactive protein (CRP) value above 20 mg/L were placed in a COMBI group.

The subjects included in this study were adult persons age ranged from 39 - 60 years old included; ten apparently healthy adult with no history of anemia and diseases and patients with history of anemia (IDA, ACD and ACD/IDA).

Certain patients were excluded from the present study as they suffered from hemolytic anemia, defined vitamin B12 or folate deficiency, blood transfusion within the past 3 months, hematologic malignancies, trauma, cancer, patients currently receiving chemotherapy or who received chemotherapy in the last 3 months, "since chemotherapy and/or radio therapy as well as bone marrow infiltration by tumor cells might alter the pathophysiology of the anemia compared with subjects with ACD on the basis of autoimmune or infectious disease" and patients who currently taking iron supplements or receiving recombinant erythropoietin or mycophenolate mofetil therapy.

All patients were subjected to full history taking with emphasis on: age, gender, time elapsed before hospital admission, history of medical diseases that include active infections and complete physical examination.

II. Samples:

♦Bone marrow aspiration

The bone marrow was collected from the sternal bone or iliac crest with the help of the bone marrow aspiration needle under local anesthesia. Immediately, many thin smears from bone marrow were done according to **Larson (12)** .The remaining of the sample was placed in EDTA tube for further investigations.

♦Blood

Ten milliliters (10mL) of freshly venous blood were withdrawn from each subject under complete aseptic precautions. Two ml of blood were placed in EDTA tube for complete blood count. The remaining of the blood was placed in sterile tubes (without anticoagulants) and left to clot for 30 minutes. Serum was then separated by centrifugation at 3000g for 15 minutes and serum was immediately subdivided into three eppendorfs; one for immediate assay of Iron profile. The second eppendorf was for immunoassay determination of ferritin .The last was frozen at -20°C for further specific investigations of serum serum transferrin receptors. Before analysis, frozen samples were allowed to thaw at room temperature. Hemolysed and lipaemic samples were discarded, repeated freezing and thawing was avoided **(7).**

III. Histological and histochemical studies:

1-Bone marrow aspiration smears were stained for iron store by using Sigma –Aldrich Perl's Prussian iron stain **(13).**

2 - Peripheral blood smears were examined by Leishman's stain **(14).**

3- Complete blood counts were determined by using automated hematology analyzer (ABX Micros 60 analyzer).

4 – Examination and count of reticulocytes were done by using Briiliant cresyl blue stain **(15).**

IV. Biochemical studies:

Biochemical studies were recorded by measuring the following:

- Serum iron determination was done by using ELTECH diagnostics kit **(16)**.
- Transferrin saturation (% Saturation value) was calculated by the following equation**:** $\frac{serum\ iron \times 100}{TIBC}$ **(12).**
- The quantitative determination of ferritin concentrations in human serum by ELISA **(17).**
- Measurement of serum transferrin receptors **[**sTfR] by ELISA **(18)**
- Calculation of serum sTFR / Log ferritin ratio **(19)**.

V. Data analysis:

Data were presented as mean ± SE. Results were analyzed using the computer program of SPSS. All statements of significance were based on probability of P≤0.05 was considered to be significant.

RESULTS

1.Estimation of stainable iron distribution in bone marrow in anemic patients and control groups:

Stainable iron distribution in bone marrow in the present studied of the three groups of anemic patients and control group was done by using Aldrich Perl's Prussian iron blue stain "gold standard". Categorization of the patients into three groups was based on presence or absence of stainable iron in bone marrow and inflammation. Patients of IDA group and patients of ACD/IDA group showed no stainable iron (negative) in bone marrow, while patients of ACD group showed presence (positive) of intense stainable iron in bone marrow as an increase and large granules of stainable iron in small clumps, and also the healthy normal control subjects showed presence (positive) of stainable iron in bone marrow (normal positive small, sparse iron particles in low power field), see Table (1) & Figs (1- 4).

All patients in the present studies were anemic with hemoglobin less than 12 g/L and normal control group were more than 12 g/L. All patients had low serum iron less than 50 mg/dl as recorded in the previous our work **(20).**

2. MCH & MCV values and blood films stain:

MCH values & MCV values: There were significant differences decrease between IDA and ACD/IDA patient groups with normal group P <0.001 and with ACD group P <0.001 whereas, there was insignificant difference between normal with ACD groups as P>0.05 and also between IDA group with ACD/IDA P>0.05 as recorded previously in our study **(20)**. The microscopic examinations of stained peripheral blood films with Leishman's stain showed normal biconcave disc-shaped of red cells with normal central pallor and lacks intra-cytoplasmic inclusions in normal control and ACD groups, but anisipoikilocytosis and microcytosis appeared with larger central pallor (Figs. 5, 6, 7&10).

3. MCHC values:

As regard to MCHC value, there was significant difference between groups P<0.001. There was significant decrease difference between IDA, ACD/IDA patient groups and control group P<0.001 and with ACD group P<0.001, but there was insignificant difference between normal group and ACD group as P>0.05 and between IDA group and ACD/IDA group P>0.05 (Fig.11).

4. Reticulocytes count (Retix):

Reticulocytes (Retix) are immature RBCs, non-nucleated and contain reminant RNA. The reticulocytes displayed a black-blue network or black-blue dots with brilliant cresyl blue stain in normal blood smears of control group (Fig. 8), while the anemic patients of the three groups demonstrated increment of reticulocytes (Fig. 9). There was significant increase between the three patient groups comparable with normal group P <0.001, and also between IDA and ACD/IDA groups with ACD group P <0.001. On the other hand, there was insignificant difference between IDA group with ACD/IDA group P>0.05 (Fig. 12) as recorded previously in our work **(20)**.

5. % Saturation value:

As regard % Saturation value, there was significant difference between groups P <0.001. There was significant difference between IDA, ACD, ACD/IDA groups and normal group P <0.001, but there was insignificant difference between group IDA group and ACD/IDA group P>0.05 (Fig.13).

6. Serum ferritin levels:

There was significant decrease difference of ferritin levels between IDA group with normal group P <0.001. On contrast, there was significant increase between ACD and ACD/IDA groups with normal group p<0.001 and p<0.05, respectively. The IDA group showed the lowest ferritin level among all groups, while the ACD group showed the highest level (Fig.14) as recorded previously in our work **(20).**

7. TfRs levels:

Data of TfRs levels demonstrated significant increase differences between IDA and ACD/IDA groups with normal group P <0.001, but there were insignificant differences between ACD group with normal group P>0.05, also there were insignificant differences between IDA with ACD/IDA group (Fig. 15) as recorded previously in our work **(20).**

8. TfRs / Log ferritin ratio:

There was significant difference between groups as regard TfRs / Log ferritin ratio P <0.001. There was significant increase difference between IDA, ACD/IDA groups and normal group P <0.001 and P <0.005 respectively but there was no significant difference between normal group and ACD group as P>0.05 (Fig.16).

Table 1): Presence or absence of stainable iron in bone marrow distribution in the studied anemic patients and control group.

Iron stain		Group				Total
		Normal	IDA	ACD	ACD/IDA	
+ve	N	10	0	27	0	37
	%	100.0%	100.0%	100.0%	.0%	50.0%
-ve	N	0	27	0	10	37
	%	100.0%	100.0%	.0%	100.0%	50.0%
Total	N	10	27	27	10	74
	%	100.0%	100.0%	100.0%	100.0%	100.0%
	P-value	0.001*				

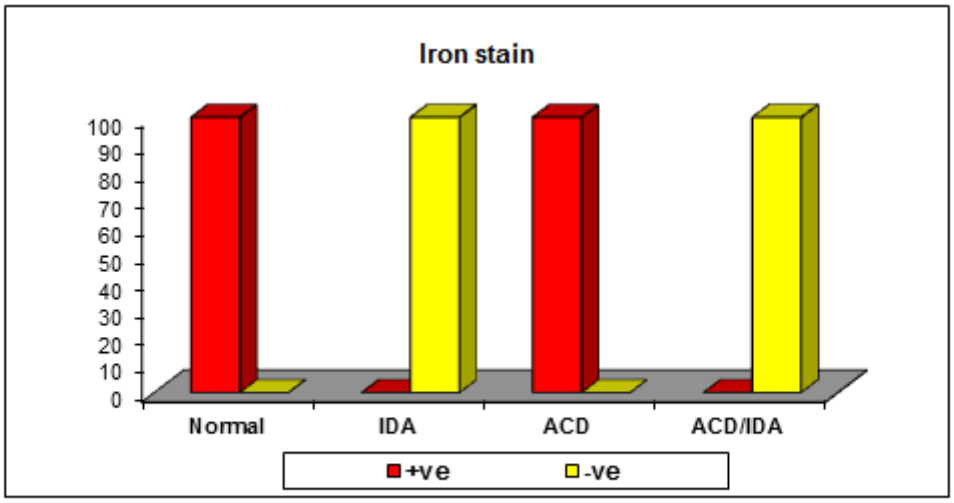

Fig (1): Statistical analysis presence (+Ve) or absence (-Ve) of stainable iron distribution in bone marrow from normal donor and anemic patient groups.

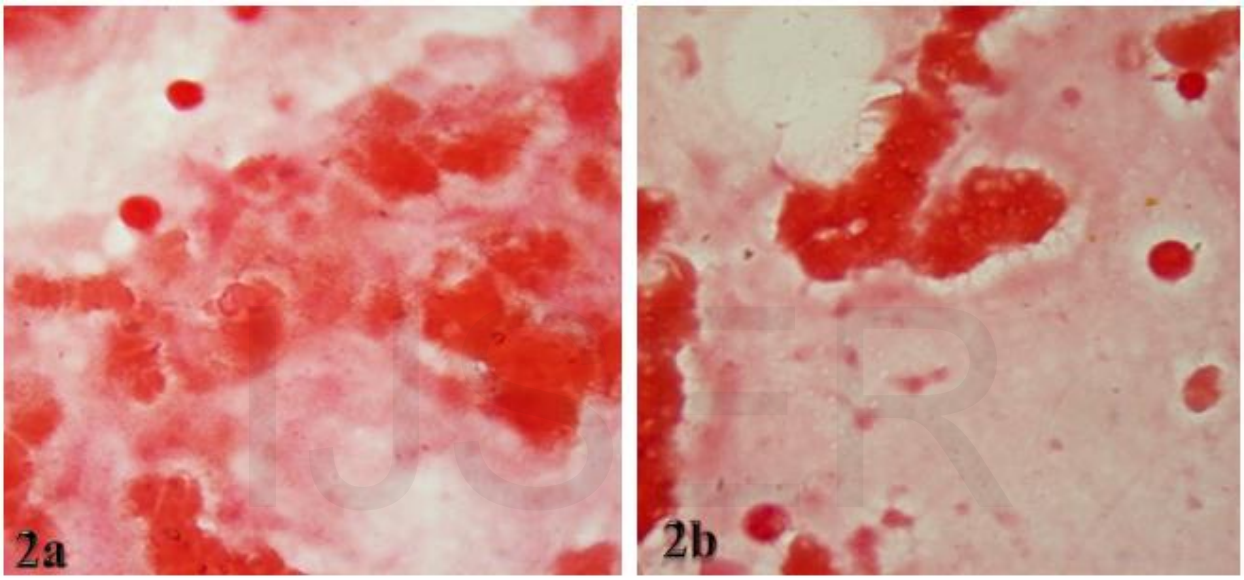

Fig. (2): Bone marrow smears of iron deficiency anemic patient (IDA) showing absence of stainable iron deposition in different areas of smears (a & b). (Perl 's Prussian blue stain), X1000.

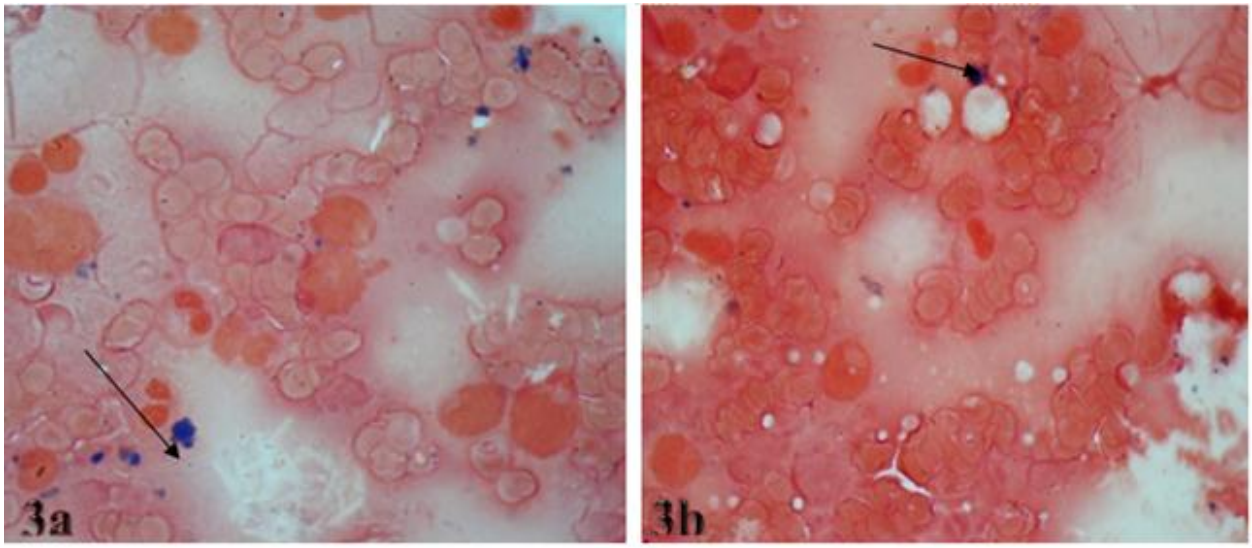

Fig. (3): Bone marrow smears of normal control subjects showing presence stainable iron deposition (arrows) in different areas of smears (a&b). (Perl 's Prussian blue stain), X1000.

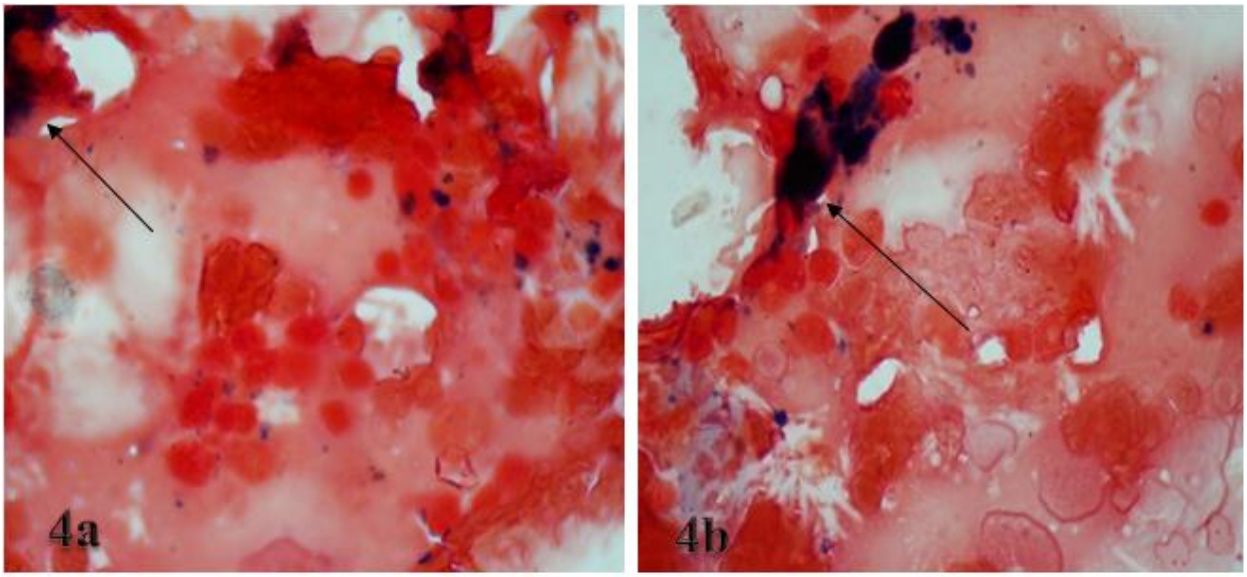

Fig. (4): Bone marrow smears of chronic disorders anemic patient (ACD) showing increased stainable iron deposition (arrows) in different areas of smear (a&b). (Perl's Prussian blue stain), X1000.

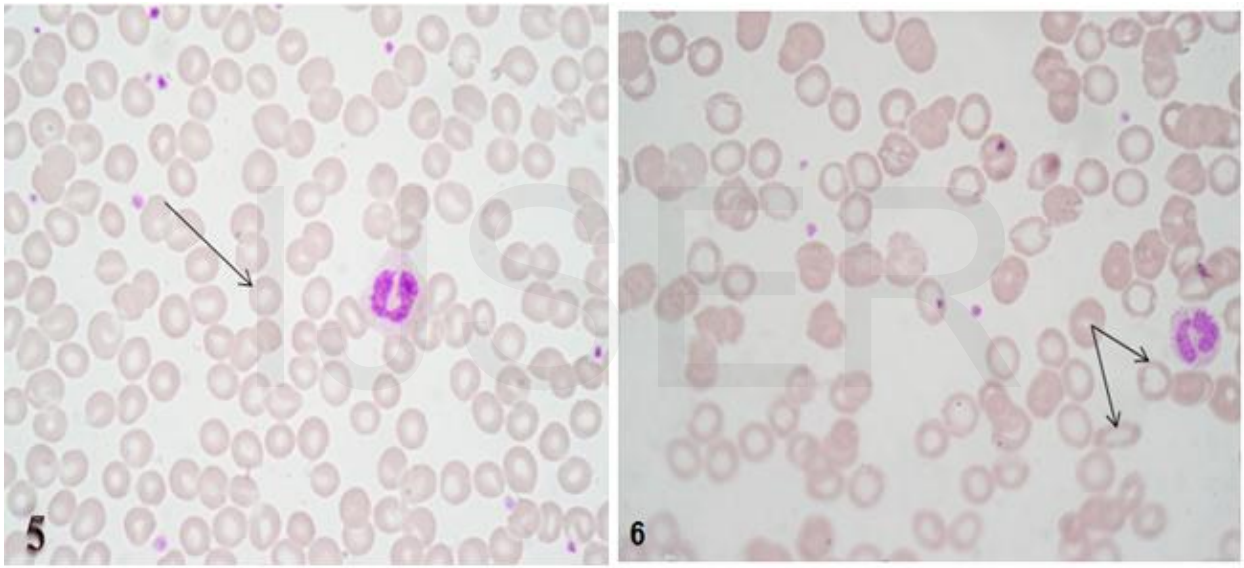

Fig. (5): Peripheral blood smear of normal control subjects showing normal mean cell hemglobin (MCH) (arrow) in red blood cells. (Leishman's stain), X1000.

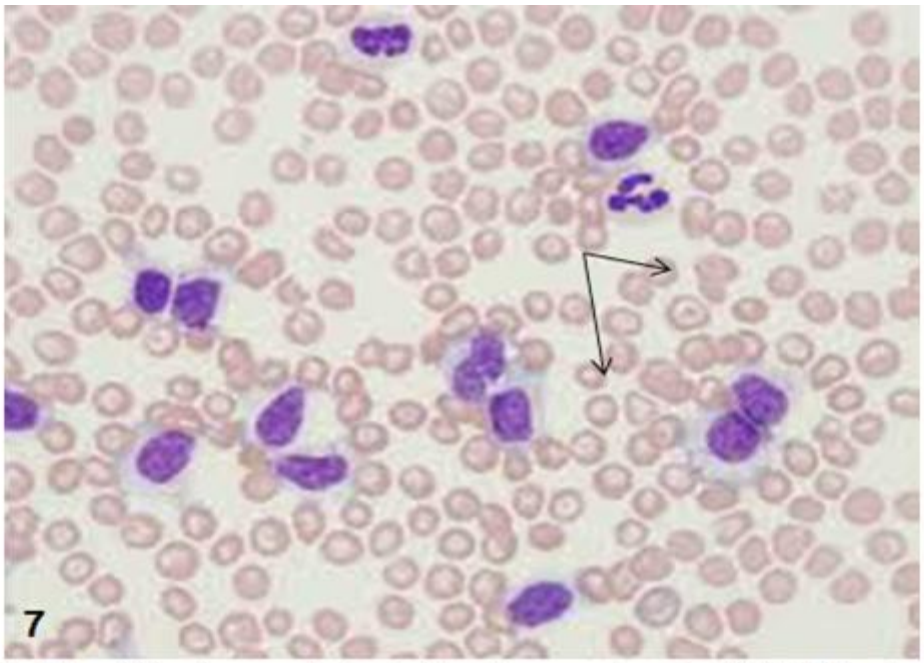

Fig. (7): Peripheral blood smear of chronic disorders anemic patient (ACD) showing mean cell hemglobin (arrows) in red blood cells. (Leishman's stain), X1000.

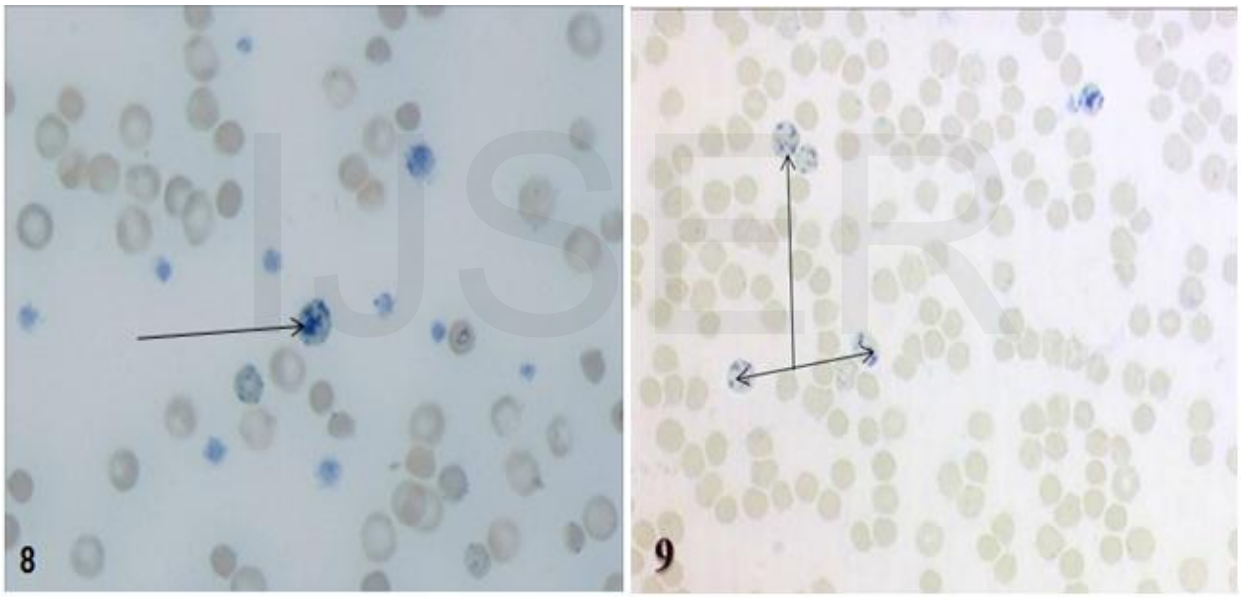

Fig. (8): Peripheral blood smear of normal healthy subjects showing normal reticulocytes (arrow). (Brilliant cresyl blue stain), X1000.

Fig. (9): Peripheral blood smear of anemic patient showing increment of reticulocytes (arrows). (Brilliant cresyl blue stain), X1000.

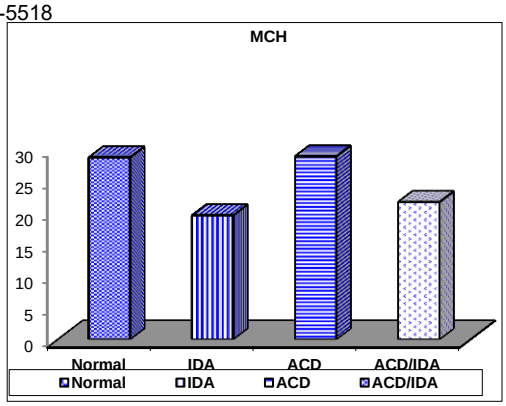

Fig. (10): MCH values in the studied anemic patients and normal group **(Al-Fadaly *et al.*, 2017).**

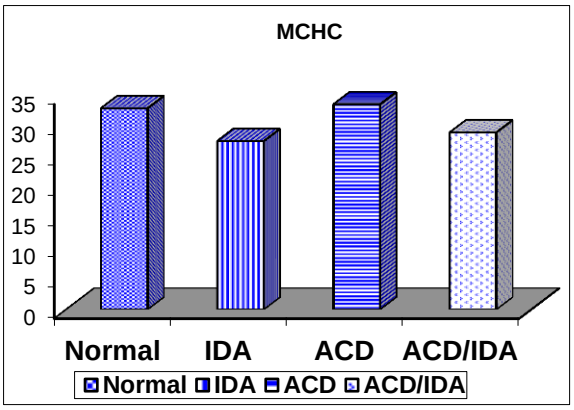

Fig. (11): MCHC values in the studied anemic patients and normal group.

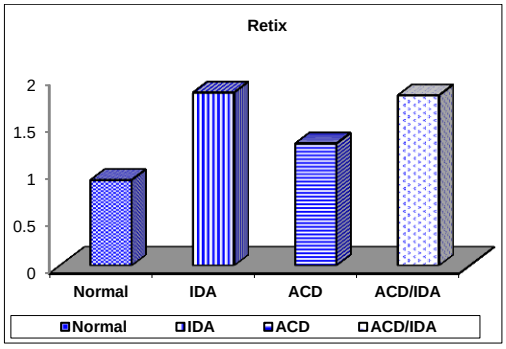

Fig. (12): Reticulocytes count in the anemic patients and normal group **(Al-Fadaly *et al.*, 2017).**

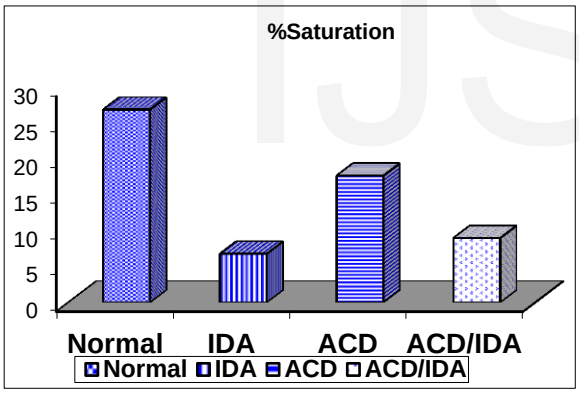

Fig. (13): % Saturation values in the studied anemic patients and normal group.

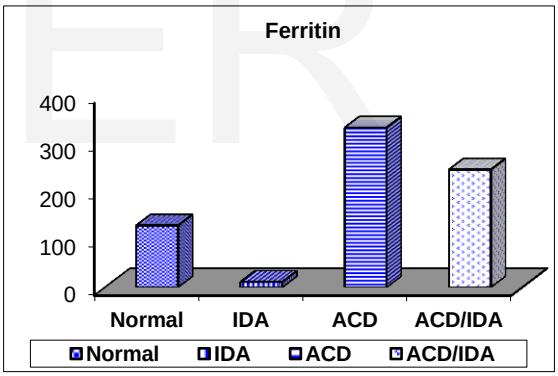

Fig. (14): Ferritin levels in the anemic patients and normal group **(Al-Fadaly *et al.*, 2017).**

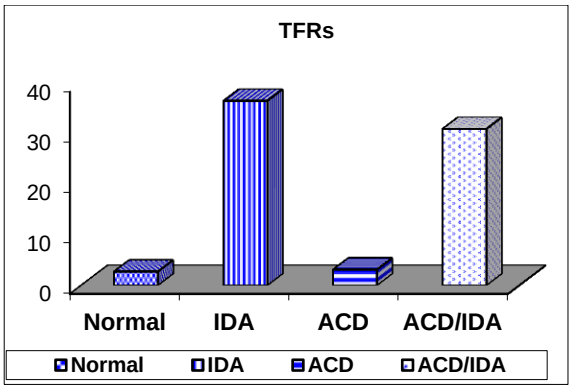

Fig. (15): Serum transferrin receptors levels in the anemic patients and normal group.

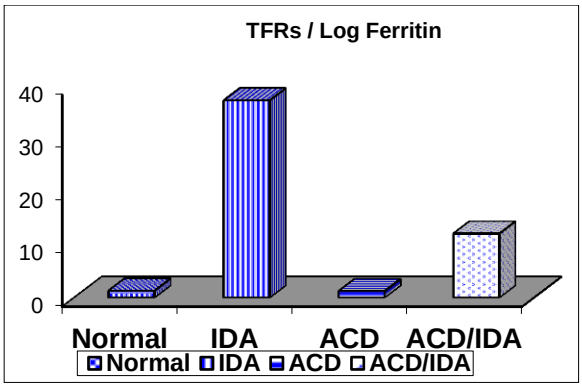

Fig. (16): TFRs/Log ferritin relation in the anemic patients and normal group **(Al-Fadaly *et al.*, 2017)**.

DISSCUSSION

Bone marrow examination is necessary to evaluate iron in stores exactly and to setup crucial diagnoses **(4).** However, bone marrow examination cannot be regularly performed because it is painful, expensive and time consuming **(6).** The microscopic examination of stainable iron in the bone marrow aspirate smear is generally considered the reference standard for determining the body iron stores **(21)**. Microscopic examination of a Perl's Prussian blue stained bone marrow aspirate smears is widely regarded as the "gold standard" for the assessment of the marrow iron stores **(22)**.

In the current study, absence of stainable iron in the bone marrow was diagnostic of iron deficiency, whereas abundance of stainable iron particles was considered as a diagnostic of ACD. Bone marrow in patients with anemia of chronic disorders is usually not hypercellular, and sTfR are also not increased in these disorders. This may be an explanation for normal sTfR in ACD **(23**).

The erythrocytes of IDA group in the present study appeared paler and smaller as well as their hemoglobin inside them were reduced than in control normal erythrocytes group. This is manifested by reduced mean erythrocyte volume (MCV) and reduced mean eryhtrocyte hemoglobin (MCH) in complete blood count. On peripheral normal blood smear, the erythrocytes are microcytic and hypochromic while in ACD, they appeared as normochromc normocytic with normal MCH and MCV. These results make RBCs indices less effective in diagnosis iron deficiency anemia in chronic disorders. These results were in accordance with **Cheng *et al.* (24) and Pasricha *et al.* (25)**.

Regard to MCHC, there was no significant difference between normal group and ACD group indicated the fact that ACD is normochromic and normocytic (although anemia may become microcytic as disease progresses and the reticulocyte count is low, reflecting the hypoproliferative nature of the anemia **(19).**

Concerning reticulocytes count (Retix), there was significant increase between the three patient groups with normal group. This result reflects the fact that an increase of immature reticulocytes in the blood of individuals with iron deficiency anemia represents a response to anemia, as long as the medullary tissue and the indispensable factors for erythropoiesis are preserved **(26).**

Considering % Saturation, There was significant difference between IDA, ACD, ACD/IDA groups comparable with normal group, but there was no significant difference between group IDA group and ACD/IDA group. As a conclusion, %Saturation is not a good marker to detect true iron deficiency in ACD/IDA group. The current results were correlated with the results of many authors **(2 &27).**

Regard to ferritin concentrations, the present data showed significant decrease in ferritin concentrations of all patients of IDA group which had no stainable iron in bone marrow, while it recorded significant increase in patients of ACD group which had an increment of stainable iron in bone marrow. Although, all patients of ACD/IDA group showed absence of stainable iron in bone marrow, the mean of ferritin concentrations showed significantly increase. This could be attributed to the fact that ferritin is considered an acute phase protein which means it can be elevated in the presence of inflammation **(20, 28 & 29).**

Serum ferritin and transferrin saturation (TSAT) are currently the main surrogate markers used in daily clinical practice for assessing iron status **(30)**. However, ferritin

levels are difficult to interpret during inflammation because sferritin expression is induced by both iron overload and inflammatory cytokines. TSAT may also mislead since it has some acute phase reactivity **(31)**.

In the current study the sTfR value in IDA patients group showed significant increase in its mean value compared with normal value. The significant increase in sTfR in IDA patients group could be attributed to the erythroid marrow hyperplasia where its concentration correlates directly with erythropoietic activity and inversely proportional with the amount of iron available for erythropoiesis. Thus, the increased sTfR measurements can effectively identify iron deficiency even in the presence of accompanying inflammatory or acute infectious conditions. As it was previously suggested the quantification sTfR has been shown to differentiate effectively between iron replete and iron deplete anemic states, irrespective of the presence of acute or chronic inflammatory conditions **(32- 35)**.

Determination of sTfR has been proposed to identify iron-deficiency anemia (IDA) in patients affected by concurrent inflammatory disease that may spuriously increase ferritin concentration **(36&37).** Thus the serum ferritin level varies with iron stores, while TfRs is assumed to reflect reliably the degree of tissue iron supply. The TfRs/ferritin ratio has been suggested to be a good estimate of body iron in individual subjects

In the present study, the sTfR /ferritin ratio was significantly increased when IDA group was compared with the control group.This was in accordance with many authors **(38-42)**. They suggested that sTfR / ferritin ratio and sTfR/ Log ferritin index are a good estimate of body iron. The usage of this ratio provides the advantage of the combining of two phenomena i.e., increase in sTfR and a decrease in the ferritin concentration. These two variables, in general, are influenced by the body iron stores, the availability of iron for erythropoiesis, and total mass of erythroid marrow. The present findings of increased sTfR and sTfR /ferritin ratio in ACD patients could be attributed to the accepted explanation of the possibility of the presence of IDA with ACD.

In conclusion, the present results illustrated that the serum ferritin is still the effective marker for diagnosis of IDA in absence of inflammation and chronic diseases, but it is not a good indicator to differentiate IDA when associated with ACD/IDA as well as %Saturation is not a good marker to detect IDA in ACD/IDA group. In contrast, results of sTfR showed no change in both IDA and ACD/IDA group. Therefore, sTfR and transferrin receptors – ferritin index (sTfR / Log ferritin) could be used as reliable parameters in differentiation between IDA, ACD and ACD/IDA.

REFERENCES

1) **Goddard, A.F.; James, M.W.; McIntyre, A.S.; Scott, B.B. and on behalf of the British Society of GastroenteroLogy (2011):** Guidelines for the management of iron deficiency anemia. Gut, 60:1309–1316.
2) **Bermejo, F.; Garcìa-Lòpez, S. (2009):** A guide to diagnosis of iron deficiency and iron deficiency anemia in digestive disease. World J. Gastroenterol., 15: 4638–4643.
3) **Guyatt GH.; Oxman, AD.; Ali, M..; Willan A.; McIlroy, W. and Patterson C. (1992):** Laboratory diagnosis of iron-deficiency anemia:an overview. J.Gen. Intern Med.,7: 145-153.
4) **Zimmermann, M.B. (2008):** Methods to assess iron and iodine status. Br. J. Nutr., 99(3): 2–9.
5) **Dignass, AU.; Gasche C.; Bettenworth, D.; Birgegård, G.; Danese, S.; Gisbert, J.P.;Gomollon F.; Iqbal T.;Katsanos**

K.; Koutroubakis I.; Magro F.; Savoye G.; Stein J.;Vavricka S. and European Crohn's and Colitis Organisation [ECCO] (2015): European consensus on the diagnosis and management of iron deficiency and anemia in inflammatory bowel diseases. J. Crohns. Colitis, 9 (3): 211-222.

6) **Ferraro, S.; Mozzi, R. and Panteghini, M. (2012):** Revaluating serum ferritin as a marker of body iron stores in the traceability era. Clin. Chem. Lab. Med., 50 (11):1911–1916.

7) **Berlin, T.; Meyer, A.; Rotman-Pikielny, P.; Natur, A. and Levy, Y. (2011):** Soluble transferrin receptor as a diagnostic laboratory test for detection of iron deficiency anemia in acute illness of hospitalized patients. Isr. Med. Assoc. J., 13: 96–98

8) **Speeckaert, M. M.; Speeckaert, R. and Delanghe, J. R. (2010):** Biological and clinical aspects of soluble transferring receptor. Crit. Rev. Clin. Lab. Sci., 47:213–228.

9) **Skikne, B.S.; Punnonen, K. and Caldron, P. H. (2011):** Improved differential diagnosis of anemia of chronic disease and iron deficiency anemia: a prospective multicenter evaluation of soluble transferrin receptor and the sTfR/Log ferritin index. Am. J. Hematol., 86: 923–927.

10) **Oustamanolakis, P.; Koutroubakisa, I. E.; Messaritakisb, I.; Niniraki, M. and Kouroumalisa, E. A. (2011):** Soluble transferrin receptor-ferritin index in the evaluation of anemia in inflammatory bowel disease: a case control study.Ann.Gastroenterol., 24:108–114.

11) **Infusino, I.; Braga, F.; Dolci, A. and Panteghini, M. (2012):** Soluble transferrin receptor (sTfR) and sTfR/Log ferritin index for the diagnosis of iron-deficiency anemia. A meta -analysis. Am. J. Clin. Pathol., 138: 642–649.

12) **Larson, D. (2005):** Clinical Chemistry -E-Book: Fundamentals and Laboratory Techniques. Elsevier Health Sciences, p: 246

13) **Bancroft, J.D. and Gamble, M. (2002):** Theory and Practice of HistoLogical Techniques. 5th edn,Churchill Livingstone, London, New York & Sydney, 377-694.

14) **Garbyal, R.S.; Agarwal N. and Kumar, P. (2006):** Leishman-Giemsa cocktail: An effective Romanowsky stain for air dried cytologic smears. Acta. Cytol., 50:403–406.

15) **Lewis, S. M.; Bain, B. J. and Bates, I. (2006):** HematoLogiaprática de Dacie e Lewis, 9th edn, Porto. Alegre. Artmed.

16) **Callahan, J. H. and Cook K. O. (1982):** Anal. Chem. 54, 59-62.

17) **Tietz, N. (1999):** Textbook of Clinical Chemistry, Carl. A. Burtis., 3rd edn, WB. Saunders, Philadelphia.

18) **Lee, E.J.; O.H, E.J.; Park, Y.J.; Lee, H.K. and Kim, B.K. (2002):** Soluble transferrin receptor (sTfR), ferritin, and sTfR/Log ferritin index in anemic patients with non-hematologic malignancy and chronic inflammation. Clin. Chem., 48: 1118-1121.

19) **Weiss, G. and Goodnough, L. T. (2005):** Anemia of chronic disease. N. Engl. J. Med., 352:1011-1023.

20) **Al-Fadaly, N.H.; Elwy, A.M.; El-Desouki N.I. and Mossaed, E.M. (2017):** Diagnostic implication of iron deficiency in the management of toxicity associated with chronic diseases. J. Am. Sci., 13(4): 51-59.

21) **Hughes, D.A.; Stuart-Smith, S.E. and Bain, B.J. (2004):** How should stainable iron in bone marrow films be assessed? J. Clin. Pathol., 57(10):1038-1040.

22) **Pujara, K. M.; Bhalara, R.V. and Dhruva, G.A. (2014):** A study of bone

marrow iron storage in hematoLogical disorder. Int. J. Health Allied Sci., 3:221-224.

23) **Beesley, R.; Filteau, S.; Tomkins, A.; Doherty, T.; Avles, H. and Reid, A. (2000):** Impact of acute malaria on plasma concentrations of transferrin receptors. Trans. R. Soc. Trop. Med. Hyg., 94(3): 295–298.

24) **Cheng, P.P.; Jiao, X. and Wang, X. (2010):** Hepcidin expression in anemia of chronic disease and concomitant iron deficiency anemia. Clin. Exp. Med., 11: 33–42.

25) **Pasricha, S. S.; Flecknoe-Brown, S.C.; Allen, K.J.; Gibson, P.R.; Mahon, L.P. and Olynyk, J.K. *(*2010):** Diagnosis and management of IDA: a clinical update. Med. J., 193:525–532.

26) **Wollmann, M.; Gerzson, B.M.; Schwert, V.; Figuera, R.W. and Ritzel-Gde, O. (2014):** Reticulocyte maturity indices in iron deficiency anemia. Rev. Bras. Hematol. Hemoter., 36 (1): 25-28.

27) **Wians, F. H.; Urban, J. E.; Keffer, J. H. and Kroft, S. H. (2001):** Discriminating between iron deficiency anemia and anemia of chronic disease using traditional indices of iron status vs transferrin receptor concentration. Am. J. Clin. Pathol., 115(1): 112-118.

28) **Kalantar-Zadeh, K.; and Lee, G.H. (2006):** The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease. Clin. J. Am. Soc. Nephrol., 1: 9-18.

29) **Wang, W.; Knovich, M. A.; Coffman, L. G.; Torti, F. M. and Torti, S.V.(2010):** Serum ferritin: Past, present and future. Biochim. Biophys. Acta., 1800 (8): 760-769.

30) **Rocha, A. L.; Daniela, V. and Fellype, B. C. (2009):** Serum ferritin level remains a reliable marker of bone marrow iron stores evaluated by histomorphometry in hemodialysis patients. Clin. J. Am. Soc. Nephrol., 4: 105–109.

31) **Theurl, M.; Nairz, M.; Schroll, A.; Sonnweber, T.; Asshoff, M.; Haschka, D.; Seifert, M.; Willenbacher, W.; Wilflingseder, D.; Posch, W.; Murphy, A.T.; Witcher, D. R.; Theurl, I. and Weiss, G. (2014):** Hepcidin as a predictive factor and therapeutic target in erythropoiesis-stimulating agent treatment for anemia of chronic disease in rats. Haematologica, 99(9):1516-1524.

32) **Das Gupta, A.; Abbi, A. (2003):** High serum transferrin receptor level in anemia of chronic disorders indicates coexistent iron deficiency. Am. J. Hematol., 72: 158-161.

33) **Pavai, S.; Jayaranee, S. and Sargunan, S. (2008):** Soluble transferrin receptor, ferritin and soluble transferrin receptor--Ferritin index in assessment of anemia in rhaeumatoidarthritis. Med. J. Malaysia, 62(4): 303-307.

34) **Koulaouzidis, A.; Said, E.; Cottier, R. and Saeed, A. A. (2009):** Soluble transferrin receptors and iron deficiency, a step beyond ferritin, a systematic review.J. Gastrointestin. Liver Dis., 18(3):345-352.

35) **Jain, S.; Narayan, S.; Chandra, J.; Sharma, S.; Jain, S. and Malhan, P. (2010):** Evaluation of serum transferrin receptor and sTfR ferritin indices in diagnosing and differentiating iron deficiency anemia from anemia of chronic disease. Indian J. Pediatr., 77(2):179-183.

36) **Braga, F.; Infusino, I.; Dolci, A. and Panteghini, M. (2014):** Soluble transferrin receptor in complicated anemia. Clin. Chim. Acta., 20; 431:143-147.

37) **Harms, K. and Kaiser, T. (2015):** Beyond soluble transferrin receptor: old

challenges and new horizons. Best Pract. Res. Clin. Endocrinol. Metab., 29 (5):799-810.

38) Rimon, E.; Levy, S.; Sapir, A. (2002): Diagnosis of iron deficiency anemia in elderly by transferrin receptor- ferritin index. Arch. Intern. Med., 162: 445-449.

39) Goyal, R.; Das, R.; Bambery, P. and Garewal, G.(2008): Serum transferrin receptor ferritin index shows concomitant iron deficiency anemia and anemia of chronic disease is common in patients with rheumatoid arthritis in north India. Indian J. Pathol.Microbiol., 51: 102-104.

40) Skikne, B.S. (2008): Serum transferrin receptor. Am. J. Hematol., 83(11):872-875.

41) Abitbol, V.; Borderie, D.; Polin, V.;Maksimovic, F.; Sarfati, G.; Esch, A.; Tabouret, T.; Dhooge, M.; Dreanic, J.;Perkins, G.; Coriat R. and Chaussade, S. (2015): Diagnosis of Iron Deficiency in Inflammatory Bowel Disease by Transferrin Receptor-Ferritin Index. Medicine (Baltimore), 94(26): e1011.

42) Mücke, V.; Mücke, M.M.; Raine T. and Bettenworth, D. (2017): Diagnosis and treatment of anemia in patients with inflammatory bowel disease. Ann. Gastroenterol., 30 (1):15-22.