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Burden of organochlorine pesticides in blood and its effect on thyroid hormones in women

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Abstract

Man has utilized a wide variety of pesticides to combat the crop pests and vectors of human diseases. However, in this process, he has overlooked the darker side of these noxious chemicals, the concentrations of which have reached the environment and pose serious threats, such as mutagenesis, teratogenesis, carcinogenesis and endocrine dysfunction in various components of the ecosystem. The present study was planned to assess the burden of organochlorine pesticides and their influence on thyroid function in women. The study included a total of 123 women from Jaipur City who visited the Thyroid clinic in SMS Medical College and Hospital. One hundred women showed normal thyroid hormone levels while the remaining 23 women had depleted T_4 and high TSH levels. The qualitative and quantitative estimation of organochlorine pesticides was carried out by gas chromatography. Out of the analyzed pesticides, the concentration of *p,p'*-DDT and its metabolites was higher in all the subjects, but dieldrin was found to be significantly high in the hypothyroid women. The correlation analysis for dieldrin and depleted T_4 levels in hypothyroid women elicited an inverse relationship between them.
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1. Introduction

Pesticides have been largely responsible for increased yields and improved quality of cereals, root crops, fruits and vegetables by making them free of insects. However, the large-scale usage of these pesticides, particularly chlorinated hydrocarbons, has led to their presence in the environment, including food, water, soil, and even air. Evidence suggests that humans and domestic and wild life species have suffered adverse health consequences from exposure of these environmental chemicals (Kavlock et al., 1996). These chemicals, being lipophilic, non-polar and relatively resistant to degradation, persist in the environment after they have performed their job. As a result, organisms that are at the top of food chain may sustain injury from these chemicals due to the gradual accumulation of residues from their food sources. During the last few decades, the residues of these noxious

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chemicals have been reported in various food commodities, air, water, soil and biological materials, such as milk, blood and fat from animal and humans (Sharma and Bhatnagar, 1996; John et al., 2001) which can pose great problems in the human body. This in turn may disturb the normal functioning of the body, including the hormones controlling the overall metabolism.

The normal functions of all the organ systems are regulated by hormones and small disturbances in endocrine function especially during certain stages of lifecycle can lead to profound and lasting effects. Reports indicate that chronic exposure of endocrine system to these pollutants result in multitude of effects in human and wildlife species (Gierthy et al., 1997). Like Rachel Carson's 'Silent Spring', 'Our Stolen Future' mentioned the names of some 50 synthetic chemical compounds that the body confuses with endogenous hormones. The synthetic hormonal 'mimics' have the power to trigger developmental abnormalities or block normal development. This hormonal disruption occurs due to compounds (which include some plastics, personal care products, contraceptive creams, detergents and pesticides) with different structures that degrade into disrupters (Johnson, 1996). Endocrine disrupters can throw off the system by sending the wrong signals or blocking the right signals. This effect may be temporary in case of adults, but small children and babies are more vulnerable and there can be permanent effects on a fetus. Therefore, endocrine disruption can result in morphologic, functional or behavioral abnormalities and certain malignancies (Kavlock et al., 1996).

It has been reported that the exposure of a variety of compounds, such as dioxin-like and non-dioxin-like (non-*ortho*-substituted polychlorinated biphenyls {PCBs}, polychlorinated dibenzodioxins/furans {PCDDs/PCDFs} and organochlorine pesticides) affects reproductive and hormonal parameters, including androgen status, sexual differentiation and thyroid functionality in humans and experimental animals (Birnbaum, 1995; Feeley, 1995). Srivastava et al. (1995) also reported that there are a number of xenobiotics, including halogenated organic chemicals, that may lead to alterations in thyroid hormone homeostasis in man and experimental animals. Therefore, the present study was conducted to assess the exposure of organochlorine pesticides in general populations.

2. Materials and method

2.1. Collection of sample

The samples were collected from Sawai Man Singh Medical College and Hospital Jaipur, which is one of the main hospitals of the state. The hospital organized a thyroid outdoor clinic once a week. The study was carried out on 123 women who attended the Thyroid Clinic and Radioisotope Laboratory in this hospital in the year 1997–1998. All the study subjects were asked to fill up a questionnaire to gather detailed information about them. The factors included in questionnaire were age, health status, family history, economic status, dietary habits, obstetrical and menstrual history, questions related to thyroid dysfunction, if any, and the use of pesticides either at home or in the work place.

2.2. Extraction and clean up of pesticides from samples

Four milliliters of blood from each subject were collected in a heparinized vial. Extraction and clean up of the samples were done using the modified method suggested by Bush et al. (1984). All the chemicals used were of analytical grade; the main solvent used for extraction was hexane of high-pressure liquid chromatography (HPLC) grade. All the glassware were cleaned with liquid soap followed by tap water, distilled water and acetone. The glassware were then kept in the oven at a temperature of 220 °C for 24 h.

To 2 ml of blood, the first extraction was carried out with a mixture of methanol (5 ml) and 1:1 diethyl ether/hexane (8 ml). The re-extraction was done with 1:1 diethyl ether/hexane. Clean up was done using a glass column packed with pre-washed glasswool, activated silica gel (5 g) and anhydrous sodium sulfate (5 g). The packed column was first rinsed with hexane followed by the extract and finally by 1% methanol/hexane. The elute was

Table 1
Serum levels of T_3, T_4 and TSH of all the subjects

	Serum T_3 ng/ml	Serum T_4 μg%	Serum TSH μU/ml
Euthyroids ($n=100$)	1.32 ± 0.04	9.09 ± 0.23	2.94 ± 0.17
Hypothyroids ($n=23$)	1.13 ± 0.09	4.95 ± 0.78	64.87 ± 15.17

Values represent mean ± S.E. n = Number of subjects.

completely evaporated with the help of a rotary vacuum evaporator, the dried extract was then dissolved in 2 ml of hexane. The sample was then ready for analysis.

2.3. Analysis

The analysis included a qualitative and quantitative estimation of organochlorine pesticides with the help of a gas chromatograph, a Hewlett Packard model 5890 series II, equipped with a ^{63}[Ni] foil electron capture detector coupled with an integrator HP 3396A. A coiled capillary column HP-1 (methyl silicon gum) 10 m × 0.54 × 2.65 μm was used. The gas chromatograph operating parameters were as follows—injector: temperature 250 °C, pneumatics—Splitless mode, purge delay—1 min; oven: initial temperature 80 °C (1 min), first ramp 30 °C/min to 190 °C, second ramp 6 °C/min to 300 °C, final temperature 300 °C (1.34 min); and detector: temperature 330 °C.

Purified nitrogen gas was used as the carrier gas and a known volume (1 μl) of the sample was injected in the column with a Hamilton syringe.

The different peaks of the sample were identified by comparing their retention times with those of the standards obtained from Pesticides, India Ltd., Udaipur, India and Aldrich Chemie, D-7924 Steinheim.

The pesticides analyzed were alpha, beta and gamma isomers of hexachloro cyclohexane (HCH), Dieldrin, Heptachlor and *p,p*′-dichlorodiphenyltrichloroethane (*p,p*′-DDT) and its metabolites *p,p*′-dichlorodiphenylethane (*p,p*′-DDE) and *p,p*′-dichlorodiphenyldichloroethane (*p,p*′-DDD).

2.4. Estimation of T_3, T_4 and TSH levels

The levels of T_3 (triiodothyronine), T_4 (thyroxine) and TSH (thyroid stimulating hormone) were estimated by the radioimmunoassay (RIA) technique. The ready-to-use kits were procured from the Radiopharmaceutical Section of BARC (Bhabha Atomic Research Centre), Mumbai (Code RIAK-4/4A for T_3, RIAK-5/5A for T_4 and IRMAK-9 for TSH). The protocols of the kits were strictly followed.

2.5. Statistical analysis

The calculations in this study were based on Student's '*t*'-test and the values were expressed as mean ± standard error (S.E.). Significant differences of mean between the residue levels of different groups were judged at the 5% level.

The coefficient of correlation was measured using Karl Pearson's method.

3. Observations

The hormone analysis of all the subjects reveals that 100 women (81.3%) had normal T_4 and TSH values and were referred as to Euthyroids, while hormone analysis of the remaining 23 women (18.6%) were towards Hypothyroidism (Table 1). On the basis of hormone analysis, two groups were made (euthyroid group and hypothyroid group) and a comparative study was done between these groups.

The total organochlorine pesticides residue in the blood samples of all the subjects is shown in Table 2. Hypothyroid females had higher levels of these pesticides (18.83 ± 2.19 ppm) than euthyroid females (14.68 ± 0.98 ppm). Out of all the pesticides detected, the total DDT (*p,p*′-DDD, *p,p*′-DDE and *p,p*′-DDT) was reported to be higher in both euthyroid and hypothyroid groups, i.e. 6.91 ± 0.55 ppm and 8.43 ± 1.15 ppm, followed by total HCH (α, β and γ isomers of HCH), which was 3.86 ± 0.35 ppm in euthyroid and 3.82 ± 0.68 ppm in hypothyroids. Dieldrin was 2.5 ± 0.31 ppm

Table 2
Pesticide residue levels in all the subjects

Pesticides (in ppm)	Normal T_4 ($n=100$)	Depleted T_4 ($n=23$)
α-HCH	1.32±0.17	1.32±0.25
β-HCH	1.69±0.15	1.56±0.26
γ-HCH	0.84±0.17	0.94±0.30
Total HCH	3.86±0.35	3.82±0.68
Dieldrin	2.5±0.31	5.38±1.23*
Heptachlor	1.41±0.15	1.18±0.24
p,p'-DDD	1.35±0.22	1.97±0.47
p,p'-DDE	2.19±0.29	2.57±0.48
p,p'-DDT	3.36±0.34	3.89±0.66
Total DDT	6.91±0.55	8.43±1.15
Total pesticides	14.68±0.98	18.83±2.19

* $P<0.05$.
Values represent mean±S.E.

in subjects with normal thyroid hormone levels, and 5.38±1.23 ppm with depleted T_4 and increased TSH values. Heptachlor was reported to be 1.41±0.15 ppm in the euthyroid group and 1.18±0.24 ppm in the hypothyroid group. Of all the pesticides, dieldrin was found to be significantly high in hypothyroid females.

When comparing age, the majority of the subjects were from the higher age group (44 subjects of 31–40 years and 32 subjects of 41–50 years), but the pesticide residues were higher in the hypothyroid females of the age group 11–20 years (25.18±1.8 ppm) and 21–30 years (23.38±6.06 ppm). None of the females of the age groups 51–60 years and 61–70 years were found to be suffering from hypothyroidism (Fig. 1).

Table 3 shows the geographical distribution of all the subjects. The majority of the women were from urban areas, followed by semi-urban and rural. The pesticide residues were highest in the women coming from the semi-urban area (16.42±2.47 ppm in euthyroid and 22.03±10.7 ppm in hypothyroids), although hypothyroid women from urban areas had significantly high levels of dieldrin.

When dietary habits were taken into consideration, the number of subjects reported to be consuming a vegetarian diet was greater than the ones consuming a non-vegetarian diet. There was not much difference observed in the pesticide residues of both of these groups (Fig. 2), although the non-vegetarian group had slightly elevated levels of pesticides.

The obstetrical history of the subjects reveals that primiparous females had slightly higher levels of organochlorine pesticides (15.65±1.64 ppm in euthyroids and 17.43±4.92 ppm in hypothyroids) than multiparous women (13.94±1.45 ppm in euthyroids and 16.37±4.92 ppm in hypothyroids). (Fig. 3) In both these groups, hypothyroid women had higher level of pesticide residues than euthyroids.

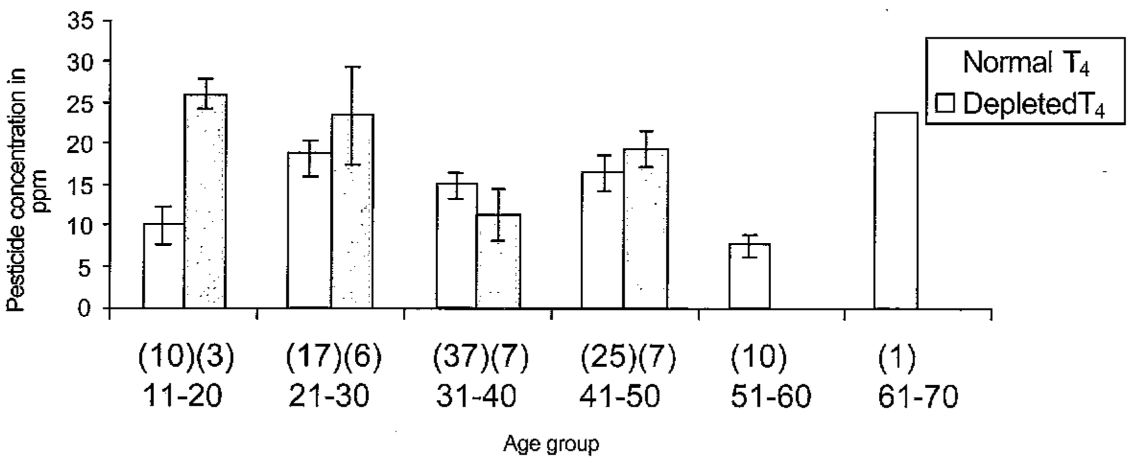

Fig. 1. Comparison of pesticide residue levels in female subjects of different age groups.