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DECREASED PSEUDOCHOLINESTERASE FOLLOWING EXPOSURE TO ORGANOPHOSPHORUS PESTICIDE IN A RURAL AREA POPULATION OF BRAŞOV COUNTY

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Abstract: *Pseudocholinesterase activity has a long history of use in monitoring both workers at risk of organophosphorus pesticide (OP) exposure and in investigating exposures to OPs. Nevertheless, the knowledge about the effect of pesticide exposure on baseline enzyme activity function is still limited. The current study evaluates the exposure to OP on 100 individuals from a rural area of Brasov county, divided into two groups: study or exposed group (n=75) and control or non-exposed group (n=25). Serum enzymatic activity level was used as markers to monitor the extent of OP exposure. Our results showed a significant decrease of pseudocholinesterases in the exposed group compared with non-exposed group (p=0.0002).*

Key words: *pesticide exposure, pseudocholinesterase, confounding factors.*

1. Introduction

The assessment of pseudocholinesterase also known as butyrylcholinesterase or plasma cholinesterase activity is widely used to investigate incidents of organophosphorus pesticide (OP) over-exposure, or as part of health surveillance in workers at risk of exposure. The first synthesis of OP was made in 1854 [6] and today more than 100 compounds are used in agriculture, households and public health. The OP is neurotoxic as it inhibits an enzyme called cholinesterase, necessary for a balanced transmission of messages at the neural junction. Organophosphorus is less powerful than nerve agents, the intoxication with OP being clinically similar and the effect is irreversible inhibition of cholinesterase. Additionally, the pathogenicity of OP intoxication is based on anti-cholinesterase effect interfering to the enzyme baseline with accumulation of acetylcholine and vagotony [6].

Routes of exposure comprise the digestive route, dermal and respiratory as well as conjunctive route. They are being absorbed rapidly and are highly distributed into the liver, kidneys and lungs and less

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distributed in muscles, myocardium and brain without accumulation. The dermal route is of equal importance as the digestive one [2].

The farm workers involved in spraying pesticides are exposed to contamination. Exposure can be made through inhalation, dermic or oral. The dermic pathway is the main gate of exposure for the farm workers [3]. To those exposed due to their profession the oral pathway is less important, although there are few reported studies of poisoning after consuming some foods held an entire day in the pocket of a jacket with which the spraying was made [4].

The aim of this study was to assess whether exposure to OP in an intensive agriculture setting may lead to changes in baseline enzyme activity parameters, using pseudocholinesterase as marker of monitoring the extent of exposure. Because of the low economic status of the peasants, the specific type of vegetable chosen for crops- the potato and due to the many OP producers on the market it was impossible to individualize the specific molecules used.

2. Material and Methods

An epidemiologic study on a 75 individuals exposed to OP compared to a 25 non-exposed was conducted. Socio-demographic characteristics (i.e. smoking, alcohol consumption, and educational level) were analysed. Moreover, we surveyed the factors which increased or decreased the risk of exposure and the possibility of interference (i.e. statin consumption and diabetes). The endpoint was the serum enzymatic activity level of pseudocholinesterase (Enzyme Commision number further on abbreviated as E.C 3.1.1.8). We used as instrument of selection a pretested questionnaire that consists of 16 items applied to 107 answerers, 7 of them being excluded due to inconsistency and presence of exclusion criteria. The exclusion criteria were presence of hepatic disease, TBC, chronic alcoholism.

Through a series of questionnaire items two groups were selected: study or exposed group (n=75) and control or non-exposed group (n=25). This chosen design allowed the subdivision of the study group into a directly exposed group (n=39) and an indirectly exposed group (n=36). The pattern of direct exposure includes working directly with pesticides; the pattern of indirect exposure implies living in an area were pesticides are spread, being in contact with directly exposed ones, handling items once in contact with these compounds.

The measurement method was kinetic, using butyrilcholine (Spinreact, Girona, Spain), performed with spectrophotometer (T80 UV/VIS, PG Instruments Ltd, Leicestershire, United Kingdom), 0 calibration with distilled water, wavelength 405 nm and fresh serum, with samples brought at a 25^0C. The method was based on the hydrolysis of butyrilthiocholine in thiocholine with the forming in presence of 5,5‘-dithio-2-nitrobenzoic acid of a coloured product spectrophotometrically analysed at 405 nm.

For the chosen method the range of normality for pseudocholinesterase (EC 3.1.1.8) into human serum is 3000-9300 U/L at 25ºC (Spinreact, Girona, Spain). The blood was collected on site (villages of Cata, Ormenis and Prejmer – Brasov county), the serum obtained and then refrigerated, transported in refrigerated conditions to the Biochemistry laboratory of the Transilvania University of Brasov from Romania. There was no need of ethical consent as each peasant filled an informed consent agreeing to participate to the study by giving blood to analysis. The samples were numbered and no identification information was request from the peasants but their agreement of giving the blood. After the blood samples were collected; a maximum period of 48 hours passed until the moment of analysis. After reaching a temperature of 25^0C, the measures were performed.

Statistical comparisons were performed using Student t test and Chi-square. Probability values < 0.05 were considered to indicate significant difference. The results were analysed using Microsoft Office Pack 2007.

3. Results and Discussions

The health risk behaviours were analysed and it was observed that in the exposed group there are less than 10 cigarettes smoked/day in contrast to the non-exposed group where there are more than 20 cigarettes smoked/day. In what concerns the consumption of 10-20 cigarettes/day in both groups are homogenous (Figure 1).

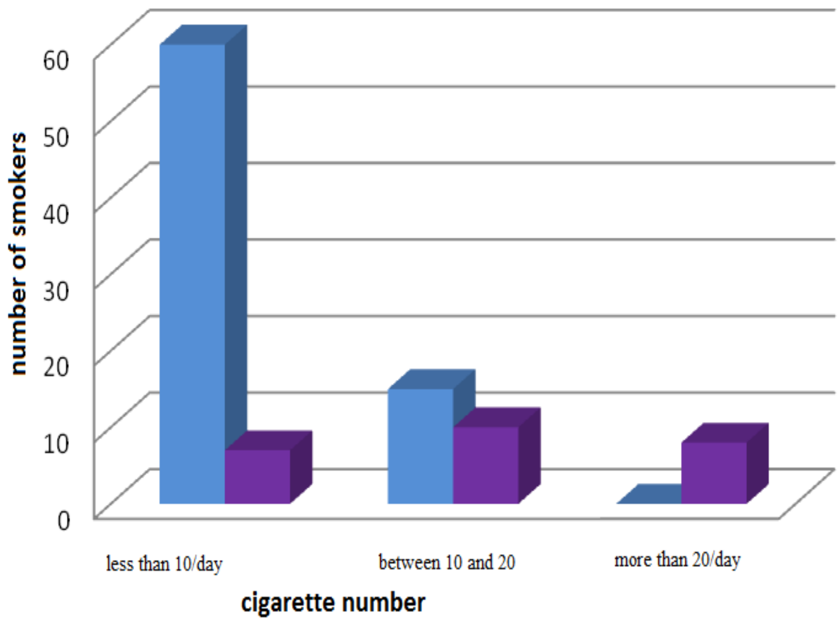

Fig. 1. *Smoking in the exposed and non-exposed groups – number of cigarettes by day*

Interestingly, regarding the alcohol consumption, both groups have registered similar levels of consumption. In the exposed group the percentage of those who do not consume alcohol at all is higher, and further showed to be favourable, having in the view that liver diseases can interfere with cholinesterase levels (Figure 2).

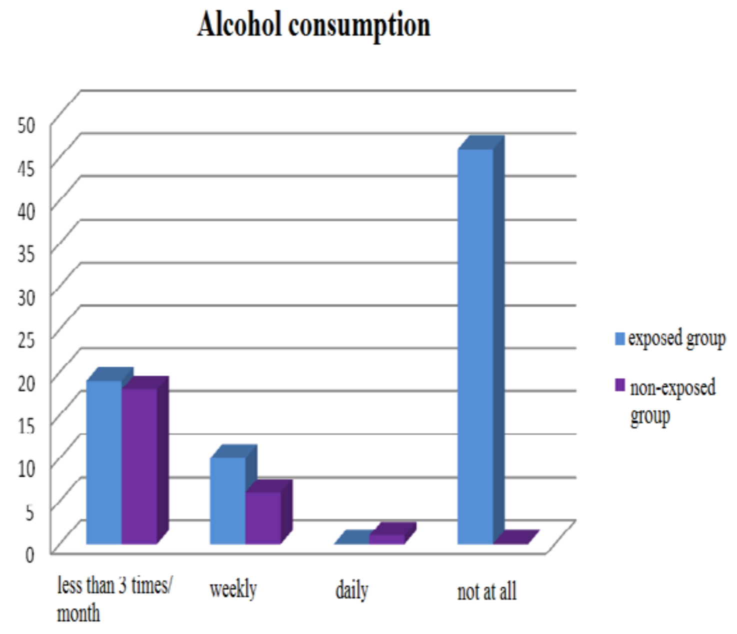

Fig. 2. *Alcohol consumption in the exposed and non-exposed groups- occasion of consumption by time*

Concerning education level in the studied population, both groups showed to be homogenous (Figure 3).

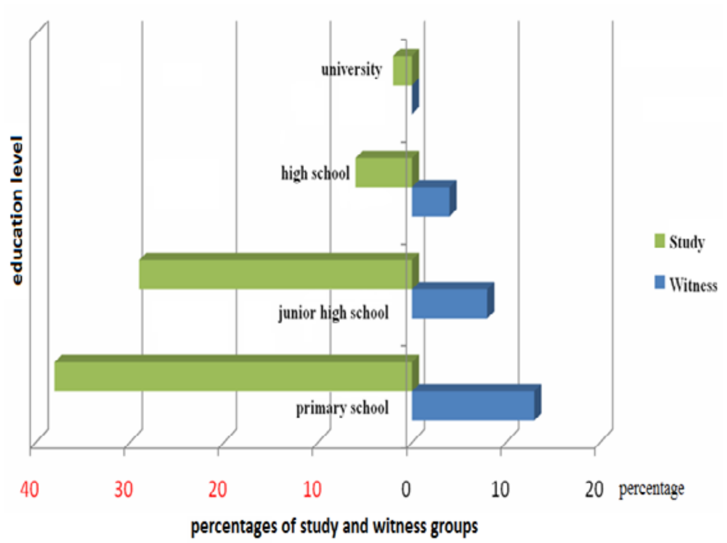

Fig. 3. *Education level in the exposed and non-exposed groups expressed in percentage OX axe-percentage of exposed and non-exposed groups, OY axe-education level*

Figure 4 shows the descriptive analysis of blood pseudocholinesterase levels in the study/exposed group compared to the levels of the control/non-exposed group. The difference between the groups was statistically different (p*=0.0002).

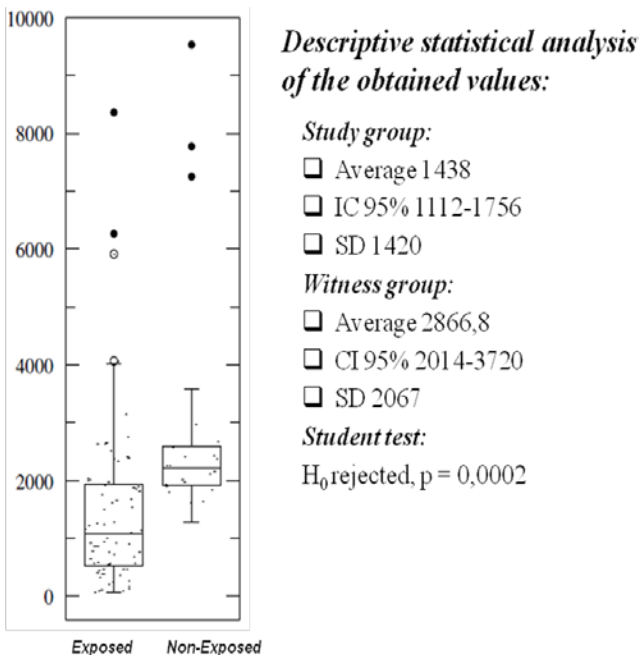

Fig. 4. *Box plot distribution diagrame*: *„blood pseudocholinesterase levels in exposed and non-exposed groups E.C. 3.1.1.8 units”*

Exposure to OP or a confounding factor that will be studied later decrease the level of pseudocholinesterase in the exposed group compared to the non-exposed group. We further analysed the difference of blood pseudocholinesterase levels between the directly exposed subgroup (n=39) and the indirectly exposed subgroup (n=36) which did not show any difference between the groups (Figure 5).

We further divided the directly exposed group into two subgroups, the first having an exposure to OP of less than 10 years (n=10) and second having an exposure to OP of more than 10 years (n=29). The differences in the blood levels of pseudocholinesterase between the two subgroups were statistically different (p*=0.0022) (Figure 6).

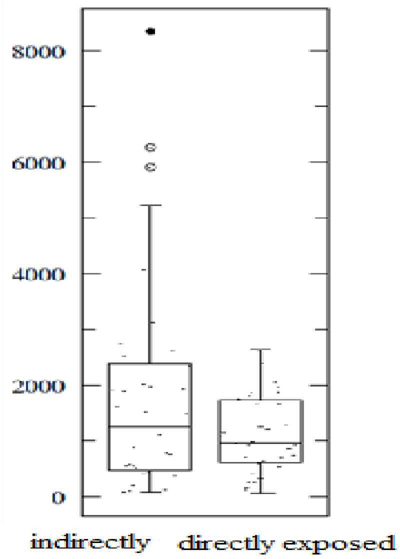

Fig. 5. *Box plot distribution*: *„blood pseudocholinesterase levels in directly exposed group and indirectly exposed group.E.C.3.1.1.8 units*

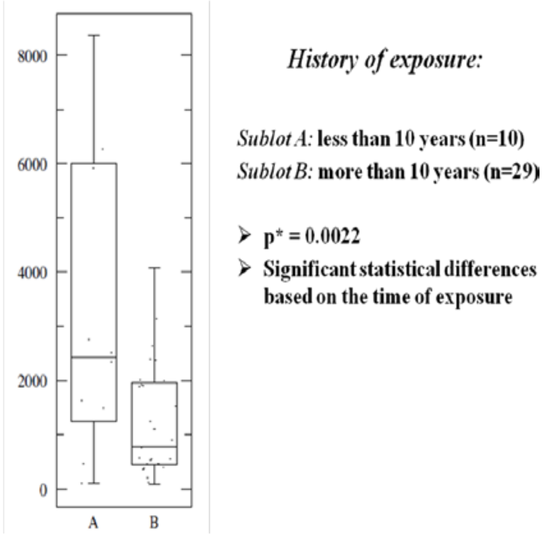

Fig. 6. *Box plot distribution*: *„Blood pseudocholinesterase levels according to history of exposure"*

Table 1 shows the factors which could decrease the risks of exposure to pesticides. Alarming is the fact that nobody from the directly exposed group wears protection mask. It increases the risk of contamination for greater exposure.

Table 1

Factors influencing the risk of exposure

Parameter	**Mask**	**Gloves**	**Boots**
Yes	0	17/1570	20/1924
No	39	22/2083	19/1528
Significance	-	p=0.36	p=0.50

Next step was to analyse the possibility of interference of some known confounding factors. The known factors that interfere with the level of pseudocholinesterase in the serum are: statins consumption, contraceptives usage, antidepressants consumption, diabetes, Alzheimer's disease [4]. In our population statins consumption and diabetes were the most common (Table 2).

Table 2

Confounding factors in exposed and non-exposed groups

Parameter	**Exposed Group**	**Non-Exposed Group**	**Significance**
Statins	33	6	p=0.076
Diabetes	55	9	p=0.082

According to $\chi 2$ test, there were no significant differences between the exposed/study group and the non-exposed control group concerning the use of statins and/or diabetes. This explains the fact that the groups were homogenous if we consider the confounding factors and the difference shown on Figure 5 are the results of exposure and not of confounding. An original article by Ohayo-Mitoko and contributors [5] on 539 farmworkers agricultors from 4 areas of Kenya showed only a minimum inhibition of acethylcholinesterase after using protection equipment in contrast to non-usage of protection equipment.

A ground-breaking study by Arcuary et al. [1] involving 9 families of farmers living in the West of North Carolina and Virginia which measured exposure to OP in urine samples, showed higher levels of metabolites in families of farm workers which did not change clothes and did not wash after the use of pesticides (parathion/ methyl parathion, chlorpyrifos/ chlorpyrifos methyl). Taken together, these findings suggest that pesticide exposure dramatically affect the serum enzymatic activity levels. Strikingly, a reduction in pseudocholinesterase activity could be associated with decreased levels of enzymatic activity, indicating that immediately after exposure anticholinesterase pesticides may interact with the enzyme molecules and alter their catalytic activities.

In our study, as occurred with non-exposed group, the exposed population showed slight but significantly decreased levels of pseudocholinesterase activity.

In addition, a reduction in pseudocholinesterase enzymatic activity was associated with increased levels of OP exposure according to the difference between directly exposed and indirectly exposed.

This finding calls for further experimental and epidemiological research.

4. Conclusions

Our results showed a significantly statistic difference of serum pseudocholinesterases in the exposed group compared with non-exposed group. ($p=0.0002$) The differences in the blood levels of pseudocholinesterase between the two subgroups, according to history of exposure, were also statistically significant. ($p=0.0022$)

These data may be useful to those health professionals who need to interpret blood pseudocholinesterase activity from individuals exposed to organophosphorus pesticide.

Exposure to pesticides showed a decreased level of pseudocholinesterase activity and this reduction may depend upon the particular pesticide, the duration of the exposure, and use of personal protective equipment by pesticide users.

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