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Management, Diagnostic and Prognostic Significance of Acetylcholinesterase as a Biomarker of the Toxic Effects of Pesticides in People Occupationally Exposed

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Abstract

AIM: The paper presents research on the most common causes of exposure that leads to disorders of cholinesterase activity, as well as an overview of the results of cholinesterase activity with the poisoned people.

MATERIAL AND METHODS: In a group of 35 acute poisoned patients by organophosphate compounds has led to inhibition of AchE. A total number of examined workers are 175 in the chemical industry and agricultural production in the area of Rasina District-Serbia.

RESULTS: The results showed that among workers who are constantly exposed to pesticides, acetylcholinesterase is within the reference value. Having examined the medical records of these workers, it is noted that, at 72%, there is a slight fall of AchE activity, each year. The workers who had been exposed to pesticides at the time of testing had acetylcholinesterase regarding reference value, but 52% of them had a few years ago significantly reduced the value of the activity of acetylcholinesterase, which was treated and then transferred to other jobs. The 48% of these workers had acetylcholinesterase regarding benchmarks or were transferred to other jobs, for a variety of other health problems.

CONCLUSION: Using each pesticide should only deal with people who are well versed in the way of its use, as well as the way of protecting them from poisoning.

Introduction

Organophosphorus compounds (OPC) inhibit both serum and erythrocyte cholinesterase. According to chemical composition, these are esters of phosphoric acid. Organophosphorus compounds smoothly penetrate the uninjured skin and all mucous membranes. Poisoning can occur at inhalation, contact through is the dermal penetration route and inhalation, and the lower character has a penetration through the digestive tract. Organophosphorus insecticides are causes about 80-90% of all cases of acute pesticide poisoning of the people. Annually, in the world is registered about 100,000 victims of acute organophosphate poisoning. About 20% were accidental poisoning, and suicidal is approximately 70%, with 30% of the cases are with fatal outcome [1-4]. Exposure to organophosphate can cause four clinical syndromes that have different pathogenesis, prognosis and requiring different treatment. They occur after a long latency different intervals after exposure to the organism of a man to organophosphorus compounds. These are: acute cholinergic crisis, intermediate syndrome, delayed peripheral neuropathy and other specific lesions of organs. Symptomatology of acute cholinergic crisis occurs immediately after exposure or to a maximum of 12 hours from the moment of exposure. How is in poisoning by organophosphates the physiological function of the enzyme acetylcholinesterase inhibited, the image of poisoning has the characteristics of intoxication by endogenous acetylcholine. Depending on the degree of inhibition of acetylcholinesterase and increase acetylcholine content, organophosphates lead to stimulation or paralysis transmission of nerve impulses in the central and neuro-effector synapses of the cholinergic nervous. In the clinical picture of poisoning are being distinguished: muscarinic effects (bradycardia, bronchoconstriction, bronchorrhea, hypotension, increased gastrointestinal motility, abdominal cramps, miosis, hypersalivation), nicotinic effects (hypertension, tachycardia, fasciculation, skeletal muscle necrosis) and the central toxic phenomena (tremor, incoordination movements, convulsions, central respiratory depression, coma and death) [5, 9-11]. The therapy of organophosphate poisoning includes aspiration of the upper respiratory tracts, cardiopulmonary reanimation, gastric lavage, provision of specific drugs and antidotes. In the context of specific therapies are delivered as atropine, as an antagonist of a muscarinic receptor and oximes as inhibited cholinesterase reactivators. Conventional therapy involves the use of benzodiazepines, which are effective anticonvulsants [1, 5-7].

Organophosphate compounds easily penetrate into the body through the skin and of all lining. These compounds then react with an acetylcholinesterase to form a complex of enzyme phosphate. The phosphorylated enzyme is very stable due to the phosphorus atom, which is not electrophilic enough to react in the presence of water, so the spontaneous reactivation does not take place or is very slow, that is, inhibition of acetylcholinesterase is practically irreversible. Therefore, there is a buildup of acetylcholine at the synapses of nerve endings and to the clinical picture of toxic effects of endogenous acetylcholine.

In this study, on workers of occupationally exposed pesticides, will be monitored biochemical and hematological parameters in evaluating the impact of pesticides on the health of exposed workers: identify the toxic effects of pesticides using appropriate standardized biochemical and hematological parameters; the activities of some specific-organ enzymes; and determine the cholinesterase activity as a biomarker of toxicity of organophosphorus compounds; and assess the importance of changes in cholinesterase activity under the influence of pesticides from the group of organophosphorus compounds, as an indicator of the degree of toxicity in exposed workers.

All the above parameters can be determined in some biochemical laboratories and for their determination is using the standard equipment. This means that they can be monitored with any systematic review which is regularly subjected to workers in occupational exposure. It is planned, therefore, that from the obtained results, by the made findings, the device protocols for monitoring the early toxic effects of pesticides when still there was no visible damage to health. Thus prepared protocols can be used in medicine work within the healthcare of the workers, with the aim of helping the employees to be timely moved from those jobs to which they are endangered, so as not to cause lasting impairment of their health and working ability.

Material and Methods

A total number of examined workers are 175 in the chemical industry and agricultural production in the area of Rasina District. Of these, the two control groups were 47 workers who were not exposed to pesticides. Other groups consisted the workers who are (or previously were) exposed to pesticides. And that: 78 workers in the chemical industry who are working in the production process of pesticides in the plant of the "Pesticides" and 50 workers working in agriculture. Workers of the "Pesticides" plant were divided into three groups according to the length and duration of occupational exposure:

Industrial workers who are constantly exposed to pesticides (N = 42);

Industrial workers who are occasionally exposed to pesticides (N = 17);

Industrial workers who have previously been exposed to pesticides, and then were transferred to other jobs (N = 19);

A control group of industrial workers (CGI, N24) consisting of 24 industrial workers who are not exposed to pesticides;

Agricultural workers who are in the process of plant protection continually exposed to pesticides (N = 50). 35 of them were acutely poisoned OPC, 40 days after the systematic review, so we get the Va subgroup; and A control group of workers in agriculture (CGa, N = 23) comprised of 23 agricultural workers who are not exposed to pesticides.

Basic data which detail the examined groups of workers are presented in Table **1**. All data were obtained during regular periodic examinations of workers, which mean that they can be monitored in a systematic review of each whom undergoes occupationally exposed workers.

Table 1: Average values of the age, of the total working experience (TWE) and the exposed working experience (EWE) of the observed groups

Group	Average age	TWE	EWE
I (N = 42)	37,4 ± 6,28	15,7 ± 4,1	9,4 ± 4,5
II (N = 17)	38,3 ± 7,7	14,2 ± 5,2	7,9 ± 5,8
III (N = 19)	48,6 ± 6,4	20,3 ± 5,2	15,3 ± 5,6
IV (N = 24)	34,9 ± 8,3	12,3 ± 5,7	9,9 ± 3,4
V (N =50)	35,7 ± 5,9	11,9 ± 2,7	8,2 ± 4,4
VI (N = 23)	36,8± 3,1	14,7 ± 8,4	11,9 ± 7,7

Termination of exposure to pesticides for the III-rd group is 5.45 years, with a range of minimum and maximum values in 1-13 year.

The study made use of standard methods:

- Hemoglobin concentration, the number of erythrocytes, the number of leukocytes, leukocyte formula, thrombosis and reticulocytes were determined by counting blood elements of the company Coulter counter. (Commercial instruction of the device).

- Chemical examination of urine was made for urine test strips of the Lachema company.
- Urine sediment was carried out microscopically.
- Blood glucose concentration was determined by GOD/PAP method.
- The concentration of cholesterol in the blood was determined using cholesterol oxidase with phenol and 4-aminoantipyrine as chromogens.
- The concentration of triglycerides in the blood was determined by glycerol oxidase/PAP without correction in blood and urine was determined by Jaffe's method with alkaline picrate.
- Urea in the blood was determined using urease and hypochlorite.
- Uric acid in blood was determined with an alkali phosphatase
- The concentration of bilirubin in the blood was determined by Jendrassik-Groff's method
- The activity of transaminases in the blood was determined by a discontinuous colourimetric method with 2,3-dinitrophenylhydrazine.
- Alkaline phosphatase activity in the blood was determined with p-nitrophenyl phosphate as a substrate.
- Glutamyltransferase activity in blood was determined by reaction with L γ glutamyl-3-carboxy-p-nitroanilide as a substrate.
- Cholinesterase activity in blood was determined by a kinetic method with Propionaldehyde as substrate and 5,5'ditio-bis-2-nitrobenzol acid (DNTB) which gives a stained reaction with tioholinom.

For the determination of the above parameters were used appliances: Counter of blood elements "Coulter Counter"; Centrifuge “Technics”***;*** Biochemical analyser "Selectra"; Microscope "Carl Zeiss" and Spectrophotometer Gilford 300 T and LKB.

Statistical methods

From the obtained results was calculated the mean value, standard deviation, coefficient of variation and medians. The significance of differences between mean values was tested by Student's t-test and analysis of variance for a probability level of 0.05.

Results

In modern agriculture, as in public health, for the destruction of pests of the plant and animal origin are used various means, all more or less toxic, known by the common name of pesticides. The chemical composition of pesticides is chlorinated hydrocarbons; organophosphorus products; dinitrophenols; nicotine; arsenical preparations; fumigation; organic mercury compounds; coumarin preparations; zinc phosphide; talium sulphate; sodium fluoroacetate; some solvents and others. Many of these are commonly known in the trade under the trade name.

Pesticides are very effective in the fight against plant and animal pests, but those, especially poisonous, are not without danger to the people who apply them. To poisoning usually comes from improper handling of pesticides, and due to an error or accident, but are known cases of deliberate poisoning. Pesticides can penetrate into the body through the digestive tract through the respiratory organs, or through the skin. For many pesticides, poisoning is possible through any of these routes, but one of them is still the most common. Signs of poisoning may, depending on the type of pesticide to be very different. Most of them, more or less, effect to the nervous system and cause a headache, dizziness, paralysis, anxiety and other nervous disorders. Many, however, cause stomach pains, vomiting and diarrhoea (organophosphorus preparations, dinitrophenols, nicotine, arsenic preparations, etc.). Some of them cause choking or increased sweating, salivation, tears, etc. Finally, a large number of pesticides and solvents irritate the skin and mucous membranes with which it comes into contact, causing a variety of changes on them.

Recovering of patients poisoned with organophosphorus compounds (OPC) is a long and slow process. Degradation of acetylcholine occurs only after the synthesis of new quantities of acetylcholinesterase (AchE), which is a small daily percentage. During the treatment, acetylcholinesterase was determined every 72 hours while monitoring the return of the acetylcholinesterase in the range of the reference value. When the enzyme activity is within the reference values and did not show the presence of the OPC is discontinued administration of an antidote. This fact points to the great importance of laboratory testing enzyme activity both in the aspect of treatment and in economic terms. In acute poisoning, the poisoned ones will be temporarily unable to work, until the rehabilitation of clinical manifestations. After complete healing, workers need to be transferred to other jobs until cholinesterase activity reaches a level that it had before exposure (approximately 2-3 months depending on the degree of inhibition). After that, workers will be able to return to their previous jobs with the rehabilitation of working conditions and adequate implementation of protection measures. In cases of poisoning with permanent consequences or recurrent poisoning, the changes are indicated in the workplace [6].

Diagnosis of poisoning organofluorine compounds can be confirmed by determination of acetylcholinesterase activity in the serum. According to data of the Poison Control Center of Serbia in 2011 examined 58 patients due to acute exposure and pesticide poisoning, and the clinic has treated 35 patients. The most common toxic agents were organofluorine insecticides (71%). There were treated approximately an equal number of men and women. Of hospitalised patients without clinical symptoms of poisoning (PSS0) were 14 people (40%). Poisoning light degree (PSS1) is manifested in 6 patients (17%) of moderate severity (PSS2) in 3 patients (9%), and because of severe poisoning (PSS3) were treated nine patients (25%). The lethal outcome of acute poisoning organophosphate insecticides was noted in 2 patients [13].

In 2012, due to acute exposure and pesticide poisoning in OPR CKT, were examined 97 patients (2.3%) of the total number of examined patients, which was significantly higher than in the previous year. At the hospital treatment was received 54 patients, accounting for 6.5% of all hospitalised patients. About gender, there were more men (65%) than women (35%). The most common toxic agents, this year it was organophosphate insecticides (54%). Of hospitalised patients without clinical symptoms of poisoning (PSS0) were 18 people (33%). Poisoning light degree (PSS1) was demonstrated in 15 patients (28%) of moderate severity (PSS2) in 6 patients (11%), and because of severe poisoning (PSS3) were treated ten patients (19%). The lethal outcome occurred in 3 patients who were poisoned by organophosphate insecticides [14].

Compared to 2012 in 2013 was recorded a decline in the number of acute pesticide poisoning (81 patients). At the hospital treatment was received, 38 patients. Were treated 60.5% men and 39.5% women. Of hospitalised patients poisoning light degree (PSS1) is manifested in 22 patients (7.9%), due to a severe poisoning (PSS3) were treated eight patients (21.1%). The lethal outcome occurred in 5 patients [15].

In acute poisoning is usually a correlation between the degree of inhibition of AchE activity and severity. In the inhibition of AChE activity by 50% relative to the initial, one can expect light poisoning, from 60% to 70% of medium-heavy, 80% and 90% in the heavy and AChE activities designed from 1 to 4% lethal. The appearance of symptoms of poisoning depends more on the speed of cholinesterase inhibition than the absolute level of found activity [24, 25, 26].

Due to the significant presence of pesticides in the Rasina district has been investigated the clinical status of 175 workers in the chemical industry and agricultural production in the area of Rasina District. Workers were divided into six groups. Of these, the two control groups were 47 workers who were not exposed to pesticides. Other groups consisted of workers who are (or were) exposed to pesticides, such as 78 workers in the chemical industry who are working in the production process of pesticides in the plant of "Pesticides" and 50 workers working in agriculture. Workers in the "Pesticides" plant were divided into three groups according to the length and duration of occupational exposure, namely: the workers who are constantly exposed to pesticides (N=42); workers who are occasionally exposed to pesticides (N=17); and workers who have previously been exposed to pesticides, and then were transferred to other jobs (N=19).

At the time of the test, the results showed that among workers who are constantly exposed to pesticides, acetylcholinesterase is within the reference value. Having examined the medical records of these workers, it is noted that, at 72%, there is a slight fall of AchE activity, each year.

In industrial workers who are occasionally exposed to pesticides acetylcholinesterase in serum is in the framework of reference values, and there is a declining trend over the past years.

The workers who had been exposed to pesticides at the time of testing had acetylcholinesterase in terms of reference value, but 52% of them had a few years ago significantly reduced the value of activity of acetylcholinesterase, which were treated and then transferred to other jobs. The 48% of these workers had acetylcholinesterase in terms of benchmarks, or were transferred to other jobs, for a variety of other health problems.

For workers who are working in agriculture being exposed to pesticides, the results showed that the activity of acetylcholinesterase is also in terms of the reference value. A review of their medical records showed that the workers during their years of work in 87% of cases have acetylcholinesterase activity in the framework of the reference value, which over the years has not significantly decreased, and 13% had over the years, the value of slight decreased activity of acetylcholinesterase.

Acute toxicity, however, caused a significant decrease in the activity of acetylcholinesterase, which is shown in Table 2. The cause of toxicity has been malathion, a preparation which is dissolved erroneously by supervisor and given to the use of workers. In 89% of workers value of the enzyme activities of AchE were lower after the use of such prepared preparation of those values that they had during the systematic review. The percentage of inhibition ranged from 1% to 59%, indicating varying degrees of poisoning. The highest degree of poisoning had older workers. Only four of them from this shift, they had reduced activity of acetylcholinesterase.

Table 2: Percentage of reduction in serum cholinesterase activity of 35 workers of your group

Percentage of AchE activity reduction in a patient group	AchE activity in a group before acute poisoning	AchE activity in a group after acute poisoning
1. - 49%	2938	1442
2. - 38%	3058	1170
3. - 58%	2458	1436
4. - 59%	3128	1842
5. - 41%	2432	1408
6. - 16%	2099	1770
7. - 19%	2338	1884
8. - 20%	2372	1908
9. - 34%	2648	2002
10. - 2%	2110	2072
11. –32%	2587	2008
12. –36%	3130	2014
13. –33%	3040	2328
14. - 18%	2858	2342
15. – 7%	2229	2070
16. - 22%	2788	2172
17. – 14%	3087	2666
18. ----	2093	2094
19. -15%	2598	2070
20. -15%	2449	2092
21. - 3%	3003	2928
22. ----	3015	3004
23. - 5%	1898	1805
24. ----	2008	2000
25.. ----	1992	1980
26. - 3%	2222	2148
27. -1%	2412	2384
28. - 3%	2143	2079
29. -17%	2998	2489
30. -4%	2089	2002
31. -29%	2924	2088
32. -5%	2118	2014
33. -23%	3008	2311
34. -11%	2525	2000
35. -18%	2768	2260

The reference values for serum cholinesterase are from 2618 to 6971 U / L.

In order to look at the general state of health of workers exposed to pesticides, we are, in addition to determining the activity of acetylcholinesterase, as a biomarker of toxicity of organophosphorus compounds (OPC), set the number of haematological and biochemical parameters as indicators: hematotoxicity; nephrotoxicity and hepatotoxicity. The results showed that the number of red blood cells are higher in industrial workers exposed to the constant action of pesticides, than in all other groups of industrial workers and that there is no statistically significant difference in the number of red blood cells between the II and III groups of industrial workers. There was a statistically significant difference between the number of red blood cells for agricultural workers who are exposed to pesticides and the control group of workers in agriculture. Increased number of erythrocyte in groups of workers compared to the control group is probably a result of higher feeding these workers. Hemoglobin concentration was not significantly different in either group of tested workers, except in the Vath subgroup in which the acute organophosphate poisoning of workers caused a statistically significant decrease in haemoglobin concentration. White blood cell count was not significantly different in either group of examined workers. In the leukocyte, the formula was observed a slight decrease in segmented granulocytes at V-th group compared to the control group of workers in agriculture. In the same group of workers are slightly increased lymphocytes.

The results lead us to conclude that exposure to pesticides has not caused permanent haematotoxicity in workers exposed to pesticides. However, acute pesticide poisonings caused a statistically significant decrease in haemoglobin concentration.

Hepatotoxicity of pesticides in our patients we evaluated by determining the activity of some enzymes (transaminases, alkaline phosphatase, gamma-glutamyl transferase) and bilirubin. The results showed that transaminase does not exceed the limit reference value in either group of respondents. Among the groups of industrial workers exposed to pesticides no significant differences in the activity of transaminases. Alkaline phosphatase does not exceed the limit of the reference value in any group of patients, and no statistically significant difference in the alkaline phosphatase activity between the control group and any group of examined workers. Gama deficient does not exceed the limit reference value in either group of respondents. No statistically significant differences in the activity of GGT between the control group and any group of examined workers. The concentration of bilirubin is in all groups within the reference value. No statistically significant differences in the concentration of bilirubin between two groups and the control group.

Discussion

The obtained results show that the exposure of workers to pesticides did not cause hepatotoxicity.

Nephrotoxicity in workers exposed to pesticides, we evaluated by determining creatinine, urea and uric acid in their serum, as well as physical-chemical analysis and the examination of their urine sediment. The obtained results showed that creatinine in the blood does not exceed the limit of the reference value in any study group. There was no statistically significant difference in creatinine concentration among the test groups. It regulates the blood does not exceed the reference value in any study group. Among the groups of workers exposed to pesticides, there is no significant difference in the concentration of urea. Uric acid in any group does not exceed the limits of reference values.

Among the groups of workers exposed to pesticides, there were no statistically significant differences in the concentration of uric acid. Chemical and microscopic examination of urine in all groups is clear. 60% of workers first and the fourth group had calcium oxalate crystals in the urine sediment. The presence of crystals of calcium oxalate is most likely a result of the so-called "hard water" you drink these workers.

Pesticide poisoning

Treatment in case of poisoning varies and also is applied to each concrete case. However, whenever it comes to some of the pesticide poisoning we should quickly call a physician or transfer the patients to the hospital, but before the doctor arrives should be taken, mainly, the following general measures:

1. It should come as soon as possible to establish which pesticides caused the poisoning. If the poisoning occurred at work, in most cases, it is not difficult, but if it comes to deliberate poisoning, then it is much more difficult. In any case, to take all necessary measures will be important to preserve the sputum of patients, the vessel in which the pesticide is found, as well as the clothes of workers, if needed chemical analysis of pesticides.

2. If poisoning is caused by the digestive tract through the mouth, should be as soon as the appropriate way to induce vomiting, and if possible and wash the stomach. Then, giving some laxative means to discharge hoses. If the poisoning is caused by some chlorinated hydrocarbons (DDT, hexachlorocyclohexane - NSN, dieldrin, toxaphene, etc.), for the cleaning should be given neither castor nor any other oil because the oil absorbs these toxins. If poisoning is caused by the skin or via the conjunctiva of the eye, then with the basic wash of contaminated skin and eyes should be removed remains of poison. For such washing are the best water and soap***.***

3. If it is poisoning some of asphyxiating, for example, hydrocyanic acid or the like, then the patient should immediately administer artificial respiration. To the patient, in this case, is much more useful artificial respiration on the spot, without losing time, then the transfer to the hospital. Of course, doctors should be in this case, immediately call or by telephone notify the measures taken and ask for advice. However, with artificial respiration in patients to continue until the patient is fully restored, or until the doctor arrives.

4. In case of poisoning at work, should urgently be taken the necessary measures to prevent the occurrence of poisoning to others, new cases;

5. Any case of pesticide poisoning at work, and otherwise, the application must be submitted to the appropriate authorities of sanitary inspection and inspection at work.

When poisoning with pesticides, as well as in other poisonings, are given if any, the so-called antidotes. Then are given drugs to maintain heart and medicines for calming of disturbances etc. In the case of choke is given oxygen, and when it is necessary and the blood transfusion.

In conclusion, the fact is that today we can hardly survive without pesticides. However, there is an alternative, which consists of biological possibilities to eliminate pests and diseases, from an attempt which is collectively called as organic agriculture. It is certain that we must try to replace as many pesticides and produce healthy food. Pesticide should be taken as a necessary evil at this point helps humanity to ensure a sufficient food source, but the real challenge is precise to seek ways of production which will not poison the land, water and air by these compounds. The main problem associated with the use of pesticides and their usage is a knowledge of a very narrow circle of negative effects because users are content with the current result (the destruction of pests) and often do not think about all the interactions and interdependencies that such action has on the long run List of unwanted operation of DDT or other pesticides to human health, or the environment would be lengthy. And while the overall use of pesticides can be individually badly affect on each of us remains to try to reduce the use of those pesticides in 12% of households. Maybe your imagination will be stimulated by the following examples.

Against weeds are recommended mechanical (e.g. surface treatment of land grabbing and pulling of weeds) and thermal methods (targeted incineration), as well as the alternate sowing of various crops on the same parcel.

Fighting insects includes a selection of healthy, robust varieties of crops that are adapted to climatic conditions, alternating sowing of different crops on the same parcel of land, the natural balance of the soil microorganisms by avoiding synthetic chemical and soil improvers, as well as surface treatment of the soil. In the case of occurrence of stronger insects are recommended mineral substances such as algae powder, and insecticides based on plant extracts.

Instead of fungicide, antifungal is recommended to use copper salts or preparations of sulphur.

About pesticide poisoning should, above all, take appropriate protective measures. It is necessary, first, that pesticides are stored properly and by the regulations on poisons stored properly. Storage should be organized in such a way that by pesticides, especially more toxic, can be reached only on permitted, regulated manner. Also, the warehouse operator and the vendor must be well acquainted with the nature of the toxic properties of each pesticide sold.

Depending on the way in which the highest possible poisoning, it is recommended for the application of any pesticide or group of pesticides with adequate protection (protective clothing, mask, gloves, boots, etc.).

Then, it is necessary that each pesticide is applied to the mandatory taking of prescribed hygienic and technical protection measures. Instructions on taking these measures should be attached to each package of each pesticide. If such instructions are missing when packaging, should be asked from the store when purchasing pesticides. - Instructions must be strictly observed.

Finally, using each pesticide should only deal with people who are well versed in the way of its use, as well as the way of protecting them from poisoning.

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