

Correlation between serum concentration of diazinon pesticide and breast cancer incidence in Mazandaran Province, northern Iran

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ABSTRACT

Growing evidences have revealed a positive association between organophosphorus pesticides and diseases like cancer. Located in northern Iran, Mazandaran Province is a region with the overuse of agricultural pesticides. Due to the extensive use of the pesticides like diazinon in rice paddies of Mazandaran for the control of *Chilo suppressalis*, and high incidence rate of cancer in this province, we analysed and compared diazinon in the serum samples of the breast-cancer patients with the healthy volunteers. This cross-sectional case-control study includes 10 breast-cancer patients and 10 healthy controls. Diazinon was extracted with a mixture of acetone and diethyl ether (1:1 v/v) in acidic medium and the residue was analyzed by GC-MS instrument. Results showed the presence of 0.151 ppm diazinon in the serum of just one healthy subject. In conclusion, despite excessive use of diazinon in Mazandaran Province, no association was found between serum level of diazinon and the incidence of breast cancer.

Keywords: Breast cancer; Diazinon; Gas chromatography, Mazandaran province.

INTRODUCTION

During the last decade, the indiscriminate use of pesticides has become one of the major global concerns. Pesticides contain a wide range of compounds used in pest control, including insecticides, fungicides, herbicides, rodenticides, molluscicides, and others (Bondarenko *et al.* 2004). According to the data of the Iranian Plant Protection Organization in 2012–2014, an average of 14,000 tons pesticides are used in Iran every year, of which approximately 60% are just applied in Guilan, Mazandaran and Golestan provinces, located in the southern coastal areas of Caspian sea (Behrooz *et al.* 2009, Morteza *et al.* 2017). Rice commonly cultivated in the northern part of Iran, is the main course of consumers and is often preferred over other cereals by most Iranians (Abdollahzadeh *et al.* 2015). Diazinon is a main insecticide widely used in Mazandaran Province for the control of *Chilo suppressalis*, the Asiatic rice borer or the striped rice stem borer, which is a serious pest of rice (Pathak and Khan, 1994, MAHDAVI, 2001). This phosphorous pesticide contaminates food chain by entering waters and the environment (Imo *et al.* 2007, Hahn-Deinstrop 2007). Diazinon gets absorbed to the body mainly through skin and eyes, or enters via inhalation or ingestion (Anwar 1997). There are several reports showing the negative effects of exposure to diazinon on human and animal health (Kamrin 1997, Fritschi *et al.* 2015). Nowadays, cancer is a global warning and its incidence is in the rise. Some reports mentioned that there is a logical link between the increase in the amount of pesticide used with cancer risk. Pesticides can trigger cancer in a variety of ways, including disrupting hormones, genotoxicity, immunotoxicity and epigenetic effects (IARC 1991, Watts 2012,

Esfahani *et al.* 2017). There are different investigations in Iran revealing that the incidence of cancer among people in the belt between Ramsar and Behshar in Mazandaran Province is 30% higher than in other parts of Iran (Heidari 2003, Khalili *et al.* 2015).

Due to the increased risk of cancer as well as indiscriminate usage of pesticide in Mazandaran Province, encouraged us to measure the levels of a commonly-used pesticide diazinon in patients with breast cancer and also to compare them with healthy people.

MATERIALS AND METHODS

Sample collection

This study was performed on 10 patients with confirmed breast cancer at referenced hospitals in Mazandaran Province from September 2012 through December 2014. The study was approved by Ethics Committee of Sari University of Agricultural Sciences and Natural Resources (*SANRU*) based on Declaration of Helsinki and its later amendments or comparable ethical standards. Patients were informed by a physician, and the protocol was explained to the subjects, who gave their consent before inclusion. The control group included 10 healthy volunteers matching based on gender, age, and ethnicity with the patient group. A 5 mL blood sample was taken from each patient and healthy volunteers, then the samples were centrifuged (2500 rpm, 10 min), and frozen at $-20\text{ }^{\circ}\text{C}$ after serum isolation.

Chemicals

Acetone, diethyl ether (GC Assay) and methanol (GC grade) were produced by Merck (Germany). Anhydrous sodium sulfate (AR), 5N hydrochloric acid, *n*-hexane (HPLC grade) were supplied by E-Merck (India). Diazinon (purity 99 %, CAS number: 333-41-5, Supelco Analytical).

Standard solutions

To determine diazinon concentrations in serum samples, at first 125.4 mg of Stock standard solutions (99%) were prepared at a concentration of 100 mL in methanol (GC grade), the stock solution was 1254 ppm (mg L^{-1}). Four depicting standard curves of standard diazinon with the concentrations of 0.1, 0.5, 1, 5, 10 ppm were made and injected into the gas chromatography instrument to determine the retention time of the pesticide.

Gas chromatography-mass spectrometry (GC/MS)

GC-MS was performed by Clarus 680 GC (PerkinElmer, Waltham, MA, USA) with a Clarus SQ 8T mass detector, identification method: Select Ionization Report (SIR) injector was operated in electron impact ionization mode with an ionizing energy of 70eV, silica, capillary column (30 m \times 0.25 mm i.d., film thickness 0.25 μm), carrier gas: helium at a flow rate of 1 mL min^{-1} . The column, injection port and detector temperatures were 280 $^{\circ}\text{C}$, 280 $^{\circ}\text{C}$ and 300 $^{\circ}\text{C}$ respectively. All compounds, including the internal standard, were eluted within 30 min.

Extraction and Preparation of the Sample

Five hundred microliter of serum was taken in a 10 mL glass stoppered tube to which was added 2 mL of a mixture of acetone and diethyl ether (1:1 v/v). The mixture was vigorously shaken overnight using a cyclomixer. The next day they were acidified with 0.1 mL of 5N HCl. The mixture was again shaken for 2 minutes, taking the caution of avoiding prolonged contact of the sample with the 5N HCl solution to prevent the decomposition of the compounds. The organic layer was separated with a pipette and transferred to a separate tube. The residual slurry was again extracted two more times with 2 mL of diethyl ether. Ten mL of organic solvents were combined in a separate beaker (25 mL) and passed through 1 gr of anhydrous sodium sulfate to remove inorganic phosphate and water contents. The organic filtrate fraction was evaporated to dryness under a gentle stream of nitrogen gas. The residue was dissolved in 100 μL of methanol (GC grade) and a 0.6 μL aliquot was injected into the gas chromatograph (Singh & Dogra 2009).

RESULT

Retention Time

Retention Time (RT) of the diazinon was observed at 9.61 minutes (9 minutes and 36.6 seconds), after injection of 0.6 μL of each standard of diazinon concentrations (0.1, 0.5, 1, 5, 10 ppm) into chromatography. Moreover, the limit of detection (LOD) and the limit of quantitation (LOQ) were measured as 1.97 and 6.58 $\mu\text{g L}^{-1}$ respectively (Fig. 1).

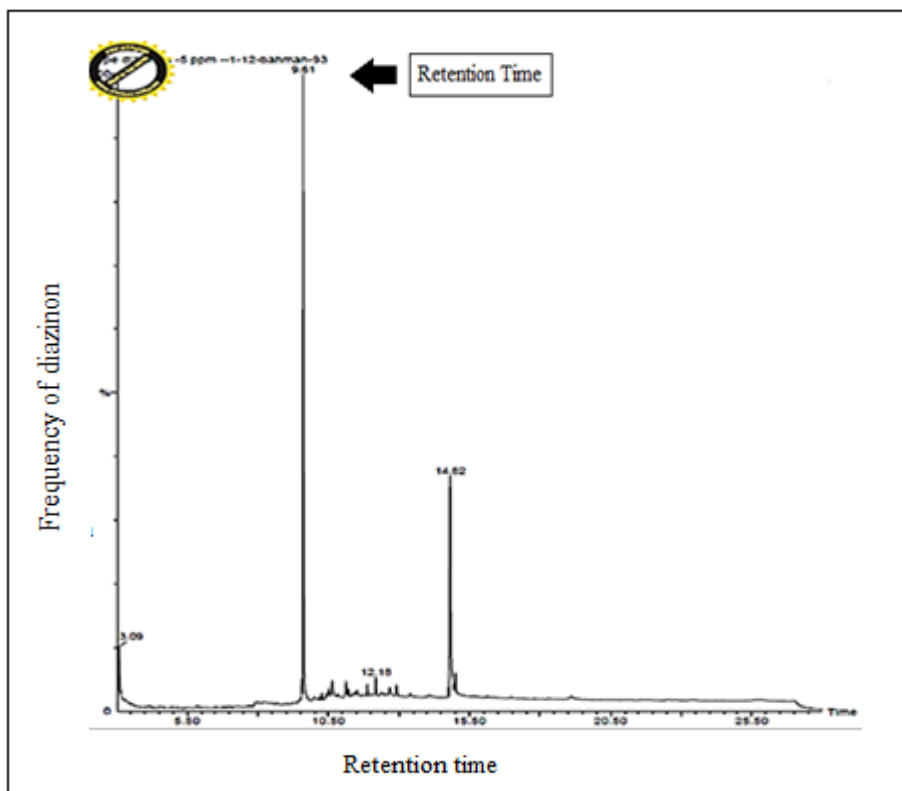


Fig. 1. Chromatogram for the retention time of diazinon at the standard pesticide diazinon concentrations (5 ppm). The horizontal axis: retention time, the vertical axis: frequency of diazinon.

A standard curve for diazinon

The standard diazinon calibration curve was plotted based on the area under the peaks of the chromatograms, then the equation of the curve was detected (Fig. 2).

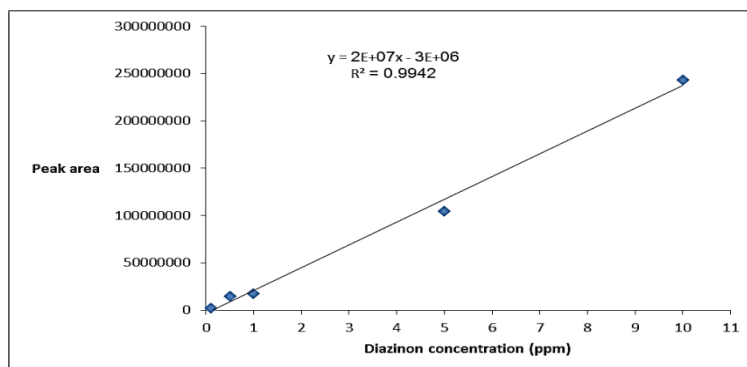


Fig. 2. Standard graph based on area under the curve to standard concentrations of diazinon. The horizontal axis: detected diazinon concentration (ppm) and the vertical axis: concentrations of diazinon standard. The equation obtained from the standard curve ($y = 2E + 07x - 3E + 06$).

Mass spectrometry of diazinon

After breaking diazinon into molecular structures, diazinon was detected based on the mass-to-charge ratio of the ions for every sample. The results showed that diazinon was present only in one healthy subject (10%) at a concentration of 0.151 ppm (Fig. 3).

DISCUSSION

According to the National Cancer Registry (NCR) report, Mazandaran Province is one of the regions with high risk of cancer in Iran. Furthermore, some studies indicated the high levels of agricultural pesticides in rivers of this province (Salehiniya et al. 2016, Marzouk et al. Darvishi et al., 2016) . Based on the available evidences, our

study aimed at measuring the levels of the most commonly used pesticide diazinon in patients with breast cancer. The results showed that diazinon was detected in only one healthy sample with the concentration of 0.151 ppm, while not observed in any patients. This result obviously revealed no logical correlation between this pesticide and risk of breast cancer. This outcome is consistent with some previous studies. A self-reported study by Niehoff *et al.* examined the association between pesticide exposure during childhood and adolescence and breast cancer risk. The results did not reveal any relationship between these factors (Niehoff *et al.* 2016). Duell *et al.* conducted a case-control study of breast cancer to examine the role of farming and pesticide exposure in American population. Their results suggested that there is no correlation between breast cancer and exposure to pesticides among farming women (Duell *et al.* 2000). Apparently, it seems that rapid degradation and the chemical instability of organophosphorus pesticides, in contrast to organochlorine pesticides, may explain these null results.

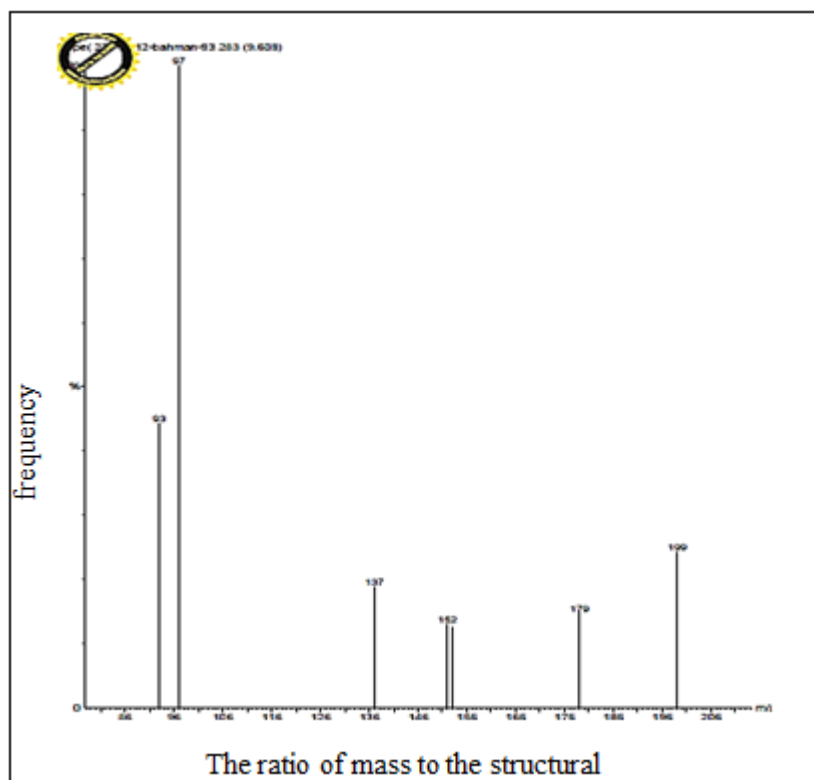


Fig. 3. Chart mass spectrometry to determine the diazinon concentration in healthy subjects. The horizontal axis: the ratio of mass to the structural components of diazinon, the vertical axis: frequency of them.

Exposure to pesticides is recognized as the environmental risk factor associated with the development of several diseases in human and animals. According to the report of Environmental Protection Agency (EPA), diazinon can induce carcinogenicity. A large body of evidence confirms the genotoxicity and mutagenicity effects of diazinon *in vitro* and *in vivo* (Barnett *et al.* 1980, Dutta *et al.* 1997, Kamalipour *et al.* 2017). Several studies have reported the association of diazinon with various cancers, including brain (Davis *et al.*, 1993b), non-Hodgkin's lymphoma (Cantor *et al.* 1992, Hu *et al.* 2017), multiple myeloma (Morris *et al.* 1986), leukemia (Beane Freeman *et al.* 2005), bladder (Mortazavi *et al.* 2019), lung (Pesatori *et al.* 1994, Beane Freeman *et al.* 2005), and prostate (Band *et al.* 2011). These chemical compounds can promote cancer through the alternative mechanisms, such as genotoxicity, disrupting hormones, immunotoxicity and epigenetic changes (Sadeghi Amiri 2018). As mentioned in previous reports, estrogenic hormones like estrogen and progesterone can lead to uncontrolled growth of breast cells, and diazinon may also affect breast cancer by imitating estrogen role and disrupting the hormonal systems (Davis *et al.* 1993a). Many studies have shown a positive link between diazinon and increased incidence of breast cancer in human and laboratory animals. A comprehensive investigation of organophosphates use and cancer risk in American population revealed that these pesticides have a strong potential for several hormonally-related cancers. So that, malathion was associated with increased risk of thyroid cancer, while diazinon was associated

with ovarian and breast cancer (Lerro *et al.* 2015). Dolapsakis *et al.* and Hopenhayn-Rich *et al.* noticed that women exposed to organophosphorous pesticides have a higher risk for breast cancer (Dolapsakis *et al.* 2001, Hopenhayn-Rich *et al.* 2002). Cabello *et al.* 2001, investigated the effects of malathion and parathion on the mammary glands of mice. They observed that these compounds enhanced the growth of the mammary gland epithelial cells and caused tumour in the animals (Brody & Rudel 2003). They also exposed mice to atrazine in the early stage of life and observed a delay in the differentiation of mammary glands followed by the occurrence of breast tumours. Moreover, rats exposed to chlorpyrifos (CPF), an organophosphorus pesticide, exhibited develop in mammary tumors with a reduced steroid receptors expression (Ventura *et al.* 2019). Methylation of CPG site in the promoter of the gene is one of most famous epigenetic mechanism which can widely induce abnormal expression pattern (Sadeghi-Amiri *et al.* 2019). Some experimental evidences displayed that diazinon may modify DNA methylation in the promoter of various genes. Zhang *et al.* 2012, for the first time examined the DNA methylation alteration in the diazinon-treated human cell. They observed that diazinon significantly change methylation pattern of 984 genes which some genes were tumor suppressor (TP53INP1 and PTEN), chromatin modification (HDAC3), DNA repair (RTEL1 and UVRAG), and proliferation /apoptosis-related genes (P53AIP1, ALX4, DAXX, MAPK11, GAS6, CDC20, CDKN1C, and CDK4). Kobatake *et al.* 2004, detected the methylation of p57KIP2 gene belonging to Cyclin-dependent kinases (CDK) family, which regulate the mammalian cell cycle, and down-regulated gene in breast and lung cancer. Larson *et al.* (2008) also reported the decrease of p57KIP2 gene, at both mRNA and protein levels, in breast cancer due to methylation of the promoter (Larson *et al.* 2008). The results of the present study revealed that despite the excessive use of diazinon in Mazandaran Province, there is no correlation between serum level of diazinon and breast cancer risk. Since pesticides play important role in causing chronic disorders like cancer, it is needed to perform more research with larger population, especially those who have occupational exposure to these pesticides such as farmers to verify the results.

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Conflicts of Interest

There are no conflicts of interest.

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بررسی ارتباط بین سطح سرمی سم دیازینون و سرطان پستان در استان مازندران

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چکیده

گزارش‌های متعددی در خصوص نقش سموم اورگانوفسفاته در بروز اختلالات مختلف از قبیل سرطان وجود دارد. استان مازندران یکی از سه استانی است که در بخش شمالی ایران قرار داشته و سموم کشاورزی به میزان زیادی در این مناطق استفاده می‌شوند. بدلیل استفاده زیاد سموم کشاورزی از قبیل دیازینون در مزارع برنج استان مازندران به منظور کنترل کرم ساقه‌خوار برنج و همچنین شیوع بالای انواع سرطان در این استان، در این تحقیق سطح سرمی دیازینون در افراد مبتلا به سرطان سینه بررسی و با افراد نرمال مقایسه گردید. این بررسی بر روی ۱۰ فرد بیمار و ۱۰ فرد سالم انجام شد. دیازینون با استفاده از مخلوطی از استون و دی‌اتیل‌اتر (۱:۱ حجمی/حجمی) در یک محیط اسیدی استخراج و میزان آن با استفاده از GC-MS اندازه‌گیری شد. نتایج نشان داد که سم دیازینون تنها در سرم خون یکی از افراد سالم به میزان ۰/۱۵۱ ppm وجود دارد. بنابراین، علیرغم استفاده زیاد از سم دیازینون در استان مازندران، ارتباط معنی‌داری بین میزان سرمی این سم و بروز سرطان سینه وجود ندارد.

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