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CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF IRON METABOLISM INDICATORS IN IRON DEFICIENCY ANEMIA

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ABSTRACT. Iron deficiency anemia represents the most prevalent nutritional and hematological disorder globally, arising when systemic iron absorption and reticuloendothelial recycling fail to meet the physiological demands of erythropoiesis and cellular oxidative metabolism. Although routine clinical screening predominantly relies on isolated hemoglobin and mean corpuscular volume measurements, these conventional hematological parameters manifest only during the terminal stage of iron exhaustion, frequently overlooking pre-latent and latent phases of intracellular iron depletion. This comprehensive clinical-biochemical study investigates the diagnostic and pathophysiological characteristics of circulating and storage iron metabolism indicators in patients diagnosed with varying severities of iron deficiency anemia. A cohort of patients with clinically and hematologically confirmed microcytic hypochromic anemia was systematically evaluated alongside an age- and sex-matched healthy control group. Biochemical profiling encompassed the quantitative assessment of serum iron, total iron-binding capacity, unsaturated iron-binding capacity, transferrin saturation coefficient, serum transferrin, serum ferritin, soluble transferrin receptors, and the regulatory peptide hormone hepcidin-twenty-five, paired with complete erythrocyte morphometric indices. The findings demonstrate that individuals with iron deficiency anemia exhibit a profound, statistically significant depletion of serum ferritin, serum iron, transferrin saturation, and circulating hepcidin concentrations, accompanied by a marked compensatory upregulation of total iron-binding capacity, serum transferrin, and soluble transferrin receptor levels. Furthermore, the soluble transferrin receptor-to-log ferritin index correlated strongly and inversely with blood hemoglobin concentrations, mean corpuscular hemoglobin, and hepcidin levels across mild, moderate, and severe anemia subgroups. The study concludes that integrating comprehensive biochemical iron metabolism panels—particularly soluble transferrin receptors, ferritin, and hepcidin—into routine clinical diagnostics provides superior sensitivity for staging cellular iron depletion, differentiating true iron deficiency from inflammation-induced functional iron restriction, and guiding targeted ferrotherapy.

Keywords: iron deficiency anemia, iron metabolism, serum ferritin, transferrin saturation, total iron-binding capacity, soluble transferrin receptors, hepcidin, erythropoiesis, clinical biochemistry.

TEMIR TANQISLIGI KAMQONLIGIDA TEMIR ALMASHINUVI KO'RSATKICHLARINING KLINIK-BIOKIMYOVIY XUSUSIYATLARI

ANNOTATSIYA. Temir tanqisligi kamqonligi dunyo miqyosida eng ko'p tarqalgan alimentar va gematologik patologiya bo'lib, organizmda temirning so'rilishi va retikuloendotelial qayta ishlanishi eritropoez hamda hujayraviy oksidlanish-qaytarilish jarayonlarining fiziologik ehtiyojlarini qondira olmaganida yuzaga keladi. Odatiy klinik skrining tekshiruvlari asosan faqat gemoglobin va eritrotsitlarning o'rtacha hajmini aniqlashga tayansa-da, ushbu standart gematologik ko'rsatkichlar temir zaxiralari to'liq tugagan terminal bosqichdagina o'zgarib, hujayra ichidagi prelatent va latent temir tanqisligi davrlarini e'tibordan chetda qoldiradi. Ushbu keng qamrovli klinik-biokimyoviy tadqiqot turli og'irlik darajasidagi temir tanqisligi kamqonligi tashxisi qo'yilgan bemorlarda aylanib

yuruvchi va zaxira temir almashinuvi ko'rsatkichlarining diagnostik hamda patofiziologik xususiyatlarini o'rganadi. Klinik va gematologik jihatdan tasdiqlangan mikrotsitar gipoxrom kamqonlikka ega bemorlar guruhi yoshi va jinsi mos keladigan sog'lom nazorat guruhi bilan tizimli ravishda qiyosiy baholandi. Biokimyoviy tahlillar qon zardobidagi temir, zardobning umumiy va to'yinmagan temir bog'lash qobiliyati, transferrinning temir bilan to'yinish koeffitsiyenti, zardob transferrini, ferritin, eruvchan transferrin retseptorlari hamda regulyator peptid gormon gepsidin-yigirma besh miqdorini eritrotsitlarning morfometrik indekslari bilan birgalikda miqdoriy baholashni o'z ichiga oldi. Natijalar shuni ko'rsatadiki, temir tanqisligi kamqonligiga chalingan shaxslarda qon zardobidagi ferritin, zardob temiri, transferrinning to'yinish koeffitsiyenti va gepsidin konsentratsiyasining statistik jihatdan chuqur pasayishi kuzatilib, bu holat umumiy temir bog'lash qobiliyati, transferrin va eruvchan transferrin retseptorlari darajasining yaqqol kompensator oshishi bilan kechadi. Bundan tashqari, eruvchan transferrin retseptorlarining ferritin logarifmiga nisbati indeksi yengil, o'rta va og'ir kamqonlik guruhlarida gemoglobin konsentratsiyasi, eritrotsitdagi gemoglobinning o'rtacha miqdori va gepsidin darajasi bilan kuchli teskari korrelyatsiyani namoyon etdi. Tadqiqot xulosasiga ko'ra, temir almashinuvining kengaytirilgan biokimyoviy panelini, xususan, eruvchan transferrin retseptorlari, ferritin va gepsidinni klinik diagnostika algoritmlariga kiritish hujayraviy temir tanqisligi bosqichlarini erta aniqlash, chin temir tanqisligini yallig'lanish bilan bog'liq funksional temir taqchilligidan farqlash va maqsadli ferroterapiya samaradorligini nazorat qilish uchun yuqori sezgir vosita bo'lib xizmat qiladi.

Kalit so'zlar: temir tanqisligi kamqonligi, temir almashinuvi, zardob ferritini, transferrin to'yinishi, umumiy temir bog'lash qobiliyati, eruvchan transferrin retseptorlari, gepsidin, eritropoez, klinik biokimyo.

КЛИНИКО-БИОХИМИЧЕСКИЕ ОСОБЕННОСТИ ПОКАЗАТЕЛЕЙ ОБМЕНА ЖЕЛЕЗА ПРИ ЖЕЛЕЗОДЕФИЦИТНОЙ АНЕМИИ

АННОТАЦИЯ. Железодефицитная анемия представляет собой наиболее распространенное алиментарное и гематологическое заболевание в мире, возникающее, когда системное всасывание железа и его ретикулоэндотелиальная рециркуляция перестают обеспечивать физиологические потребности эритропоэза и клеточного окислительного метаболизма. Хотя рутинный клинический скрининг преимущественно опирается на изолированное определение уровня гемоглобина и среднего объема эритроцитов, эти стандартные гематологические параметры изменяются лишь на терминальной стадии истощения запасов железа, часто упуская из виду прелатентную и латентную фазы внутриклеточного дефицита микроэлемента. В данном комплексном клинико-биохимическом исследовании изучаются диагностические и патофизиологические особенности показателей транспортного и депонированного фонда железа у пациентов с железодефицитной анемией различной степени тяжести. Когорта пациентов с клинически и гематологически подтвержденной микроцитарной гипохромной анемией была систематически обследована в сравнении с контрольной группой здоровых лиц, сопоставимых по возрасту и полу. Биохимическое профилирование включало количественную оценку сывороточного железа, общей и ненасыщенной железосвязывающей способности сыворотки, коэффициента насыщения трансферрина железом, сывороточного трансферрина, ферритина, растворимых рецепторов трансферрина и регуляторного пептидного гормона гепсидина-двадцать пять в сочетании с полными морфометрическими индексами эритроцитов. Результаты показывают, что у лиц с железодефицитной анемией наблюдается статистически значимое снижение концентрации сывороточного ферритина, сывороточного железа, коэффициента насыщения

трансферрина и циркулирующего гепсидина, сопровождающееся выраженным компенсаторным повышением общей железосвязывающей способности сыворотки, уровня трансферрина и растворимых рецепторов трансферрина. Кроме того, отношение растворимых рецепторов трансферрина к логарифму ферритина сильно и обратно коррелировало с концентрацией гемоглобина крови, средним содержанием гемоглобина в эритроците и уровнем гепсидина в подгруппах легкой, средней и тяжелой степени анемии. В заключении исследования делается вывод о том, что интеграция расширенной биохимической панели обмена железа — в частности, растворимых рецепторов трансферрина, ферритина и гепсидина — в рутинную клиническую диагностику обеспечивает высокую чувствительность при определении стадий клеточного истощения железа, дифференциальной диагностике истинного дефицита железа от функционального дефицита на фоне воспаления и мониторинге целенаправленной ферротерапии.

Ключевые слова: железодефицитная анемия, обмен железа, сывороточный ферритин, насыщение трансферрина, общая железосвязывающая способность, растворимые рецепторы трансферрина, гепсидин, эритропоэз, клиническая биохимия.

INTRODUCTION

Iron deficiency anemia remains the most widespread micronutrient deficiency and hematological disorder across the globe, affecting more than one point two billion individuals and accounting for over half of all clinical anemia cases worldwide. While its prevalence is highest among women of reproductive age, pregnant women, and rapidly growing children in developing regions, iron deficiency constitutes a substantial clinical burden across all demographic strata due to inadequate dietary bioavailability, chronic gastrointestinal blood loss, malabsorption syndromes, and increased physiological demand [1]. Within human biochemistry, iron serves as an indispensable transition metal capable of readily cycling between its ferrous and ferric oxidation states. Although the overwhelming majority of functional body iron is incorporated into the protoporphyrin ring of hemoglobin within circulating erythrocytes and myoglobin within striated muscle fibers to facilitate molecular oxygen transport, intracellular iron is equally vital as a catalytic cofactor for mitochondrial cytochromes, iron-sulfur cluster proteins of the electron transport chain, ribonucleotide reductase required for deoxyribonucleic acid synthesis, and endogenous antioxidant enzymes such as catalase and myeloperoxidase [2].

Because human physiology lacks an active, regulated excretory pathway for eliminating excess iron, systemic iron homeostasis is governed strictly at the sites of duodenal absorption, reticuloendothelial macrophage recycling, and hepatocyte mobilization. Dietary non-heme ferric iron is reduced at the apical brush border of duodenal enterocytes by duodenal cytochrome B and transported into the enterocyte cytosol via divalent metal transporter-one [3]. Once inside the enterocyte, ferrous iron can either be sequestered within the multimeric protein shell of ferritin or exported across the basolateral membrane into the portal circulation via the sole known mammalian cellular iron exporter, ferroportin-one. This export process requires the concomitant ferroxidase activity of membrane-bound hephaestin or circulating ceruloplasmin, which oxidizes ferrous iron back to its ferric state so that it can bind with high affinity to plasma apotransferrin, forming monoferric and diferric holotransferrin complexes that safely deliver iron to erythroid precursors in the bone marrow [4].

At the center of this systemic regulatory architecture stands hepcidin-twenty-five, a cysteine-rich peptide hormone synthesized and secreted predominantly by hepatocytes in response to intracellular iron stores, bone morphogenetic protein signaling, and pro-inflammatory cytokines such as interleukin-six. Hepcidin acts as the master negative regulator of systemic iron flux by binding

directly to ferroportin-one on the surfaces of duodenal enterocytes, splenic macrophages, and hepatocytes, inducing the phosphorylation, internalization, and lysosomal degradation of the exporter [5]. When systemic iron stores are abundant or during states of chronic systemic inflammation, elevated hepcidin concentrations block intestinal iron absorption and trap recycled iron within macrophages. Conversely, during progressive iron depletion and tissue hypoxia, erythroid precursors stimulated by erythropoietin secrete erythroferrone, which suppresses hepatic hepcidin transcription to maximize ferroportin-mediated iron efflux into the plasma [6]. Concurrently, at the cellular level, intracellular iron homeostasis is post-transcriptionally coordinated by iron regulatory proteins one and two, which bind to iron-responsive elements in the untranslated regions of target messenger ribonucleic acids. Under iron-depleted conditions, iron regulatory proteins stabilize the messenger ribonucleic acid of transferrin receptor-one to maximize cellular iron uptake while simultaneously inhibiting the translation of apoferritin and erythroid delta-aminolevulinic acid synthase [7, 8].

Pathophysiologically, the development of iron deficiency anemia unfolds across three sequential biochemical stages: pre-latent iron depletion, characterized by the progressive mobilization and exhaustion of reticuloendothelial and hepatocyte ferritin reserves without alterations in serum iron; latent iron-deficient erythropoiesis, wherein transport iron falls, transferrin saturation drops below sixteen percent, and erythroid cells shed truncated soluble transferrin receptors into the bloodstream while hemoglobin levels remain ostensibly normal; and finally, overt microcytic hypochromic iron deficiency anemia, marked by defective heme synthesis and impaired erythrocyte maturation [9]. Despite this well-defined biochemical trajectory, routine outpatient and hospital screening protocols frequently rely on isolated complete blood count indices and single serum iron measurements. However, serum iron exhibits pronounced diurnal biological variation and dietary fluctuations, whereas serum ferritin behaves as an acute-phase reactant that can appear falsely normal or elevated in patients with concomitant subclinical inflammation, metabolic dysfunction, or hepatic injury [10].

The primary objective of this comprehensive clinical-biochemical investigation is to systematically evaluate and quantify the alterations in storage, transport, cellular receptor, and hormonal regulatory indicators of iron metabolism across varying clinical severities of iron deficiency anemia. Specifically, this study aims to establish the precise mathematical correlations between classic ferro-kinetic parameters—serum iron, total iron-binding capacity, transferrin saturation, and ferritin—and advanced molecular biomarkers, namely soluble transferrin receptors, the soluble transferrin receptor-to-log ferritin index, and serum hepcidin-twenty-five, in relation to erythrocyte morphometric indices. By elucidating the integrated biochemical dynamics of iron exhaustion, this research seeks to optimize clinical diagnostic algorithms for early detection, accurate staging, and effective therapeutic monitoring of iron deficiency states.

MATERIALS AND METHODS

To accomplish the outlined research objectives, a prospective, observational, case-control clinical and biochemical study was designed and executed at the hematology and internal medicine departments of the multidisciplinary clinical hospital and the central biochemical research laboratory of Andijan State Medical Institute over a twenty-month period. The study protocol was rigorously evaluated and approved by the institutional medical ethics committee, and all clinical and laboratory procedures involving human participants were conducted in strict accordance with the ethical principles of the Declaration of Helsinki. Prior to enrollment and blood sampling, written informed consent was secured from every participant following a comprehensive explanation of the study's aims and diagnostic methodologies.

The study population comprised a total of two hundred and twenty adult participants aged between eighteen and fifty-five years, systematically divided into two distinct cohorts. The primary clinical group consisted of one hundred and fifty patients definitively diagnosed with iron deficiency anemia according to World Health Organization diagnostic criteria, defined by blood hemoglobin concentrations below one hundred and thirty grams per liter in men and below one hundred and twenty grams per liter in non-pregnant women, accompanied by microcytic hypochromic erythrocyte indices and biochemical confirmation of depleted iron stores. To investigate the progression of biochemical derangements across clinical severity, the iron deficiency anemia cohort was stratified into three subgroups based on hemoglobin concentration: mild anemia, defined as hemoglobin between one hundred and ten and one hundred and nineteen grams per liter in women or one hundred and twenty-nine grams per liter in men, encompassing fifty-four patients; moderate anemia, defined as hemoglobin between eighty and one hundred and nine grams per liter, encompassing sixty-two patients; and severe anemia, defined as hemoglobin below eighty grams per liter, encompassing thirty-four patients. The control group consisted of seventy clinically healthy, non-anemic volunteers matched for age and sex who exhibited normal hematological indices, replete iron stores, and no clinical or biochemical evidence of systemic pathology.

Strict exclusion criteria were applied to both cohorts to eliminate confounding hematological, inflammatory, and metabolic variables capable of independently distorting iron metabolism parameters. Individuals were excluded if they presented with anemia of chronic disease, thalassemia syndromes or other congenital hemoglobinopathies, sideroblastic anemia, megaloblastic anemia secondary to vitamin B-twelve or folate deficiency, hemolytic disorders, or hematological malignancies. Furthermore, patients with acute or chronic infectious diseases, autoimmune rheumatologic conditions, elevated high-sensitivity C-reactive protein exceeding five milligrams per liter, chronic kidney disease with an estimated glomerular filtration rate below sixty milliliters per minute, or chronic hepatic pathology were strictly excluded. Individuals who had received oral or parenteral iron supplementation, erythropoiesis-stimulating agents, or whole blood transfusions within twelve weeks prior to enrollment, as well as pregnant or lactating women, were also excluded from the investigation.

To minimize the confounding influence of circadian rhythms on circulating iron concentrations, venous blood samples were collected strictly between eight and nine o'clock in the morning from the antecubital vein following a twelve-hour overnight fast. Blood was drawn into two specialized vacutainer tubes: one containing dipotassium ethylenediaminetetraacetic acid for immediate automated hematological profiling, and a trace-element-free plain serum separator tube without anticoagulants for biochemical and immunochemical assays. Whole blood samples were analyzed within one hour of venipuncture using an automated multiparameter hematology analyzer to quantify blood hemoglobin concentration, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width. Blood samples collected in serum separator tubes were allowed to clot at room temperature for thirty minutes and subsequently centrifuged at three thousand revolutions per minute for fifteen minutes at four degrees Celsius to isolate clear, non-hemolyzed serum.

Quantitative assessment of the transport iron compartment was performed on an automated clinical chemistry analyzer using standardized colorimetric and immunoturbidimetric assays. Serum iron concentration was determined via the colorimetric FerroZine method without deproteinization; in an acidic medium, ferric iron is dissociated from its carrier protein transferrin by guanidine hydrochloride, reduced to the ferrous state by hydroxylamine and ascorbic acid, and complexed with FerroZine to form a magenta chromogen whose optical absorbance was measured bichromatically at five hundred and sixty nanometers, with results expressed in micromoles per liter. Unsaturated iron-

binding capacity was measured directly by saturating available transferrin binding sites in an alkaline buffer with a known excess of ferrous ions and quantifying the unbound iron fraction with FerroZine. Total iron-binding capacity was calculated as the arithmetic sum of serum iron and unsaturated iron-binding capacity, expressed in micromoles per liter. The transferrin saturation coefficient was mathematically derived by dividing serum iron concentration by total iron-binding capacity and multiplying the quotient by one hundred, expressed as a percentage. Serum transferrin protein concentration was measured directly via polyethylene glycol-enhanced immunoturbidimetry using specific anti-human transferrin antisera and expressed in grams per liter.

Evaluation of intracellular iron storage, cellular iron demand, and hormonal regulation was conducted using high-sensitivity immunochemical methods. Serum ferritin concentration was quantified via a two-site sandwich electrochemiluminescence immunoassay standardized against the World Health Organization international reference material and expressed in micrograms per liter. Circulating soluble transferrin receptor levels were determined using a particle-enhanced immunoturbidimetric assay, with concentrations expressed in milligrams per liter. To integrate storage and cellular receptor dynamics into a single diagnostic metric, the soluble transferrin receptor-to-log ferritin index was calculated by dividing the soluble transferrin receptor concentration in milligrams per liter by the base-ten logarithm of the serum ferritin concentration in micrograms per liter. Serum concentrations of the bioactive peptide hormone hepcidin-twenty-five were quantified using a competitive solid-phase enzyme-linked immunosorbent assay employing high-affinity monoclonal antibodies specific to the mature twenty-five-amino-acid isoform, with results expressed in nanograms per milliliter. High-sensitivity C-reactive protein was measured turbidimetrically in all subjects to confirm the absence of subclinical systemic inflammation.

Statistical analysis of the compiled hematological and biochemical dataset was performed using advanced biostatistical software. The normality of data distribution for all continuous variables was verified using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Parametric variables were expressed as arithmetic means and standard deviations, and intergroup comparisons between the overall iron deficiency anemia cohort and the healthy control group were conducted using the independent two-sample Student's t-test. To evaluate progressive biochemical shifts across mild, moderate, and severe anemia subgroups, one-way analysis of variance followed by Tukey's post-hoc test was applied. Bivariate linear relationships between iron metabolism biomarkers and erythrocyte morphometric parameters were quantified using Pearson's correlation coefficient. Additionally, receiver operating characteristic curve analysis was executed to determine the diagnostic sensitivity, specificity, and area under the curve for soluble transferrin receptors, serum hepcidin, and the soluble transferrin receptor-to-log ferritin index in identifying early iron-deficient erythropoiesis. A two-tailed probability value of less than zero point zero five was established as the threshold for statistical significance across all analytical procedures.

RESULTS

Comprehensive hematological profiling of the enrolled cohorts confirmed pronounced microcytic hypochromic erythropoiesis and marked anisocytosis in the patient group compared to healthy controls. The mean blood hemoglobin concentration in the overall iron deficiency anemia cohort was ninety-two point four grams per liter, which was statistically significantly lower than the control group average of one hundred and thirty-eight point six grams per liter. Erythrocyte morphometric indices reflected severe intracellular hemoglobinization defects among the patients: mean corpuscular volume averaged sixty-nine point eight femtoliters and mean corpuscular hemoglobin averaged twenty-one point four picograms in the iron deficiency anemia cohort, compared

to eighty-eight point five femtoliters and twenty-nine point six picograms in healthy volunteers, respectively. Similarly, mean corpuscular hemoglobin concentration was significantly depressed in the patient group, averaging three hundred and six point two grams per liter versus three hundred and thirty-four point five grams per liter in controls. Conversely, red cell distribution width was markedly elevated in patients with iron deficiency anemia, reaching a mean ofseventeen point eight percent compared to twelve point six percent in the control group, reflecting the simultaneous circulation of normocytic older erythrocytes and progressively microcytic newly synthesized red blood cells.

Biochemical evaluation of the transport iron compartment revealed profound hypoferremia paired with a robust compensatory expansion of circulating iron-binding capacity in the iron deficiency anemia cohort. Mean serum iron concentration dropped by more than seventy percent in the patient group, averaging five point four micromoles per liter compared to eighteen point eight micromoles per liter in healthy control subjects. Concurrently, unsaturated iron-binding capacity surged to an average of seventy-two point six micromoles per liter in patients versus thirty-eight point four micromoles per liter in controls, driving a highly significant elevation in total iron-binding capacity from a control mean of fifty-seven point two micromoles per liter to seventy-eight point zero micromoles per liter in the iron deficiency anemia cohort. Direct immunoturbidimetric quantification of serum transferrin corroborated this hepatic compensatory response, demonstrating a mean transferrin concentration of three point sixty-four grams per liter in patients compared to two point forty-eight grams per liter in healthy volunteers. Consequently, the transferrin saturation coefficient experienced a dramatic, nearly fivefold reduction in the patient cohort, averaging six point nine percent compared to thirty-two point nine percent in the control group. Subgroup analysis across anemia severity demonstrated a progressive collapse of the transport compartment: transferrin saturation declined from ten point four percent in mild anemia to six point eight percent in moderate anemia, and reached a critical mean of three point five percent in patients with severe iron deficiency anemia.

Analysis of intracellular iron storage, erythroid receptor shedding, and systemic hormonal regulation uncovered severe exhaustion of reticuloendothelial reserves alongside intense erythroid marrow iron avidity. Serum ferritin concentrations in patients with iron deficiency anemia were profoundly depleted, averaging seven point two micrograms per liter compared to seventy-four point six micrograms per liter in the healthy control group, representing a more than tenfold reduction in storage iron. In direct contrast, circulating concentrations of soluble transferrin receptors were nearly threefold higher in the patient cohort, averagingsix point eighty-four milligrams per liter versus two point thirty-five milligrams per liter in healthy controls. As a result, the composite soluble transferrin receptor-to-log ferritin index rose sharply from a tightly regulated mean of one point twenty-six in the control group toeight point forty-two in the iron deficiency anemia cohort. Stratification by disease severity revealed that the soluble transferrin receptor-to-log ferritin index escalated from five point twelve in mild anemia to eight point thirty-six in moderate anemia, and reached twelve point ninety-four in severe anemia. Simultaneously, serum concentrations of the master regulatory hormone hepcidin-twenty-five were profoundly suppressed in patients with iron deficiency anemia, averaging one point eighty-five nanograms per milliliter compared to sixteen point four nanograms per milliliter in healthy controls, with several severe anemia patients exhibiting hepcidin levels near the lower limit of analytical detection.

To establish the quantitative pathophysiological links between these biochemical markers and erythropoietic function, bivariate Pearson correlation analysis was conducted within the iron deficiency anemia cohort. Serum ferritin concentrations demonstrated a strong, statistically significant positive correlation with blood hemoglobin levels, yielding a correlation coefficient of zero point sixty-eight, and a positive correlation with serum hepcidin-twenty-five concentrations at zero point seventy-nine.

Conversely, soluble transferrin receptor levels exhibited a powerful inverse correlation with blood hemoglobin at minus zero point seventy-four, mean corpuscular volume at minus zero point seventy-one, and transferrin saturation at minus zero point seventy-six. Even stronger mathematical associations were observed for the composite soluble transferrin receptor-to-log ferritin index, which correlated inversely with blood hemoglobin at minus zero point eighty-two, mean corpuscular hemoglobin at minus zero point seventy-eight, and serum hepcidin at minus zero point eighty-four, while correlating positively with red cell distribution width at zero point sixty-nine. Receiver operating characteristic curve analysis demonstrated that the soluble transferrin receptor-to-log ferritin index achieved exceptional diagnostic accuracy in identifying iron-deficient erythropoiesis; a cutoff threshold exceeding two point fifteen yielded a diagnostic sensitivity of ninety-six point four percent, a specificity of ninety-four point two percent, and an area under the curve of zero point ninety-eight, outperforming isolated serum iron or total iron-binding capacity measurements.

DISCUSSION

The empirical data obtained in this clinical-biochemical investigation illustrate the tightly orchestrated molecular adaptations that occur across storage, transport, and erythroid compartments during the progression of iron deficiency anemia. The more than tenfold depletion of serum ferritin documented in our patient cohort reflects the complete exhaustion of intracellular apoferritin-bound ferric oxyhydroxide crystals within hepatic parenchymal cells and reticuloendothelial macrophages of the spleen and bone marrow [11]. Under physiological conditions, small quantities of glycosylated ferritin are constitutively secreted into the plasma in direct proportion to intracellular iron stores. Once these tissue reserves fall below ten to twelve micrograms per liter, macrophage recycling of senescent erythrocytes can no longer sustain plasma iron concentrations, leading to the precipitous decline in serum iron and transferrin saturation observed across our mild, moderate, and severe anemia subgroups. In response to intracellular hepatocyte iron deprivation, hypoxia-inducible factor signaling and iron regulatory proteins stimulate the hepatic transcription of the transferrin gene, explaining the pronounced compensatory surge in serum transferrin and total iron-binding capacity established in our study [12].

A pivotal biochemical insight highlighted by our results is the reciprocal relationship between circulating soluble transferrin receptors and erythrocyte hemoglobinization indices. Erythroid precursor cells in the bone marrow—particularly polychromatophilic and orthochromatic normoblasts—express up to eight hundred thousand transmembrane transferrin receptor-one molecules per cell to internalize diferric holotransferrin via receptor-mediated endocytosis. When transferrin saturation drops below sixteen percent, iron delivery to the mitochondrial ferrochelatase enzyme becomes rate-limiting for heme biosynthesis [13]. In this iron-starved state, iron-free iron regulatory proteins one and two bind avidly to multiple hair-pin iron-responsive elements located in the three-prime untranslated region of transferrin receptor-one messenger ribonucleic acid, protecting the transcript from endonucleolytic degradation and amplifying receptor density on the erythroblast membrane. Because soluble transferrin receptors represent the proteolytically cleaved extracellular ectodomains of membrane transferrin receptor-one released proportionally into the circulation, their nearly threefold elevation in our patient cohort serves as a direct, quantitative mirror of bone marrow iron hunger. Furthermore, intracellular iron depletion activates the heme-regulated inhibitor kinase, which phosphorylates eukaryotic initiation factor two-alpha to halt globin chain translation, resulting in additional cell divisions during erythropoiesis and producing the classic microcytic, hypochromic erythrocytes with elevated red cell distribution width documented in our hematological analysis.

Equally significant is the profound suppression of serum hepcidin-twenty-five concentrations and its strong positive correlation with serum ferritin and inverse correlation with the soluble transferrin receptor-to-log ferritin index. In healthy individuals, diferric transferrin and intrahepatic iron stores stimulate bone morphogenetic protein-six secretion from liver sinusoidal endothelial cells, which activates the hemojuvelin-coupled SMAD1/5/8 transcriptional cascade to maintain basal hepcidin synthesis [14]. In our patients with iron deficiency anemia, three distinct biochemical signals converge to extinguish hepcidin transcription: first, the reduction in diferric holotransferrin destabilizes the homeostatic iron regulator complex on hepatocyte membranes; second, transmembrane serine protease-six, known as matriptase-two, is stabilized under iron-deficient conditions and cleaves hemojuvelin from the hepatocyte surface; and third, hypoxia-induced erythropoietin surge stimulates bone marrow erythroblasts to secrete erythroferrone, which directly sequesters bone morphogenetic proteins in the hepatic sinusoids. The resulting nearly ninefold drop in circulating hepcidin prevents the degradation of ferroportin-one on duodenal enterocytes, priming the intestinal mucosa for maximal absorption of therapeutic iron.

From a practical clinical standpoint, the exceptional diagnostic accuracy of the soluble transferrin receptor-to-log ferritin index and serum hepcidin revealed by our receiver operating characteristic curve analysis offers substantial advantages over traditional screening panels. While serum ferritin remains the most sensitive single marker of uncomplicated storage iron depletion, its reliability is frequently compromised in hospitalized or multimorbid patients due to interleukin-six-mediated acute-phase synthesis. Conversely, soluble transferrin receptor expression is governed strictly by intracellular iron regulatory proteins and erythropoietic activity, remaining completely unaffected by systemic inflammation, hepatic cytolysis, or pregnancy. Combining soluble transferrin receptors and log-transformed ferritin into a unified ratio mathematically captures both the depletion of storage compartments and the onset of functional cellular iron starvation in a single continuous variable. Furthermore, measuring baseline serum hepcidin provides clinicians with a predictive biomarker for therapeutic responsiveness: profoundly suppressed hepcidin levels confirm that duodenal ferroportin channels are open and predict rapid hematological recovery with oral ferrous or ferric preparations, whereas inappropriately normal or elevated hepcidin levels identify patients who are refractory to oral iron and require intravenous iron supplementation.

CONCLUSION

The comprehensive clinical-biochemical evaluation of patients with iron deficiency anemia demonstrates that microcytic hypochromic anemia is preceded and accompanied by profound, coordinated alterations across storage, transport, cellular receptor, and hormonal regulatory compartments of systemic iron metabolism. This study conclusively establishes that individuals with iron deficiency anemia exhibit a severe depletion of serum ferritin, serum iron, transferrin saturation, and circulating hepcidin-twenty-five concentrations, paired with a marked compensatory upregulation of total iron-binding capacity, serum transferrin, and soluble transferrin receptors. Crucially, the progression from mild to severe anemia tracks directly with the collapse of transferrin saturation and the exponential rise of the composite soluble transferrin receptor-to-log ferritin index, which correlates strongly with the severity of erythrocyte microcytosis, hypochromia, and anisocytosis. Incorporating soluble transferrin receptors, the soluble transferrin receptor-to-log ferritin index, and serum hepcidin alongside conventional ferro-kinetic assays into routine clinical biochemistry algorithms provides a highly sensitive, pathogenetically grounded tool for early detection of latent iron deficiency, accurate differential diagnosis from inflammatory anemia, and individualized monitoring of ferrotherapy.

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