

**BIOCHEMICAL STUDY OF HEMATOLOGICAL DISEASES IN CHILHOOD***Gordana Bjelaković<sup>1</sup>, Tatjana Jevtović<sup>1</sup>, Ivana Stojanović<sup>2</sup>, Bojko Bjelaković<sup>2</sup>, Mira Ilić**<sup>1</sup>Institute of Biochemistry, University School of Medicine, Niš**<sup>2</sup>Paediatric Department, Clinical Centre, Niš*

*Summary:* Hematological diseases in childhood occur frequently. Analysis of hematological status as well as standard biochemical analysis of blood, as a result of hematopoietic system function, are of great importance in detection and recognition of these diseases. All hematological diseases appear as disturbance of hematopoietic system function at the level of the production and viability in blood flow; this disturbance is reflected on the hemogram and biochemical analyses of blood plasma and serum. The basic hematological parameters of blood diseases (except disturbance of blood coagulation) are: Total blood count cells (erythrocytes, leukocytes with differential leukocytes formulae, trombocytes) hemoglobin concentration, amount of hematocrit (PCV, packed cell volume), erythrocytes sedimentation rate, erythrocytes osmotic fragility. Among the standard biochemical analysis the determination of total bilirubin in the calculation fraction of indirect bilirubin, the amount of serum iron and transferrin (TIBC), serum copper and ceruloplasmin are in use in these diseases. Hemoglobin electrophoresis is also use. The measurement of lactate dehydrogenase activity and other enzymological analyses are rarely performed. The basic idea of this report is the evaluation of another biochemical possibility with the aim to understand the nature of hematological disorders by the use a large spectrum of enzyme analyses, which are present in different blood cells and with direct reflection on the serum enzyme activities.

*Key words:* hematological diseases, enzymological examination, pyridoxal-phosphate, cobalamin, folic acid

**Introduction**

Hematopoiesis is a process of blood cell production, differentiation and development. In childhood the total blood volume is about 3,5–4,5 L. Almost half of this volume refers to blood cells – erythrocytes, leukocytes and platelets.

Normal human erythrocytes are small (6 to 8  $\mu\text{m}$ ) biconcave disk. They have no nuclei, mitochondria, endoplasmatic reticulum or other organelles. The biconcave shape of erythrocytes allows for maximum surface area and greatest flexibility. The cell is soft and pliable and can therefore easily move through tissue capillaries and in splenic microcirculation. As the erythrocytes move through circulation their shape changes. Hemoglobin is the principal protein in erythrocytes, in quantitative and functional aspect. The blood

red color is caused by hemoglobin content in erythrocytes.

Biological membranes separate cells from their external environment and divide the interior of the cell into compartment. The plasma membrane of erythrocytes is composed of lipid bilayer in which some proteins (intrinsic) are imersed while the other proteins (extrinsic) are loosely attached to the surface of the membrane. Each layer of lipid bilayer has a different composition with respect to individual glycerophospholipids and sphingolipids. Cholesterol is an integral part of plasma membrane.

The membrane has two principal glycoproteins, glycophorin and band 3 protein a, the protein of the anion channel. Glycophorin is a glycoprotein that contains 131 amino acids but whose function is unknown. Band 3 contains over 900 amino acids and is involved in interacting with ankyrin and possibly in the facilitated diffusion of  $\text{Cl}^-$  and  $\text{HCO}_3^-$ . These are the only integral membrane proteins that are exposed to the

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outer surface of the membrane and they provide the erythrocytes with a complex carbohydrate surface. The integral membrane proteins-spectrin, ankyrin and actin – are exposed to the cytoplasmic side of the membrane and form the cytoskeleton of the cell (1).

Hemoglobin is synthesized during most of the erythrocytic maturation process. About 65% of hemoglobin is synthesized in the nucleus before it is extruded and the remaining 35% is synthesized in the early reticulocyte

Globin synthesis occurred on ribosome according to the process of protein synthesis. Production of polypeptide chains in hemoglobin molecule is under genetic control. At least five genetic loci direct globin synthesis. Chromosome 11 (non-alpha chain type) and 16 (alpha type) contain gene loci for globin synthesis. Globin synthesis is highly coordinated with heme synthesis.

Heme synthesis occurs in most body cells except in mature erythrocytes. Heme synthesis begins with condensation succinyl-CoA and glycine in mitochondria in the presence of pyridoxal phosphate under the influence of enzyme amino levulinat synthase (ALA synthetase) which is the key regulatory enzyme in this metabolic pathway. Following the formation of delta-ALA in mitochondria the synthesis continues in the cytoplasm. Two molecules of  $\delta$ -amino levulinic acid condense to form the monopyrrole porphobilinogen (PBG). Four molecule of PBG condense into a cyclic tetrapyrrole to form uroporphyrinogen type III or I. Type III isomer is converted through coproporphyrinogen III and protoporphyrinogen to protoporphyrin. In this conversion pyridoxal phosphate participates. The final step occurring in mitochondria involve the formation of protoporphyrin and incorporation of iron by the action of ferrochelatase (or heme synthase) to form heme (2). This step completes the formation of heme, a colored compound consisting of four pyrrole rings connected by the methene bridges into a larger tetrapyrrole structure with ferrous ions ( $Fe^{2+}$ ) in the centre.

The major function of the hemoglobin molecule is a reversible transport of oxygen from lungs to tissue and carbon dioxide from tissues to lungs. Hemoglobin of erythrocytes in arterial blood when passing from the lungs to the peripheral tissues is about 96% saturated with oxygen. In the venous blood returning to the heart, hemoglobin is only about 64% saturated. Thus, blood passing through a tissue releases about one third of oxygen it carries.

The transport function of hemoglobin also includes support to carbon dioxide, transport from the tissues to the lungs.

### Metabolic characteristics of erythrocytes

Hemoglobin is a predominant, red color, protein in erythrocytes. The erythrocytes are more limited

in metabolic activity than are other body cells. Energy production is mainly glycolytic. In erythrocytes 90% of glucose is metabolised by anaerobic glycolysis to lactic acid with the net production of two moles of ATP. ATP is necessary for energy-requiring reactions in the cell: for active transport (cation and anions) across the membrane, for maintaining membrane fluidity, and for preservation for cell biconcave shape.

The specificity of erythrocyte glycolysis is the formation of 2,3-diphosphoglycerate (2,3-DPG). 2,3-DPG combines with beta chains of hemoglobin and decreases the affinity of hemoglobin for oxygen. At a given partial pressure of oxygen, therefore, increased 2,3-DPG allows more oxygen to leave hemoglobin and go to the tissue. 10% of glucose is metabolised through the pentose phosphate pathway. Probably, the most important function of hexose-monophosphate pathway (HMP) is the maintenance of glutathione in the reduced state (GSH). In the first two steps, through the activity of glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase generates NADPH + H. Production of NADPH + H is linked to glutathione reduction ( $GS - SG \rightarrow 2 \text{ mol GSH}$ ) and in that way to preservation of vital enzymes and hemoglobin from oxidation. As a coenzyme of methemoglobin reductase NADPH+H prevent oxidation of hemoglobin to methemoglobin; while as a coenzyme of glutathione reductase NADPH+H prevents the increase of hydrogen peroxide and so destruction of cell membrane by the process of lipid peroxidation (3).

The pentose phosphate pathway is in direct relationship with *de novo* biosynthesis of purine nucleotide mostly adenine-containing, through the production of ribose-5 phosphate. The most important enzymes in this metabolic pathway are adenosine deaminase and AMP deaminase (4).

Erythrocytes have high activity of *arginase* an enzyme who catalyses degradation of arginine to urea and ornithin (5). Arginine may be also cleaved by the action of nitric oxide synthase (NOS) into nitric monoxide (NO) and citrulline. The significance of arginase and NOS activities in physiological condition and in some diseases of erythrocytes are now under investigation (6). This metabolic pathway is connected with biosynthesis of polyamines-spermine, spermidine and putrescine-aliphatic nitrogen bases which are important for the function of erythrocytes plasma membrane due to their linking to phospholipides present in the membrane (7–9).

The average life span of normal human erythrocytes is approximately 120 days. Exception for this normal erythrocyte life span occurs in premature infants, whose erythrocytes have a mean life span of only 35 to 50 days and in the fetus where the erythrocytes have an average life span of 60 to 70 days. At the end of this period the aged red blood cells are selectively removed from the circulation and destroyed. Sequestration of old or damaged erythrocytes takes place mainly in phagocyte cells that line the sinusoids

of the spleen, Kupffer liver cells and bone marrow, namely macrophagal cells of reticuloendothelial system. After processes of haemolysis are finished the catabolism of hemoglobin and synthesis of bilirubin begin.

Bilirubin is a product of heme catabolism. Approximately 85% of bilirubin are derived from senescent erythrocytes. Most of the remainder is produced by intracorpuscular degradation of immature erythrocytes in the bone marrow (ineffective erythropoiesis). Very small amount is derived from the degradation of heme-containing enzymes and proteins like myoglobine, catalase, peroxidase, cytochromes, cytochrome oxidase, cytochromes P 450, and NO synthetase (10).

### Erythrocyte diseases

Among the most common diseases of erythrocytes (RBC) are anemias. Anemia may be defined as any condition resulting as significant decrease in total body erythrocytes mass caused tissues hypoxia (11). Three general sings of anemia are: decrease in total hemoglobin concentration, erythrocytes count and hematocrit.

Anemias can be classified by cytometric shemes (i.e., those that depend on cell size and hemoglobin-content parameters, such as MCV and MCHC); erythrokinetic schemes (those that account into the rates of rbc production and destruction), and biochemical/molecular schemes ( those that consider the etiology of anemia at the molecular level). In clinical biochemistry the most useful classification of childhood anemia is in two group: a) nutritive anemias and b) hemolytic anemias.

Nutritive anemias include:

1. iron deficiency anemia
2. vitamin B<sub>12</sub> deficiency
3. folat deficiency
4. vitamin B<sub>6</sub> deficiency

Hemolytic anemias may be a consequence of many different metabolic abnormality:

1. cellular membrane defect – hereditary spherocytosis
2. erythrocytes enzymes defect:
  - a) pyruvate kinase deficiency
  - b) glucose-6-phosphate deficiency
3. Hemoglobin abnormalities – hemoglobinopathies:
  - a) Qualitative hemoglobinopathies
  - b) Quantitative hemoglobinopathies – thalassemias

#### Nutritive anemias

*Iron deficiency anemia* occurs when the body iron stores became inadequate for the needs of normal erythropoiesis (11). Anemia occurs at a late stage

of iron deficiency. In its fully developed form iron-deficient erythropoesys is characterized by hypochromia and microcytosis of he circulating erythrocytes.

A child has 3,5 to 4,5 g of total iron in average. Seventy percent of iron is functional or essential and 30% is stored as nonessential iron. The metabolism of iron is dominated by its role in hemoglobin synthesis.

A typical iron deficiency belongs to hypochromic, microcytic anemias, usually with decreased the red blood cells count. Hemoglobin concentration and hematocrit (Hct or PCV = packed cell volume) are decreased. Biochemical analysis shows marked low serum iron, TIBC (total iron-binding capacity) may be normal or increased, UIBC (unbound iron-binding capacity) is increased, serum ferritin decreased. The amount of serum copper is often lower than are normal values, but in some case it may be normal.

Low RBC count and hemoglobin amount (a consequence of low serum iron) cause hypoxia of body tissue and in acido-base status acidosis may appear. Because of hypoxia in tissues anaerobic metabolism predominates with an increase in lactate dehydrogenase activity and formation and liberation of a greater amount of lactic acid (Lactate) in blood, and in some cases, greater activity of total LDH.

*Laboratory findings.* The degree of anemia is variable and depends on the duration of iron-limited erythropoiesis. Because of hypochromia, the hemoglobin concentration is usually reduced to a greater degree than hematocrit. The plasma iron concentration is generally less than 8,95 g/L and plasma TIBC (transferrin concentration ) is greater than 62 g/L. As a result, the transferrin saturation is less than 15%. The plasma ferritin concentration is generally less than 10 ng per milliliter. During the biosynthetic pathway of heme last enzymatic reaction catalyzed by ferrochelatase need iron. In iron deficiency excess of protoporphyrin IX accumulates in the developing erythrocytes and is retained by the circulating erythrocytes. As a result the free erythrocyte protoporphyrin (FEP) is increased, usually about five times of normal. The osmotic fragility of the erythrocytes may be normal, but more often there is increased resistance to hemolysis in hypotonic salt solution.

*Vitamin B<sub>12</sub> and folic acid deficiency anemia.* Cobalamin (vitamin B<sub>12</sub>) deficiency and folate deficiency are two common cause of megaloblastic anemias. Inadequate amounts of these vitamins in human body cause abnormality in DNA synthesis that lead to a common set of hematological abnormality of cell nuclei that is readily recognizable by microscopic examination. Cobalamin has a certain role as an essential cofactor for only two enzymes in human cells, methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase catalyzes the recycling of homocystein to methionine using 5-methyltetrahydrofolate as a required coenzyme (12).

Folic acid take parts in many enzymatic reactions that are intimately related to the synthesis of DNA and RNA. In folate deficiency all forms of folate are reduced within the cells which impaired the growth and maturation of rapidly growing bone marrow cells.

Metabolic role of cobalamin is in direct relationship with folic acid intracellular functions. In cobalamin deficiency increasing amounts of intercellular folate are converted to 5-methyltetrahydrofolate in an attempt to prevent intracellular methionine deficiency. Thus, cobalamin deficiency results in a secondary intracellular deficiency of all forms of folate except for 5-methyltetrahydrofolate. As a result, the activities of all enzymes that utilize folate to transfer one-carbon moieties, including thymidilate synthase are impaired. This concept of »methylfolate trapping« explains why cobalamin deficiency and folate deficiency produce hematological abnormalities and why these disorders, seen in vitamin B<sub>12</sub> can be completely reversed by pharmacological amounts of folic acid (12).

The specific biochemical methods for proving the inadequate amount of cobalamin and folate in the body base on biochemical places of these vitamins in intermediary metabolism: determination of quantity of L-methylmalonyl acidaemia and methylmalonyl aciduria for cobalamin (13); determination of formiminogulamic acid (Figlu) quantity in blood and urine (18) for folic acid, by using specific electrophoretic methods and measurement of homocystein for both vitamins (13).

*Deficiency of vitamin B<sub>6</sub> (pyridoxine).* Vitamin B<sub>6</sub> (pyridoxine) in active form as a pyridoxal phosphate (B<sub>6</sub>P) appears in many enzymatic reactions during biosynthesis of heme. As a principal compound in amino acid metabolic pathways B<sub>6</sub>P is also important for globin biosyntheses. Literature data mention the appearance of microcytic anemias caused by deficiency of pyridoxine (14).

Pyridoxal phosphate is a coenzyme of transaminase. During *in vitro* supplementation of serum with pyridoxal phosphate the measurement of alanine and aspartate transaminase activities without and in the presence of the excess vitamin amount, may be a useful method in justifying the low quantity of vitamin B<sub>6</sub> in human body.

Pyridoxal phosphate is a coenzyme of kinureninase, the enzyme which participates in catabolism of triptophane. In patients with pyridoxine deficiency as a consequence of the lower activity of kinureninase, there is accumulation of xanthurenic acid in blood and urine. The estimation of xanthurenic acid has been used to detect B<sub>6</sub>P deficiency. Several method are in use, a colorimetric, paper chromatography and fluorimetry as a method of choice (11).

### *Hemolytic anemia*

All hemolytic anemias characterize by premature destruction of erythrocytes (15). A characteristic sign of hemolytic anemias is jaundice as a consequence of indirect hyperbilirubinemia.

With advancing cell age the activities of various red cell enzymes decline and cells become denser and less deformable. Phagocytic cells of the spleen and liver recognize and destroy the oldest erythrocytes. Hemoglobin derived from destroyed red cells catabolized by reticuloendothelial cells to bilirubin which leaves the spleen, enter in the blood and binds to albumin of blood plasma forming prehepatic, unconjugated indirect-reacting bilirubin. The unconjugated bilirubin in the patient, s serum reflects the quantity of heme catabolized and rate at which the liver is able to convert it into direct-reacting, water soluble bilirubin. Serum level of conjugated, direct bilirubin is usually normal in a child with uncomplicated hemolytic disorders, and bilirubinuria does not accrue.

General laboratory signs of anemia are decreased amounts of rbc, low hemoglobin and pcv values.

Hemolytic anemias may be divided to *congenital* or *acquired* (16).

*Congenital hemolytic anemias* appear as a consequence of:

- a) membrane defects
- b) enzyme defects
  - Embden-Meyerhof pathway defects
  - Hexose monophosphate shunt defects
- c) hemoglobin abnormalities
  - defects in globin structure, hemoglobinopathies (qualitative or quantitative)
  - defects in heme synthesis – porphyries.

*Congenital hemolytic anemia as a membrane defects is hereditary spherocytosis (HS).* This disease is an inherited hemolytic anemia characterized by osmotically fragile, partially spherical, spectrin-deficient red blood cell. The primary physiologic defect appears to be membrane instability. Membrane of red blood cell of most HS patients fragment more easily than normal when stressed. Most HS red cells are spectrin deficient and many are also ankyrin deficient. The degree of deficiency correlates closely with the degree of spherocytosis, as measured by osmotic fragility test. As a result bone marrow produces spherocytes, round cell, resulting in faster hemolysis. It is speculated that HS red cells gradually loss portions of the lipid bilayer and become progressively more spherocytic as they age in the circulation (17).

The hallmarks of HS are anemia, jaundice and splenomegaly. Reticulocyte count is always prior increased. The diagnosis is made by the use of osmotic fragility test by which it is demonstrated that spherocytes are more fragile when placed in a hypoosmotic environment than are normal erythrocytes.

The disorder of red cell membrane permeability cause the disbalance between the ration of  $K^+$  and  $Na^+$  in erythrocytes – potassium loss and sodium gain; the results is total cation content and cell water decrease. The measurement of sodim and potasium in erythrocytes is a useful laboratory sign of spherocytosis.

*Enzyme deficiencies.* Glucose, the principal metabolic substrate of the cells, is metabolized in erythrocytes via two major pathways : by anaerobic glycolysis and by pentose-phosphate pathway. Approximately 90 to 95 of glucose converts to lacatae by glycolysis. This is the major pathway of ATP synthesis in circulating red blood cells. Abnormality of most glycolytic enzymes defects caused hemolysis due to the lack of ATP. Laboratory diagnosis confirm the presence of *indirect hyperbilirubinemia* with lack of specific enzyme deficiency (pyruvate kinase, hexokinase, phosphofructo kinase).

*Pyruvate kinase deficiency.* Pyruvate kinase is one of three key regulatory enzymes in glycolysis in which ATP is formed. Pyruvate kinase (PK) deficiency accounts for about 90% of the cases associated with hemolysis. Deficiency of PK causes mild or sever hemolytic anemia which is followed by mild or excess amount of indirect-reacting bilirubin in blood.. Laboratory examination confirm that the level of hemoglobin generally fall in range of 60 to 120 g Hb per liter and packed red cell volume (PCV) of 17 to 37 % PCV=Htc). Serum iron is normal or slightly increased with a normal iron-binding capacity ( TIBC). The osmotic fragility test of fresh red cells is normal, but the incubated fragility test may show varying degrees of abnormality (4).

*Glucose-6-phosphate dehydrogenase deficiency.* Glucose-6-phosphate dehydrogenase (G6PD) deficiency together with PK deficiency is the most common of the known causes of hereditary hemolytic anemias (18). The basic abnormality in G-6-PD deficiency is the decreased of enzyme activity in mature cells. The gene for G6PD is located on X chromosome, so its inheritance is sex linked. Males have one type of G6PD and females have two types.

The red blood cells protect themselves against the oxidative denaturation of their Hb largely through the function of the pentose phosphate pathway. This metabolic pathway is the major source of red cell nicotine-amide-adenine dinucleotide phosphate, a reduced form ( $NADPH + H^+$ ), a coenzyme of glutathione reductase, important enzyme in maintaince adequate amount of reduced glutathione (GSH). GSH protects red blood cells from injury by oxidants, primarily by hydrogen peroxide.

Deficiency of G6PDH activity can be due to decreased production of enzyme molecules, formation of enzyme molecules with decreased catalytic activity, or production of enzyme molecules with reduced stability.

Laboratory diagnosis confirm that the red blood cells count, hemoglobin and hematocrit levels fall rapidly, and a number of reticulocytes in peripheral blood increases. Heinz bodies appear in many of the red cells (18). Indirect hyperbilirubinemia may be mild or sever. The estimation of enzyme activity in red blood cells confirm lower glucose-6-phosphate dehydrogenase activity than in control, healthy childrens.

The diagnosis of G-6-PD deficiency is complicated by the fact that normal red cells coexist in the circulation with deficient cells. Thus, enzyme activity in the whole blood may be only modestly reduced in spite of the fact that severely deficient erythrocytes are present. Detection of G-6-PD deficiency is also difficult in the immediate post-hemolytic period in a child with G-6-PD. During hemolysis, the older members of the red cells population are destroyed, leaving the relatively enzyme-rich young red cells in the circulation (19).

*Disorders in glutathione metabolism.* Almost all the nonprotein sulfhydryl groups of erythrocytes are in the form of glutathione. In the steady state about 99,8% of the glutathione is in the reduced forms (GSH). The maintenance of glutathione in the reduced state (GSH) is probably the most important function of hexose-monophosphate pathway (HMP).

Abnormalities of GSH metabolism, the first line of defense against oxidants can also be associated with hemolysis. Clinically, the disorders in activities of enzymes participating in glutathione metabolism are similar to G6PD deficiency; they are characterized by mild to moderate hemolytic anemia.

Defects in either glutathione synthetase or  $\gamma$ - glutamyl-cysteine synthetase, the two enzyme responsible for the synthesis of GSH, leads to hemolytic anemias (18). Activities of NADP dehydrogenases from pentose phosphate pathway are in direct connection with glutathione metabolism participating in the activity of glutathione reductase ( $GS-SG + NADPH + H^+ \rightarrow 2 GSH + NADP$ ). Glutathione peroxidase uses GSH to decompose hydrogen peroxid ( $2GSH + H_2O_2 \rightarrow GS-SG + 2 H_2O$ ).

*Disorders of hemoglobin biosynthesis.* Disorders in the syntheses of hemoglobin may appears as a disorder in globin synthesis or in heme synthesis. All disorders may be either inherited, congenital and acquired. Inherited disorders are frequently present. Congenital alteration in globin synthesis results in the appearance of pathological hemoglobins in the form of hemoglobinopathies (qualitative or quantitative). Congenital disorders in some enzyme activity on the pathway of heme synthesis result in the appearance of diseases in the form as hepatic or erythropoetic porphyries. Porphyrries of various types demonstrate higher excretion of ALA and PBG in urine as a result of greater activity of enzyme ALA synthase.

*In qualitative hemoglobinopathies* the structure of one type of the four types of formed polypeptide

chains is abnormal; the genetic defect may be due to substitution of one amino acid for another. In clinically significant diseases either the beta chain or the alpha chains are affected. Depending on the type of amino acid and site involved hemoglobin may be functionally abnormal and have altered chemical and physical properties.

Sickle cell disease is a hemoglobinopathy of beta chain: glutamic acid of beta chains in position sixth is substitute with valin (HbS =  $\alpha_2\beta_2^{6\text{Glu} \rightarrow \text{Val}}$  + Heme). Sickle-cell anemia is the prime example of a molecular disease (20). A single amino acid substitution in the hemoglobin molecule leads to severe disease in homozygous individuals. Hemoglobin S has the peculiar characteristic of expressing its biochemical instability by precipitating out of solution and forming up into long microtubular arrays called tactoids. The erythrocytes which contain the HbS stretch around the tactoids to form the characteristic long, pointed, slightly curved cells called (with somewhat liberal imagination) sickle cells. Only the deoxygenated form of HbS (deoxy-HbS) makes tactoids and have characteristic of easy hemolysis. Since Hb S is  $\beta$  chain mutation the diseases does not manifest until six months of age. HbC does not form tactoids but intracellular blunt ended crystalloids. The result is a decreased RBC survival time; however, hemolysis is not as severe as in sickle cell disease.

*Quantitative hemoglobinopathies (thalassemias)* comprise a group of disorders which result from an inherited abnormality of globin production; globin chains of normal structure are formed but the rate of production of one type of polypeptide chains is diminished.

Beta thalassemia refers to decreased production of beta chains; therefore HbF ( $\alpha_2\gamma_2$ ) or HbA<sub>2</sub> ( $\alpha_2\beta_2$ ) would be expected to be relatively increased with respect to HbA<sub>1</sub> ( $\alpha_2\beta_2$ ). The level of fetal hemoglobin increases ranging from less than 10 percent to over 90 percent. Affected children are well at birth. Anemia is usually noticed during the first few months of life and becomes progressively severe. Hemoglobin levels may be in the 20 to 30 g/L range. The reticulocyte count is moderately elevated. Iron kinetic studies indicate a gross degree of ineffective erythropoiesis, the red cell survival is usually shortened and in blood appears higher level of indirect-reacting bilirubin.

Alpha thalassemia refers to decreased production of alpha chains. Unlike  $\beta$  thalassemia  $\alpha$  thalassemia is present even before birth since the  $\alpha$  chain is integral to all hemoglobins. HbA<sub>1</sub> ( $\alpha_2\beta_2$ ), HbA<sub>2</sub> ( $\alpha_2\beta_2$ ), and HbF ( $\alpha_2\gamma_2$ ) are proportionally decreased. The major clinical disorders resulting from alpha thalassemia are the hemoglobin Bart's ( $\gamma_4$ ) hydrops fetalis syndrome and hemoglobin H disease ( $\beta_4$ ).

## Leukocytes

On the basis of function, leukocytes can be divided into granulocytic, monocytic and lymphoid series. The function of the entire leukocytic system is to defend the body against disease, with each type of leukocyte having a unique function. Lymphocytes are the blood cells primarily concerned with antigen recognition and antibody production.

Granulocytic leukocytes can be subdivided on the basis of morphology into neutrophilic, eosiphils and basophis. The major function of the granulocytes is a body defense mechanism. Phagocytosis is an active process that requires a large expenditure of energy by the cells. The required energy is primarily provided by anaerobic glycolysis in which lactate dehydrogenase participate. The vacuole formed during the engulfment process fuses with one or more lysosomal granules that contain various lytic enzymes like lysosomal hydrolases, lysosime and myeloperoxidase (11).

### *Enzymological characteristics of leukocytes*

*Acid phosphatase* is one of many acid hydrolases that have demonstrated in lysosomes. This enzyme takes part in intracellular digestive processes. Most leukocytes exhibit a positive acid phosphatase reaction to varying degrees. Monocytes demonstrates a more intensive positive reaction than neutrophils. Lymphocytes display less acide phosphatase activity than other leukocytes (11).

All species of leukocytes have *alkaline phosphatase* activity. Leukocyte alkaline phosphatase activity can be increased, normal or decreased in a variety of conditions. The determination of alkaline phosphatase activity is frequently used to distinguish between leukemoid reactions and chronic granulocytic (myelogenous) leukemia.

Esterases are a very diverse group of enzymes. Several types of esterase enzyme reactions can be used in leukocytes to differentiate neutrophilic granulocytes and earlier forms from monocytic cell lines.

*Alpha-naphthyl acetate esterase* activity is detected primarily in monocytes and is almost absent in granulocytes. Lymphocytes may occasionally exhibit some activity. Determination of this enzymes in leukocytes may be used to distinguish cells of granulocytic series from cells of monocytic series. This is particularly useful in the differentiation of leukemias.

*Chloracetate esterase* is usually considered specific for cells of granulocytic lineage. Activity is weak or absent in monocytes and lymphocytes. Under defined conditions determination of chloroacetate esterase activity provides a means to distinguish cells of granulocytic series from cells of monocytic series. *Butyrate esterase* and *beta-glucuronidase* are specific lymphocytic enzymes.

Peroxidase is a specific enzyme of granulocytes (neutrophiles, eosinophiles and basophiles), and monocytes but lymphocytes do not show peroxidase activity. *Myeloperoxidase* is located in the primary, azurophilic granules. However, primitive blasts that are committed to the myeloid cell line demonstrate myeloperoxidase activity in areas such as the endoplasmic reticulum and Golgi region. Cells of the myelogenous series exhibit positive reactions that intensify as the cells mature, whereas cells in monocytic cell line display a less intense positive reaction (by steaning) that is characterised by fine granular deposits scattered throughout the cell. Other cell types demonstrate negative reactions. For all of these enzymes there are cytochemical staining methods.

*Lactate dehydrogenase* (specific enzyme for anaerobic glycolysis) may be higher in some leukocytes diseases.

*Arginase*, the key enzyme of the urea-ornithine cycle, catalyzes the cleavage of L-arginine to ornithine and urea. It was reported that leukocytes possess arginase activity. Granulocytes, as opposed to mononuclear or blast cells, contribute primarily to the total arginase activity of leukocytes. The physiological function of arginase of normal leukocytes is uncertain (21).

*Cytidine deaminase* catalyses the deamination of cytidine to uridine, thus allowing metabolic conversion of cytidine to any pyrimidine ribo or deoxyribonucleotide. It also deaminates deoxycytidine to deoxyuridine (22, 23). Chabner et al. (24) have reported that cytidine deaminase activity in mature granulocytes was greater than the activity found in immature cells.

Leukocytes have nucleus and active endoplasmic reticulum.

Alkaline ribonuclease and acid ribonuclease participate in degradation of foreign ribonucleic acid and also in maintenance of total amount of RNA in these blood cells (25–27).

Leukocytes, like all metabolic active cells have great amount of polyamines. *Polyamine oxidase (PAO)* catalyzes oxidative cleavage of polyamine spermine and spermidine producing  $H_2O_2$ , corresponding amino aldehydes and malondialdehyde (MDA). Granulocytic series of leukocytes are very rich in this enzyme (28).

#### *Diseases of blood white cells*

Disorders of blood white cells, leukocytes, may be nonmalignant and malignant. Nonmalignant disorders may be quantitative (leukocytosis or leukocytopenia), morphological abnormalities (toxic granulation,

hypersegmentation of mature granulocytes, binuclear lymphocytes, etc) and qualitative disorders (defective locomotion and hemotaxis, myeloperoxidase deficiency etc). Among malignant leukocytes disorders leukemias are most frequent diseases. Leukemias are neoplastic proliferative diseases that are characterized by an overproduction of immature or mature cells of various leukocytes types in the bone marrow and/or blood. The maturity of affected cells, the total leukocyte count and clinical symptoms determine whether a leukemia will be classified as an acute or chronic disease. Acute leukemias are characterized in an elevated total leukocyte count and by the presence of blasts and immature leukocytes forms in the peripheral blood and bone marrow.

Chronic leukemias characterize have total leukocyte counts that range from extremely elevated to less than normal and with mostly mature cell forms in the peripheral blood or bone marrow. Laboratory analyses of leukemias begin with careful examination of a peripheral blood smear and bone marrow stained. Additional information regarding various leukemias can be gained through electron microscope observation, cytogenetics analysis of chromosomes and cytochemical staining. Special cytochemical stains are a frequently used supplementary source of information in the identification of malignant white cell diseases (11). These stains reflect the biochemical composition of cells. Parallel with cytochemical staining a biochemical examination concerning the examination of specific enzyme analysis depending of the data of peripheral blood smear can be done. Among the classical biochemical determination of alkaline phosphatase, acid phosphatase, lactate dehydrogenase, aldolase, gamma-glutamyl transpherase, also some specific enzyme analyses may be done. This biochemical examination may be done in serum or in leukocytes after their separation from total blood. It is known that specific classes of leukocytes, especially their young, blast forms, possess characteristic enzyme properties. Leading by the characteristic of peripheral blood smear, in biochemical laboratory additional specific enzymatic examinations may be done, like peroxidase, myeloperoxidase, catalase, superoxide desaminase, esterase (with specific substrates), arginase, cytidine deaminase, polyamine oxidase, alkaline ribonuclease, acid ribonuclease. This enzymatic assay may be useful as examination of serum, total leukocytes or some specific classes of leukocytes after their separation from the blood.

Practical hematological analysis, together with the mentioned biochemical possibilities, may be very useful medical clinical diagnosis and prognosis at the present time.

## BIOHEMIJSKA DIJAGNOSTIKA HEMATOLOŠKIH BOLESTI DEČIJEG UZRASTA

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*Kratak sadržaj:* Hematološke bolesti u dečijem uzrastu jesu veoma česte. Analiza hematološkog statusa kao i standardnih biohemijskih analiza krvi, koje predstavljaju odraz funkcije hematopoeznog sistema, jesu od vitalnog značaja u otkrivanju i prepoznavanju oboljenja. Sva hematološka oboljenja javljaju se bilo zbog poremećaja funkcije hematopoeznog sistema na nivou produkcije, stepena vitalnosti krvnih ćelija i preživljavanja u cirkulaciji što ima odraza na hemogram, kao i biohemijske karakteristike krvne plazme i seruma. Osnovni hematološki pokazatelji postojanja krvnih oboljenja (osim poremećaja koagulacije krvi) jesu: ukupan broj krvnih ćelija (eritrocita, leukocita sa utvrđivanjem leukocitarne formule, ukupan broj trombocita), koncentracija hemoglobina, vrednost hematokrita, brzina sedimentacije eritrocita, osmotska rezistencija eritrocita. Od standardnih biohemijskih analiza kod ovih bolesti određuje se vrednost ukupnog bilirubina uz posebno sagledavanje vrednosti indirektnog bilirubina, nivo gvožđa u serumu, transferina (TIBC), serumskog bakra, ceruloplazmina. Elektroforeza hemoglobina se takođe koristi. Enzimološka ispitivanja, sem katkada merenja ukupne aktivnosti laktat dehidrogenaze, retko se primenjuju. Osnovna ideja ovog prikaza je sagledavanje većih biohemijskih mogućnosti u rasvetljavanju prirode hematoloških poremećaja, prvenstveno kroz širi dijapazon enzimoloških analiza, enzima specifično prisutnih u krvnim ćelijama što ima direktni odraz na enzimsku aktivnost seruma.

*Cljučne reči:* hematološke bolesti, enzimološka ispitivanja, piridoksal-fosfat, kobalamin, folna kiselina

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