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LEAD – A PREANALYTICAL/ANALYTICAL VARIABLE IN CLINICAL CHEMISTRY

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Abstract. *Lead is one of the most studied clinically important metals due its high toxicity and a high number of workers exposed to it. The interest toward Pb is elevated by the fact that children are especially susceptible to lead poisoning. Research regarding lead poisoning requires a complex, multi-disciplinary (clinical medical and clinical chemical) approach. Monitoring human exposure to lead (intake, i.e. poisoning) may be achieved by quantification of Pb in tissues and body fluids. For that reason, a number of accurate and reliable analytical methods for the determination of Pb (analytical/preanalytical variable) were developed. An objective of this review paper is to provide key information necessary for proper interpretation of results of lead related clinical/laboratory tests.*

Key words: *lead, toxicity, maximum allowed concentration, clinical chemistry, work ability estimation*

1. INTRODUCTION

Lead (Lat. *Plumbum*, Pb) or Saturn (as referred to by alchemists) is a soft gray-blue metal. Lead has had widespread use in trade and industry since the ancient times due to its stainless properties and pliability. Ancient Greeks and Romans used lead alloys for the production of plumbing systems, household dishes, in shipbuilding, in making wine grails, *etc*; some lead compounds were used in pharmacy, while others, due to their vivid coloring, were used as pigments. Lead application was a demonstration of economical power in old times, and lead poisoning was one of the oldest professional hazards. According to some historians, one of the reasons for the downfall of Roman Empire could be sought in high mortality rate and psychological illness and sterility of its citizens; these were, apparently, caused by consumption of lead-containing food, wine, and water. One of the outcomes of industrial revolution (18^{th} century) was automerization of production process; thanks to this, exposure of workers to Pb (and consequentially Pb induced

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poisonings) was significantly reduced. Sterility, abortion, premature delivery and high number of stillborns were some of the consequences of exposure of female workers to lead and its compounds. Mentioned problems were observed even in the case of women whose husbands were involved in lead production. For these reasons, at the end of the 19th century, total female workforce was withdrawn from Pb-based industries.

Despite its well documented and scientifically proven toxicity, lead (40%), its alloys (25%) and compounds (35%) are still widely used in industry. For example, lead oxides are constituents of electric battery plates, some types of rubber, protective coatings, enamels, pigments and glass; some Pb-containing salts are used in agriculture (arsenate is an insecticide), rubber and chemical industries; some organolead compounds (tetraethyl and tetramethyl lead) are anti-knock additives in gasoline; lead alloys are used for the production of letters, pipes, can sheets, cable covers, ammunition *etc*.

Lead evaporation or formation of contaminated dust, which may occur during lead processing and application, might be regarded as sources of professional lead exposure and intoxication. Non-professional risk source is contaminated food, water and air, lead item usage or usage of items coated with paint that contains lead. Larger lead content in agricultural land can be found near industries that process lead, and near traffic roads due to the consumption of gasoline with lead that contains tetraethyl and tetramethyllead. An increased lead content has been observed in plants (vital in the food chain) grown on contaminated soil [1-5].

Nowadays, the cases of acute lead intoxication are relatively rare. However, the amount of atmospheric lead is constantly increasing, mainly due to the exhaust gases from vehicles. It has been shown that the level of Pb in Greenland ice was 0.0005 mg kg^{-1} in 800 AD and has progressed to 0.21 mg kg^{-1} till 1965. Lead is still believed to be a main cause of various medical disorders. For example, it had been shown (in the study of synthesis of hepatic proteins) that laboratory animals with elevated lead load have developed fatty liver condition; some renal diseases and hypertension have been related to mild lead exposure; intake of even relatively small amounts of lead (lead level in whole blood, Pb_B, 0.5-1.0 μmol L^{-1}) seems to be capable to seriously affect mental development and decrease intelligence of pre-school children. Thus, no wonder lead is still one of the most commonly studied toxic metals (chemical features, biochemical effects, and human and environment health issues) [6-9].

Diagnosis of lead poisoning is highly complex. Most frequently, its relays on both clinical symptoms and the results of laboratory test (here, lead is analytical/preanalytical variable). Lead absorption induces changes in endogenous factors. Some factors are rapidly modified, while others are modified gradually, depending on the intoxication intensity and general health status. Needless to say, the latter precede clinical symptoms and offer quantitative information about the level of exposure/absorption and harmful lead effects; therefore, they are relevant for diagnosis, both toxicological-clinical and analytical-toxicological one and should always be considered in conjunction with the patient's history and physical findings, not in isolation. An objective of this review paper is to provide key information necessary for proper interpretation of the results of lead related clinical/laboratory tests.

2. Basic Concepts of Clinical Chemistry

Generally speaking, clinical chemistry is nothing else but qualitative and/or quantitative chemical analysis of highly complex samples (body fluids, cells, tissues *etc.*), which should enable/facilitate medical diagnosis, treatment control and prevention of health disorders. As such, it requires highly multidisciplinary approach and includes the following:

- Comprehensive analytical methodology (separation methods, optical, immuno-chemical, hematological, endocrine, special toxicology methods, instrumental analysis methods, error theory, information systems etc.)
- Clinical-chemical identification in clinical medicine (diagnosis, prognosis, prevention, therapy control etc.), and
- Evaluation of the results of clinical-chemical analysis (identification), with the aim of identifying interference and other types of impact.

Proper clinical and chemical identification is provided by:

- Preanalytical phase, based on the choice of parameters that are to be determined, patient preparation for testing and biological material sampling,
- Analytical phase, which includes the choice of method and analysis process,
- Postanalytical phase, which includes analytical and medical evaluation of the results, and
- Assessment and consulting with a physician [10, 11].

Parameter type that is determined quantitatively is referred to as variable, and each separate result is value or data. Parameter that is directly determined is always called analytical variable. When analytical variable affects other parameters, it is named preanalytical variable. These effects may be uncovered by monitoring differences in the results of an appropriate analysis, regarding appropriate endogenous factor. Experimental results remain unchanged if preanalytical variable has no significant impact on analytical variable [12]. Thus, the results of appropriate chemical tests should be compared to reference values, and not only to those for healthy individuals, but also for individuals with disorders. Reference values in clinical chemistry are the results of quantitative analysis of a substance taken from an individual or a selected group of people chosen on clearly defined criteria with risk factors, certain physiological conditions, disorders, pharmacologically active agents, and so on. Group reference values are frequently required – age, gender, race/nationality *etc*. Certain variables have reference intervals. The concept of reference values has been developed by a specific committee: IFCC (International Federation of Clinical Chemistry). Biological monitoring results are especially estimated (continuous or periodical measurements and study of potentially hazardous substances or their metabolites, or biological effects on tissues, secretions, excretions, exhaled air or other, in order to estimate professional or ecological exposure and health risk), and they are compared to adequate reference values. Reference values are those in use for professionally unexposed population and biologically tolerant values for professionally exposed population [13, 14].

3. Lead: Exposure, Intake and Toxicity

Maximum allowed concentration (MAC) of lead in living and working environment has been established in accordance with standards; their values are as follows:

- working environment air < 0.1 mg m^{-3};
- flowing water < 0.2 mg m^{-3};
- groundwater < 0.06 mg m^{-3};
- residential area atmosphere (mean value) < 0.0007 mg m^{-3};
- soil 5-25 mg kg^{-1};
- human diet 0.1-0.3 mg per day.

Inorganic lead compounds are absorbed by human organism primarily by inhaling air and ingesting food, and penetration through intact skin is negligible. Skin absorption is possible only in case of organic compound mediation, i.e. tetraethyl and tetramethyllead that are easily dissolved in fat. Ingested lead absorption varies from 10-40% and depends on other food ingredients. The intestinal absorption of lead may be modified by certain dietary constituents. Tyrosine, arginine and amino acids with sulfhydryl groups (cystine, cysteine and methionine), together with certain reducing substances (e.g. ascorbic acid, citric acid), increase intestinal lead absorption. Milk, on the contrary, failed to influence intestinal lead absorption to any significant degree. But it is also reported that diet with low vitamin D, iron, zinc and phosphorus along with milk intake increases lead absorption. The effect of bivalent cations such as Ca^{2+}, Fe^{2+}, Zn^{2+} is to decrease intestinal Pb^{2+} absorption. The reasons for this interference are not yet fully explained. Some substances are thought to enhance the solubility of lead, while certain bivalent cations (Ca^{2+}, Fe^{2+}) may well compete with lead for absorptive sites in the intestinal mucosa. Lead compounds convert into soluble lead chlorides under influence of hydrochloride acid of gastric juice, and by bile they are transformed into lead chelates, that are absorbed in intestines. Main elemental lead, lead alloy, and lead compound hazard occurs due to its cumulative toxicity in which Haber's law ($K = C \times T$) is not applicable, where the product of toxic matter concentration (C) and exposure time (T) is constant. In accordance with the toxicity scale (from 0 to 4), lead and its derivatives are highly toxic (level 3), and the effects can be acute and chronic. Lead has hemotoxic, nephrotoxic and neurotoxic impact.

Lead absorption by inhalation and ingestion is the cause of metal deposition in soft tissues (kidney, liver and nervous system), and bones (about 95% of total lead). This leads to hematopoiesis disorder and causes biochemical lesions in many stages of the heme synthesis pathway. Lead toxicity is proportional to its great capacity to make chemical bonds with sulfhydryl protein groups. Enzymes that are involved in heme biosynthesis of are lead-sensitive. Heme biosynthesis is performed in every human tissue cell, with the highest intensity in erythrocyte cell line in bone marrow (85% of the total heme) where hemoglobin is formed. Disorder heme synthesis leads to the accumulation of delta-aminolevulinic acid (δ-ALA) which creates free radicals by autoxidation.

Nephrotoxic lead effects, lead nephropathy, occur in professional exposure. Chronic irreversible nephropathy may be induced by repeated acute poisoning with high lead concentration or long-term exposure (lead concentration in blood 3.38 μmol L^{-1} over a period of 10 years). These effects are characterized by progressive loss of renal function, often followed by hypertension. The impairment mechanism is not completely understood, but the presence of hypertension may be explained with renal disorder and spasmogenic lead effect on the smooth muscles of blood vessels.

Changes in the nervous system (central, peripheral and autonomous) arise in the presence of Pb_B concentration of 0.8-1.2 mg L^{-1}.

Chronic lead poisoning (saturnism) is important from the toxicological point of view. Lead poisoning occurs due to the inability of an organism to compensate for the increased absorption. Many pathological conditions influence the biological capacity and absorption, thus increasing the risk of poisoning. These include: infectious diseases, acute and chronic bronchitis, anemia, nervous system anomalies, gastrointestinal system effects, personal hygiene level, *etc*.

Firstly, lead poisoning safety aim is to prevent lead vapor inhalation by applying technical prevention measures in industries, and secondly, to prevent lead intake via contaminated food and water. The role of health protection is to prevent diseases, which is conducted by occupational medicine via the following:

- medical examination prior to employing workers (screening for alcohol addicts or individuals who suffer from mental illnesses or hypotension, in order to prevent their professional engagement on locations with possible Pb contact),
- periodical medical examination for each employee (in order to evaluate physical and emotional reactions in general, reactions to the environment), and in particular
- targeted medical examination of employees under special surveillance, such as workers recuperated from lead poisoning or patients recovering from certain diseases.

In case of identifying a disease, clinical testing and monitoring is required. The role of clinical laboratory is to assess each requirement (hematological, toxicological, biochemical) in accordance with analytical parameters determined in human samples (blood, serum, plasma, urine, etc.), chosen by a clinical toxicologist [15-18].

4. Lead in Human Material: Reference Values

Reference values for Pb in various biological (human) samples are as follows:

- hair and nails < 29 μg g^{-1};
- skin (tetraethyl- and tetramethyllead) < 0.005 mg m^{-3};
- bones < 70 μg g^{-1} wet mass;
- urine < 50 μg g^{-1} creatinine;
- urine (biological value) < 150 μg g^{-1} creatinine;
- urine (Pb-elemental and inorganic compounds) < 0.39 μmol L^{-1};
- urine (tetraethyl-lead exposure) < 0.53 μmol L^{-1};
- urine (biological value, Pb-inorganic) < 0.63 μmol L^{-1};
- whole blood in children < 1.21 μmol L^{-1};
- whole blood in adults < 1.93 μmol L^{-1};
- whole blood (Pb-elemental, and inorganic compounds) < 2.88 μmol L^{-1};
- whole blood (biological value, Pb-inorganic at the end of shift) < 3.38 μmol L^{-1};
- whole blood (toxic effects) ≥ 4.83 μmol L^{-1};
- stable lead isotope distribution in whole blood: 204 (1.4%), 206 (24.1%), 207 (22.1%) and 208 (52.3%), and
- mobilized by chelates: 600 μg per 48 h.

The amount of lead higher than appropriate reference value in certain biological sample (most commonly in whole blood (Pb_B) and urine (Pb_U)) is biological marker for lead exposure.

As already mentioned, the main entry point of lead is the respiratory system. Certain amount of lead can be absorbed at this level, but greater amount is absorbed by the

pulmonary blood circulation and transported to organs and tissues. Lead distribution to tissue is the result of concentration gradient and specific tissue affinity for lead. About 95% of the total lead load in a human organism is accumulated in bone tissue where it partially replaces calcium, out of which the greater part (3/4 of the total lead amount in the bones) is unchangeable, irreversible fraction, and the remaining part of 1/4 is changeable, reversible fraction that can be redistributed by modifying pH, chelates, alcohol, trauma and so on. Lead concentration in bones increases due to aging, especially in male individuals. Significantly lower amount of lead is accumulated in soft tissue: liver (1.4%), kidney (0.9%), lungs, aorta, grey matter of the cortex and basal ganglia. About 95% of the total amount of lead in blood is attached to erythrocytes out of which 90% attaches to hemoglobin, especially to its γ–chain. Lead bound in erythrocytes does not diffuse easily. Lead in ionized state (about 0.3 to 0.4% of lead in blood) is responsible for toxic effects. Total lead amount in whole blood is 2% of the total lead amount in the body. Biological half life of lead in blood is about 20 days, in soft tissues and irreversible fraction about 30-40 days, and in irreversible fraction in bones about 20-30 years. Lead passes through the placental membrane and can be found in the fetus (12 to 14 weeks after gestation with similar distribution as in adults).

Lead is excreted via kidneys, primarily glomerular filtration (75-80%), and by the gastrointestinal system (about 16%). In a balanced state, the amount of lead excreted by urine reflects the amount of lead in blood, and can be used as a biomarker in assessing exposure. Lead elimination by the gastrointestinal system is performed either by active secretion or passive loss through the appropriate glands (salivary glands, pancreas, intestinal glands), by exfoliation and by bile. One portion of lead is reabsorbed and submitted to the enterohepatic circulation. The total remaining amount of secreted, unabsorbed and reabsorbed lead is discharged through feces. Lead content in feces of 4 mg per 100 g after a four-week lead exposure proves continuous lead intake by food. Other elimination pathways (hair, nails, sweat, milk, teeth, sperm and menstrual bleeding) constitute less percentage (about 8%) and less significance, except for the secretion by breast milk which poses a risk for the newborn [15, 19, 20].

Pb_B concentration in a balanced state is best shown in soft tissue Pb content (in case of recent exposure), and it is not correlated with total lead load in the organism. Pb_U concentration test is a very good indicator of the level of organic lead exposure and increased lead absorption. The results of this test are important for monitoring of $Na_2CaEDTA$ therapy that is used in case of chronic intoxication in order to accelerate lead elimination. $Na_2CaEDTA$ is distributed mostly in extracellular medium, where ionic exchange of Pb with Ca occurs. Newly formed complex Pb-EDTA is soluble, weakly dissociated and is excreted by glomerular filtration. Formation of complex with lead bonded to cells is considerably slower. Application of chelates in prevention of lead intoxication is not indicated, while in lead alkyls poisoning some insignificant improvements are achieved. Calcium gluconate, barbiturates, mannitol and BAL (dimercaptol) can also be used in lead elimination therapy. Pb_U concentration is not significant as an early test for inorganic lead exposure. It is important to note that a 24-hour aliquot urine sample should be used when determining Pb_U content, without insisting on the numerical value of the volume. Correlation can be established between lead and endogenous factors excreted in a 24-hour urine, on the one hand, and clinical symptoms related to diuresis, on the other.

Determination of microconcentration of Pb_B, Pb_U and Pb in hair or nails requires the following methods: spectrophotometry of colored complexes with dithizone (diphenylthiocarbazone) with previous sample mineralization (the method is adequate in terms of sensitivity and precision; the disadvantage is time-consuming analysis, chemical and solution purification, and easily contaminable samples), polarographic analysis (–0.25 to –0.60 V) of previously mineralized sample and lead sedimentation (higher operating speed compared to dithizone, but requires complete mineralization of sample). Electrothermal-atomic absorption spectrophotometry (ET-AAS) and flame-atomic absorption spectrophotometry (AAS) are also used. Combined method of inductively coupled plasma/mass spectrophotometry (ICP/MS) is used for the determination of stable lead isotope content and distribution in blood and urine [18, 19, 21, 22].

Level of organism failure cannot be determined based on lead content tests that serve as isolated indicators. Parameters listed above provide direct and reliable indication on lead exposure and absorption; however, they offer only indirect information about possible toxic effects on organism in general [20, 23-25].

5. (INDIRECT) MARKERS OF LEAD POISONING

Changes in the activity of δ-aminolevulinic acid dehydratase (D-δALA) or porphobilinogen (PBG) synthase, or in levels of free erythrocyte protoporphyrin (FEP), zinc-protoporphyrin (Zn-PP), pyrimidine-5'-nucleotidase, δ-ALA, uroporphyrin (UP) and coproporphyrin (CP) may be used as indirect biological markers of lead poisoning/intake. Reference levels/intervals for these are as follows:

- D-δALA in erythrocytes > 200 nmol PBG mg^{-1} HGB min^{-1};
- D-δALA in erythrocytes = 139-211 kU L^{-1};
- δ-ALA in serum = 1.14-1.75 μmol L^{-1};
- δ-ALA in urine = 9.9-53.4 μmol per day;
- δ-ALA in urine (biological value, Pb-inorganic) < 76.3 μmol L^{-1};
- CP in urine = 51-351 nmol per day;
- CP in urine (male) < 368 nmol per day;
- CP in urine (female) < 336 nmol per day;
- PBG in urine < 8.8 μmol per day;
- UP in urine < 50 μg per day;
- FEP < 0.4-1.6 μmol L^{-1} erythrocytes.

Various reference intervals for FEP might be expressed in various ways: as porphyrine level per dl of whole blood, dl of erythrocytes, g of hemoglobin, or per mol of heme.

It seems that the relative amount of lead in blood sufficient to inhibit almost every enzyme involved in heme biosynthesis (especially D-δALA and ferrochelatase (heme synthetase)) is Pb_B 0.4 mg L^{-1} (1.93 μmol L^{-1}). As the result of the inhibition of mentioned enzymes, δ–ALA and PBG amounts in urine are elevated (low renal threshold causing their low blood concentration levels), along with CP in urine and feces, and uroporphyrin (UP) in urine.

The most sensitive test for early lead exposure is based on its inhibition of D-δALA enzyme in erythrocytes. Negative correlation between D-δALA and Pb_B was found even in city residents exposed to very low lead concentrations; it appears that even the lowest

possible concentration of Pb_B can have an impact on the enzyme. Its activity was reduced by 50% at Pb_B 0.77 μmol L^{-1}. Spontaneous reactivation of D-δALA inhibited by lead was very slow. Results of the analysis can prove increased lead exposure in the past. This enzyme can also be inhibited by tetraethyllead. D-δALA activity is measured by determination of the amount of generated PBG in a specific period of time. The study of D-δALA enzyme activity is performed by a standardized internationally verified and accepted enzyme-spectrophotometry method applicable in inorganic lead exposure control (biomonitoring). Interpretation of the result has to be thorough because both acute and chronic alcoholism and smoking have a significant impact on the reduction of activity of D-δALA [26-29].

Physiological increases of δALA in serum and urine, and CP (ether-extracted coproporphyrin III) in urine are in correlation with the increase of lead content in blood:

- $Pb_B < 0.678$ μmol L^{-1},
  $\delta\text{-}ALA_S = 0.328 \pm 0.025$ μmol L^{-1} and
  $\delta\text{-}ALA_U = 0.8 \pm 0.2$ mg g^{-1} creatinine;
- $Pb_B = 1.207\text{-}1.641$ μmol L^{-1},
  $\delta\text{-}ALA_S = 0.403 \pm 0.090$ μmol L^{-1} and
  $\delta\text{-}ALA_U = 0.9 \pm 0.4$ mg g^{-1} creatinine, and
- $Pb_B > 2.65$ μmol L^{-1},
  $\delta\text{-}ALA_S = 1.117 \pm 0.565$ μmol $L^{-1}$and
  $\delta\text{-}ALA_U = 4.5 \pm 2.9$ mg g^{-1} creatinine.

δALA evaluation in poisoning is a more specific and sensitive test than coproporphyrin quantification, because coproporphyrin is the most commonly excreted porphyrin during secondary porphyrinopathy. Hexachlorobenzene, alcohol, sedatives, hypnotics and certain diseases, such as liver disease, myocardial infarction, tyrosinemia and so on, can also be the cause of coproporphyrinuria. Quantitative determination of δ- ALA, PGB, CP and UP in urine might be achieved by spectrophotometric analysis of the purified (ion exchange/ chromatography/extraction with diethyl ether) sample [21, 24].

Metabolism disorders related to erythrocytes include inhibition of glucose-6-phosphat-dehydrogenase, phosphofructokinase, arginase, nicotinamide-adenine-dinucleotide synthetase and Na/K adenosine-triphosphatase. ATPase inhibition impairs the function of ionic pump in maintaining ionic gradient and leads to ATP energy accumulation; its increase inhibits the activity of PBG-synthase, and the accumulated δ–ALA creates free radicals by autoxidation. Stimulation of lipid peroxidation dependent on iron is present. Concentration of reduced or oxidized glutathione in erythrocytes is lowered and the activity of enzymes glutathione peroxidase, catalase, and superoxide-dismutase decreases.

Lead inhibits iron (Fe) incorporation into heme and competitively excludes copper (Cu) out of ceruloplasmin inhibiting its catalytic role in the process of oxidation Fe(II) into Fe(III). As a result, iron concentration in serum and copper in erythrocytes are increased physiologically. Low selenium (Se) in serum, decrease of mercury (Hg) concentration in urine, and triple cadmium (Cd) increase are also the consequences of lead poisoning. In the concentration of 1 mg L^{-1}, lead does not interfere with the determination of iron in serum [30, 31].

Quantity of protoporphyrin in erythrocytes is a very good indicator of professional lead exposure. D-δALA and FPP activities can be used to observe the intensity (shown by the enzyme) and duration (shown by porphyrin) of lead exposure. Zn-PP is formed during erythropoiesis due to iron deficiency induced by various etymologies: malnutrition, blood

loss, chronic inflammation or chronic lead exposure. A significant increase in free erythrocyte protoporphyrin IX or as zinc-chelate (Zn-PP) is present one to three months after lead exposure, which confirms the level of metal effect on bone marrow. An increase in protoporphyrin in urine has been observed in exposed workers. Before the Zn-PP and hematofluorometry based methods, determination of porphyrin in erythrocytes was done via acid extraction and formation of free protoporphyrin (free erythrocyte protoporphyrin or FEP method). Elevated Zn-PP levels can nowadays be determined directly by means of hematofluorometry. Laboratory results must be interpreted cautiously because other preanalytical variables (iron deficiency and erythropoietic protoporphyria) may cause increase of erythrocyte protoporphyrin [32-34].

Besides inhibition of hemoglobin synthesis, lead may cause shortening of the erythrocyte life. Erythrocyte number in blood (RBC, red blood cell), hemoglobin content (HGB), MCHC (Mean Corpuscular Hemoglobin Concentration), MCV (Mean Corpuscular Volume), and HCT (HematoCriT) are reduced due to hemolytic anemia. Because of stimulated hemolysis (accelerated erythrocyte degradation), the number of reticulocytes is increased, and due to pancytopenia, the number of leucocytes and thrombocytes is decreased. The manifestation of degenerative regeneration of erythrocytes causes an increase in the number of basophilic stippling of erythrocytes (BSE); they are created by the aggregation of partially degraded ribosomes as the consequence of pyrimidine-5'-nucleotidase enzyme inhibition. Anemia is present at Pb_B = 0.8 mg L^{-1} or 3.86 μmol L^{-1} [15, 35-37].

Erythrocyte number in urine is increased due to nephrotoxicity, and hemoglobin content is increased in acute hemolytic crisis. Urobilinogen content in urine is also increased. Red-brown urine color is due to porphyrin and hemoglobin presence. Lead-sulfide poisoning may cause occult bleeding with diarrhea. Bilirubin content in serum is increased due to hemolytic anemia; glucose, alanine and β-aminoazobenzene acid in urine, and urea in serum are increased as well due to nephrotoxicity [37]. In case of lead encephalopathy, protein content in cerebrospinal liquid is increased. The number and motility of spermatozoids are reduced in men who are exposed to large concentrations of lead. Uric acid in serum is increased, but uric acid concentration in urine is reduced due to nephropathy and low secretion by nephrons. Lead impact on creatinine concentration has shown the following results [30, 38-43]:

- serum creatinine - no change
- urine creatinine is increased by 30%, and
- mean value of creatinine clearance is decreased in men and women.

Medium lead exposure (mean concentration Pb_B = 1.9 μmol L^{-1}) showed significantly lower FSH (follicle stimulating hormone) in plasma compared to the control group who showed mean Pb_B = 0.2 μmol L^{-1}. In female workers under 40 who were professionally exposed to lead, low LH (luteinizing hormone) concentrations in plasma were within reference values or without confirmed physiological effects. Experimental data has shown that there is no significant physiological impact of professional exposure on testosterone levels, TSH (thyroid stimulating hormone) and T_3 (triiodothyronine), but in severe exposure, a negative poisoning impact on T_4 (thyroxine) and fT_4 (free, unbound thyroxine) is noted [39, 40, 44].

Lead alkyls do not impact heme biosynthesis; therefore, no increase of porphyrin concentration or their precursors can be found during poisoning. There is no anemia or basophilic stippling. Diagnosis is confirmed by elevated Pb_U concentrations and no other toxic marker findings.

6. LEAD POISONING: CONCLUDING REMARKS

Laboratory tests for lead poisoning status examining, as preanalytic variable can be classified into two groups of identifying the analytic variables: first group - metal content evaluation, and second group - lead-dependent variable evaluation. Analytical processes for limited human sample analysis must be distinctly sensitive, precise and reliable. Procedures must be done with great caution in order to make the differences between reference and experimental values as unbiased as possible, in order to observe, identify and monitor lead-detrimental changes during exposure or treatment. These requirements can be fulfilled only by laboratories with specialized experience and technological capacity for measuring trace metals.

Fully skilled personnel and continuous additional training of employees, analytical procedure and laboratory equipment selection for multidisciplinary analyses for determining lead poisoning status - all depend on education and skill levels for applying wide-range analytic variable tests. Attention should be paid to selection and modernization of methods which can be applied conveniently and beneficially for clinic practitioners. Depending on the number and features of endogenous parameters to be determined, and available laboratory conditions, the time consumed for result acquisition may cover a few minutes to several hours, up to 24 hours; longer period than this is usually of little use to clinicians.

Laboratory search results in analysis and biological marker evaluation for lead exposure and its effects (Pb_B, Pb_U, D-δALA, FEP or Zn-PP, δ-ALA, CP and other), besides patient history and clinical manifestation, should help dictate the classification of worker ability that can be temporarily or permanently lost because of lead exposure or threat.

Long-term disorders of markers suffice to affirm professional lead poisoning in legal manner in accordance with other criteria stated by law. Since their quantitative changes are determined exclusively by applying substantial analytic methodology in laboratories, it is a task and obligation for laboratories to provide accurate clinical-chemical testing by means of correct application of preanalytic, analytic and postanalytic phases. Laboratories should also be responsible for the process of communicating and distributing results [8, 23, 45].

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OLOVO - PREANALITIČKA/ANALITIČKA VARIJABLA U KLINIČKOJ HEMIJI

Olovo spada u red najizučavanijih klinički relevantnih metala zbog svoje toksičnosti i broja radnika izloženih ovom metalu. Povećano interesovanje prema ovom metalu je posledica i toga što su deca naročito podložna trovanju njime. Ispitivanja koja se tiču trovanja olovom zahtevaju kompleksan, multidisciplinaran (kliničko-medicinski i kliničko-hemijski) pristup. Monitoring izlaganja olovu (unos, odnosno trovanje) se može ostvariti kvantifikacijom Pb u tkivima i telesnim tečnostima. Iz tog razloga je razvijen veliki broj preciznih i pouzdanih metoda za njegovo određivanje (analitička/preanalitička varijabla). Cilj ovog preglednog članka je da sumira ključne informacije neophodne za valjanu interpretaciju rezultata kliničkih/laboratorijskih testova vezanih za olovo.

Ključne reči: *olovo, toksičnost, maksimalna dozvoljena koncentracija, klinička hemija, ocena radne spospobnosti*