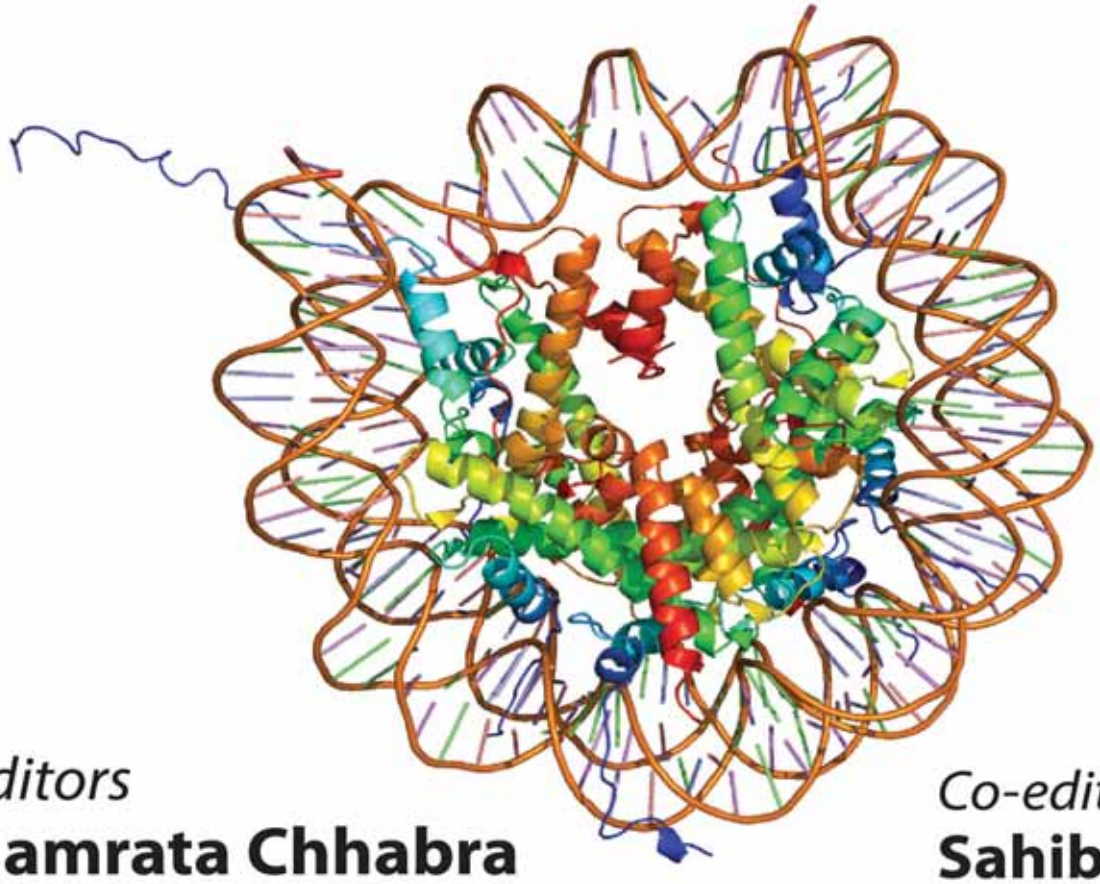
ers.com, for detailed information on biochemistry books, visit our website www.jaypeebrothers.com, for detailed information on biochemistry books

diginerve
A Jaypee Initiative
Your Guide at Every Step
Video Lectures | Notes | Self-Assessment
Scan code to check out courses

A Case Oriented Approach Towards BIOCHEMISTRY

SECOND EDITION



Editors
Namrata Chhabra
Sahil Chhabra

Co-editors
Sahiba Kukreja
Sarah Chhabra

Forewords
Sona Vasudevan
Binita Goswami
Ranjit Patil
Navjot Bajwa

JAYPEE

Contents

Chapter 1: Carbohydrate: Chemistry and Clinical Significance 1

- Case Study 1: Chemistry and Significance of Glycosides *1*
- Case Study 2: Clinical Significance of Heparin and an Overview of Chemistry and Significance of Glycosaminoglycans *3*
- Case Study 3: Mucopolysaccharidoses *7*

Chapter 2: Carbohydrate: Metabolism and Related Disorders 10

- Case Study 1: Clinical Implications of Impaired Digestion of Lactose *10*
- Case Study 2: Clinical Significance of Glycolysis *12*
- Case Study 3: Implications of Impaired Conversion of Pyruvate to Acetyl CoA *15*
- Case Study 4: Clinical Significance of HMP Pathway *18*
- Case Study 5: Glycosuria—Causes and Laboratory Diagnosis *22*
- Case Study 6 : Clinical Implications of Impaired Fructose Metabolism *24*
- Case Study 7: Glucose Homeostasis and Hypoglycemia *26*
- Case Study 8: Clinical Implications of Impaired Galactose Metabolism *30*
- Case Study 9: Biological and Clinical Significance of Glycogen Metabolism *33*
- Case Study 9: An Overview of Glycogen Storage Diseases *38*

Chapter 3: Lipid: Chemistry and Clinical Significance 42

- Case Study 1: Clinical significance of Cerebrosides (Glycolipids) *42*
- Case Study 2: Clinical significance of "Gangliosides" (Glycolipids) *45*
- Case Study 3: Chemistry and significance of Prostaglandins and NSAIDs versus Celecoxib *48*
- Case Study 4: Significance of Low-dose Aspirin in Preventing Ischemic Heart Disease *51*

Chapter 4: Lipid: Metabolism and Related Disorders 52

- Case Study 1: Role of Carnitine in Beta-oxidation of Fatty Acids and Clinical Implications of Carnitine Deficiency *52*
- Case Study 2: Fatty Liver—Causes and Clinical Implications *55*
- Case Study 3: Alcoholic Liver Disease *57*
- Case Study 4: Alcohol-induced Liver Cirrhosis *60*
- Case Study 5: Clinical Implications of Impaired Peroxisomal Fatty Acid Oxidation *62*
- Case Study 6: Clinical Implications of Impaired Alpha-oxidation of Fatty Acids *64*
- Case Study 7: Clinical Implications of Impaired Metabolism of Bile Salts *66*
- Case Study 8: Gallstones—Causes and Clinical Implications *68*
- Case Study 9: Clinical Implications of Impaired Cholesterol Uptake *70*
- Case Study 10: Regulation of Cholesterol Biosynthesis and Management of Hyperlipidemia *74*
- Case Study 11: Metabolism of Lipoproteins and Hyperlipoproteinemia *77*

Chapter 5: Protein: Chemistry and Significance 80

- Case Study 1: Clinical Implications of Protein Misfolding *80*
- Case Study 2: Clinical Implications of Abnormal Gamma-globulins *83*
- Case Study 3: Clinical Significance of Disruption of Normal Collagen Structure *85*
- Case Study 4: Clinical Implications of Genetically Altered Type I Collagen Structure *87*
- Case Study 5: Clinical Implications of Genetically Altered Type II Collagen Structure *89*
- Case Study 6: Clinical Implications of Genetically Altered Dystrophin *92*
- Case Study 7: Biological Role of Alpha-1-antitrypsin and Clinical Implications of its Deficiency *94*

Chapter 6: Amino Acid Metabolism and Related Disorders 97

- Case Study 1: Urea Cycle Disorders *97*
- Case Study 2: Clinical Implications of N-Acetyl Glutamate Synthase Deficiency *102*
- Case Study 3: Clinical Significance of Impaired Tyrosine Metabolism *104*
- Case Study 4: Clinical Implications of Impaired Melanin Synthesis *106*

JAYPEE BROTHERS

- Case Study 5: Clinical Implications of Impaired Tyrosine Synthesis *108*
- Case Study 6: Clinical Implications of Tryptophan Absorption Defect *110*
- Case Study 7: Clinical Implications of Hyperactive Serotonin Pathway *112*
- Case Study 8: Cystine Absorption Defect *114*
- Case Study 9: Clinical Implications of Abnormal Lysosomal Storage of Cystine *116*
- Case Study 10: Disorders of Homocysteine Metabolism *118*
- Case Study 11: Metabolic Disorder of Branched-Chain Amino Acids *121*
- Case Study 12: Synthesis and Significance of Nitric Oxide *122*

Chapter 7: Nucleotide: Chemistry and Significance **125**

- Case Study 1: Significance of cAMP in the Causation of Cholera *125*

Chapter 8: Nucleotide—Metabolism and Related Disorders **131**

- Case Study 1: Biochemical Basis of Severe Combined Immunodeficiency in Adenosine Deaminase Enzyme Defect *131*
- Case Study 2: Clinical Significance of Pathway of Purine Nucleotide Degradation *133*
- Case Study 3: Clinical Significance of Salvage Pathway of Purine Nucleotide Biosynthesis *139*
- Case Study 4: Clinical Significance of Pathway of De Novo Synthesis of Pyrimidine Nucleotides *143*
- Case Study 5: Clinical Role of Ribonucleotide Reductase *146*

Chapter 9: Clinical Enzymology **148**

- Case Study 1: Diagnostic Significance of Isoenzymes *148*
- Case Study 2: Clinical Significance of Enzymes in Acute Pancreatitis *151*
- Case Study 3: Mechanism of Enzyme Inhibition *154*
- Case Study 4: Mechanism of Enzyme Action *157*

Chapter 10: Biological Oxidation and Oxidative Phosphorylation **160**

- Case Study 1: Clinical Implications of Cyanide Induced Inhibition of Electron Transport Chain *160*
- Case Study 2: Implications of Carbon Monoxide Induced Inhibition of Electron Transport Chain *163*
- Case Study 3: Mechanism of Action of "Uncouplers of Oxidative Phosphorylation" *166*
- Case Study 4: Role of Calcium as an Uncoupler of Oxidative Phosphorylation *167*
- Case Study 5: Salicylate-induced Hyperthermia and Respiratory Alkalosis *170*

Chapter 11: Vitamins **172**

- Case Study 1: Causes and Clinical Implications of Thiamine Deficiency *172*
- Case Study 2: Thiamine—Chemistry, Metabolism, Coenzyme Role, and Clinical Implications of Thiamine Deficiency in Alcoholics *175*
- Case Study 3: Chemistry, Metabolism, Coenzyme Role, and Clinical Implications of Vitamin B_2 Deficiency *180*
- Case Study 4: Chemistry, Metabolism, Coenzyme Role, and Clinical Implications of Niacin Deficiency and Toxicity *182*
- Case Study 5: Folic Acid—Chemistry, Metabolism, Functions, and Clinical Implications of Folic Acid Deficiency *185*
- Case Study 6: Chemistry, Metabolism, Coenzyme role, and Clinical Implications of Deficiency of Vitamin B_{12} *191*
- Case Study 7: Differential Diagnosis of Megaloblastic Anemia *196*
- Case Study 8: Chemistry, Metabolism, Coenzyme Role, Functions, and Clinical Significance of Vitamin C *199*
- Case Study 9: Chemistry, Metabolism, Functions, and Clinical Significance of Vitamin A *203*
- Case Study 10: Chemistry, Metabolism, Functions, and Clinical Significance of Vitamin D *209*
- Case Study 11: Chemistry, Metabolism, Functions, and Deficiency of Vitamin E *215*
- Case Study 12: Chemistry, Metabolism, Functions, and Deficiency of Vitamin K *217*

Chapter 12: Minerals **223**

- Case Study 1: Iron Metabolism, Function, and Iron Deficiency Anemia *223*
- Case Study 2: Causes and Clinical Implications of Iron Overload *229*
- Case study 3: Asymptomatic Hypercalcemia *232*
- Case Study 4 : Clinical Implications of Hypercalcemic Crisis *233*
- Case Study 5: Calcium Homeostasis and Clinical Implications of Hypocalcemia *237*
- Case Study 6 : Neonatal Hypocalcemia *241*
- Case study 7: Copper Metabolism and Clinical Implications of Copper Deficiency and Toxicity *243*
- Case Study 8: Zinc—Metabolism, Functions, and Significance *246*
- Case Study 9: Selenium—Functions, Deficiency, and Toxicity *251*
- Case Study 10: Lead Poisoning *255*
- Case Study 11: Mercury Poisoning *258*
- Case Study 12: Arsenic Poisoning *261*

JAYPEE BROTHERS

Chapter 13: Heme Synthesis and Degradation 264
- Case Study 1: Bilirubin Metabolism and Differential Diagnosis of Jaundice *264*
- Case Study 2: Steps and Significance of Heme Synthesis *271*

Chapter 14: Hemoglobinopathies 280
- Case Study 1: Clinical Implications of Altered Hemoglobin Structure—Sickle Cell Disease (Qualitative Defect) *280*
- Case Study 2: Clinical Implications of Altered Hemoglobin Structure—Thalassemia (Quantitative Defect) *286*
- Case Study 3: Clinical Implications of Altered Hemoglobin Methemoglobinemia (Qualitative Defect) *293*

Chapter 15: Hormones 297
- Case Study 1: Biological and Clinical Significance of Growth Hormone *297*
- Case Study 2: Clinical Implications of Deficiencies of Adrenal Cortical Hormones *303*
- Case Study 3: Clinical Implications of Hyperaldosteronism *307*
- Case Study 4: Clinical Implications of Congenital Hypothyroidism *311*
- Case Study 5: Clinical Implications of Excess of Glucocorticoids *313*
- Case Study 6: Clinical Implications of Antidiuretic Hormone (ADH) Deficiency *316*
- Case Study 7: Clinical Implications of Excess of Antidiuretic Hormone *319*
- Case Study 8: Clinical Implications of Hyperthyroidism (Autoimmune Disease) *322*
- Case Study 9: Clinical Implications of Hypothyroidism (Autoimmune Disease) *326*
- Case Study 10: Clinical Implications of Hypothyroidism *329*
- Case Study 11: Clinical Implications of Hyperparathyroidism *332*
- Case Study 12: Clinical Implications of Hypoparathyroidism *335*
- Case Study 13: Clinical Implications of Excess Release of Catecholamines *338*
- Case Study 14: Causes, Clinical Implications, Diagnosis, and Treatment of Type 1 Diabetes Mellitus *343*
- Case Study 15: Causes, Pathogenesis, Clinical Manifestations, Diagnosis, and Treatment of Type 2 Diabetes Mellitus *354*
- Case Study 16: Biochemical Basis and Clinical Implications of Diabetic Ketoacidosis *363*
- Case Study 17: Biochemical Basis and Clinical Implications of "Metabolic Syndrome" *366*
- Case Study 18: Clinical Implications of Excess Release of Insulin (Insulinoma) *370*
- Case Study 19: Causes and Clinical Implications of Rebound Hypoglycemia *372*

Chapter 16: Detoxification 374
- Case Study 1: Phase 1 and 2 Reactions of Detoxification *374*
- Case Study 2: Entoxification of Xenobiotics *377*
- Case Study 3: Isoniazid-induced Pyridoxal-phosphate Deficiency *379*

Chapter 17: Diet and Nutrition 382
- Case Study 1: Protein-energy Malnutrition—Causes and Consequences *382*
- Case Study 2: Obesity—Causes and Consequences *387*
- Case Study 3: Biochemistry of Starvation *392*
- Case Study 4: Biological and Clinical Significance of Dietary Fiber *395*

Chapter 18: HIV Infection/Acquired Immunodeficiency Syndrome 400
- Case Study: Human Immunodeficiency Virus Infection—Causes and Consequences *400*

Chapter 19: Acid-Base Balance and Imbalance 411
- Case Study: Regulation of Plasma pH in Normal Health *411*
- Case Study 1: Diagnosis of Acid-base Imbalance *416*
- Case Study 2: Causes, Compensation, and Clinical Implications of Metabolic Acidosis (Lactic Acidosis) *417*
- Case Study 3: Causes, Compensation, and Clinical Implications of Metabolic Alkalosis *419*
- Case Study 4: Anion Gap and Causes, Compensation, and Clinical Implications of Metabolic Acidosis *423*
- Case Study 5: High Anion Gap Metabolic Acidosis *426*
- Case Study 6: Causes, Compensation, and Clinical Implications of Respiratory Acidosis *429*

Chapter 20: Molecular Biology 434
- Case Study 1: Types and Effects of Mutations *434*
- Case Study 2: Effects of Mutations (Codon Deletion) *438*
- Case Study 3: Mechanism of Action and Clinical Applications of Topoisomerases *441*
- Case Study 4: DNA Damage and Repair System *445*

JAYPEE BROTHERS

Chapter 21: Organ Function Tests 452
- Case Study 1: Diagnosis and Management of Acute Renal Failure *452*
- Case Study 2: Diagnosis and Management of Chronic Renal Failure *457*
- Case Study 3: Diagnosis and Management of Diabetic Nephropathy *463*
- Case Study 4: Diagnosis and Management of Nephrotic Syndrome *465*
- Case Study 5: Renal and Liver Function Tests in Preeclampsia *469*
- Case Study 6: Diagnosis and Management of Acute Myocardial Infarction *470*
- Case Study 7: Diagnostic Significance of Liver Function Tests *476*
- Case Study 8: Interpretation of Impaired Liver Function Tests *481*

Chapter 22: Water and Electrolyte Balance and Imbalance 483
- Case Study 1: Normal Water and Electrolyte Balance and the Implication of Dehydration *483*
- Case Study 2: Causes and Consequences of Hyponatremia *488*
- Case Study 3: Causes and Consequences of Hypernatremia *492*
- Case Study 4: Causes and Consequences of Hyperkalemia *497*
- Case Study 5: Causes and Consequences of Hypokalemia *502*

Chapter 23: Biochemistry of Cancer 506
- Case Study 1: Tumor Suppressor Genes and Oncogenes *506*
- Case Study 2: Mechanism of Action of Anticancer Drugs *511*
- Case Study 3: Tumor Markers *515*

Chapter 24: Biochemical Techniques 518
- Case Study 1: Principle, Types, Mechanism, and Applications of Electrophoresis *518*
- Case Study 2: Principle, Types, Mechanisms, and Applications of Chromatography *522*
- Case Study 3: Principle, Procedure, and Applications of Western Blotting Technique *531*
- Case Study 4: Southern Blotting—Principle, Steps, and Applications *534*
- Case Study 5: Polymerase Chain Reaction (PCR)—Principle, Procedure, Types, and Applications *538*
- Case Study 6: ELISA—Enzyme-linked Immunosorbent Assay *542*
- Case Study 7: Principle, Procedure, and Significance of Colorimetry *545*
- Case Study 8: Newborn Screening Tests *550*

Chapter 25: Case Studies for Self-Assessment 555

Chapter 26: Explanations of Case Studies 571

Chapter 27: Multiple Choice Questions for Self-Assessment 577

Chapter 28: Quick Revisions 584

Chapter 29: Normal Laboratory Reference Values 606

Index *613*

JAYPEE BROTHERS

Chapter 12

Minerals

JAYPEE BROTHERS

LEARNING OBJECTIVES

To understand:

- Chemistry, metabolism, and functions of iron
- Clinical implications of iron deficiency and overload
- Calcium metabolism and homeostasis
- Clinical implications of hyper- and hypocalcemia
- Copper metabolism, functions, and clinical implications of copper deficiency and toxicity
- Zinc—metabolism, functions, and significance
- Selenium—functions, deficiency, and toxicity
- The sources of exposure, pathogenesis, clinical manifestations, laboratory diagnosis, and management of heavy metal toxicity

CASE STUDIES

CASE STUDY 1

Theme: Iron Metabolism, Function, and Iron Deficiency Anemia

An 18-year-old female reported to the physician for consultation. She complained of generalized weakness, lethargy, and inability to do the routine work from the previous few months. On further questioning, she revealed that she was having excessive bleeding during menstruation from the previous 6 months. She complained of breathlessness and palpitations while climbing stairs for her house. She also had experienced periods of lightheadedness, though not to the point of fainting. Other changes she had noticed were cramping in her legs, a desire to crunch on ice. There was no history of any fever, drug intake, or abdominal discomfort. Her appetite had also decreased and she was taking meals only once a day.

Upon examining, her physician found that she had tachycardia, pale gums, and nail beds, and her tongue was swollen. Given her history and the findings on her physical examination, the physician suspected that the patient was anemic and ordered a sample of her blood for examination. The results were as shown below:

Hb—8.0 g/dL Serum iron—32 μg/dL

What is the cause of anemia in this patient?
What are the possible complications in the untreated cases?

CASE DETAILS

The most likely diagnosis is iron deficiency anemia.

Generalized weakness, exercise intolerance, dyspnea, palpitations, history of blood loss during menstruation, tachycardia, and low Hb all are suggestive of iron deficiency anemia. Iron deficiency is the most prevalent single deficiency state on a worldwide basis. Iron deficiency is related in part to abnormal iron metabolism.

Overview of Iron Metabolism

The balance of iron in humans is tightly controlled and designed to conserve iron for reutilization. There is no regulated excretory pathway for iron, and the mechanisms by which iron is lost from the body are blood loss (via gastrointestinal bleeding, menses, or other forms of bleeding) and the loss of epithelial cells from the skin, gut, and genitourinary tract. Normally, the only route by which iron comes into the body is via absorption from food or from medicinal iron taken orally. Iron may also enter the body through red-cell transfusions or injection of iron complexes. The margin between the amount of iron available for absorption and the requirement for iron in growing infants and the adult female is narrow; this accounts for the great prevalence of iron deficiency worldwide—currently estimated at one-half billion people.

Iron Requirement

The amount of iron required from the diet to replace losses averages about 10% of body iron content a year in men and 15% in women of childbearing age. Dietary iron content is closely related to total caloric intake (approximately 6 mg of elemental iron per 1,000 calories). Iron bioavailability is affected by the nature of the foodstuff, with heme iron (e.g., red meat) being most readily absorbed. In the United States, the average iron intake in an adult male is 15 mg/day with 6% absorption; for the average female, the daily intake is 11 mg/day with 12% absorption. An individual with iron deficiency can increase iron absorption to about 20% of the iron present in a meat-containing diet but only 5–10% of the iron in a vegetarian diet. As a result, one-third of the female population in the United States has virtually no iron stores. Vegetarians are at an additional disadvantage because certain foodstuffs that include phytates and phosphates reduce iron absorption by about 50%. When ionizable iron salts are given together with food, the amount of iron absorbed is reduced. When the percentage of iron absorbed from individual food items is compared with the percentage for an equivalent amount of ferrous salt, iron in vegetables is only about one-twentieth as available, egg iron one-eighth, liver iron one-half, and heme iron one-half to two-thirds.

Infants, children, and adolescents may be unable to maintain normal iron balance because of the demands of body growth and lower dietary intake of iron. During the last two trimesters of pregnancy, daily iron requirements increase to 5–6 mg. That is the reason why iron supplements are strongly recommended for pregnant women in developed countries.

Iron absorption takes place largely in the proximal small intestine and is a carefully regulated process. For absorption, iron must be taken up by the luminal cell. That process is facilitated by the acidic contents of the stomach **(Fig. 12.1)**, which maintains the iron in solution. At the brush border of the absorptive cell, the ferric iron is converted to the ferrous form by a ferrireductase. Transport across the membrane is accomplished by divalent metal transporter 1 (DMT1, also known as Nramp2 or DCT-1). DMT1 is a general cation transporter. Once inside the gut cell, iron may be stored as ferritin or transported through the cell to be released at the basolateral surface to plasma transferrin through the membrane-embedded iron exporter, ferroportin. The function of ferroportin is negatively regulated by hepcidin, the principal iron regulatory hormone. In the process of release, iron interacts with another ferroxidase, hephaestin, which oxidizes the iron to the ferric form for transferrin binding. Hephaestin is similar to ceruloplasmin, the copper-carrying protein.

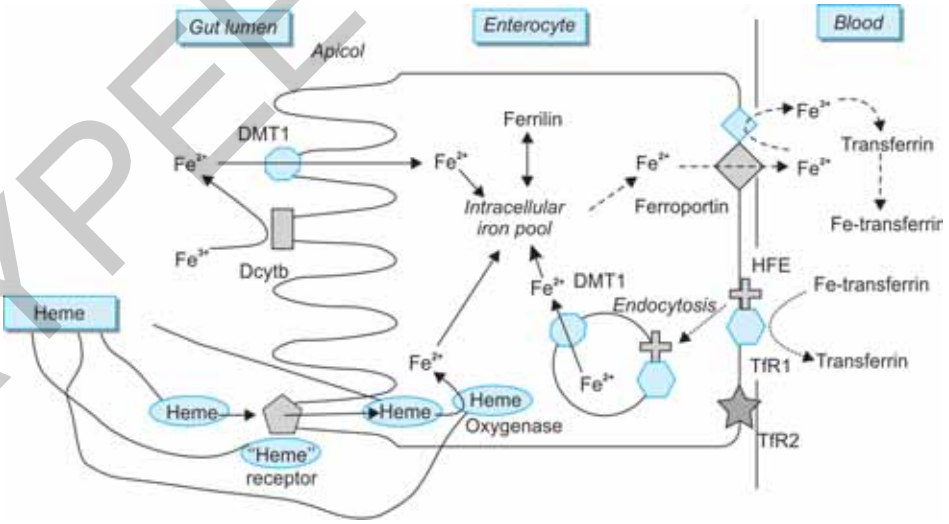


Fig. 12.1: Mechanism of iron absorption.

Iron absorption is influenced by a number of physiological states erythroid hyperplasia, for example, stimulates iron absorption, even in the face of normal or increased iron stores, and hepcidin levels are inappropriately low. The molecular mechanism underlying this relationship is not known. Thus, patients with anemias associated with high levels of ineffective erythropoiesis absorb excess amounts of dietary iron. Over time, this may lead to iron overload and tissue damage. In iron deficiency, hepcidin levels are low and iron is much more efficiently absorbed from a given diet; the contrary is true in states of secondary iron overload. The normal

JAYPEE BROTHERS

individual can reduce iron absorption in situations of excessive intake or medicinal iron intake; however, while the percentage of iron absorbed goes down, the absolute amount goes up. This accounts for the acute iron toxicity occasionally seen when children ingest large numbers of iron tablets. Under these circumstances, the amount of iron absorbed exceeds the transferrin-binding capacity of the plasma, resulting in free iron that affects critical organs, such as cardiac muscle cells.

Free Iron Toxicity

Iron is a critical element in the function of all cells, although the amount of iron required by individual tissues varies during development. At the same time, the body must protect itself from free iron, which is highly toxic because it participates in chemical reactions that generate free radicals, such as singlet O_2 or OH^-. In chemical reactions that generate free radicals, such as singlet O_2 or OH^-. Consequently, elaborate mechanisms have evolved that allow iron to be made available for physiological functions while at the same time conserving this element and handling it in such a way that toxicity is avoided.

Iron Transport

Iron absorbed from the diet or released from stores circulates in the plasma bound to *transferrin*, the iron transport protein. Transferrin is a bilobed glycoprotein with two iron-binding sites. Transferrin (Tf) is normally about one-third saturated with iron transferrin that carries iron, which exists in two forms—*monoferric* (one iron atom) or *diferric* (two iron atoms). The turnover (half-clearance time) of transferrin-bound iron is very rapid—typically 60–90 minutes. Because almost all of the iron transported by transferrin is delivered to the erythroid marrow, the clearance time of transferrin-bound iron from the circulation is affected most by the plasma iron level and the erythroid marrow activity. When erythropoiesis is markedly stimulated, the pool of erythroid cells requiring iron increases and the clearance time of iron from the circulation decreases. The half-clearance time of iron in the presence of iron deficiency is as short as 10–15 minutes. With suppression of erythropoiesis, the plasma iron level typically increases and the half-clearance time may be prolonged to several hours. Normally, the iron bound to transferrin turns over 10–20 times per day. Assuming a normal plasma iron level of 80–100 µg/dL, the amount of iron passing through the transferrin pool is 20–24 mg/day.

The iron-transferrin complex circulates in the plasma until it interacts with specific *transferrin receptors* on the surface of marrow erythroid cells. Diferric transferrin has the highest affinity for transferrin receptors; apotransferrin (transferrin not carrying iron) has very little affinity. While transferrin receptors are found on cells in many tissues within the body—and all cells at some time during development will display transferrin receptors—the cell having the greatest number of receptors (300,000–400,000/cell) is the developing erythroblast.

Utilization of Iron

Once the iron-bearing transferrin interacts with its receptor, the complex is internalized via clathrin-coated pits and transported to an acidic endosome, where the iron is released at the low pH. The iron is then made available for heme synthesis, while the transferrin-receptor complex is recycled to the surface of the cell, where the bulk of the transferrin is released back into circulation and the transferrin receptor reanchors into the cell membrane. At this point, a certain amount of the transferrin receptor protein may be released into circulation and can be measured as soluble transferrin receptor protein. Within the erythroid cell, iron in excess of the amount needed for hemoglobin synthesis binds to a storage protein, apoferritin, forming ferritin. This mechanism of iron exchange also takes place in other cells of the body expressing transferrin receptors, especially liver parenchymal cells where the iron can be incorporated into heme-containing enzymes or stored. The iron incorporated into hemoglobin subsequently enters the circulation as new red cells are released from the bone marrow. The iron is then part of the red cell mass and will not become available for reutilization until the red cell dies.

Conservation of Iron

In a normal individual, the average red cell lifespan is 120 days. Thus, 0.8–1.0% of red cells turn over each day. At the end of its lifespan, the red cell is recognized as senescent by the cells of the reticuloendothelial (RE) system, and the cell undergoes phagocytosis. Once within the RE cell, the hemoglobin from the ingested red cell is broken down, the globin and other proteins are returned to the amino acid pool, and the iron is shuttled back to the surface of the RE cell, where it is presented to circulating transferrin. It is the efficient and highly conserved recycling of iron from senescent red cells that supports steady state (and even mildly accelerated) erythropoiesis. Persistent errors in iron balance lead to either iron deficiency anemia or hemosiderosis. Both are disorders with potential adverse consequences.

Storage of Iron

Ferritin and hemosiderin are iron-containing compounds meant for storage of iron. Ferritin is a protein-bound, water-soluble, and mobilizable storage compound and is the major source of storage iron. Hemosiderin is a water-insoluble form that is less readily available for use. When the amount of total body iron is relatively low, storage iron consists predominately of ferritin. When iron stores are high, hemosiderin predominates. Unlike ferritin, hemosiderin stains with the Prussian blue stain (Pens reaction) and may be observed in tissues. Storage forms normally comprise approximately 30% of total body iron. Iron stores provide a source of iron when physiological demand is high, e.g., blood loss, pregnancy, and periods of rapid growth.

JAYPEE BROTHERS

Metabolic Role of Iron

Iron is vital for all living organisms because it is essential for multiple metabolic processe, including oxygen transport, DNA synthesis, and electron transport.

The major role of iron in mammals is to carry O_2 as part of hemoglobin. O_2 is also bound by myoglobin in muscle. Iron is a critical element in iron-containing enzyme, including the cytochrome system in mitochondria. Without iron, cells lose their capacity for electron transport and energy metabolism. In erythroid cells, hemoglobin synthesis is impaired, resulting in anemia and reduced O_2 delivery to tissue.

Demand and Supply Imbalance

Since each milliliter of red cells contains 1 mg of elemental iron, the amount of iron needed to replace those red cells lost through senescence amounts to 16–20 mg/day (assuming an adult with a red cell mass of 2 L). Any additional iron required for daily red cell production comes from the diet. Normally, an adult male will need to absorb at least 1 mg of elemental iron daily to meet needs, while females in the childbearing years will need to absorb an average of 1.4 mg/day. However, to achieve a maximum proliferative erythroid marrow response to anemia, additional iron must be available. With markedly stimulated erythropoiesis, demands for iron are increased by as much as six- to eightfold. With extravascular hemolytic anemia, the rate of red cell destruction is increased, but the iron recovered from the red cells is efficiently reutilized for hemoglobin synthesis. In contrast, with intravascular hemolysis or blood loss anemia, the rate of red cell production is limited by the amount of iron that can be mobilized from stores. Typically, the rate of mobilization under these circumstances will not support red cell production >2.5 times normal. If the delivery of iron to the stimulated marrow is suboptimal, the marrow's proliferative response is blunted, and hemoglobin synthesis is impaired. The result is a hypoproliferative marrow accompanied by microcytic, hypochromic anemia.

Menstrual blood loss in women plays a major role in iron metabolism. The average monthly menstrual blood loss is approximately 50 mL, or about 0.7 mg/day. However, menstrual blood loss may be five times the average. To maintain adequate iron stores, women with heavy menstrual losses must absorb 3–4 mg of iron from the diet each day. This strains the upper limit of what may reasonably be absorbed, and women with menorrhagia of this degree will almost always become iron deficient without iron supplementation.

Pregnancy may also upset the iron balance, since requirements increase to 2–5 mg of iron per day during pregnancy and lactation. Normal dietary iron cannot supply these requirements, and medicinal iron is needed during pregnancy and lactation. Repeated pregnancy (especially with breastfeeding) may cause iron deficiency, if increased requirements are not met with supplemental medicinal iron. Decreased iron absorption can on very rare occasions cause iron deficiency and usually occurs after gastric surgery, though concomitant bleeding is frequent.

By far, the most important cause of iron deficiency anemia is blood loss, especially gastrointestinal blood loss. Chronic aspirin use may cause it even without a documented structural lesion. Iron deficiency demands a search for a source of gastrointestinal bleeding, if other sites of blood loss (menorrhagia, other uterine bleeding, and repeated blood donations) are excluded.

Chronic hemoglobinuria may lead to iron deficiency since iron is lost in the urine; traumatic hemolysis due to a prosthetic cardiac valve and other causes of intravascular hemolysis (e.g., paroxysmal nocturnal hemoglobinuria) should also be considered. Frequent blood donors may also be at risk for iron deficiency.

While blood loss or hemolysis places a demand on the iron supply, conditions associated with inflammation interfere with iron release from stores and can result in a rapid decrease in the serum iron level.

SUMMARY OF CAUSES OF IRON DEFICIENCY

Conditions that increase demand for iron, increase iron loss, or decrease iron intake or absorption can produce iron deficiency:

- **Increased demand for iron and/or hematopoiesis:**
  - Rapid growth in infancy or adolescence
  - Pregnancy
  - Erythropoietin therapy
- **Increased iron loss:**
  - Chronic blood loss
  - Menses
  - Acute blood loss
  - Blood donation
  - Phlebotomy as treatment for polycythemia vera.
- **Decreased iron intake or absorption:**
  - Inadequate diet
  - Malabsorption from disease (sprue, Crohn's disease)
  - Malabsorption from surgery (postgastrectomy)
  - Acute or chronic inflammation

JAYPEE BROTHERS

IRON DEFICIENCY ANEMIA

Iron deficiency is defined as decreased total iron body content. Iron deficiency anemia occurs when iron deficiency is sufficiently severe to diminish erythropoiesis and cause the development of anemia. Iron deficiency is the most prevalent single deficiency state on a worldwide basis. It is important economically because it diminishes the capability of individuals who are affected to perform physical labor, and it diminishes both growth and learning in children.

Frequency

In countries where little meat is in the diet, iron deficiency anemia is 6–8 times more prevalent than in North America and Europe. This occurs despite consumption of a diet that contains an equivalent amount of total dietary iron because heme iron is absorbed better from the diet than nonheme iron. In certain geographic areas, intestinal parasites, particularly hookworm, worsen the iron deficiency because of blood loss from the gastrointestinal tract. Anemia is more profound among children and premenopausal women in these environs.

Mortality/Morbidity

Chronic iron deficiency anemia is seldom a direct cause of death; however, moderate or severe iron deficiency anemia can produce sufficient hypoxia to aggravate underlying pulmonary and cardiovascular disorders. In children, the growth rate may be slowed, and a decreased capability to learn is reported.

Clinical Manifestations

As a rule, the only symptoms of iron deficiency anemia are those of the anemia itself (easy fatigability, tachycardia, palpitations, and tachypnea on exertion). Severe deficiency causes skin and mucosal change, including a smooth tongue, brittle nails, and cheilosis. Dysphagia because of the formation of esophageal webs (Plummer–Vinson syndrome) also occurs. Many iron-deficient patients develop pica, craving for specific foods (ice chips, etc.) often not rich in iron.

Laboratory Findings

- **Complete blood count (CBC):**
  - This documents the severity of the anemia. In chronic iron deficiency anemia, the cellular indices show a microcytic and hypochromic erythropoiesis, i.e., both the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) have values below the normal range for the laboratory performing the test. Reference range values for the MCV and MCHC are 83–97 fL and 32–36 g/dL, respectively.
  - Often, the platelet count is elevated (>450,000/µL), which normalizes after iron therapy.
  - The white blood cell (WBC) count is usually within reference ranges (4,500–11,000/µL).
  - If the CBC count is obtained after blood loss, the cellular indices do not enter the abnormal range until most of the erythrocytes produced before the bleed are destroyed at the end of their normal lifespan (120 days).
- **Peripheral smear:**
  - In the early stages, the MCV remains normal. Subsequently, the MCV falls and the blood smear shows hypochromic microcytic cells (blood smear; **Figure 12.2**). With further progression, anisocytosis (variations in red blood cell size) and poikilocytosis (variation in shape of red cells) develop. Severe iron deficiency will produce a bizarre peripheral blood smear, with severely hypochromic cells, target cells, hypochromic pencil-shaped cells, and occasionally small numbers of nucleated red blood cells **(Fig. 12.2)**. The platelet count is commonly increased.
  - Combined folate deficiency and iron deficiency are commonplace in areas of the world with little fresh produce and meat. The peripheral smear reveals a population of macrocytes mixed among the microcytic hypochromic cells. This combination can normalize the MCV.

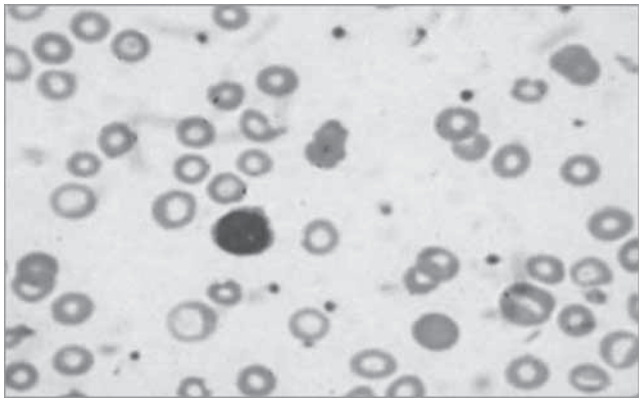


Fig. 12.2: Microcytic hypochromic cells in peripheral smear in a patient suffering from iron deficiency anemia.

- **Serum iron, total iron-binding capacity (TIBC), and serum ferritin:** Iron deficiency develops in stages. The first is depletion of iron stores. At this point, there is anemia and no change in red blood cell size. The serum ferritin will become abnormally low. A ferritin value <30 mcg/L is a highly reliable indicator of iron deficiency. The serum TIBC rises. After iron stores have been depleted, red blood cell formation will continue with deficient supplies of iron. Serum iron values decline to <30 mcg/dL and transferrin saturation to <15%. A low serum iron and ferritin with an elevated TIBC are diagnostic of iron deficiency. While a low serum ferritin is virtually diagnostic of iron deficiency, a normal serum ferritin can be seen in patients who are

JAYPEE BROTHERS

27 mL/kg; a 50 kg woman will thus have 1,350 mg of iron circulating in her red blood cells. Thus, a woman whose hemoglobin is 9 g/dL would be treated with a total of 1,315 mg of parenteral iron, 315 mg for the increased red blood cell mass and 1,000 mg to provide iron stores. The entire dose may be given as an intravenous infusion over 4–6 hours. A test dose of a dilute solution is given first, and the patient should be observed during the entire infusion for anaphylaxis.

Red Cell Transfusion

Transfusion therapy is reserved for individuals who have symptoms of anemia, cardiovascular instability, continued and excessive blood loss from whatever source, and require immediate intervention. The management of these patients is less related to the iron deficiency than it is to the consequences of the severe anemia. Not only do transfusions correct the anemia acutely, but the transfused red cells provide a source of iron for reutilization, assuming they are not lost through continued bleeding. Transfusion therapy will stabilize the patient while other options are reviewed.

Prognosis

Iron deficiency anemia is an easily treated disorder with an excellent outcome; however, it may be caused by an underlying condition with a poor prognosis,, such as neoplasia. Similarly, the prognosis may be altered by a comorbid condition, such as coronary artery disease.



CLINICAL NOTE

The margin between the amount of iron available for absorption and the requirement for iron in growing infants and the adult female is narrow; this accounts for the great prevalence of iron deficiency worldwide—currently estimated at one-half billion people.



CLINICAL PEARLS

1. Iron deficiency anemia occurs when iron deficiency is sufficiently severe to diminish erythropoiesis and cause the development of anemia.
2. Symptoms of iron deficiency anemia are easy fatigability, tachycardia, palpitations, and tachypnea on exertion. Severe deficiency causes skin and mucosal change, including a smooth tongue, brittle nails, and cheilosis.
3. The diagnosis of iron deficiency anemia can be made either by the laboratory demonstration of an iron-deficient state or evaluating the response to a therapeutic trial of iron replacement.
4. CBC, peripheral blood smear, serum iron, ferritin, and serum total iron-binding capacity estimations are diagnostic of iron deficiency anemia.
5. Treatment of Fe deficiency anemia is based on treatment of the primary cause and iron supplementation orally, parenterally or by red cell transfusion depending upon the state of the patient.

CASE STUDY 2

Theme: Causes and Clinical Implications of Iron Overload

A 56-year-old Caucasian woman visited her family physician for her annual physical checkup. She stated that she was in generally good health but was experiencing some fatigue, mild depression, and mild-to-moderate joint pain from the previous several months. She was holding a full-time job as an accountant. Her medical history was significant only for hormone replacement therapy from the past 12 years. The patient stated that her consumption of alcohol was limited to one to two glasses of wine each week. She had been taking ibuprofen occasionally for joint pain but denied use of any other over-the-counter medication except daily vitamin tablets. Family history revealed that her father died 10 years ago of liver disease at age of 70. The patient was uncertain about the exact nature of her father's illness. Her mother was alive and was in good health. She also stated that she had two siblings—a brother and a sister who was recently diagnosed with diabetes.

Physical examination revealed a well-appearing female with normal vital signs. There was a mild hepatomegaly and joint tenderness. Laboratory studies were ordered on a fasting specimen and are as reported:

Laboratory data included—random blood glucose of 253 mg/dL, normal creatinine and electrolytes, aspartate aminotransferase (GOT) of 66 U/L (normal <44), alanine aminotransferase (ALT) of 133 U/L (normal <31 U/L), normal bilirubin and alkaline phosphatase levels, and negative hepatitis antigen screen.

Her iron level was 306 µg/dL (normal <155), with an iron-binding capacity of 315 µg/dL (normal <400), and percent saturation of 97% (normal <50%). Serum ferritin was 2,920 µg/L (normal <160). The polymerase chain restriction assay demonstrated

JAYPEE BROTHERS

homozygosity for the C282Y chromosome. Referral to the hepatology clinic resulted in a liver biopsy, which identified increased iron stores and early periportal fibrosis.

What is the probable diagnosis?

What is the basis for all the laboratory findings?

CASE DETAILS

The lady in question is most probably suffering from hemochromatosis. Hereditary hemochromatosis (HH) is a common inherited disorder of iron metabolism. This disease is usually diagnosed by elevated iron saturation, serum ferritin, a positive family history, hepatic abnormalities and cirrhosis, congestive heart failure, hypogonadism, arthritis, and *HFE* gene mutation (usually C282Y/C282Y) which is found in most cases.

The given patient has arthritis, a positive family history, raised transaminases, raised serum iron, iron-binding capacity, and high serum ferritin levels. PCR analysis also reveals the HFE gene mutation and the liver biopsy is revealing the increased iron stores. All these features are strongly in favor of hemochromatosis as a diagnosis.

BASIC CONCEPT

Hereditary hemochromatosis: Hereditary hemochromatosis (HH) is a fairly common disease in whites and is a result of iron deposition in hepatocytes, myocardial fibers, and other visceral cells. The classic tetrad of manifestations resulting from hemochromatosis consists of (1) cirrhosis, (2) diabetes mellitus, (3) hyperpigmentation of the skin **(Fig.12.3)**, and (4) cardiac failure. Clinical consequences also include hepatocellular carcinoma, impotence, and arthritis.



Fig. 12.3: Hyperpigmentation in hereditary hemochromatosis.

Biochemical Defect

The *hemochromatosis* gene is located on the long arm of chromosome 6 where it codes for a membrane protein, HFE, which is 343 amino acids in size. The exact mechanism of the role of HFE in iron metabolism is not completely understood. A popular hypothesis is that HFE, along with a second protein, beta-2 microglobulin (beta-2M), interacts with TfR on cell membranes. This interaction represses the affinity of Tf for the receptor, thus lowering the uptake of Tf into the cell. TfR have been found on the surface of a variety of cells, with the greatest concentration on the basolateral membrane of the intestine, hepatocytes, and erythrocyte precursors.

In HH, two mutations of the HFE gene have been described—*C282Y* and *H63D*. In the more prevalent *C282Y* mutation, there is a base substitution leading to a change in the amino acid in position 282 from cysteine (C) to tyrosine (Y).The loss of the sulfhydryl-containing amino acid disrupts the tertiary structure of HFE, so that it no longer binds to beta-2M. Beta-2M appears to act along with other proteins to chaperone the newly synthesized HFE out of the Golgi and to the cell surface where it can then bind to TfR. In the *C282Y* mutation, HFE remains in the Golgi, never making it to the cell surface. The result is that Tf binding to TfR is enhanced and excessive amounts of iron are absorbed in the small intestine, hepatocytes, and other tissues.

The second mutation, *H63D*, occurs when a histidine (H) residue in position 63 is replaced by an aspartic acid (D). The mechanism by which this mutation leads to increased iron uptake is less well understood when compared to the *C282Y* mutation. Unlike the *C282Y* mutation, the *H63D* mutation does not seem to affect the binding of beta-2M and intracellular trafficking, since detectable concentrations of the mutated protein are found on cell membranes. Some researchers speculate that the *H63D* mutation affects the binding of proteins involved in iron regulation and uptake at the cell surface.

A third *HFE* mutation, S65C, has also been described which appears to lead the mild to moderate hepatic iron overload without fibrosis in some cases.

Pathophysiology

Hemochromatosis is an autosomal recessive genetic disorder characterized by iron overload. Persons homozygous for mutations of the *hemochromatosis* gene, *HFE*, absorb 3–4 mg of dietary iron daily, a rate two to three times greater than normal. Normal storage sites become overloaded with iron, resulting in ferritin levels as much as ten times normal. With no mechanism to dispose of the excess, iron is deposited in the parenchymal cells of the liver, pancreas, pituitary, heart, synovium, and other tissues. Iron in excess of normal cellular ferritin stores contributes to the generation of free radicals and reactive oxygen intermediates that cause cell damage to organs and tissues. The amount of time needed for iron to increase in levels causing organ damage is variable and most likely partially dependent on gender and dietary factors. Blood loss through menstruation and pregnancy are thought to delay the onset of iron overload, and therefore symptoms, in women. Similarly, regular blood donation may confer some degree of protection. As levels of storage iron increase, clinical features of iron overload, including hepatic dysfunction or failure, diabetes, hypogonadism, arthritis, cardiomyopathy, hyperpigmentation and fatigue may become evident. Hyperpigmentation and fatigue may become evident. Symptomatic patients typically present in middle age between the ages of 30 and 60, although this is quite variable. Persons as young as 20 years of age may show clinical signs and symptoms of HH.

JAYPEE BROTHERS

Treatment

1. Affected patients should avoid foods rich in iron (such as red meat), alcohol, vitamin C, raw shellfish, and supplemental iron.
2. Weekly phlebotomies of 1 or 2 units (250–500 mL) of blood (each containing about 250 mg of iron) are indicated in all symptomatic patients—those with a serum ferritin level of at least 1,000 mcg/L and those with an increased fasting iron saturation, and should be continued for up to 2–3 years to achieve depletion of iron stores.
3. The hematocrit and serum iron values should be monitored. When iron store depletion is achieved (iron saturation <50% and serum ferritin level <50 mcg/L), maintenance phlebotomies (every 2–4 months) are continued (although compliance has been reported to decrease with time).
4. The chelating agent, deferoxamine, is indicated for patients with hemochromatosis and anemia or in those with secondary iron overload due to thalassemia who cannot tolerate phlebotomies, treatment is painful and time-consuming.
5. An oral chelator, deferasirox—20 mg/kg once daily has been approved for treatment of iron overload due to blood transfusions.
6. Complications of hemochromatosis—arthropathy, diabetes, heart disease, portal hypertension, and hypopituitarism—also require treatment.
7. Liver transplantation for advanced cirrhosis associated with severe iron overload including hemochromatosis, has been reported to lead to survival rates that are lower than those for other types of liver disease because of cardiac complications and an increased risk of infections.

Prognosis

The major determinant of prognosis is the degree of organ damage from iron overload at the point of diagnosis. The presence of cirrhosis reduces life expectancy. Hemochromatosis patients with cirrhosis at the time of diagnosis are over five times more likely to die compared to hemochromatosis patients without cirrhosis.



CLINICAL NOTE

Although rarely seen in clinical practice, the classic triad of skin hyperpigmentation (bronzing), type 2 diabetes, and hepatic cirrhosis has long been recognized as evidence of advanced iron overload.



CLINICAL PEARLS

1. HH is a fairly common disease in whites and is a result of iron deposition in hepatocytes, myocardial fibers, and other visceral cells.
2. In HH, two mutations of the *HFE* gene have been described—*C282Y* and *H63D*.
3. Persons homozygous for mutations of the *hemochromatosis* gene, *HFE*, absorb 3–4 mg of dietary iron daily, a rate two to three times greater than normal. Normal storage sites become overloaded with iron, resulting in ferritin levels as much as ten times normal. With no mechanism to dispose of the excess, iron is deposited in the parenchymal cells of the liver, pancreas, pituitary, heart, synovium, and other tissues.
4. This disease is usually diagnosed by elevated iron saturation, serum ferritin, a positive family history, hepatic abnormalities and cirrhosis, congestive heart failure, hypogonadism, arthritis, and *HFE* gene mutation (usually *C282Y/C282Y*), which is found in most cases.
5. Weekly phlebotomies of 1 or 2 units (250–500 mL) of blood (each containing about 250 mg of iron) are indicated in all symptomatic patients—those with a serum ferritin level of at least 1,000 mcg/L and those with an increased fasting iron saturation, and should be continued for up to 2–3 years to achieve depletion of iron stores.

CASE STUDY 3

Theme: Asymptomatic Hypercalcemia

An asymptomatic 55-year-old woman on a routine physical examination was noted to have the following abnormalities in her biochemical profile:
Serum calcium = 11.3 mg/dL (normal: 8.4–10.2 mg/dL)
Serum phosphorous = 2.9 mg/dL (normal: 3.0–4.5 mg/dL)
Serum sodium = 140 mEq/L (normal: 135–147 mEq/L)
Serum potassium = 4.0 mEq/L (normal: 3.5–5.0 mEq/L)
Serum chloride = 106 mEq/L (normal: 95–105 mEq/L)
Serum bicarbonate = 20 mEq/L (normal: 22–28 mEq/L)

JAYPEE BROTHERS

for detailed information on biochemistry books, visit our website www.jaypeebrothers.com, for detailed information on biochemistry books, visit o

A Case Oriented Approach Towards BIOCHEMISTRY

Highlights of the Book

- 300 case studies
- Based on new CBME curriculum allowing horizontal, spiral, vertical and nested integration
- Full coverage of all chapters of biochemistry with complete clinical correlations
- Illustrations of clinical cases in sections, namely history, physical examination, laboratory work-up and questions related to the diagnosis of the case. This will help sharpen the clinical skills by simulating a clinical reaching at a diagnosis experience
- Each case study ends-up in a clinical note and a short summary in the form of important points, titled as Clinical Pearls. This will help the readers to quickly revise the contents
- Many alternative unsolved case studies (72) have been provided at the end of the book which would help the students to evaluate their problem-solving skills gained through the course of the book
- Detailed description of molecular and biochemical techniques, including electrophoresis; chromatography; western blotting; southern blotting; polymerase chain reaction; enzyme-linked immunosorbent assay (ELISA); colorimetry, and newborn screening
- Case-based multiple choice questions for self-assessment (with answers)
- Quick revisions (summary charts): Acid-base imbalance; disorders of amino acid metabolism; disorders of carbohydrate metabolism; regulatory mechanisms of carbohydrate metabolism; disorders of lipid metabolism; regulatory mechanisms of lipid metabolism; electrolyte imbalance; clinical implications of vitamin deficiencies, and clinically important hemoglobinopathies
- Nine new case studies including—clinical role of ribonucleotide reductase, tumor markers, enzyme action and inhibition, functions and significance of zinc and selenium, and heavy metal toxicity
- Pictures (37 new pictures), tables and flowcharts for meaningful explanations of content details.

Namrata Chhabra is a Specialist in Biochemistry (MD) with a work experience of more than 33 years. She has worked at Government Medical College, Amritsar, Punjab, India, with Ministry of Health and Medical Education, Iran; and is currently working as a Principal-in-Charge, Professor and Chair, Department of Biochemistry, Sir Seewoosagur Ramgoolam Medical College, Belle Rive, Mauritius. She has to her credit a multitude of research publications, award-winning presentations, and professional laurels. She has carved a niche with her simplified yet thought-provoking teaching by her expertise in the teaching field. A writer with an inherent perfective tense is yet again manifested.

Sahil Chhabra MBBS MAS (Scholar) is a medical graduate from Dayanand Medical College and Hospital, Ludhiana, Punjab, India. He honed his skills as a lecturer in Sir Seewoosagur Ramgoolam Medical College, Belle Rive, Mauritius. He worked in the Department of Immunohematology and Blood Transfusion, Dayanand Medical College and Hospital, Ludhiana, Punjab, India and at the Department of Anesthesiology and Perioperative Medicine, University of Louisville, Louisville, Kentucky, United States of America. Currently, he is a MAS (Scholar), Leadership of Health Care Organization, University of California, San Diego, United States of America. His substantial educational background and excellent writing skills have fetched him numerous accolades in his professional carrier. He has always rendered exquisite interest in the field of biochemistry, and his work reflects an excellent caliber as a writer. He has wielded himself as an author efficaciously by these endowments.

Printed in India

Available at all medical bookstores
or buy online at www.jaypeebrothers.com



JAYPEE BROTHERS
Medical Publishers (P) Ltd.
EMCA House, 23/23-B, Ansari Road,
Daryaganj, New Delhi - 110 002, INDIA
www.jaypeebrothers.com

Join us on facebook.com/JaypeeMedicalPublishers

Shelving Recommendation
BIOCHEMISTRY

