ORIGINAL ARTICLE

THE EFFECTS OF PESTICIDE EXPOSURE ON SERUM TOTAL SIALIC ACID LEVELS

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Summary: Pesticides used to increase agricultural production have hazard effects for human being health. The present study reports the serum levels of total sialic acid (TSA) in the pesticide applicators in the agriculture areas of the Kahramanmaras, Turkiye. This study included a total of 79 volunteer individuals. Pesticide group included 47 men who were working as pesticide applicators. Control group comprised healthy males (n=32), nonsmokers and nondrinkers and also nonexposured to pesticide and other chemicals chronically. Serum TSA level was measured with the Warren method modified by Pönniö et al. The TSA concentrations were significantly higher in the pesticide groups ($p<0.001$) than those of control subjects. We can conclude from the results obtained that serum TSA was affected by pesticide exposure. This finding may be an indication of harmful effects of pesticides. Increased serum TSA levels in pesticide exposure might be related to various diseases e.g. various cancers, which are also often associated with elevated serum TSA levels. However further studies are necessary to evaluate the concentrations of serum TSA in pesticide exposure.

Key words: *Sialic acid; Pesticide exposure*

Introduction

The use of pesticides has increased dramatically in both developed and developing countries during the last few decades. Pesticides used to increase agricultural production have hazard effects for human being health (6). Several studies concerning pesticide residues in foods, foodstaffs, and also animal and human milk and tissues have been published, indicate that pesticides may have a long term effects in biological system (2). Pesticides with an extremely high acute toxicity may be easily metabolized and eliminated from the body; following long-term low exposure, they may be less toxic and without carcinogenic or mutagenic properties. On the other hand, pesticides with low acute toxicity can accumulate in the body and chronic toxicity after long-term exposure even in comparatively low doses (6). In several studies, it has been observed that pesticides have hazard effects for human being health. Such effects may vary from an influence on the reproductive system and immunosuppressive effects, to the promotion of carcinogenic activity (6,18,19). On the other hand, serum total sialic acid (TSA) and lipid bound sialic acid have been used as useful markers for human cancer (4,11). Also, elevated concentrations of serum TSA were suggested as a potent cardiovascular risk factor in the general population (13,14).

Sialic acid (SA) is the common name for compounds of N-acetylated derivates of neuraminic acid, which mainly occur as nonreducing terminal residues of carbohydrate chains of glycoproteins (GP) or glycolipids (GL) in biological fluids and cell membranes. They have a central role for the functioning of biological systems: Stabilizing the conformation of glycoproteins and cellular membranes; assisting in cell-cell recognition and interaction, and serving as chemical messengers in tissue and body fluids; affecting the function of membrane receptor molecules by developing binding sites for ligands, enzymes, etc., or by blocking such; affecting the functioning, stability, and survival of GP in blood circulation. Although a small portion of the TSA is free in tissues and body fluids, most is bound to glycocongutes. In human plasma a large quantitiy of SA is found in orosomucoid, α_1 antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, complement proteins, and transferrin. Some of these sialylated glycoproteins are called acute phase reactants, and such substances rapidly increase in concentration after the onset of an inflammatory reaction or injury (4,13,21,27). Several studies have been performed on serum TSA in patients with cardiovascular disease (CVD) and cancer. Elevated serum TSA has been reported in patients with CVD (13) and cancers of the lung, prostate, bladder, brain, and gastrointestinal system (7,11). But there is no report available on serum TSA levels in subjects who exposured to pesticide. Therefore, the present study has been undertaken to investigate whether there is any relationship between pesticide exposure and serum TSA levels.

Materials and Methods

This study included a total of 79 volunteer individuals. Pesticide group included 47 men who were working as pesticide applicators, in the cotton growing areas of the Kahramanmaras, a city and agriculture area in southern Turkiye, and lived in same area. The mean duration of this occcupation was 20.93±12.09 years (range 6–50 years). Also, pesticide group was divided according to the habits of subjects as smoking and alcohol consumption. Blood samples were obtained from the subjects at the application season of pesticide. Control group comprised healthy males, nonsmokers and nondrinkers and also nonexposured to pesticide and other chemicals chronically. This group consisted 32 subjects. There was no significant difference between the ages of participants.

Information on used pesticides in the agriculture region was obtained. Although, in this study the subjects were exposed to some certainly an undefined mixture of pesticides, these pesticides were principally herbicides including trifluralin and synthetic pyrethroids including cypermethrin and oxydemeton-methyl.

Informed consent was obtained from all subjects, who were not suffering from any disease and were not on any medications. Venous blood was (5 mL) were collected by venipuncture in vacutainer tubes and serum was separated by centrifugation. All samples were stored at -20 °C until analysis. Serum TSA was measured with the Warren's colorimetric method (26) modified by Pönniö and et al. (17). In brief, GP and glycolipid-bound SA were dissociated by acid hydrolysis. Periodate was then used under strongly acidic conditions to oxidise N- acetylneuraminic acid to form β-formylpyruvic acid. The reaction was stopped by sodium arsenite, after the thiobarbiturate was added to obtain a red chromophore. Extraction of this into cyclohexanone was used to intensify the color with a maximum absorbance at 549 nm.

All chemicals in this study were of analytical grade and purchased from Sigma (Germany) or Merck Chemicals Co. (Germany). All solutions were prepared in deionized water.

Statistical analyses were performed with the SPSS pocket programme for windows. The data were expressed as mean values ± standard deviation (X ± SD). The mean values in the groups were compared with Mann Whitney U test. The level of statistical significance was defined as $p < 0.05$.

Results

In this study, serum TSA level was measured in pesticide group and compared with healthy control subjects who nonexposure to pesticide and also nonsmokers and nondrinkers. Results are given in Tab. 1. As seen from the table, mean serum TSA level of pesticide group was significantly increased compared to those of controls ($p < 0.001$). In pesticide group, there was no significant difference in TSA levels between smokers-drinkers and nonsmokers-nondrinkers ($p > 0.05$).

Discussion

A wide range of different pesticides have become important in agriculture, mainly in the developed countries, but also increasingly in the developing countries. Pesticides have been used in Turkiye as well as in other parts of the world. This study was undertaken to search for the effects of pesticide exposure on TSA levels; but, no similar study has been reported. Therefore, this is the first study specificially aimed at this subject.

In our study, the results obtained indicate that serum TSA levels elevated in pesticide groups compared with control subjects who non-exposured to pesticide and also nondrinkers and non-smokers. Elevated serum TSA has been reported in alcoholics (12,17) and also smokers (12,14). Therefore smokers and drinkers were excluded in the control group. In the pesticide group, there was no significant difference in TSA levels between smokers-drinkers and nonsmokers-nondrinkers. The finding shows that elevated serum TSA levels in our study were directly related to the effects of pesticide on serum TSA levels. However, the mechanisms that generate the elevated TSA levels in the serum of pesticide group are unknown.

In animal studies, many pesticides are carcinogenic while others are tumor promoters. Some contaminants in commercial pesticide formulations also may pose a carcinogenic risk (6).

Tab.1: Serum TSA levels in pesticide and control groups, X±SD.

Group	n	Age (years)	Duration of pesticide exposure (years)	TSA (μg/mL)
Pesticide	47	36.62±11.88	20.93±12.09	749.12± 45.57*
Pesticide (Smokers-Drinkers)				
Smokers but nondrinkers	14	39.14±13.39	24.84±14.95	753.09±69.17*
Both smokers and drinkers	11	37.30±10.47	16.00±7.07	752.60±21.46*
Smokers or drinkers	25	34.84±7.11	20.64±12.63	752.87±52.76*
Pesticide (Nonsmokers and nondrinkers)	22	36.63±11.37	21.00±11.75	744.854±36.48*
Control (Nonsmokers and nondrinkers)	32	35.46±13.27	-	581.95±134.36

*$p < 0.001$ vs control group

In several studies, occupational exposure to pesticides in agriculture and cancer has been studied (9,28). Some studies on occupations associated with pesticide exposure have shown excess risk for: leukemia, and cancers of the brain and connective tissue, prostate, stomach, and testis (2,5,6,22). In addition, cancers of the breast, endometrium, kidney, liver, bladder, ovary, stomach, and thyroid also have been associated occasionally with pesticide exposure (6). However, the results of the investigations are different according to used pesticide. Because, pesticides may be greatly different in mode of action, uptake by and elimination from the body, and toxicity to humans so their chemical and functional properties are different. Also, inconsistencies between epidemiological studies may result from differences in study design, variation in pesticide exposure between different populations, or other underlying characteristics of the population, including both environmental factors and inherited susceptibility. The limited assessment of pesticide exposure, smaller sample size, and inadequate control of cigarette smoking in many of previous studies, underscores the need for improved investigations.

In our study, it was not possible to investigate the risk of the specific pesticides because, the subjects were exposed to some certainly an undefined mixture of pesticides. In addition, the effect of pesticide exposure may be diluted if non-carcinogenic pesticides are considered together with other carcinogenic pesticides. However, in our study, some used pesticids in the agriculture region were herbicides including trifluralin and synthetic pyrethroid including cypermethrin. Trifluralin (a dinitroaniline: -trifluoro-2,6-dinitro-N, N-dipropyl-p-toluidine) is an herbicide used on a wide range of plants. In several studies, health effects of trifluralin investigated in animals however related reports are somewhat controversial. Trifluralin has been tested in six long-term (two year) dietary studies, four on rats and two on mice. In the first two bioassays, carried out in the early 1960s on Harlan and on Cox rats, no treatment-related tumorigenic effects were observed. In a National Cancer institute (NCi) study on Osborne-Mendel rats and on B6C3F1 mice in which technical trifluralin (later shown to be contaminated with 84 to 86 ppm dipropylnitrosamine [NDPA]) was administered in the diet, there were no tumorigenic effects in rats of either sex or in male mice, but hepatocellular carcinomas and alveolar/bronchiolar adenomas were observed in female mice (15,24). However, this could have been related to nitrosamine contamination of the trifluralin, as many nitrosamines have been demonstrated to be tumorigenic in mice (15). In addition United States Environmental Protection Agency (EPA) has classified trifluralin as a Group C, possible human carcinogen (25). On the other hand, also, EPA has classified cypermethrin [(*RS*)-α-cyano-3-phenoxybenzyl(1*RS*)-*cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] as a possible human carcinogen, as a Group C, because it causes lung tumors in female mice (3). Two recent studies have demonstrated molecular mechanisms by which cypermethrin might be involved in causing cancer. One study looked at "gap junctional" intercellular communication (23). This process plays "important roles in maintenance, growth, and differentiation of cells" and is inhibited by many carcinogenic agents (23). A second study showed that, in addition to inhibiting intercellular communication, cypermethrin also increased the number of "altered foci" in rat livers (10). Both are characteristics of tumor promoters (10,23).

On the other hand, serum TSA has also been used as a tumor marker for a number of different cancers including colorectal, prostate, and breast cancers (7,16). Elevated serum TSA has been reported in patients with cancers of the lung, prostate, bladder, brain, and gastrointestinal system (7,11). Cell surfaces and membrane components play a prominent role in neoplastic behavior. Neoplasms often have an increased concentration of TSA on the tumor cell surface, and sialoglycoproteins are shed or secreted by some of these cells, which increases the concentration in blood. TSA concentrations have been reported to be related not only to diagnosis, but also to staging, prognosis, and detection of early recurrence (7). On the other hand, cancer cells have been associated with an increased activity of sialyltransferase, leading to an increased amount of TSA on the cell surface, thus increasing the plasma concentration (20,21). Increased serum TSA levels in pesticide exposure might be related to various diseases e.g. various cancers, which are also often associated with elevated serum TSA levels.

From the results obtained we can conclude that serum TSA was affected by pesticide exposure. This finding is possibly related to be harmful effects of pesticides. Because, the first reactions of organisms to toxic compounds take place at the molecular and cellular level, before the effects become visible at higher levels of biological organization (8). In addition, this study is important because the finding draw attention to a significant potential occupational health hazard. However further studies are necessary to evaluate the concentrations of serum TSA in pesticide exposure.

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