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Organochlorine pesticide concentrations in serum from patients with thyroid diseases and effects of endosulfan I and o,p'-DDT on lymph node metastases of thyroid cancer

Xinyu Lou[1,#], Dan Chen[1,#], Zheng Dong[2], Yuhao Fan[1], Ye Wu[1], Qianqian Wang[3], Ying Wang[1,4], Jun Jin[1,4], Zhiyan Liu[3]

[1]College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China.
[2]Medical Science and Technology Innovation Center, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117, Shandong, China.
[3]Department of Pathology, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200233, China.
[4]Engineering Research Center of Food Environment and Public Health, Beijing 100081, China.

Correspondence to: Prof. Jun Jin, College of Life and Environmental Sciences, Minzu University of China, 27 Zhongguancun South Street, Haidian District, Beijing 100081, China. E-mail: junjin3799@126.com; Prof. Zhiyan Liu, Department of Pathology, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 600 Yishan Road, Xuhui District, Shanghai 200233, China. E-mail: zhiyanliu@shsmu.edu.cn

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Abstract

Organochlorine pesticides (OCPs) are common environmental pollutants. OCPs are detected in human tissues. Many studies have found that OCPs are associated with thyroid diseases, but the specific effects of OCPs on thyroid disease are not clear. We measured and analyzed the concentration of OCPs in the serum of individuals with thyroid disorders. The main components of OCPs in the serum of individuals with thyroid disorders were p,p'-DDE, β-hexachlorocyclohexane (HCH), and hexachlorobenzene (HCB). However, the concentration levels of endosulfan I and o,p'-dichlorodiphenyltrichloroethane (DDT) showed a tendency to be higher in young adults. In addition, the appearance of thyroid cancer lymph node metastasis also showed a trend of younger age. The serum concentrations of endosulfan I and o,p'-DDT were higher in patients without signs of lymph node metastasis cancer than in individuals diagnosed with thyroid cancer without cancer spread to the lymph nodes. The scratch

healing assay showed that endosulfan I and o,p′-DDT-treated experimental group had faster scratch wound healing, which indicated that endosulfan I and o,p′-DDT could promote the enhancement of the migration ability of the thyroid cancer cells, and similar results were obtained by the Transwell migration assay. This study suggests that endosulfan I and o,p′-DDT can promote lymph node metastasis of thyroid cancer.

Keywords: Thyroid disease, OCPs, lymph node metastasis, endosulfan I, o, p′-DDT

INTRODUCTION

Organochlorine pesticides (OCPs) can be divided into two categories: cyclopentadiene and benzene synthesis types. OCPs are highly toxic, stable, and lipid-soluble and readily accumulate in biota, making them a significant concern in the environmental health field. In 2001, nine OCPs, including dichlorodiphenyltrichloroethane (DDT) and hexachlorobenzene (HCB), were included in the Stockholm Convention along with three other substances, collectively known as the "dirty dozen". Later, α-, β-, and γ-hexachlorocyclohexane (HCH), endosulfan, and methoxychlor were also added to the Convention, leading to restrictions on their production and use. OCPs were the first synthetic organic pesticides to be produced and widely used. DDTs and HCHs were popular for pest control from the 1940s onward. Various OCPs have been released into environmental media, and their presence in the environment poses significant risks to human health[1-4]. Furthermore, various OCPs are already detectable in the human body.

Environmental media, including soil, water, and ambient air, contain OCPs[5-7]. OCP accumulation in human serum has also attracted attention[8]. Some OCPs have been found, in experiments, to be endocrine disruptors[9,10]. These pesticides have been linked to various health risks, including cancer, neurotoxicity, genotoxicity, diabetes, and adverse effects on the endocrine, reproductive, and immune systems[11-16]. The thyroid is an important endocrine gland in humans. Thyroid disease incidence has been increasing in recent years[17,18]. In 2020, an estimated 586,202 people were diagnosed with thyroid cancer worldwide[19]. The American Cancer Society estimated that, in 2023, 43,720 new cases of thyroid cancer (12,540 in men and 31,180 in women) would occur in the USA. Previous studies have indicated that exposure to OCPs is associated with an increased risk of thyroid disease in humans[17,20,21]. Thyroid nodules are prone to developing into thyroid cancer[22], and the progression of thyroid cancer is often accompanied by lymph node metastasis, which is a common route of cancer spread. Existing research has focused on factors influencing lymph node metastasis in thyroid cancer[23]. Whether there is an association between OCP exposure and lymph node metastasis in thyroid cancer requires further analysis.

The role of OCPs in the development of thyroid diseases deserves further investigation. The scratch wound healing assay is a laboratory technique for studying cell migration movements, repair capacity, and cell-cell interactions, and is commonly used to assess cell migration capacity. The speed of cell migration can be compared to the speed of scratch healing[24]. A number of studies have been done to verify the effect of certain influencing factors on the progression of papillary thyroid carcinoma (PTC) through the scratch wound healing assay. Transwell migration assay is widely used in the study of cancer cell metastasis, which helps to understand the mechanism of cancer metastasis and is important for the investigation of the factors affecting the migration and invasion of thyroid cancer cells. Tang *et al.* used the Transwell migration assay to explore the regulatory effects of miR-152 on thyroid cancer and its mechanism[25].

In this study, the concentrations of 14 OCPs were measured in the serum of patients with thyroid disease. Additionally, the differences in OCP levels between male and female patients, as well as across various age groups, were evaluated. Associations between OCP concentrations and thyroid diseases were investigated.

The aim was to determine which OCPs strongly affect thyroid diseases. After finding that endosulfan I and o,p'-DDT may promote the development of lymph node metastasis in thyroid cancer, the present study attempted to validate this speculation using cell experiments *in vitro*.

MATERIALS AND METHODS

Sample collection

Serum samples from those suffering from thyroid diseases were collected from the Sixth People's Hospital in Shanghai in 2022. All participants in the study are adults. Due to the higher incidence of thyroid diseases in women, there are more females than males among the participants. Additionally, participants are required to have no other related endocrine diseases and to have maintained stable lifestyle habits over the past three months. The population samples had no endocrine disorders other than thyroid disease and they had a stable lifestyle. Every patient gave informed consent after the study had been clearly explained and also approved by the Ethics Committee of the School of Basic Medical Sciences, Shandong University. Serum from fresh blood samples was stored at -20 °C. A total of 59 serum samples were collected. The characteristics of the people who provided the serum samples are summarized in Table 1.

Most of the subjects were female, consistent with the sex ratio of the patients with thyroid diseases treated by the hospital. The participants were 20-70 years old, and most lived in the Yangtze River Delta area. Of the 59 patients, 56 had been diagnosed with tumors (11 benign and 45 malignant). The malignant tumors had been confirmed by medical examination, and 24 had lymph node metastases, 19 had no lymph node metastases, and it could not be determined if two patients had lymph node metastases.

Materials and instruments

^{13}C-labeled OCPs ($^{13}C_{12}$-p,p'-DDE, $^{13}C_{10}$-dicarboxylate, $^{13}C_6$-HCB, $^{13}C_6$-β-HCH, $^{13}C_6$-γ-HCH, $^{13}C_{10}$-mimetron, and $^{13}C_{10}$-*trans*-nachloride) were purchased from Cambridge Isotope Laboratory (Andover, MA, USA). Standards of 14 OCPs (HCB, endosulfan, α-HCH, β-HCH, γ-HCH, δ-HCH, o,p'-dichlorodiphenyldichloroethane, p,p'-dichlorodiphenyldichloroethane, o,p'-DDE, p,p'-DDE, o,p'-DDT, p,p'-DDT, and methoxychlor) were purchased from AccuStandard (New Haven, CT, USA).

Human thyroid papillary carcinoma cell lines BHP 10-3 and TPC-1 were purchased from the Shanghai Cell Bank Type Culture Collection (Shanghai, China).

Ultrapure water was prepared using a Milli-Q system (Merck, Darmstadt, Germany). Pipettes were obtained from Eppendorf (Hamburg, Germany). A TDL-40B centrifuge was supplied by Shanghai Anting Scientific Instrument Factory (Shanghai, China). A BF2000 nitrogen evaporation system was obtained from Bafang Century (Beijing, China). An N-1300D-WB evaporation instrument was purchased from EYELA (Tokyo, Japan). A Trace 1310-TSQ 8000 gas chromatograph mass spectrometer was supplied by Thermo Fisher Scientific (Waltham, MA, USA). A GloMax Luminometer was procured from Promega (Madison, WI, USA). A Primovert inverted microscope was made by Zeiss (Oberkochen, Germany).

A J&W DB-5-MS gas chromatography column (30 m long, 0.25 mm i.d., 0.25 μm film thickness) was obtained from Agilent Technologies (Santa Clara, CA, USA). RPMI 1640 media were supplied by Gibco (Grand Island, NY, USA). CellTiter-Glo Luminescent Cell Viability Assay kits were purchased from Promega (Madison, WI, USA). 24-well Transwell chambers (8 μm pores) were obtained from Corning (Corning, NY, USA).

Table 1. Demographic and baseline characteristics of the study population (*n* = 59)

Variable		Number of participants (*n* = 59)
Sex	Male	17
	Female	42
Age	Minimum	20
	Maximum	70
	Mean (standard deviation)	47 (12)
Thyroid tumor	Benign	11
	Malignant	45
Lymph node metastasis		24
No lymph node metastasis		19

Determining OCP concentrations in serum

Sample preparation

A serum sample for analysis was removed from the refrigerator and allowed to thaw for one day, then 2 mL was transferred by pipette to a centrifuge tube. A 40 μL aliquot of a solution containing ^{13}C-labeled OCPs, each at a concentration of 50 pg/μL, was added to the serum sample. Each centrifuge tube received 1 mL of 6 mol/L hydrochloric acid, followed by swirling, and then 6 mL of isopropyl alcohol was added and the mixture was swirled again. A 6 mL aliquot of a 1:1 v/v mixture of n-hexane and methyl tert-butyl ether was subsequently added, followed by oscillation of the tube using a vortex device.

After centrifugation at 2,000 rpm for 5 min, the supernatant was collected into a fresh centrifuge tube. Subsequently, 3 mL of a 1:1 n-hexane and methyl tert-butyl ether mixture was added to the aqueous residue, and the mixture was agitated. The mixture was centrifuged and the supernatant was mixed with the previously removed supernatant. The extraction procedure was repeated once more. The mixed supernatants were evaporated to 7 mL using a nitrogen evaporation system, then 4 mL of 1% KCl in water was added, and the mixture was oscillated.

Following centrifugation at 2,000 rpm for 5 min, the supernatant was transferred into a centrifuge tube that had been pre-weighed. Then, 4 mL of a 1:1 v/v mixture of n-hexane and methyl tert-butyl ether was added to the aqueous residue, and the mixture was agitated. The mixture was then centrifuged and the supernatant transferred to the weighed centrifuge tube. This process was repeated, and the supernatants were mixed and then evaporated to dryness using flowing nitrogen. The centrifuge tube was weighed and the difference between the clean tube and tube containing the residue was defined as the mass of lipid extracted from the serum sample. A 4 mL aliquot of *n*-hexane and 2 mL of 0.5 mol/L KOH in a 1:1 v/v mixture of ethanol and water were added to the centrifuge tube, and then the mixture was oscillated using the vortex instrument.

The tube was then centrifuged at 2,000 rpm for 5 min. The supernatant was transferred to a fresh centrifuge tube, then 3 mL of *n*-hexane was added to the aqueous residual liquid, and the mixture was oscillated. The mixture was centrifuged again and the supernatant was mixed with the previous supernatant. The *n*-hexane extraction process was then repeated and the supernatant was mixed with the previous supernatant mixture.

The mixed supernatant was evaporated to 1 mL under flowing nitrogen and then passed through a gel permeation chromatography column.

The eluate was evaporated, transferred to a 15 mL centrifuge tube, and then evaporated to 1 mL using flowing nitrogen. The extract was then passed through an acidified silica gel chromatography column

containing, from bottom to top, glass wool, 0.5 g of neutral silica gel, 0.5 g of acidified silica gel, and 1.5 g of anhydrous Na_2SO_4. The purified extract was collected in a 15 mL centrifuge tube.

Using evaporation under flowing nitrogen, the purified extracts were concentrated to 100 μL of n-nonane. The clean extract was stored cold until it was analyzed.

Gas chromatography-mass spectrometry analysis

A Trace 1310 Gas Chromatograph coupled with a TSQ 8000 Evo triple quadrupole mass spectrometer (Thermo Fisher Scientific, USA) was employed to analyze the OCP concentrations in the samples. The mass spectrometer operated in electron impact ionization (EI) mode, with selective ion monitoring (SIM) employed to detect the characteristic ions of the target compounds. The ion source and transmission line were maintained at 250 °C. Helium of high purity served as the carrier gas, with a flow rate of 1.0 mL/min, while high-purity argon was used as the collision gas. The temperature program for the oven began at 80 °C, held for 3 min, then ramped at 6 °C/min to 260 °C, where it was held for 7 min. Separation of the compounds was achieved using a DB-5-MS gas chromatography column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness).

Quality control

The R^2 values for the standard calibration curves plotted using an internal standard method were all > 0.999. The recoveries were 31.6%-156.3%. A method blank was analyzed alongside each batch of samples. The analytes were detected at very low levels in the blank samples (approximately 10% of the concentrations observed in the test samples), with signal-to-noise ratios below 10. As a result, the sample concentrations were not blank corrected. The limit of quantification was 0.08 ng/g lw. If an analyte concentration was below the detection limit, it was labeled "not detected".

Cell experiments *in vitro*

Cell culture and cell viability assessments

Both cell lines were cultured in RPMI 1640 medium, supplemented with 100 U/mL penicillin/streptomycin and 10% fetal bovine serum, in a 37 °C cell incubator with a 5% CO_2 atmosphere.

Cell viability of BHP 10-3 and TPC-1 cells, exposed to various concentrations of endosulfan I or o,p'-DDT for 24 h, was assessed using the CellTiter-Glo Luminescent Cell Viability Assay, according to the manufacturer's instructions. The results of the cell viability assessment are detailed in Supplementary Figure 1.

Scratch wound healing assay

BHP 10-3 or TPC-1 cells were seeded in 12-well culture plates and allowed to grow to full confluence. The cell monolayer was then scratched in a straight line with a sterile pipette tip and washed with phosphate-buffered saline to remove any cellular debris. The cells were then treated with 5 or 10 μM endosulfan I or o,p'-DDT, and dimethyl sulfoxide was used as the solvent control. Images of the migrated cells were acquired using a Primovert inverted microscope at 0, 12, and 24 h after the wound was inflicted[24].

Transwell migration assay

BHP 10-3 or TPC-1 cells, suspended in serum-free medium, were plated in the upper chambers of 24-well Transwell plates, with medium supplemented with 20% fetal bovine serum placed in the lower chambers. The cells were exposed to 5 or 10 μM of endosulfan I or o,p'-DDT for 12 or 24 h. After incubation, the migrated cells on the underside of the upper chambers were fixed with 4% paraformaldehyde, stained with

0.25% crystal violet solution for 30 min, and subsequently imaged using a Primovert inverted microscope[26].

Statistic analysis

The raw data obtained from the GC-MS/MS assay were processed by TraceFinder software (Thermo Fisher Scientific, Waltham, MA, USA) to obtain OCP concentration values. The data were statistically analyzed using SPSS software (International Business Machines Corporation, Armonk, NY, USA). It was examined that the OCP concentration data did not conform to the normal distribution law, so non-parametric tests were used to assess the differences between different categories of data. The images obtained from the scratch wound healing assay were processed using Image J software (National Institutes of Health, Bethesda, MD, USA) to identify the scratch area and record the scratch area, and then the healing rate was calculated. The images obtained from the Transwell migration assay were also processed using Image J software to identify the number of cells in the same area of the experimental groups in different categories. The results of the scratch healing assay and the Transwell migration assay were analyzed together to determine the effects of endosulfan I and o,p'-DDT on the migration of thyroid cancer cells.

RESULTS AND DISCUSSION

OCP concentrations in serum from patients with thyroid diseases and differences between people of different sexes and different ages

In this study, the concentrations of 14 OCPs, including HCB, endosulfan I, endosulfan II, α-HCH, β-HCH, γ-HCH, δ-HCH, o,p'-DDD, o,p'-DDE, o,p'-DDT, p,p'-DDD, p,p'-DDE, and p,p'-DDT, were measured, along with Methoxychlor. The maximum concentrations of 13 OCPs exceeded the detection limit, with the exception of endosulfan II, which was not detected. The detection rate of $\sum_{14}$OCPs in the serum of patients with thyroid disease was 100%. The concentrations of the seven OCPs that were detected in > 50% of the samples are shown in Figure 1. The p,p'-DDE, β-HCH, and HCB detection rates were 100%, and these OCPs contributed 64.4%, 23.7%, and 7.7%, respectively, of the total OCP concentrations in the serum samples. Endosulfan I was heavily used as a pesticide and had a detection rate of 78%, even though it has been banned by many countries. The total OCP concentrations in the serum samples were 118.7-6,576.7 ng/g lw, and the mean concentration was 1,061.9 ng/g lw.

Wang *et al.* determined the concentration of OCPs in human blood from five cities in China, including Yitong, Weifang, Ganzi, Huaihua, and Lingshui, and 13 OCPs were detected, with a mean total OCP concentration of 767 ng/g lw, and the range of the mean total OCP concentration levels in the different cities was 560-975 ng/g lw. p,p'-DDE, HCB, β-HCH, and endosulfan I were the major OCP constituents in serum samples, accounting for 46%, 28%, 14%, and 5.2%, respectively[27]. Polachova *et al.* assessed the concentrations of 11 OCPs in serum from Czech police officers, with total OCP concentration levels ranging from 0.106 to 1,016 ng/g lw, and p,p'-DDE and HCB being the major constituents[28]. Achour *et al.* determined the concentrations of DDTs, HCHs, and HCB in human serum in Tunisia, with total OCP concentration levels ranging from 79 to 343 ng/g lw, with a mean value of 189 ng/g lw, and p,p'-DDE as the main constituent accounting for about 88% of the DDTs[29]. Han *et al.* determined the concentrations of DDTs, HCHs, and HCB in patients with type 2 diabetes mellitus in East China, as well as in a non-diseased population 29 OCPs in serum, of which six were detected at more than 75%, namely β-HCH, trans-chlordane, trans-nine-chlorine, p,p'-DDE, p,p'-DDT, and mirex/chlordecone, with the highest concentration also found for p,p'-DDE[11]. Attaullah *et al.* examined serum samples from patients with confirmed cancer and healthy residents of Karachi, Pakistan, for residues of 14 OCPs. They found that OCPs were detected in 97.59% of cancer cases and 93.75% of healthy subjects, with a mean concentration of 322 ng/g lw of total OCPs in healthy residents and a somewhat higher concentration of 606 ng/g lw in cancer patients, with the main constituents being endosulfan and p,p'-DDE[12]. The determination of the main components of OCPs and their percentage within serum in the present study is similar to the results

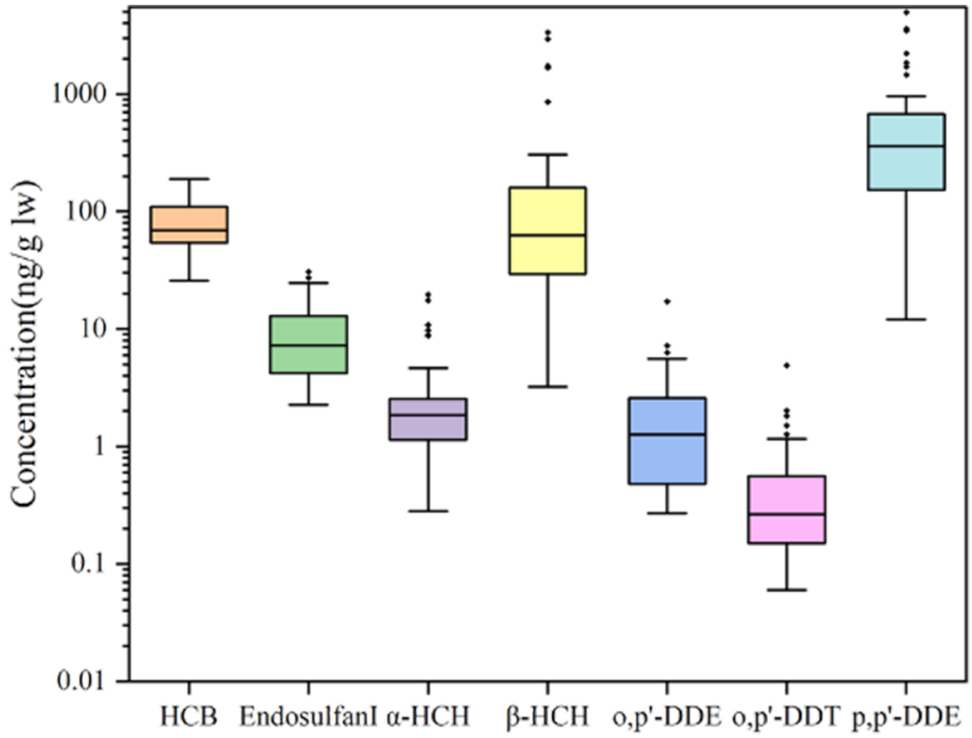

Figure 1. Concentrations of OCPs in the serum of patients with thyroid diseases. HCB: Hexachlorobenzene; HCH: hexachlorocyclohexane; DDE: dichlorodiphenyldichloroethylene; DDT: dichlorodiphenyltrichloroethane; OCPs: organochlorine pesticides.

of studies conducted around the world in recent years. Comparing the composition of human serum OCPs in different studies, the concentration level of p,p'-DDE was the highest, followed by HCB and HCHs. Endosulfan was also considered a major component in a few studies. The percentage of other OCPs was relatively low. The above is also consistent with past studies, in which samples from China tended to have higher concentrations of p,p'-DDE and β-HCH, including in breast milk[30]. p,p'-DDE is the main degradation and metabolite of DDTs. p,p'-DDE has the highest percentage of concentration, presumably because malaria is endemic in most of the southern part of China, so the use of DDTs is more widespread in the place where the subjects of the present study live[27]. A comparison between patients with thyroid disease and non-diseased populations showed that patients with thyroid disease had higher serum levels of OCPs. These results indicate that OCP concentrations may be related to thyroid diseases. It is therefore necessary to continue to monitor OCP concentrations in serum samples collected from patients diagnosed with thyroid diseases and differences between OCP concentrations in serum samples collected from patients diagnosed with thyroid diseases and the general population.

The ages of the patients with thyroid diseases involved in this study ranged from 20 to 70 years, and according to the latest age classification criteria organized by the World Health Organization, the participants were divided into young adults (< 45 years old) and middle-aged and elderly adults (> 45 years old). The OCP concentrations in the serum samples from the different age groups are shown in Figure 2. Upon comparison, p,p'-DDE concentration levels were found to show strong variability between young adults with thyroid disease and middle-aged and elderly patients with thyroid disease. Serum p,p'-DDE concentrations were significantly higher in middle-aged and elderly adults than in young adults ($P < 0.05$). Similar trends were observed for HCB, α-HCH, β-HCH, and o,p'-DDE, but it did not show significant

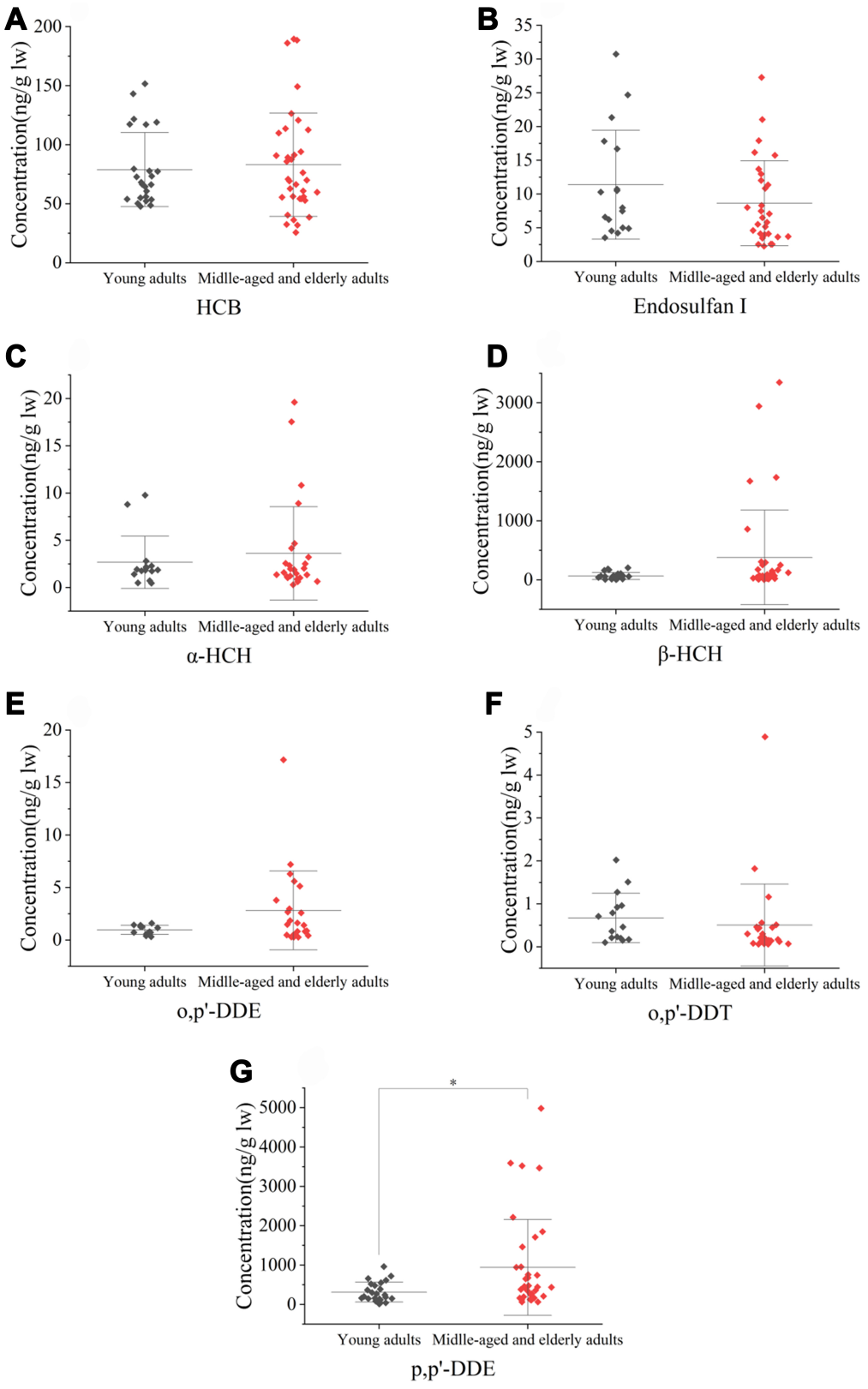

Figure 2. Concentrations of OCPs in the serum of individuals from different age groups. OCPs: Organochlorine pesticides.

variability between age groups. The concentration levels of total OCPs showed higher levels in middle-aged and elderly adults than in young adults, which is similar to the results of some previous studies, where a positive correlation between the concentration levels of most of the OCPs and age was found in the studies of Arrebola *et al.*, Ibarluzea *et al.*, and Wittsiepe *et al.*[13,31,32]. Higher p,p'-DDE concentrations have previously been found in serum from cancer patients than in healthy people, but we did not find higher p,p'-DDE concentrations in serum from patients with lymph node metastases than in serum from individuals diagnosed with thyroid cancer but no lymph node metastases[12]. The endosulfan I and o,p'-DDT concentrations were higher in the serum samples from the young adults than the middle-aged and elderly adults. The mean and median endosulfan I and o,p'-DDT concentrations were higher in serum from the young adults than the middle-aged and elderly adults. The mean and median endosulfan I concentrations in serum from the young adults were 11.4 and 8.0 ng/g lw, respectively, and the mean and median endosulfan I concentrations in serum from the middle-aged and elderly adults were 8.6 and 6.5 ng/g lw, respectively. The mean and median o,p'-DDT concentrations in serum from the young adults were 0.7 and 0.5 ng/g lw, respectively, and the mean and median o,p'-DDT concentrations in serum from the middle-aged and elderly adults were 0.5 and 0.2 ng/g lw, respectively. In a study by Wang *et al.*, serum concentrations of endosulfan I were found to be slightly higher in young people than in older individuals[27]. Tsatsakis *et al.* collected hair samples from residents of Greece for testing and found higher o,p'-DDT concentration levels in younger age groups[33]. Given the increasing prevalence of thyroid disease among younger individuals, exposure to endosulfan I and o,p'-DDT in this population is a growing concern.

OCP concentrations in serum from people of different ages and thyroid cancer patients with and without lymph node metastases

The results indicated that lymph node metastasis of thyroid cancer is more likely to occur in young than in older people. As shown in Supplementary Figure 2, the individuals diagnosed with thyroid cancer with lymph node metastases were significantly younger than the individuals diagnosed with thyroid cancer but without lymph node metastases ($P = 0.015$). The mean age of the individuals diagnosed with thyroid cancer lymph node metastases was 41, which was lower than the mean age of 49 for individuals diagnosed with thyroid cancer but without lymph node metastases. This suggested that the risk of lymph node metastasis is higher for younger than older people with thyroid cancer. Do *et al.* found that the thyroid cancer and lymph node metastasis incidences were significantly higher for people < 50 years old than people > 50 years old, and the statistical differences were $P = 0.0310$ for thyroid cancer and $P = 0.0496$ for lymph node metastasis[34]. Similar results were found in our study, with lymph node metastasis of thyroid cancer being more common in young adults than middle-aged and elderly adults. Thyroid diseases and lymph node metastasis of thyroid cancer are more common in younger than older people, and there is an urgent need to investigate the factors involved.

Endosulfan I and o,p'-DDT concentrations were higher for serum from patients with lymph node metastases of thyroid cancer than patients without lymph node metastasis of thyroid cancer. This was particularly true for o,p'-DDT, for which the difference was significant ($P = 0.039$). The concentrations of seven OCPs in serum from patients with and without lymph node metastasis of thyroid cancer are shown in Figure 3. The mean endosulfan I concentration in serum from patients with and without lymph node metastases of thyroid cancer were 10.0 and 8.6 ng/g lw, respectively. The mean o,p'-DDT concentrations in serum from patients with and without thyroid cancer lymph node metastases were 0.6 and 0.3 ng/g lw, respectively, and the medians were 0.4 and 0.2 ng/g lw, respectively. We performed statistical tests and found that the o,p'-DDT concentrations in serum from patients with lymph node metastases of thyroid cancer were significantly higher than the o,p'-DDT concentrations in serum from patients without metastasis ($P < 0.05$). Higher OCP concentrations have previously been found in serum from thyroid cancer patients than in healthy people[17]. A mean endosulfan concentration of 214 ng/g has been found in serum

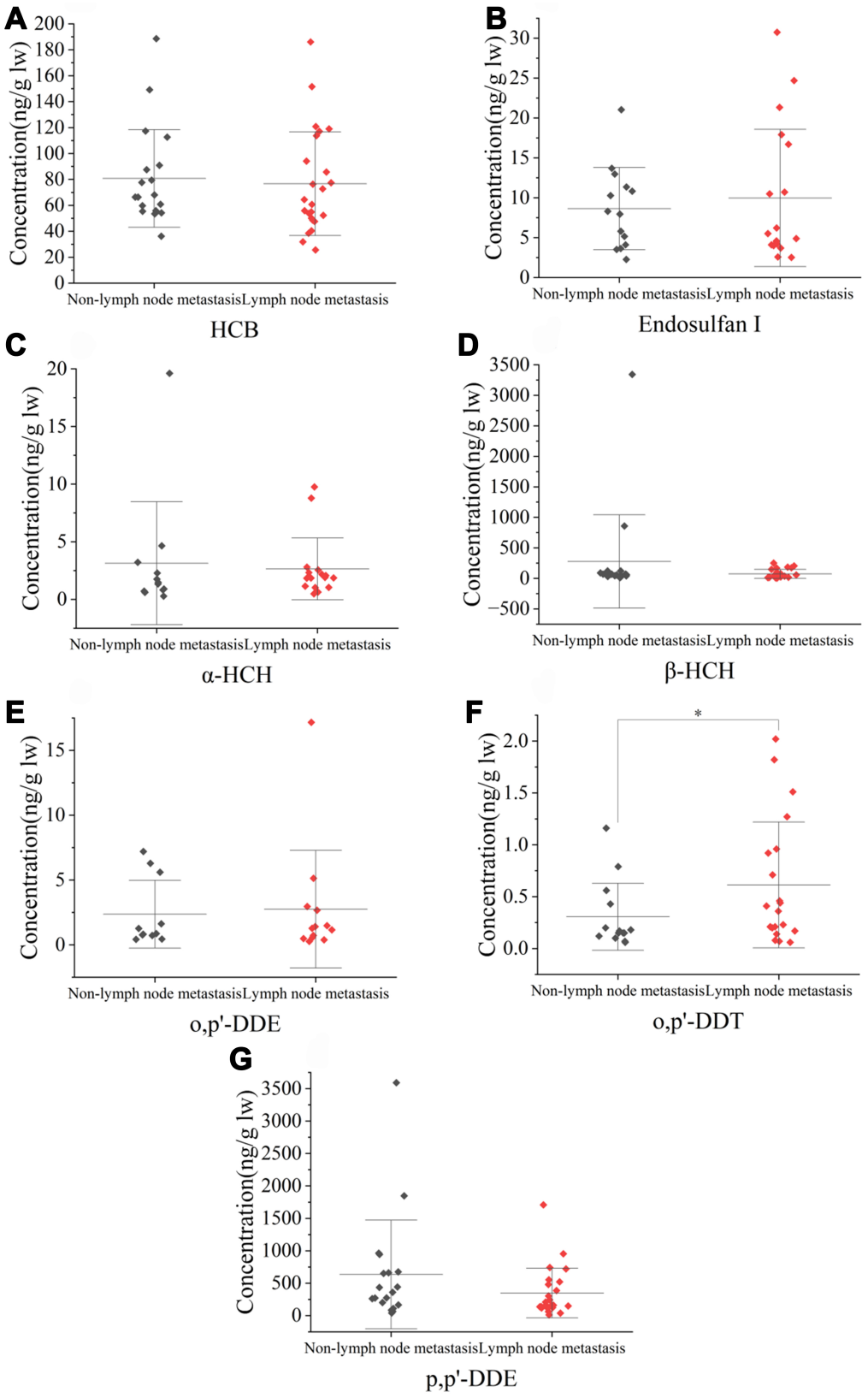

Figure 3. Concentrations of OCPs in the serum of individuals diagnosed with thyroid cancer, with or without lymph node metastases. OCPs: Organochlorine pesticides.

from cancer patients, and this was significantly higher than the concentration of 166 ng/g found in serum from healthy people[12]. However, associations between o,p'-DDT and cancers are still not understood. This study was therefore focused on the effects of endosulfan I and o,p'-DDT on lymph node metastases of thyroid cancer.

Promotion of thyroid cancer cell migration by endosulfan I and o,p'-DDT

The results described above led us to speculate that endosulfan I and o,p'-DDT could affect lymph node metastasis in people with thyroid cancer. We, therefore, performed *in vitro* experiments to investigate the effects of endosulfan I and o,p'-DDT on thyroid cancer cell migration. Given that almost all of the thyroid cancer patients involved in this study were patients with PTC, two PTC strains, BHP 10-3 and TPC-1 cells, were used in the cell migration tests.

It was verified by scratch wound healing assay that endosulfan I and o,p'-DDT could stimulate the accelerated migration of papillary thyroid cancer cells of both BHP10-3 and TPC-1. The experimental phenomenon [Figure 4] was more pronounced for BHP10-3 than for TPC-1, especially at 24 h. In the scratch healing experiments of BHP10-3, the mean values of scratch healing rate in the blank control group at 12 and 24 h were 26.4% and 82.1%, respectively, the mean values of scratch healing rate in the 5 μM endosulfan I experimental group at 12 and 24 h were 51.7% and 100.0%, respectively, the mean values of scratch healing rate in the 10 μM endosulfan I experimental group at 12 and 24 h were 54.5% and 100.0%, the average values of scratch healing rate for 12 and 24 h in the 5 μM o,p'-DDT experimental group were 58.1% and 100.0%, respectively, and the average values of scratch healing rate for 12 and 24 h in the 10 μM o,p'-DDT experimental group were 46.3% and 100.0%, respectively.

In the Transwell migration assay, this study found that the number of cells migrated in the presence of endosulfan I and o,p'-DDT was greater than that of the blank control group at the same moment. As shown in Figure 5, the experimental phenomenon of the TPC-1 cell group was especially remarkable. In the TPC-1 group, the number of cells migrated in the blank control group, 5 μM endosulfan I, 10 μM endosulfan I, 5 μM o,p'-DDT, and 10 μM o,p'-DDT at 12 h was 207, 323, 275, 256, and 210, respectively; and the number of cells migrated in the blank control group, 5 μM endosulfan I, 10 μM endosulfan I, 5 μM o,p'-DDT, and 10 μM o,p'-DDT at 24 h was 410, 843, 722, 568, and 572, respectively. Based on the results of the Transwelll migration assay, it can be concluded that endosulfan I and o,p'-DDT can promote the enhancement of the migration ability of BHP 10-3 and TPC-1 cells.

In summary, we determined that endosulfan I and o,p'-DDT have a direct promotional enhancement effect on the migration ability of thyroid cancer cells. Invasion and migration of thyroid cancer cells is a complex process. Reduced cellular matrix, intercellular adhesion, and extracellular matrix degradation may affect thyroid tissue metastasis. Similar to this study, Chang *et al.* used BHP10-3 and TPC-1 to investigate how miR-873-5p inhibits the malignant behavior of thyroid cancer cells by targeting CXCL5 and regulating the P53 pathway using scratch healing assay and Transwell migration assay, and the results of the experiments showed that the downregulation of CXCL5 significantly inhibited the malignant behavior of thyroid cancer cells[35]. Although the present study verified the promotional effect of endosulfan I and o,p'-DDT on PTC lymph node metastasis in thyroid cancer using scratch healing assay and Transwell migration assay, there are various pathways contributing to cancer metastasis, and the specific mechanism by which endosulfan I and o,p'-DDT stimulate lymph node metastasis in thyroid cancer is still unclear and needs to be verified by more experiments in the next studies.

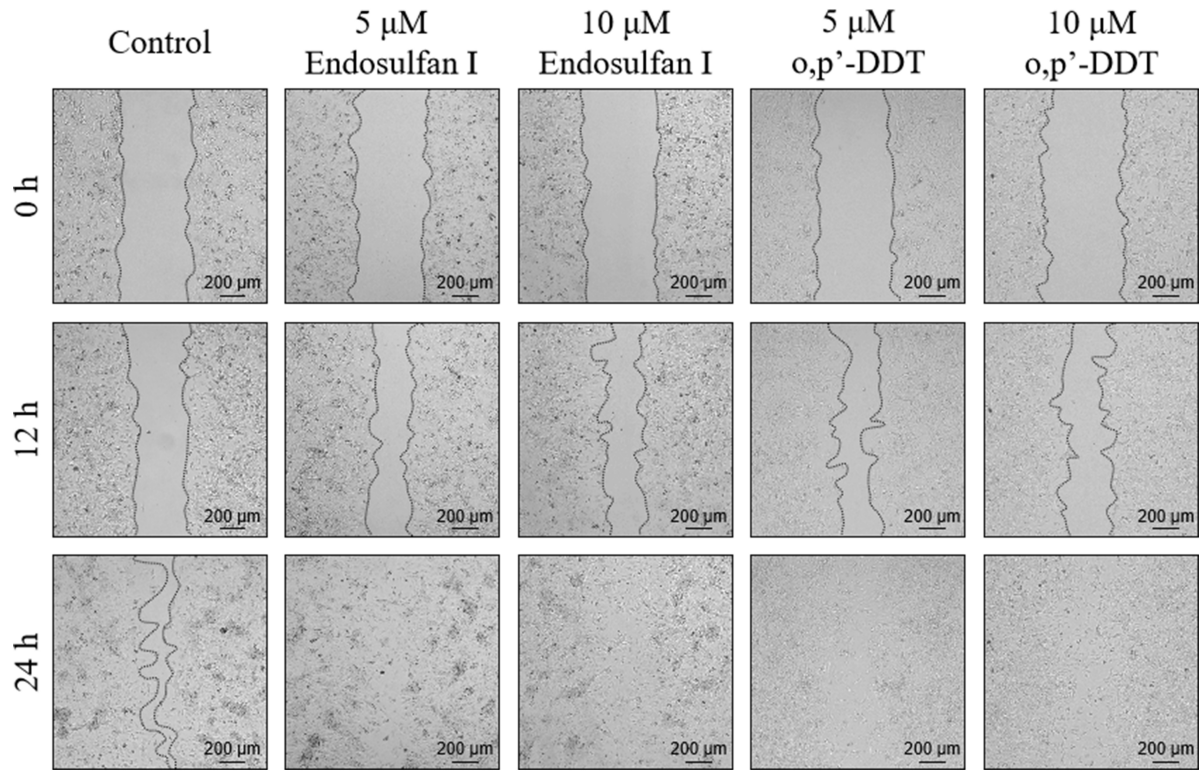

Figure 4. Effects of endosulfan I and o,p′-DDT on BHP 10-3 cell migration in the scratch wound healing assays. BHP 10-3 cell migration after exposure to 5 or 10 μM endosulfan I or o,p′-DDT was evaluated by performing a cell-scratch-wound healing assay. Images from the same area were acquired at 0, 12, and 24 h after the wound was inflicted. The solvent control was dimethyl sulfoxide. The black dotted lines are the scratch borders. DDT: Dichlorodiphenyltrichloroethane.

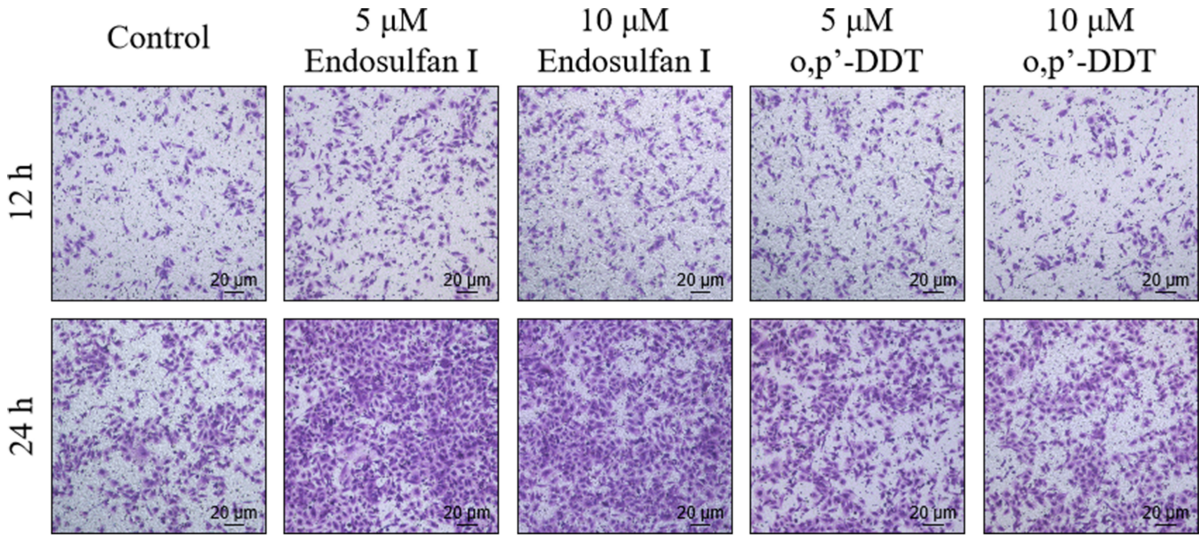

Figure 5. Effects of endosulfan I and o,p′-DDT on TPC-1 cell migration in the Transwell migration assays. TPC-1 cell migration after exposure to 5 or 10 μM endosulfan I or o,p′-DDT was evaluated by performing Transwell assays. The migrated cells were visualized at 12 and 24 h by staining with crystal violet. The solvent control was dimethyl sulfoxide. DDT: Dichlorodiphenyltrichloroethane.

CONCLUSION

The mean $\sum_{14}$ OCP concentration in serum from patients with thyroid diseases was 1,061.9 ng/g lw, which was higher than the concentrations in healthy people. p,p'-DDE, β-HCH, and HCB were the dominant OCPs in patients with thyroid diseases, contributing 64.4%, 23.7%, and 7.7%, respectively, of the $\sum_{14}$ OCP concentration. No sex differences were found in the concentration of OCPs. The total concentrations of OCPs were significantly higher in middle-aged and elderly adults compared to young adults. Furthermore, the concentrations of endosulfan I and o,p'-DDT were higher in the serum of young adult patients than in that of middle-aged and elderly patients. Lymph node metastases were found to be more prevalent in younger thyroid cancer patients than in older ones. Additionally, endosulfan I and o,p'-DDT concentrations were higher in the serum of thyroid cancer patients with lymph node metastases. Scratch wound healing and Transwell migration assays confirmed that endosulfan I and o,p'-DDT directly promote thyroid cancer cell migration. These findings support the conclusion that endosulfan I and o,p'-DDT facilitate lymph node metastasis of thyroid cancer cells. Due to the limitation of sample size in this study, future research will increase the sample size to further investigate the concentrations of OCPs within the thyroid disease population. Additionally, further studies are needed to explore the underlying pathways and mechanisms through which endosulfan I and o,p′-DDT promote the metastasis of thyroid cancer cells.

DECLARATIONS

Authors' contributions

Conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, writing - original draft: Lou, X.; Chen, D.
Data curation, investigation, methodology, visualization: Dong, Z.
Formal analysis, methodology, visualization: Fan, Y.
Formal analysis, validation, visualization: Wu, Y.
Data curation, investigation, resources, validation: Wang, Q.
Conceptualization, formal analysis, methodology, visualization, writing - review and editing: Wang, Y.
Conceptualization, funding acquisition, project administration, supervision, writing - review and editing: Jin, J.
Funding acquisition, investigation, project administration, resources, supervision, writing - review and editing: Liu, Z.

Availability of data and materials

The data that support the findings of this study are available in the Supplementary Materials of this article.

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Conflicts of interest

Jin, J. is an Editorial Board member of *Journal of Environmental Exposure Assessment*. Jin, J. was not involved in any steps of editorial processing, notably including reviewer selection, manuscript handling, and decision making. The other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study protocol has been reviewed and approved by the Ethics Committee of the School of Basic Medical Sciences, Shandong University (ECSBMSSDU2019-1-024). All participants signed a written informed consent document prior to enrollment.

Consent for publication

Not applicable.

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