ERYTHROCYTE SUPEROXIDE DISMUTASE AND CATALASE ACTIVITIES IN PEOPLE CHRONICALLY EXPOSED TO PESTICIDES

PESTİSİTLERİN KRONİK ETKİSİNE MARUZ KALAN YÖRE HALKINDA ERİTROSİT SÜPEROKSİT DISMUTAZ VE KATALAZ AKTİVİTELERİ

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ABSTRACT

Purpose: *In this study, we aimed to investigate the effects of chronic exposure to pesticides on erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities in residents who have lived near agricultural areas.* ***Methods:*** *Blood samples were taken from residents (n=50) who had long-term exposure to pesticides (15.03±2.25 years) in Mersin and erythrocyte SOD and CAT activities were measured using spectrophotometric techniques. These antioxidant system enzymes were also evaulated in persons not exposed to pesticides in any known way (n=30).* ***Results:*** *Erythrocyte SOD levels were significantly higher (p<0.001) but erythrocyte catalase activities were significantly lower (p<0.001) in residents than in the control group.* ***Conclusion:*** *We have found that long term exposure to pesticides slightly changed antioxidant enzyme activities in residents.*

Key Words: *Pesticide Exposure, Resident, Superoxide Dismutase, Catalase, Erythrocyte.*

ÖZET

Amaç: *Bu çalışmada tarım alanlarına yakın yerleşim bölgelerinde yaşayan ve pestisitlerin kronik etkisine maruz kalan yöre halkında eritrosit süperoksit dismutaz ve katalaz aktivitelerinin incelenmesi amaçlanmıştır.* ***Gereç ve Yöntem:*** *Mersin ve çevresi tarım alanlarının yakınındaki yerleşim bölgelerinde yaşayan (15.03±2.25 yıl) ve pestisitlerin kronik etkisine maruz kalan kişilerden (n=50) kan örnekleri alınmış ve bu örneklerden elde edilen eritrositlerde süperoksit dismutaz (SOD) ve katalaz (CAT) aktiviteleri spektrofotometrik olarak ölçülmüştür. Aynı ölçümler pestisitlere herhangi bir şekilde maruz kalmayan kişilerde (n=30) de yapılmıştır.* ***Bulgular:*** *Yöre halkında eritrosit SOD aktiviteleri kontrol grubuna oranla daha yüksek bulunurken (p< 0.001), CAT aktivitesinde anlamlı bir azalma gözlenmiştir (p<0.001).* ***Sonuç:*** *Bu çalışma sonucunda pestisitlerin uzun süreli etkisine maruz kalan yöre halkında antioksidan enzim aktivitelerinin değişikliğe uğradığı saptanmıştır.*

Anahtar Sözcükler: *Pestisit, Eritrosit, Antioksidan Sistem, Kronik Etki.*

INTRODUCTION

Pesticides have an impact on the environment because of their widespread use in agriculture to enhance crop production. Pesticides are unique in that they are intentionally released into the environment to protect agricultural products from pests. However most of them are neither specific nor selective to pests, and therefore are hazardous to nontarget organisms, as well as humans (1). They affect reproductive function, immune system, nervous system, liver, endocrine system, blood and other systems in nontarget organisms (2-7).

Exposure to several classes of pesticides is known to increase lipid peroxidation, a metabolic endpoint of reactive oxygen species produced in vivo. Under normal conditions, endogenous reactive oxygen species are the by-products of metabolism that include the generation of cellular enegy in mitochondria, peroxisomal oxidations

and hemoglobin oxidation in erythrocytes. These reactive oxygen species are suppressed by endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and heme oxygenase and redox molecules such as glutathione. Generally, oxidative stress occurs when the cellular antioxidant capacity is overwhelmed by the reactive oxygen species generated by the metabolism of pesticides and other xenobiotics, some of which can either inhibit or modulate the expression of antioxidant enzymes. Reactive oxygen species interact with cellular targets such as proteins, DNA and lipids, activate cell signaling molecules and induce oxidative stress, which eventually lead to lipid peroxidation, cell proliferation and subsequently to preneoplastic lesions (1).

More than a thousand pesticides were authorized by the Ministry of Agriculture and Rural Affairs (M.A.R.A.) to be used against pest in Turkey (8). As a consequence of agricultural activities, a very wide range of plant species are grown in the Mersin region. Due to the variety of cultivated plant species, the number of pests that infest these plants are also very high in Mersin. Therefore, a long pesticide list (azodrine, basudin, malathion, methyl parathion, cypermethrin, delthametrin, captan, lindane etc.) is available.

In order to determine pesticide effects on erythrocyte antioxidant enzymes, we investigated SOD and CAT activities in residents who had had chronic exposure to a mixture pesticides in Mersin.

MATERIALS AND METHODS

We investigated 50 residents, living near the agricultural areas and having long-term exposure to pesticides in Mersin (exposed group). Thirty persons who have not been exposed to pesticides in any known way, were evaluated as healthy controls.

Information related to age, smoking and drinking habits, medication and other systemic diseases were obtained from each subject in both the experimental and the control groups using a questionnaire. Subjects who had chronic health problems such as emphysema, cancer, cardiovascular and inflammatory diseases and tobacco smoking or irradiation history were excluded from the study.

Blood samples for measurement of enzymes of antioxidant system were drawn by venipuncture using 10 ml vacutainer tubes, containing ethylenediaminetetra-acetic acid (EDTA) as anticoagulant. After collection, blood samples were transported to the laboratory in a cool box and analysed on the same day. After centrifugation at 2500 rpm for 5 minutes, the plasma was removed and erythrocytes were washed four times with 5 ml of 0.9 % NaCl.

Enzyme Analysis

Hemoglobin (Hb) concentration of the erythrocyte hemolysate was determined by the cyanmethemoglobin method using Sigma Kit 512# (Sigma chemicals, St Louis. MO). SOD activity was assessed by the method of Mc Cord (9). The determination of SOD was based on the oxidation of xanthine with xanthine oxidase to generate superoxide radicals (O2-) which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-fenyltetrazolium chloride to form a red formazan dye. The SOD activity was followed through the inhibition of this reaction. The absorbance change was measured by a Shimadzu UV-1601 spectrophometer (Japan) at 505 nm. SOD activity was expressed as units per g Hb (U/gHb). One unit of SOD activity represents a 50 % inhibition of production of free radicals.

CAT activity was determined by the method of Beutler (10). The rate of decomposition of H_2O_2 by catalase is measured spectrophotometrically at 230 nm, since H_2O_2 absorbs light at this wavelength. One unit of CAT activity corresponds to the amount of enzyme, decomposing the reaction of one micromole H_2O_2 per minute.

Statistical analysis

The significant differences between the means of exposed and control groups were compared using Student's t -test. p value <0.001 was considered to be statistically significant.

RESULTS

The average age of residents were 33.64 ± 5.55 years. The average age of the control group was 30.80 ± 4.86 years, and the age difference between exposed and control groups was not significant ($p>0.05$). The mean exposure time of

Table - 1: The mean ± SD and 95% CI values of the SOD and CAT activities in the exposed and control groups.

Parameters	Exposed group (n=50)			Control group (n=30)		
	mean	SD	95 % CI	mean	SD	95 % CI
SOD (U/gHb)	1709.8*	840	1441-1978	884.5	447.2	720.5-1048.5
CAT (x10^4 U/gHb)	12.2 *	4.72	10.68-13.71	21.16	3.0	20.06-22.26

* p<0.001; SD: Standard deviation ; CI: Confidence interval

the exposed group was 15.03 ± 2.25 years.

Erythrocyte SOD and CAT levels of residents and control group are shown in Table I. Erythrocyte SOD levels were significantly higher but erythrocyte CAT activities were significantly lower ($p<0.001$) in residents than in the control group. SOD activity in residents was about 2-fold higher than the control group. The activity of CAT in residents was 1.7 fold lower that in controls. Figure 1 and Figure 2 show the calculated means (with confidence intervals) for the SOD and CAT activity.

DISCUSSION

In this study we assessed the erythrocyte SOD and CAT activities of 50 residents who were living near the agricultural areas and 30 controls who have not been exposed to pesticides in any known way.

The results of our studies indicate that antioxidant enzymes were modified by long term exposure to pesticides. Cellular antioxidative defense mechanisms protect cells from the potentially harmful effects of reactive oxygen metabolites (9). SOD catalyses the dismutation of O_2^- to yield H_2O_2 and O_2. GSH-Px and CAT catalyse the decomposition of H_2O_2 to yield O_2 and H_2O. These free radical scavenging enzymes are the first line of cellular defense against oxidative injury, decomposing O_2^- and H_2O_2 before they interact to form the more reactive OH radicals (11, 12).

Mature mammalian erythrocytes contain no nuclei or mitochondria, nor they can synthesize proteins or membrane lipids. Since they survive for an average of 120 days in the human blood circulation without membrane or protein renewal, they must carefully protect themselves against O_2^- and H_2O_2 using free radical scavenging enzymes (13, 14).

In our study erythrocyte SOD levels were significantly higher, but CAT was significantly lower in exposed residents than those of the control group. The increased SOD and reduced CAT in exposed residents would lead to

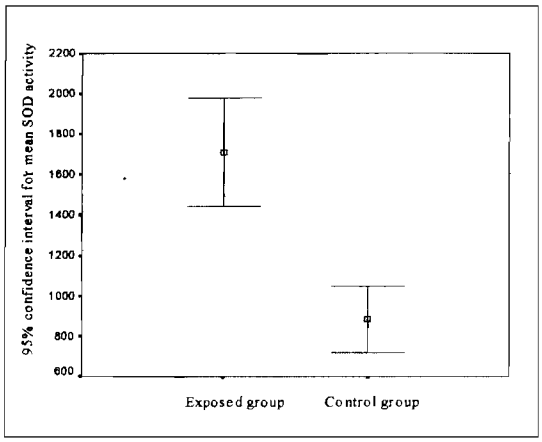

Fig. 1: Level of erythrocyte SOD activity in exposed and control groups.

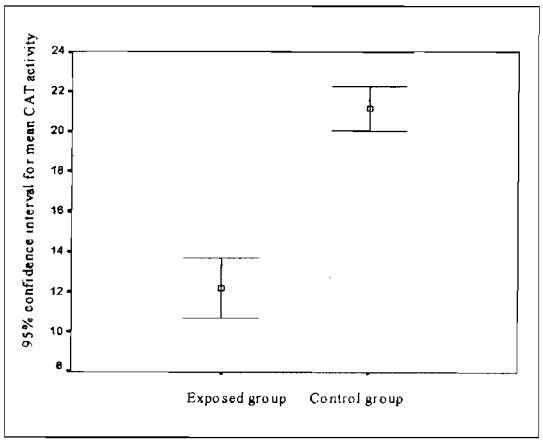

Fig. 2: Level of erythrocyte CAT activity in exposed and control group.

accumulation of H_2O_2 (15,16) a highly toxic metabolite for cells (17, 18). H_2O_2 may be converted to H_2O either by CAT or GSH-Px. H_2O_2 is decomposed to O_2 and H_2O by CAT at high concentrations, while GSH-Px serves for H_2O_2 degradation at lower concentrations (14). Observation of lower CAT levels in exposed residents than that of the controls suggests that pesticides may inhibit CAT activity and cause H_2O_2 accumulation and thus resulting in the hemolysis of erythrocytes. GSH-Px converts H_2O_2 or other lipid peroxides to water or hydroxy lipids, and during this process glutathione (GSH) is converted to oxidized glutathione (GSSG). To recycle GSSG, the cell utilizes the enzyme NADPH-dependent GSH reductase, the NADPH being supplied to the reaction by glucose-6 phosphate dehydrogenase (19). There are some reports in the literature stating that GSH-Px activities change after treatment of pesticides (25), but GSH-Px activity was not determined in this study.

Similar results were observed in the research of farm workers who had been spraying for years (20). There are very few studies showing the changes in the antioxidant system of residents who have been chronically exposed to pesticides, although some studies investigated the acute effects of pesticides on both in vitro isolated cells and in vivo animal models. Oral exposure to carbamate pesticide, benomyl (21) and organochlorine dieldrin have been shown to induce oxidative stress as indicated by malondialdehyde accumulation and/ or glutathione depletion in tissues of rats. In hepatocytes, exposure to certain organochlorine herbicides and lindane are reported to increase phospholipid hydroperoxide levels, reduce glutathione concentrations and increase superoxide dismutase activity (22). It is apparent from these and other reports (23-29) that the reactive metabolites of pesticides modify the activities of antioxidant enzymes in tissues after acute exposure. In these studies different pesticides have been tested for their effects on the antioxidant systems by the measurement of the antioxidant enzyme (SOD and CAT) activities. It has been found that under some conditions some pesticides inhibit these enzymes while under some other conditions induce their activity. The factors that change these enzyme activities depend on the type of pesticide, duration of exposure, and the way (e.g. oral, dermal, by inhalation) that pesticides are given to the animal.

In this study, we evaluated the erythrocyte SOD and CAT activities in residents who had been chronically exposed to pesticides. We found an increase in SOD activity while CAT activity was diminished, indicating that the antioxidant system is affected by chronic pesticide exposure. The endpoint chemicals, such as reactive oxygen species, produced by these activity changes may in fact cause an increase in heart diseases, aging, cataract and cancer which will be the subject for our future studies.

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