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Research Article

Novel Diagnostic Parameters in the Differentiation of Isolated Iron Deficiency and Iron Deficiency Accompanying Chronic Disease before Progressing Anemia

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Abstract

Objectives: The study aimed to investigate the patients with isolated iron deficiency (IID) and chronic disease-accompanied iron deficiency (CDID) and to analyze the predictive values of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) that can help us to distinguish IID from CDID.

Methods: Three hundred patients joined the present cross-sectional thesis study. Half had chronic diseases+ID (without anemia) called as CDID, while the other half did not have any chronic disease or anemia called IID in the text. Laboratory parameters and patient histories were obtained from the automation system and analyzed.

Results: The CDID was associated with ferritin increase (Odds Ratio [OR]=1.123; 95% Confidence Intervals [CI]=1.084–1.165). Increased sedimentation was associated with an increased risk of having a CDID (OR=1.023; 95% CI = 1.003–1.045). Ferritin showed a predictive potential for CDID with 67.2% specificity and 71.1% sensitivity at a 12.1 cutoff value (Auc:0.781; p<0.0001). NLR was the second strong predictor of CDID against IID, with 64.1% specificity and 63.5% sensitivity at a 2.09 cutoff (Auc:0.629; p<0.0001). PLR had no significance for discrimination of CDID and IID.

Conclusion: NLR can provide diagnostic support like ferritin in predicting CDID against IID and benefit physicians in the clinical use of differentiation, unlike PLR.

Keywords: Iron deficiency, neutrophil lymphocyte ratio, platelet lymphocyte ratio

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Iron is an element that is critical for many enzymatic reactions in the human body.[1] It is involved in oxygen transport, energy production, mitochondrial respiration, inactivation of harmful oxygen radicals, and DNA synthesis.[2, 3] These functions cannot be fulfilled in cases of iron deficiency with a wide cause of nutritional health problems worldwide.[4] Symptoms may develop due to isolated iron deficiency (IID), which is involved in many metabolic and enzymatic events in the body.[5] The early diagnose of IID is crucial to prevent patients from progressing anemia.[6]

Iron deficiency occurs in the following two forms: absolute and functional IID. There is no storage iron in absolute IID,[7] while storage iron is normal or increased and iron cannot be supplied to erythroid precursors functional in functional type.[8] In IID, storage iron decreases first,

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functional iron compartments are normal. Then, functional iron compartments and transferrin saturation decrease, and finally, anemia occurs.[9] True IID is defined by a ferritin <15–30 ng/mL in the absence of inflammation. In the presence of inflammation, functional ID is defined as ferritin concentration >100 ng/mL. Chronic diseases can cause clinical diagnosis confusion by causing inflammation and functional ID.[10] This because ferritin, which is considered the most valuable among the classical parameters used in the diagnosis of IID, rises as an acute phase reactant, it is difficult to diagnose both in IID anemia and in cases where inflammatory conditions are present.[11] Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are novel parameters, which are calculated from routine hematological tests, have recently demonstrated their power as a diagnostic tool in many diseases associated with inflammation.

Detection of new parameters in differential diagnosis may provide more reliable estimates in determining functional iron status and erythropoietic activity in adult patients. The study aimed to investigate the variability of parameters in patients with chronic disease-accompanied iron deficiency (CDID) and to analyze the predictive values of PLR and NLR that can distinguish IID and CDID.

Methods

Study Design

A total of 2987 patients who applied to outpatient clinics were retrospectively screened in the hospital automation system and 300 patients in this cross-sectional thesis study. Of the 300 patients included in the study, half had chronic diseases+ID (without anemia) called as CDID, while the other half did not have any chronic disease or anemia but only ID that called as IID in the text. Laboratory parameters and patient histories were obtained from the automation system and statistical analysis was performed.

Participant Selection

Inclusion criteria were as follows: Ferritin <50 ng\l in patients with chronic disease, ferritin <23.9 ng\l in men and <11 ng\l in women in patients with pure iron deficiency, and age>18 years. Exclusion criteria were as follows: Ferritin >50 ng\l in patients with chronic diseases, ferritin>23.9 ng\l in men and >11 ng\l in women in patients with pure iron deficiency, patients treated for B12\folate deficiency, patients with bone marrow-infiltrating malignancy, and age<18 years. According to Turkish Hematology Association iron deficiency criteria, ferritin <15 is accepted as iron deficiency.[13]

Laboratory Analysis

For the purpose of standardization, the reference range of ANEAH laboratory was used for iron deficiency (ferritin <23.9 in men and ferritin <11 in women). According to ANEAH laboratory values, hemoglobin (HBG) <14 in men and HGB <12 in women were accepted as anemia. For the purpose of standardization, the reference range of the Turkish Hematology Association anemia criteria (HGB <13 in men, HGB <12 in women, and <11 in pregnant women) was used. Total protein, albumin, serum iron, serum iron-binding capacity, and CRP were analyzed by spectrophotometric method in the biochemistry laboratory (AU5800 Beckman Coulter). Ferritin was analyzed by immunoassay method (DXI800). Complete blood count parameters (Hgb, MCV, RDW, PCT, PDW, MCH, MCHC, and MPV) were analyzed with (LH780) and sedimentation with (Alifax). NLR and PLR were calculated manually.

Statistical Analysis

Statistical analyses were performed using SPSS version11. The conformity of the variables to normal distribution was examined visually (histogram and probability graphs) and analytically (Kolmogorov–Smirnov/Shapiro–Wilk). Descriptive analyses were presented with means and standard deviations for normally distributed variables. 2×2 cells were compared with Pearson Chi-square and Fisher's Exact Tests. Bonferroni correction and post hoc analyses were performed in eyes with more than 2×2. Laboratory parameters were evaluated by Mann–Whitney U-test for non-normally distributed variables and by independent samples t-test for normally distributed variables. The relationship between the measured variables was examined with Spearman correlation. The relationship between the presence of CDID and age, sex, HGB, MCV, MCH, MCHC, RDW, NLO, PLO, SD, SIBC, ferritin, sedimentation, CRP, and ALB was analyzed by logistic regression (Backward LR). $p<0.05$ was considered significant.

Results

Demographics

Female and male ratio was 46% and 54% in patients CDID, and 54% and 46% in patients with IID (Table 1). No difference was found in gender between CDID and IID ($p>0.05$). Mean age was higher in CDID (56±16, median: 34) compared to IID (37±14, median: 59) ($p<0.001$). Upper endoscopy revealed pathology in 53 patients, in which the most common pathologies were antral gastritis (n=12) and pangastritis (n=12). Three patients had celiac disease, one patient had fecal parasite, five patients had previous GIS surgery, 24 patients had malignancy, 26 patients had hypothyroidism, two

Table 1. Characteristics and outputs of chronic disease with iron deficiency and only iron deficiency

Variable	CDID	IID	p
Age, year	56±16	37±14	0.0001
Male/Female	81/70	68/81	0.149
Hemoglobin, g/dL	12.8±2.4	12.1±2.7	0.018
MCV, fL	82.1±10.9	77.9±10.5	0.0009
MCH, pg	28.7±9	25.9±4	0.0005
MCHC, mg/dl	32.7±2	32.6±1.8	0.594
RDW, %	15.2±3.6	16±3.6	0.057
MPV, fL	8.9±1.3	9.2±1.4	0.073
PCT, ng/mL	0.3±0.9	0.2±0.1	0.411
PDW, %	15.9±2.4	16.4±2	0.064
NLR, ratio	3±1.9	2.2±0.9	0.0001
PLR, ratio	158±115	137±60.6	0.055
SI, µg/dL	59±33	53±42	0.18
TIBC, µg/dL	306±81	352±84	0.0001
TS, %	0.16±0.1	0.13±0.11	0.011
Ferritin, g/mL	21.5±13.3	8.7±6	0.0001
ESR, mm/h	24±21	14±14	0.0001
CRP, mg/L	11.9±26.8	3.13±6.2	0.0002
Albumin, g/dL	41.6±7.2	44.2±4.3	0.0001

Significant at the 0.05 level (two-tailed) with Mean±Standard Division (Mean ± SD); Red Blood Cell Count (RBC); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC); Red Cell Distribution Width (RDW); Mean Corpuscular Volume (MCV); Serum Iron (SI); Total Iron Binding Capacity (TIBC); Transferrin saturation (TS); Erythrocyte Sedimentation Rate (ESR); Platelet / lymphocyte ratio (PLR); Neutrophil/lymphocyte ratio (NLR).

patients had hyperthyroidism, 37 patients had coronary artery disease, 11 patients had nodular goiter, 27 patients had chronic renal failure, 60 patients had diabetes mellitus, and 17 patients had collagen tissue disease.

Chronic Disease Analysis

MCV, MCH, PDW, NLR, serum iron, transferrin saturation, ferritin, sedimentation, CRP, and albumin were higher in CDID compared to IID (p<0.05). RDW and SIBC were lower in CDID (p<0.05). NLR (6.36±7.71) was higher in malignancy (2.47±1.28) compared to those without any malignancy (p<0.001). PLR was higher in malignancy (284.5±343.6) compared to those without any malignancy (139±126) (p=0.004). RDW and ferritin were also higher in malignancy (16.2 vs. 14.4, p=0.009; 16.9 vs. 10.3, p=0.010; respectively). NLR was higher in CVD (median 2.5) compared to those without (median 2.1) (p<0.001). PLR was higher in CVD (mean 252) compared to those without CVD (mean 144) (p<0.001). NLR was higher in DM (median 2.45) compared to without DM (median 2.09) (p=0.018). NLR was higher in CAD (median 4.31) compared to those without CAD (median 2.56) (p<0.001). Ferritin was higher in DM (median 24.0) compared to those without DM (median 9.25) (p<0.001).

Logistic Regression

The significance of the model was evaluated with Hosmer and Lemeshow (p>0.05). Accordingly, the probability of having a chronic disease increases 1.06 times with 1 unit increase in age (95% Confidence Intervals [CI]=1.041–1.085). The presence of CDID was associated with ferritin increase (Odds Radio [OR]=1.123; 95%CI=1.084–1.165). Increased sedimentation was associated with an increased risk of having a CDID (OR=1.023; 95%CI= 1.003–1.045). No correlation was found between age, ferritin, and sedimentation.

ROC Analysis

Area under curve (Auc) according to ROC predicting chronic disease with iron deficiency is shared in Table 2. In the ROC analysis, we did for the predictability of CDID, we found a diagnostic potential of ferritin and NLR, as we see in Figure 1. It showed a predictive potential for CDID with 67.2% specificity and 71.1% sensitivity at a 12.1 cutoff value (Auc:0.781; p<0.0001). NLR was the second strong predictor of CDID against IID, with 64.1% specificity and 63.5% sensitivity at a 2.09 cutoff (Auc:0.629; p<0.0001). PLR had no significance for discrimination of CDID and IID.

Discussion

The importance of early detection of IID gives us a chance to intervene before it leads to anemia. In this regard, it is very important to distinguish CDID from IID. Although ferritin is partially effective in eliminating this deficiency, it is clear that new supportive markers such as NLR and PLR will be of great benefit. In this sense, our study has reached results that will benefit physicians in this distinction.

Table 2. Area under curve according to ROC predicting chronic disease with iron deficiency

Variables	AUC	SE	p	95% Confidence Interval	
				Lower	Upper
NLR	0.629	0.033	0.0001	0.565	0.693
PLR	0.526	0.034	0.438	0.460	0.593
SI	0.579	0.034	0.009	0.513	0.645
TIBC	0.351	0.032	0.001	0.288	0.414
TS	0.611	0.033	0.001	0.545	0.675
Ferritin	0.781	0.027	0.0001	0.729	0.834

Variables: NLR: Neutrophil to Lymphocyte Ratio, PLR: Platelet to Lymphocyte Ratio, SI: Serum Iron, TIBC: Total Iron-Binding Capacity, TS: Transferrin Saturation has at least one tie between the positive actual state group and the negative actual state group. Abbreviations. Area under curve (AUC), Standart Error (SE).

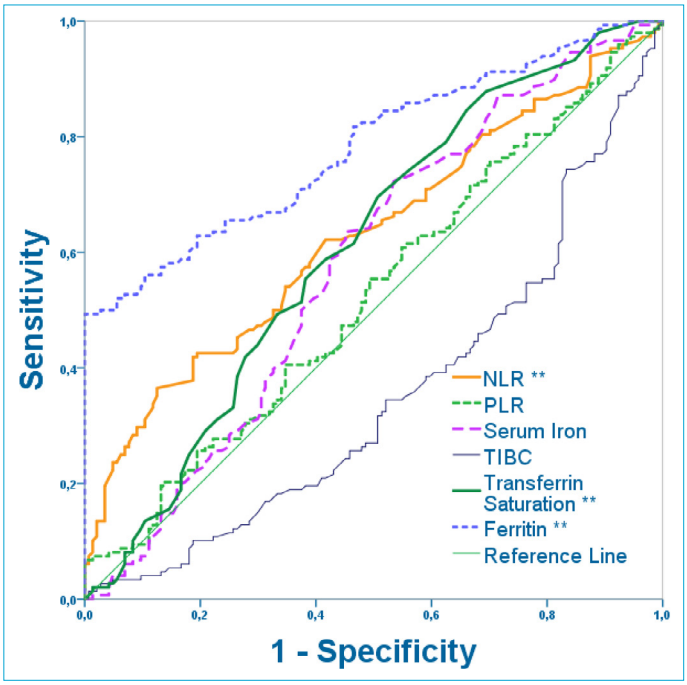

Figure 1. The ROC analysis for predicting chronic disease accompanying iron deficiency.

The iron deficiency anemia assessment, prevention, and control states IID develops as a result of long-term negative iron balance.[12] Iron deficiency mostly results in anemia, and IID has been shown to be the cause of most cases of anemia. In the same report, the WHO draws attention to the economic losses caused by anemia due to IID.[13] Considering the problems with adequate and balanced nutrition in today's social life, it is necessary to carry out screening and intervention studies on the most common nutritional problem in the world.[14] Early detection of IID is critical to distinguish it from CDID before starting treatment.[15]

Complete blood count is a routinely applied, simple, easily accessible, and economical test, and its importance is just beginning to be understood.[16] Recent studies have shown a correlation between various clinical conditions, especially with new indices such as calculated PLR and NLR. New studies[17] drew attention to the inflammation that plays a role in the formation and progression of microvascular and macrovascular complications of type 2 diabetes and the increase in NLR ratio during this inflammation process. In their study, the authors stated that changes in leukocyte values can be used as cheap, fast, and sensitive markers in evaluating the inflammation process, and concluded that leukocyte subtypes can provide information about different aspects of the inflammation process. Osadnik et al.[18] based their study on the relationship between high PLR ratio and mortality due to cardiovascular disease. They reported that high platelet, high neutrophil values, and relative low lymphocyte levels are negative indicators in patients with CAD. In a follow-up study, the authors showed that there was a relationship between an increase in PLR ratio and deaths due to cardiovascular causes. They showed that PLR ratio was a potential diagnostic marker in stable coronary disease, with HBG value. Türkmen et al.[19] reported that PLR ratio and NLR ratio correlated with inflammatory markers in cardiac and non-cardiac patients and examined the relationship of PLR and NLR ratio with inflammation in the end-stage renal disease. The authors showed that PLR ratio was significantly correlated with NLR ratio and inflammation markers interleukin-6 and TNF-α. In the present study, no significant difference was found in PLR and NLR. Sari et al.[20] compared PLR ratio and NLR ratio with the severity of coronary artery disease, which is the biggest cause of mortality and morbidity in the community and during the development, of which inflammation plays an important role.

We analyzed hematological parameters with PLR and NLR and compared CDID with IID. NLR and PLR were higher in malignancy compared to those without any malignancy. NLR and PLR were higher in CVD compared to those without CVD. NLR and ferritin were higher in DM compared to those without DM. NLR was higher in CAD compared to those without CAD. Accordingly, the probability of having a chronic disease increases 1.06 times with 1 unit increase in age. The presence of CDID was associated with ferritin increase. Increased sedimentation was associated with an increased risk of having a CDID. The correlation analysis showed a very weak but significant positive correlation among ferritin, NLR, and PLR. There was a weak but significant positive correlation between age and MCV, NLR, ferritin, sedimentation, and CRP. A weak but significant negative correlation was found between age and MCHC, MPV, SDBC, and albumin. In the ROC, we did for the predictability of CDID, we found a diagnostic potential of ferritin. NLR was the second strong predictor of CDID against IID. PLR had no significance for discrimination of CDID and IID.

The strongest aspect was that the present study has been the first novel analysis comparing CDID to IID in terms of NLR and PLR, while the weakest aspect was that we analyzed chronic diseases together as a single holistic group. Since each disease has different effects on NLR and PLR, the bias on the results is the first limitation of the study. The second limitation is that the age factor could not be excluded and we did not have long-term follow-up data of the patients.

Conclusion

The importance of the early detection of IID gives us a chance to intervene before it leads to anemia. In this regard, it is very important to distinguish CDID from IID. NLR behaviors as a parameter that will provide support like ferritin in the predicting CDID from IID and can benefit physicians in clinical use of differentiation. We cannot say that the same benefit applies to PLR that NLR was much stronger predictor than PLR and was diagnostically the closest parameter to Ferritin. To obtain stronger results, there is a need for further studies with a large number of participants in terms of iron deficiency in separated chronic disease groups.

Disclosures

Ethics Committee Approval: This thesis study was approved by the Institution's Ethics Committee with ID: 2016-1169.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Data Access Statement: The data supporting this study's findings are available from the corresponding author on reasonable request.

Authorship Contributions: Concept – M.S.D.; Design – M.S.D.; Supervision – M.S.D.; Materials – M.S.D.; Data collection &/or processing – M.S.D., S.K.; Analysis and/or interpretation – M.S.D., S.K.; Literature search – S.K; Writing – M.S.D., S.K.; Critical review – M.S.D., S.K.

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