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CLINICO PATHOLOGICAL EFFECTS OF PESTICIDES EXPOSURE ON FARM WORKERS

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ABSTRACT
The present study had been carried out to examine the acute symptoms of pesticide in the farm workers of three villages in Nagda tehsil of Ujjain district of Madhya Pradesh. Workers of different age group work seasonally in soybean and other vegetable fields applying pesticides. In this study a total of 52 intensive agriculture workers were assessed twice during the course of a spraying season for changes in serum biochemistry (AST, ALT, and CK) and other biochemical parameters, such as markers of nephrotoxicity (urea, creatinine) . The results were compared with non-sprayers of same age group who served as controls. A Significant decrease was observed in serum cholinesterase, and hematological parameters viz. Hb, Hct and RBC. Significant increase AST, ALT, was observed in exposed group when compared with control. These results provide support for a very slight impairment of the liver and renal function and indicate that pesticide sprayers working in farms are at the risk of developing serious health problems. Exposure of multiple pesticides for prolong period has also affected the health of exposed persons and produced dermatological, hepatic, nephritic, respiratory and other clinical disorders reflecting the toxic effects of pesticides. Our findings indicate that indiscriminate use of pesticides in farming environments must be regularly assessed and farm workers must be trained for safe use of pesticides.

KEY WORDS: Aspartate aminotransferase, Alanine aminotransferase, Acetyl cholinesterase, blood indices, lipid peroxidation.

INTRODUCTION

The use of chemical pesticides in India has increased by more than seventeen times since 1955. The widespread use of pesticides in agriculture, public health and household environments results in continuous exposure of human populations. A higher proportion of pesticide poisonings and deaths occur in developing countries where there are inadequate occupational safety standards, ineffective protective clothing and washing facilities, insufficient enforcement, and poor labeling of pesticides, illiteracy and insufficient knowledge of pesticide hazards (Pimental and Greine, 1996). Because farmers and farm workers directly handle seventy to eighty percent of the pesticides they use, they are at the greatest risk of exposure (McDuffie, 1994). Most people do not realize that they are being poisoned by the pesticides because many symptoms of pesticide poisoning are similar to other health problems, for example, skin rashes and dizziness. Apparently, therefore a large number of acute pesticide poisonings each year go undiagnosed and unreported. Due to heavy pesticide exposure, various chronic effects such as brain and nervous system damage, cancer, birth defects, miscarriages and still births have been reported. Few surveillance studies have been conducted in India on high risk population groups involved in the spraying of pesticides in field conditions (Rupa *et al*. 1991; Gupta *et al.* 1995; Chaudhuri 2000).Exposure to low-level of pesticides is known to produce a variety of biochemical changes, some of which may be responsible for the adverse biological effects reported in human and experimental studies (Banerjee *et al.,* 1999). Oxidative stress can also be induced by pesticides, either by over production of free radicals or by alteration in antioxidant defense mechanisms, including detoxification and scavenging enzymes (Abdollahi *et al.,* 2004). In blood, normal erythrocyte function depends on the intactness of cell membrane which is the target for many toxic factors including pesticides. Although a huge number of studies have addressed the association between exposure to pesticides and health which are heterogeneous. We therefore, conducted this study to examine the hepatic, renal and neuromuscular signs in workers exposed to pesticides and the relationship between these effects and AChE levels and work experience.

MATERIAL AND METHODS

For evaluation of immediate effects of pesticides, a total of 52 farmers in the age group of 25-45 years from three villages, namely, Nagda (n=25), Nayan (15) and piploda (n=12) of Ujjain district of Madhya Pradesh, were visited during peak spraying season of September to October, 2009. They were interviewed for various acute symptoms of poisoning such as nausea, dizziness, chest tightness, eye itchiness, discolored nails, nails dropping off, sleeplessness, excessive sweating and excessive salivation etc. mostly in the evening hours while returning home after whole day's spraying. Addiction of various types of drugs such as alcohol, smoking, and tobacco was very common. During the study period of one month, eight cases of hospitalization were recorded. Brief questions about occupation, household exposures and any other potential exposures to fumes, dust or gases will allow rapid assessment of the likelihood that an illness could be related to pesticides or other toxic chemicals. Biochemical studies: After an overnight fasting period, two samples of venous blood were collected in tubes with clot activator and citrate-treated tubes, respectively, and preserved cooled less than 2 h until they reached the laboratory. Plasma was separated by low-speed centrifugation and analyzed for toxicological analysis immediately .Written consent was obtained from all the workers. Serum AchE

activity, ALT and AST activity was assayed following the modified International Federation for Clinical Chemistry (IFCC) method laid down in monoenzyme kits (Siemens Diagnostics, Ltd, India). Urea and creatinine was determined by kit supplied by Span Diagnostic Ltd. (India). Erythrocytes (RBC) and WBC were counted immediately after blood collection in hemocytometer (Improved Neubauer. Weber scientific Ltd.) according to Wintrobe (1934). To measure hematocrit (HCT), ammonium heparinized hematocrit capillary tubes (Fisher scientific co.) were filled with blood and centrifuged for 5-min at 5000 x g in a micro capillary centrifuge (Haematokrit 24. Hettich). The percentage of hematocrit was determined by the use of a micro capillary reader. Hemoglobin concentration was measured by the cyanmethaemoglobin method (Larsen and Snieszko, 1961) using a commercially available kit (Span, India). *Student's t* test was used to examine the difference between means in case of the normally distributed variables.

RESULTS AND DISCUSSION

The health survey of farmers associated with pesticides is presented in Table 1.The maximum intensity of impact was seen in the form of skin rashes and itchiness as the primary route of pesticide exposure is the skin, and not the respiratory system as is commonly believed. Pesticides remain persistent on skin for many months after the last known exposure. Besides, cleaning equipment's, disposing off empty containers, spraying farmers also have to mix pesticides and load them into spray containers, which pose even more serious health risk, since they are handling the concentrated products. Almost all the pesticide sprayers studied did not practice precautionary principles. It shows that 94.4% of farmers interviewed were reported with skin rashes and itchiness, followed by nails dropping off discolored nails ,nausea and eye itchiness , excessive sweating , blurred vision , dizziness , sleeplessness , headache and breathing difficulty .These are various acute health effects of a combination of pesticides which in low level over a long period of time lead to chronic effects such as cancer, reproductive and endocrine disruption, neurological and immune system damages etc? The present study demonstrated a significant decrease in serum cholinesterase ($p < 0.001$) in pesticide spraying workers (Table 1).

Table 1. Changes in serum biochemical and haematological parameters in exposed and unexposed to pesticide population

Parameter	Exposed	Control
RBC count (106/µL)	4.1±0.2 *	5.11±0.6
Haematocrit %	44.11±2.2*	38.3±1.1
Hemoglobin (g/dL)	12.1±1.2 *	13.8±1.0
Leukocytes (103/µL)	6.5±0.3 *	7.2±0.4
AST (IU/L)	29.7±2.1*	24.4±2.7
ALT (IU/L)	48.8±3.0 *	38.3±3.6
ALP (IU/L)	170.0±58.0 *	154.7±57.0
Blood Urea Nitrogen (mg/dL)	24.8±5.0 **	18.4±4.4
Creatinine (mg/dL)	1.1±0.2	1.0±0.3
AchE (U/L)	2241 ± 252*	2764 ± 209

* Values are statistically significant when compared to control.

Several studies on volunteers reported that repeated long term exposures of organophosphorus pesticide and carbamate decrease the blood cholinesterase activities without clinical manifestation (Aldridge W.N. (1971). The normal function of AchE is to terminate neurotransmission due to acetylcholine, liberated at cholinergic nerve ending in response to nervous stimuli. Loss of AchE activity may lead to a range of effects resulting from excessive nervous stimulation and culminating in respiratory failure and death (Costa, 1988). Tolerance to the cholinergic over stimulation may be observed following repeated exposure to cholinesterase inhibiting chemicals. But the cellular mechanisms responsible for the development of tolerance may also lead to the development of other effects i.e. cognitive dysfunction not present at the time of initial exposure (Bushnell, *et al*, 1991). Generally the acute cholinergic effects of ant cholinesterase compounds are viewed as reversible (14), although longer lasting effects have been reported in animals (Tendon et al, 1994). Serum AST and ALT were increased in the exposed group as compared to control (Table 1). Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures to pesticides may lead these toxins to accumulate in the liver and cause pathological alterations (Braun beck, 1994). Moreover, cell injury of certain organs like liver leads to the release of tissue specific enzymes into the bloodstream (Burtis and Ashwood, 1996). Significant increase in transaminases (AST and ALT) activity in farm workers exposed to pesticides in could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver. The increased activity of ALT and AST may be due to leakage of hepatic cells which was also evidenced by our histopathologial studies (Roxana et al, 2010). Research indicates that ALT and AST can be used as biomarkers of cellular damage in blood plasma, protein degradation and liver damage (Reddy et al, 2010, Reddy and Baghel, 2012). In this study, the hematological parameters like Hb, Hct, and RBC were significantly decreased in exposed group as compared to control (Table 1).The effect of Hb on humans exposed to organophosphorus pesticides has been observed by several workers. (Illahi *et al,* 1986; Ray, 1992). The decrease in the

Hb along with the decrease in the RBC might be due to the effect of pesticides on Haemopoeisis. Many steps in heme biosynthesis are inhibited by pesticidal residues and this might be a possible physiological reason for the inverse proportion of the result obtained. The poisoning by pesticide residues is the development of anemia due to interference of Hb biosynthesis and shortening of the life span of circulating erythrocytes (Ray (1992). Most of the measured hematologic, hepatic and renal function indices were significantly different between the pesticide applicators and control groups. RBC count, Hb, and WBC count were significantly decreased ($p<0.001$). The decrease in haematological variables (Hb and RBC) of the exposed fish may be due to haemolysis of red blood cells by pesticide leading to significant decrease in haematocrit value which results in anemia. It is expected also that in the toxification, this disruption can be implemented on some elements of mitochondrial heme biosynthesis and some disturbance on the globin gene structure and ribosomal agents. It should be mentioned that the regulating factor in hemoglobin synthesis is due to negative feedback inhibition of aminolevulinic acid which may disrupted by the toxic agents (Hamid Reza et al 2007). Blood urea nitrogen (BUN) as an indicator for the kidney function which is also significantly higher in the exposed than the control group ($p<0.05$) indicates acute renal injury. However one parameter is insufficient to define early renal toxicity and that a battery of several parameters would provide a better evaluation of the onset of renal toxicity. The only way to ensure the correct diagnosis of acute or immediate pesticide poisoning is to maintain high index of suspicion and take a screening of occupational and environmental history from any patient who presents suggestive symptoms. In conclusion, workers exposed to pesticides show toxic response, hence it is compulsory to use gloves, face shields, aprons, shoes and other protective devices and supplementation of antioxidants viz Vit. C, Vit E in the food to relieve from toxic effect.

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