

CLINICAL STUDY

The genotoxicity and cytotoxicity among patients diagnosed with organophosphate poisoning*

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Abstract: The genotoxicity and cytotoxicity were investigated in 40 patients (20 females aged 21.57 ± 1.42 and 20 males aged 29.35 ± 3.59) diagnosed at the Emergency Department with organophosphate poisoning. Chromosome aberrations (CAs), sister chromatid exchanges (SCEs), micronucleus (MN), mitotic index (MI), replication index (RI) and nuclear division index (NDI) were evaluated in peripheral bloods of patients. The blood samples were collected from the patients on admission to the emergency department before treatment and after treatment before being discharged from the intensive care unit. The CA, MI and NDI values were increased before the discharge when compared to the levels measured on admission. However, there are no differences in mean SCE, frequency of MN and RI (Tab. 2, Ref. 42). Full Text (Free, PDF) www.bmj.sk.

Key words: organophosphate, genotoxicity, cytotoxicity, lymphocytes, chromosome aberration, poisoning.

The extensive use of pesticides results in environmental pollution. Pesticides have been used extensively in Cukurova region located in the South Eastern region of Turkey along the Mediterranean coast, which is the most important agricultural region in Turkey (1). The pesticides used in this region cause intoxication during spraying of fields by oral, dermal and respiratory routes (2).

Around the world, acute pesticide poisoning has become a major public health problem with more than 300,000 deaths each year (3). The easy availability of highly toxic pesticides in the homes of farming communities has made pesticides a preferred choice for suicide with extremely high case fatality. Similarly, the extensive use of pesticides exposes the community to both long-term and acute occupational health problems such as mutations (4–8), cancer and birth defects (9–16) as well as cause perinatal mortality in women occupationally exposed to organophosphates and other pesticides (17).

An estimated amount of 12,198,917 kg of pesticides was used in Cukurova region of Turkey in 2002 (18). Organophosphates were the most extensively used pesticides. Organophosphate pesticides may cause genotoxicity by inducing CAs, SCEs and MN frequency in human peripheral lymphocytes and in bone marrow cells in rats (19, 20, 21) as well as in humans occupationally or accidentally exposed to organophosphate pesticides (22, 23, 24). Organophosphates also cause mutations in other organisms (25, 26). Genotoxic and cytotoxic effects of pesticides in humans have been evaluated mostly for chronic exposures. There are only a few studies on acute intoxication (2, 3, 4).

In the present study, the CA, SCE, MN, MI, RI and NDI values were investigated in peripheral blood of patients who were diagnosed with organophosphate poisoning on admission to the emergency department.

Material and methods

This prospective study was performed at a university-based emergency department between June 2007 and November 2008. Inclusion criteria were as follows: documented exposure to organophosphate compound, signs and symptoms of cholinergic syndrome and decreased plasma cholinesterase activity. Exclusion criteria were as follows: the history of organophosphate ingestion without signs of cholinergic syndrome, previous treatment for organophosphate ingestion and follow-up at other institutions, history of chronic illness and any other known chromosomal abnormalities that can affect the method of the study. The ethic committee approval was obtained for the study. The first blood samples were collected from the patients diagnosed with organophosphate poisoning on admission (untreated) and

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Tab. 1. The CA, SCE, MN, MI, RI and NDI value in peripheral lymphocytes.

| | Admission to the Emergency Department (untreated) | Discharged from the Hospital (treated) |
|------------|---|--|
| CA Female | 0.014±0.003 | 0.022±0.008 ^{a1} |
| CA Male | 0.015±0.005 | 0.026±0.005 ^{a1} |
| SCE Female | 1.854±0.322 | 2.248±0.445 |
| SCE Male | 1.566±0.371 | 1.737±0.409 |
| MN Female | 9.061±2.178 | 13.315±2.251 |
| MN Male | 8.611±2.298 | 12.764±2.532 ^{b1} |
| MI Female | 0.0320±0.006 | 0.0533±0.014 ^{a2} |
| MI Male | 0.021±0.006 | 0.052±0.009 ^{a3} |
| RI Female | 1.291±0.176 | 1.466±0.117 |
| RI Male | 1.085±0.189 | 1.140±0.198 ^{b1} |
| NDI Female | 0.181±0.043 | 0.286±0.051 ^{a1} |
| NDI Male | 0.106±0.041 | 0.137±0.049 ^{b2} |

a – significant between untreated-treated females and untreated-treated males, b – significant between females and males, ^{a1b1} – p≤0.05; ^{a2b2} – p≤0.01; ^{a3b3} – p≤0.001.

the second samples were collected before the discharge of the patients (treated). Patients were treated immediately following the admission with nasogastric lavage, activated charcoal (1 g/kg), atropine, pralidoxime, and intravenous fluid administration. Pralidoxime 1 to 2 g and atropine ad libitum were administered until bronchial secretions were scant to absent.

The methods of Evans (27), Perry, and Thompson (28) were followed in preparation of CA and SCE tests with minor modifications. This study was conducted according to IPCS guidelines (29).

The first and the second whole blood samples (0.2 ml) were added to 2.5 ml of chromosome medium B (Biochrom, F5023) supplemented with 10 µg/ml of bromodeoxyuridine (Sigma, B5002). The cultures were incubated at 37 °C for 60–72 h. The cells were exposed to colchicine (0.06 µg/ml, Sigma C9754) 2 h before harvesting. The cells were harvested by 0.4 % KCl as hypotonic solution and methanol: glacial acetic acid (3:1) as fixative. The staining of air-dried slides were performed following the standard methods using 5 % Giemsa stain for CA and modified fluorescence plus Giemsa method for SCE³⁴. The slides were irradiated with 30 W, 254 nm UV lamp at 15 cm distance in Sorensen buffer, then incubated with 1xSSC (standard saline citrate) at 60 °C for 45–60 min and stained with 5 % Giemsa prepared with Sorensen buffer.

The number of CA was obtained by calculating the percentage of metaphases from each patient who showed structural and/or numerical alterations. CA was classified according to ISCN (International System for Human Cytogenetic Nomenclature) (24). Chromosome aberrations were evaluated in 100 well-spread metaphases per patient. The scoring of SCE was carried out according to IPCS guidelines (29). In order to score SCE, 25 second-division metaphases per patient were analyzed. The results were used to determine the mean number of SCE (SCE/cell). In addition, a total of 100 cells for each patient were scored for replication index (RI). The mitotic index (MI) was also determined by scoring 3000 cells from each donor. Gaps were not evaluated as CA according to Mace et al (30).

Tab. 2. The CA, SCE, MN, MI, RI and NDI value in peripheral lymphocytes.

| | Admission to the Emergency Department (untreated) | Discharged from the Hospital (treated) | p |
|-----|---|--|-------|
| CA | 0.014±0.002 | 0.024±0.005* | 0.004 |
| SCE | 1.718±0.242 | 2.006±0.303 | 0.242 |
| MN | 8.786±1.537 | 13.039±1.844* | 0.050 |
| MI | 0.027±0.004 | 0.052±0.008* | 0.000 |
| RI | 1.193±0.128 | 1.311±0.114 | 0.366 |
| NDI | 0.144±0.030 | 0.216±0.037* | 0.026 |

For the analysis of micronucleus in binucleated lymphocytes, 0.2 ml of fresh blood from treated and untreated patients were used to establish cultures. Cytochalasin B (Sigma, C6762) was added after 44 h of incubation to the final concentration of 6 µg/ml to block cytokinesis. After the additional 24-hour incubation at 37 °C, cells were harvested by centrifugation and processed for micronucleus test in peripheral lymphocytes. In all subjects, 2000 binucleated lymphocytes were scored from each patient. Total 1000 viable cells were scored to determine the frequency of cells with 1, 2, 3 or 4 nuclei and to calculate the NDI (nuclear division index) for cytotoxicity of agents using the formula: $NDI = (M1) + (2 \times M2) + (3 \times M3) + (4 \times M4) / N$; where M1–M4 represent the number of cells with one to four nuclei and N is the total number of viable cells scored (31).

The t-test was used for the statistical significance for the percentage of structural CA, mean SCE, MN, MI, RI, NDI.

Results

During the study period, a total of 62 patients were admitted to emergency department with the diagnosis of organophosphate poisoning. Twenty-two of the patients were excluded from the study because of prior treatment (12 patients), existence of chronic illness (5 patients), lack of cholinergic signs (3 patients), or lack of informed consent (2 patients). A total of 40 patients enrolled, of which 20 were males and 20 were females. All of the patients had taken organophosphates intentionally for suicidal purpose. The mean age of 20 females was 21.57±1.42 and that of 20 male was 29.35±3.59. The mean duration of hospitalization was 5.4±1.3 days (range: 4–8 days).

Table 1 compares the CAs, SCEs, MN, RI, and MI between the admission and discharge as well as female and male parameters evaluated in the present study.

The CAs were induced in treated female patients when compared to results obtained from untreated females patients on admission to the Emergency Department. The same results were obtained for male patients. However, there are no differences in mean SCE, frequency of MN and RI for both male and female patients. MI and NDI were increased in treated female and male patients when compared to untreated patients. The plasma AchE enzyme level was also increased in treated patients when compared to untreated patients (Tab. 1). There are no differences for CAs, SCEs, MN, MI, RI, NDI and AchE between untreated fe-

male and female patients. However, the frequencies of MN, RI, NDI and AchE enzyme level were increased in treated females when compared to treated male patients.

Table 2 shows the results of CA, SCE, MN, MI, RI, NDI values and AchE enzyme levels in untreated and treated patients (females and males). All parameters were increased in treated patients when compared to untreated patients except for mean SCE and RI values. Mean SCE and RI were not significantly increased in treated patients when compared to untreated patients.

Discussion

There exists an interrelation among populations exposed to organophosphate (OP), acute OP toxicity, neurobehavioral effects, depression, suicide, and fatality. A potential risk of depression or suicide certainly exists from OP toxicity, largely depending on the epidemiology or sociodemographics of these disorders (32). Organophosphate-induced neurobehavioral effects result in depression. A potential risk of depression and suicide exists in farm workers exposed to Ops (32, 33). Intentional insecticide intoxication was found in adolescents (44 %) and in adults (56 %) mostly due to failing at school, matrimonial impediment and financial depression (2).

In the present study, the CA, MI, NDI values and AchE enzyme levels were increased in treated female patients when compared to untreated female patients as well as in treated male patients when compared to untreated male patients. However, there are no differences in mean SCE, frequency of MN and RI for both male and female patients. All the parameters were increased in treated patients (female and male) when compared to untreated patients (female and male) except for mean SCE and RI values. Prabhavathy-Das et al (21) reported that the OP insecticide profenofos induced the chromosomal aberration in metaphase analyzing and caused DNA single-strand breaks by comet-assay in human lymphocytes. Karabay and Oguz (19) reported that OP pesticides imidacloprid and methamidophos were genotoxic in rats when orally administered. OP also induced the CA in patients occupationally or accidentally exposed (2, 22, 23, 24). OP had a mutagenic effect in Ames test (25) and in *Drosophila* wing spot test (26).

OP had significant clastogenic effects however no aneugenic effect. OP also induced the DNA single strand and double strand breaks as well as caused chromosomal aberrations and micronucleus frequencies. Results can be concluded that OPs most probably bear a genotoxic risk. Not all of parameters discussed in the present study were induced in untreated patients; however, the abnormalities were induced in treated patients whose blood samples were collected after their treatment had been completed. These results indicate that it takes a few days to show the genotoxicity and cytotoxicity of OPs by inducing CA and MN. OPs metabolized to oxon in liver microsomes are covalently bound to esterase, which is one of carboxylesterase isozymes in liver, and cause cleavage of esterase-glucuronidase complex as well as serious toxicity in both acute and chronic exposures (34).

OP had a cytotoxic effect via decreasing the mitotic index (MI) and nuclear division index (NDI) in untreated patients when compared to treated patients. Epel (35), Jain and Andsorbhoy (36) found that the decrease in MI or the inhibition of DNA synthesis might be caused by the decrease in ATP level and pressure from the functioning of energy production center. It was reported that the chemical substances caused cytotoxicity by inducing the chromosomal abnormalities and DNA double-strand breaks (37–41). In this study, OPs most probably had a cytotoxic effect by inducing the structural CA. In addition, Madle et al (42) reported mitotic selection of cells that had chromosome abnormalities capable to decrease the MI.

Recommendations for exposure reduction include the acceleration of the already existent process of declining the use of pesticides in general, and organophosphates in particular; promotion of the shift from more to less toxic organophosphates and other pesticides; and introduction of rigid performance specifications for closed systems in loading and mixing at end-user sites. Preventing environmental OP exposure and increasing the awareness of pesticide toxicity would reduce acute OP poisoning and protect human health.

References

- Topaktas M, Rencuzogullari E.** Chromosomal aberrations in cultured human lymphocytes treated with Marshal and its effective ingredient carbosulfan. *Mutation Res* 1993; 319: 103–111.
- Alptekin D, Luleyap HU, Yilmaz L, Demirhindi H, Gökel Y, Pazarbasi A, Dokur M, Kasap M, Kasap H.** The Sister-Chromatid Exchange and Acetylcholine Esterase Enzyme Levels among Patients with Insecticide Intoxication in the Çukurova Region, Turkey. *Acta Med Okayama* 2006; 60: 121–126.
- Konradsen F.** Acute pesticide poisoning-a global public health problem. *Dan Med Bull* 2007; 54: 58–59.
- Cuenca P, Ramirez V.** Chromosomal aberrations in female workers exposed to pesticides. *Rev Biol Trop* 2004; 52: 623–628.
- Costa C, Silva S, Coelho P, Roma-Torres J, Teixeira JP, Mayan O.** Micronucleus analysis in a Portuguese population exposed to pesticides: preliminary survey. *Int J Hyg Environ Health* 2007; 210: 415–418.
- Lee WJ, Alavanja MC, Hoppin JA, Rusiecki JA, Kamel F, Blair A.** Mortality among pesticide applicators exposed to chlorpyrifos in the agricultural health study. *Environ Health Perspect* 2007; 115: 528–534.
- Bohlin P, Jones KC, Strandberg B.** Occupational and indoor air exposure to persistent organic pollutants: A review of passive sampling techniques and needs. *J Environ Monit* 2007; 9: 501–509.
- Leverton K, Cox V, Battershill J, Coggon D.** Hospital admission for accidental pesticide poisoning among adults of working age in England, 1998–2003. *Clin Toxicol (Phila)* 2007; 45: 594–597.
- Kristensen P, Irgens LM, Andersen A, Bye AS, Sundheim L.** Birth defects among offspring of Norwegian farmers, 1967–1991. *1997; 8: 537–544.*
- Garcia AM, Benavides FG, Fletcher T, Orts E.** Paternal Exposure to Pesticides and Congenital Malformations. *Scand J Work Environ Health* 1998; 24: 473–480.

11. Heeren GA, Tyler J, Mandeya A. Agricultural chemical exposures and birth defects in the Eastern Cape Province, South Africa: a case-control study. *Environ Health* 2003; 2: 11.
12. Jaga K, Dharmani C. The epidemiology of pesticide exposure and cancer: A review. 2005; 20: 15—38.
13. Reynolds P, Von-Behren J, Gunier RB, Goldberg DE, Harnly M, Hertz A. Agricultural pesticide use and childhood cancer in California. 2005; 16: 93—100.
14. Jurewicz J, Hanke W. Exposure to pesticides and childhood cancer risk: has there been any progress in epidemiological studies. 2006; 19: 152—169.
15. De Silva HJ, Samarawickrema NA, Wickremasinghe AR. Toxicity due to organophosphorus compounds: what about chronic exposure. 2006; 100: 803—806.
16. Calvert GM, Alarcon WA, Chelminski A, Crowley MS, Barrett R, Correa A, Higgins S, Leon HL, Correia J, Becker A, Allen RH, Evans E. Case report: three farmworkers who gave birth to infants with birth defects closely grouped in time and place-Florida and North Carolina, 2004—2005. *Environ Health Perspect* 2007; 115: 787—791.
17. Taha TE, Gray RH. Agricultural pesticide exposure and perinatal mortality in central Sudan. 1993; 71: 317—321.
18. Delen N, Guncan A, Gungor N, Tyrgut C, Burcak A. Türkiye'de Pestisit Kullanimi, Kalinti ve Organizmalarda Duyarlilik Azalisi Sorunlarý. Türkiye Ziraat Mühendisli-i 6. Teknik Kongre Ankara, 2005.
19. Karabay NU, Oguz MG. Cytogenetic and genotoxic effects of the insecticides, imidacloprid and methamidophos. 2005; 4: 653—662.
20. Chauhan LK, Chandra S, Saxena PN, Gupta SK. In vivo cytogenetic effects of a commercially formulated mixture of cypermethrin and quinalphos in mice. 2005; 587: 120—125.
21. Prabhavathy-Das G, Pasha-Shaik A, Jamil K. Cytotoxicity and genotoxicity induced by the pesticide profenofos on cultured human peripheral blood lymphocytes. 2006; 29: 313—322.
22. Titenko-Holland N, Windham G, Kolachana P, Reinisch F, Parvatham S, Osprio AM, Smith MT. Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay in vitro and in vivo: a study of malathion-exposed workers. 1997; 388: 85—95.
23. Lieberman AD, Craven MR, Lewis HA, Nemenzo JH. Genotoxicity from domestic use of organophosphate pesticides. 1998; 40: 954—957.
24. Paz-y-Mino C, Bustamante G, Sanchez ME, Leone PE. Cytogenetic monitoring in a population occupationally exposed to pesticides in Ecuador. 2002; 110: 1077—1080.
25. Hour TC, Chen L, Lin JK. Comparative investigation on the mutagenicities of organophosphate, phthalimide, pyrethroid and carbamate insecticides by the Ames and lactam tests. 1998; 13: 157—166.
26. Cakir S, Sarikaya R. Genotoxicity testing of some organophosphate insecticides in the *Drosophila* wing spot test. 2005; 43: 443—450.
27. Evans HJ. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. In Kilbey BJ, Legator M, Nichols W, Ramel C (eds.). *Handbook of Mutagenicity Test Procedures*. Second edition, Elsevier: Amsterdam, 1984.
28. Perry PE, Thomson EJ. The methodology of sister chromatid exchanges. In Kilbey BJ, Legator M, Nichols W, Ramel C (eds.). *Handbook of Mutagenicity Test Procedures*. 2nd Edition, Elsevier: Amsterdam, 1984.
29. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG, Tice R, Waters MD, Aitio A. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutation Res* 2000; 463: 111—172.
30. Mace MLJ, Daskal Y, Wray W. Scanning electron microscopy of chromosome aberrations. *Mutation Res* 1978; 52: 199—206.
31. Fenech M. The in vitro micronucleus technique. *Mutation Res* 2000; 455: 81—95.
32. Jaga K, Dharmani C. The interrelation between organophosphate toxicity and the epidemiology of depression and suicide. 2007; 22: 57—73.
33. London L, Flisher AJ, Wesseling C, Mergler D, Kromhout H. Suicide and exposure to organophosphate insecticides: cause or effect. 2005; 47: 308—321.
34. Satoh T, Hosokawa M. Organophosphates and their impact on the global environment. 2000; 21: 223—227.
35. Epel D. The effects of carbon monoxide inhibition of ATP level and the date of mitosis in Sea Urchin egg. *J Cell Biol* 1963; 17: 315—319.
36. Jain A, Andsorbhoy RK. Cytogenetical studies on the effects of some chlorinated pesticides III. Concluding remarks. *Cytologia* 1988; 53: 427—436.
37. Armstrong MJ, Bean CL, Galloway SM. A quantitative assessment of cytotoxicity associated with chromosomal aberration detection in Chinese hamster ovary cells. *Mutation Res* 1992; 265: 45—60.
38. Hillard CA, Armstrong ML, Bradt CI, Hill RB, Greenwood SK, Galloway SM. Chromosome aberrations in vitro related to cytotoxicity of non-mutagenic chemicals and metabolic poisons. *Environ Mol Mutagen* 1998; 31: 316—326.
39. Galloway SM, Miller JE, Armstrong MJ, Bean CL, Skopek TR, Nichols WW. DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens. *Mutation Res* 1998; 400: 169—186.
40. Vock EH, Lutz WK, Hormes P, Hoffmann HD, Vamvakas S. Discrimination between genotoxicity and cytotoxicity in the induction of DNA double-strand breaks in cells treated with etoposide, melphalan, cisplatin, potassium cyanide, Triton X-100 and X-irradiation. *Mutation Res* 1998; 413: 83—94.
41. Kirkland DJ, Muller L. Interpretation of the biological relevance of genotoxicity test results: the importance of thresholds. *Mutation Res* 2000; 464: 137—147.
42. Madle S, Beek B, Nowak C. Zum verständis von chromosomenmutationstest an somazellen. In Fahrig R. (ed.). *Mutationforschung und Genetische Toxikologie* Wissenshaftliche Buchgesellschaft: Dramstat, Germany, 1993.

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