

Original article**Pesticide exposure and thyroid function in adult male sprayers**Farzaneh Farokhi^{1*}, Ali Taravati²

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Abstract: Pesticides are often used indiscriminately in a large amounts causing environmental pollution. Pesticides cause adverse effects on different body systems, including hematological and endocrine systems. Animal studies have shown the ability of organophosphorus and organochlorine pesticides to disturb thyroid function, but evidence of such effects on human remains unclear.

The purpose of this study was to investigate the association between exposure to pesticide and serum levels of thyroid hormones in pesticide sprayers.

Blood samples were obtained from 40 cases and 20 controls after overnight fasting. Glucose, urea, acid uric and lipid profile were measured by enzymatic methods. Immunoassay methods were used for determining of TSH, T₃ and T₄ levels.

The TSH level in sprayers (2.64 ± 1.50 mIU/L, $P=0.007$) was significantly increased compared to control subjects (1.33 ± 0.83 μ M). Significant decreased in T₃ and insignificant decreased in T₄ level of sprayers were observed.

These results suggest that exposure to organophosphorus and organochlorine pesticides may be responsible for increasing TSH level and decreasing T₃ and T₄ serum hormone levels, therefore supporting the hypothesis that organophosphorus and organochlorine pesticides cause hypothyroidism in pesticide sprayers. So administration of supplementary nutrients or improving defense system in these peoples is advised.

Keywords: organochlorine, organophosphate, thyroid hormones, hypothyroidism, pesticide sprayers

1. Introduction

During the past 50 years, pesticides have had an essential role in the agricultural world. Irrational and wrong use of pesticide has considerably increased with increased consumer demand.

Widespread use of pesticides in agriculture causes environmental pollution and severe health risks such as acute and chronic human poisoning (1, 2). In addition to spraying, other way of exposure to pesticides including formulation and handling without consideration of safety tips are also contribute to the emergence of toxic side effects (3).

There is evidence, indicating that environmental contact, especially with pesticides, is considered to be a risk factor for thyroid diseases. It has been reported that special insecticides, herbicides and fungicides

are endocrine disruptors. Their functions performed by different mechanisms such as inhibition of iodine absorption, intervention in thyroid hormone receptor, binding to transfer proteins, intervention with iodothyronine deiodinases, increased thyroid hormones clearance and intervention in expression of thyroid hormone (4, 5).

In a study conducted to evaluate thyroid function in the workers of a Danish greenhouse, 32% increase of thyroid stimulating hormone, TSH, and 5-9% decrease of T_3 and T_4 in serum were observed in spring compared with fall (6). Both lindane and chlordane are among organochlorine insecticides, which have been reported to affect the level of thyroid hormones in humans (7-9). Increase in chlordane level of breast milk is related to congenital hypothyroidism (10). Studies on the manufacturers of organochlorine pesticides, especially lindane and organophosphorus insecticides, have demonstrated increase of TSH and decrease of T_3 in these workers compared with the control ones (11). Malathion, which is an organochlorine insecticide, affects level of thyroid hormone in catfish and tadpoles of fresh waters (12, 13). Maneb/Mancozeb fungicides have a strong relationship with the probability of thyroid diseases so that causes hypothyroidism in rabbits (14). In another study on the workers who were in severe contact with ethylene bis dithiocarbamate without any protection, TSH level increased but T_4 level did not show any significant difference from that of the control people (15).

Some compounds such as polychlorinated biphenyls, Bisphenol A and dioxin are shown to inhibit or control cellular absorption of thyroid hormones and lead to the possible increase in biliary excretion of T_3 and T_4 and change in the activity of thyroid nuclear receptor, which changes expression of genes. Polychlorinated biphenyl, triclosan, pentachlorophenol and dioxins inhibit sulfotransferase activity, which result in decreased sulfation and dysregulation of peripheral levels of T_3 and T_4 .

People who are in touch with pesticides, especially those who spray farmlands and gardens, do not use sufficient protective instruments or do consider safety guidelines. Unfortunately, farmers are not aware of right amount of pesticides and its correct spraying method; they usually spray their crops experimentally and without knowing its side effects. The present study was aim to determine the lipids profile and thyroid function in a population involved in spraying in some villages of Mazandaran Province, north of Iran, and likely effects of pesticides on thyroid gland.

2. Material and Methods

The people selected for this study were spraying men, 40 peoples, who were involved in spraying for a long time. These people had been exposed to some kinds of organophosphorus and organochlorine pesticides. An informed consent was obtained from all subjects before measurements. Among the selected ones, a group with no experience of chronic diseases,

alcoholism and diabetes was sampled. 20 age and sex matched control people were selected, considering the absence of chronic diseases, alcoholism and diabetes.

Blood sample of the participants was taken between 6 and 7 am. Fasting blood sugar, urea, creatinine, uric acid, triglyceride (TG), total cholesterol (TC), HDL and LDL were measured using kits of Pars Azmoon Company.

The final diagnosis was done based on the following definitions:

- 1- Ideal level for total cholesterol was defined as <200 mg/dl. The moderate risk was between 200 and 239 mg/dl and high risk level was ≥ 240 mg/dl.
- 2- For LDL cholesterol, ideal level was below 130 mg/dl, the moderate risk was between 130 and 159 mg/dl and high risk level was determined as ≥ 160 mg/dl.
- 3- Ideal level for triglyceride was <200 mg/dl, the moderate risk was between 200 and 399 mg/dl and high risk level was considered ≥ 400 mg/dl.
- 4- HDL level was defined at two levels of ≤ 35 mg/dl (risk level) and >35 mg/dl (desirable level).

TSH, T_3 and T_4 tests were conducted using radioimmunoassay method (Patan Elm Company). TSH quantitative measurement kit was designed based on immune enzymatic reaction on solid phase. T_3 and T_4 quantitative measurement kit was designed based on the measurement of competitive immune enzymatic reaction on solid phase.

In the current research, the following cases were considered the criterion for the presence of thyroid disorders.

- A- Clinical hyperthyroidism: $T_4 > 12.4$ μ g/dl or $T_3 > 2.8$ nmol/l and $TSH \leq 0.1$ mIU/l
- B- Subclinical hyperthyroidism: $TSH \leq 0.5$ mIU/l with normal T_3 and T_4 levels
- C- Clinical hypothyroidism: $T_4 < 4.5$ μ g/dl and $TSH \geq 10$ mIU/l
- D- Subclinical hypothyroidism: TSH of between 5 and 10 mIU/l and normal T_4

The results were showed in the form of mean \pm standard deviation that the values $P < 0.05$ were considered statistically significant. Statistical analyses were performed to compare the groups using t-test analysis in SPSS software version 16.0 (SPSS Inc. Chicago, IL).

3. Results

In this study, effect of pesticides used in spraying the farmlands of Mazandaran Province, north of Iran, which were studied on the function of thyroid gland. For this purpose, the people lacking any history of

diseases were sampled after conducting preliminary investigations.

Mean age of the control and spraying peoples were 38.46 ± 4.86 and 38.59 ± 10.35 years, respectively,

Table 1: Mean and standard deviation of biochemical characteristics relating to age and lipid profile in the control and spraying peoples

which were not significantly different. The amount of fasting serum lipids (lipid profile) was measured in the control and sprayers, as given in Table 1. The levels of total cholesterol were 161.31 ± 25.07 mg/dL

	Sprayers (n = 40)	Controls (n = 20)	P-Value
Age (y)	38.59 ± 10.35	38.46 ± 4.86	0.965
Cholesterol (mg/dL)	154.19 ± 38.58	161.31 ± 25.07	0.47
Triglyceride (mg/dL)	127.77 ± 76.03	123.00 ± 57.58	0.823
HDL (mg/dL)	41.06 ± 9.21	39.77 ± 10.07	0.693
LDL (mg/dL)	78.07 ± 21.27	96.92 ± 26.16	0.034
LDL /HDL	1.99 ± 0.63	2.69 ± 1.21	0.017
Cholesterol /HDL	3.90 ± 1.13	4.38 ± 1.50	0.314

HDL, high

-density lipoprotein; LDL, low-density lipoprotein.

Data are presented as mean \pm SD

Table 2: Mean and standard deviation resulting from CBC results among the control and spraying peoples

	Sprayers (n = 40)	Controls (n = 20)	P-Value
WBC(Cumm)	6131.25 ± 1462.97	6215.38 ± 1448.47	0.862
RBC (mil/Cumm)	4.80 ± 0.48	4.86 ± 0.51	0.71
Hb (g/dl)	13.52 ± 1.31	13.57 ± 0.94	0.561
HCT %	40.54 ± 3.44	41.32 ± 2.26	0.461
MCV fl	84.98 ± 8.37	85.54 ± 7.28	0.824
MCH (pg)	28.20 ± 3.37	28.50 ± 2.29	0.77
MCHC %	33.32 ± 1.26	34.14 ± 3.21	0.388

WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Plt, platelet.

Table 3: Mean and standard deviation of biochemical parameters of blood among the control and spraying peoples

	Sprayers (n = 40)	Controls (n = 20)	P-Value
Fasting blood sugar (mg/dl)	74.26 ± 7.22	73.77 ± 5.36	0.806
Urea (mg/dL)	35.31 ± 7.37	33.54 ± 6.28	0.422
Creatinine (mg/dL)	1.04 ± 0.15	1.12 ± 0.10	0.047
U.A (mg/dL)	4.90 ± 1.07	4.88 ± 0.82	0.94
U.A/ Creatinine	4.76 ± 0.94	4.33 ± 0.61	0.094

Table 4: Mean and standard deviation of thyroid hormones of blood among the control and spraying peoples

	Sprayers (n = 40)	Controls (n = 20)	P-Value
TSH (mIU/l)	2.64 ± 1.51	1.33 ± 0.83	0.007
T ₃ (nmol/l)	1.12 ± 0.52	1.55 ± 0.57	0.018
T ₄ (ug/dl)	9.29 ± 2.12	10.85 ± 2.82	0.091

in the control people and 154.19 ± 38.58 mg/dL in the spraying people. Mean level of triglyceride in the control and spraying people was 123.00 ± 57.58 mg/dL and 127.77 ± 76.03 mg/dL, respectively. Based on the statistical analyses (Table 1), there was no significant difference between these two study groups in terms of blood cholesterol and triglyceride levels. HDL values did not show any significant differences between the two groups, but LDL in the spraying group was significantly lower than control group ($P < 0.05$). However, LDL/HDL ratio in the spraying population was lower than that in control population ($P = 0.017$).

In order to investigation of the pesticides destructive effects on hematological systems complete blood count (CBC) were analyzed. Complete blood count was measured in both groups, which no significant difference were found between two groups. These data are presented in Table 2. According to this table, leucocytes and erythrocytes, hemoglobin, hematocrit and other parameters were not significantly different between the control and spraying people.

Analysis of the data related to blood sugar and urea did not show any significant difference between the control and case groups (Table 3). There was insignificant increase in ureic acid level among the case group. On the other hand, the results of serum creatinine showed significantly decrease ($P < 0.05$) in the case group compared with the control one.

Low level of creatinine among spraying people can result from their malnutrition, which causes the reduction of creatinine production in these people (16). The results obtained from the study of thyroid function are shown in Table 4. T_3 hormone levels in the spraying and control people were 1.12 ± 0.52 nmol/l and 1.55 ± 0.57 nmol/l, respectively. Considering the results, it can be stated that T_3 hormone level in the spraying people was significantly less than that in the control group ($P = 0.018$). In addition, T_4 hormone level in the spraying people (9.29 ± 2.12 µg/dl) decreased compared with the control people (10.85 ± 2.82 µg/dl), which was not significant ($P = 0.091$). TSH level was 2.64 ± 1.50 mIU/l in the spraying people and 1.33 ± 0.83 mIU/l in the control people, which demonstrated significant increase compared with the control group ($p = 0.007$). This increase of TSH level confirms the occurrence of hypothyroidism reported in many studies.

The results obtained from TSH measurement revealed that the percentage of spraying people with TSH level > 6 mIU/l has been around 7%, which is relatively higher than that of the control group.

4. Discussions

Pesticides are widely used all over the world to increase agricultural yield. Therefore, every person maybe exposing to these compounds to some extent; however, farming workers are the group with the maximum exposure. Although acute effects of exposure to pesticides have been specified, their role in chronic diseases has not been well established. Pesticides have destructive effects on different organs, and consequently cause hematological and biochemical changes (17). Effect of some of these chemical compounds on animals has been well identified, but there is little information about humans. Farmers and spraying people are close in touch with pesticides in different ways, which are susceptible to endocrine dysfunction (18). Despite using protective equipment in pesticides involved industries, it is possible that people are exposed to particles, liquid or powder of these pesticides at their workplaces. Most of the studies focused on the effect of pesticides on acetyl cholinesterase and many papers have referred to the inhibition of this enzyme by pesticides. Some documents have demonstrated that exposure to the environment with pesticides is a risk factor for thyroid diseases (6, 11).

TSH measurement is the first useful test for evaluating thyroid function. Other measurements such as T_3 and T_4 would complement diagnosing of thyroid disorders. High sensitive measurement of TSH along with T_3 and T_4 estimation in this study allowed the evaluation of thyroid function in the spraying groups exposed to pesticides.

Some insecticides, herbicides and fungicides disrupt endocrine system. Thyroid disruptors affect through different mechanisms (19). It has been shown that some thyroid disruptors inhibit thyroperoxidase; thereby they change ability of follicular cell in producing T_4 and then T_3 , even at sufficient iodine concentration. Animal studies have revealed that amitrol (herbicide), ethylenethiourea (fungicide), Mancozeb (fungicide), bean isoflavones and benzophenone 2 inhibit production of thyroperoxidase and prevent thyroglobulin synthesis. Therefore, T_3 and T_4 synthesis is reduced (20). Many chemical compounds have high structural similarity to thyroxine and T_3 thereby they disrupt the binding of thyroid hormones to their receptors or transfer proteins. This case can in turn result in subclinical hypothyroidism, which is randomly diagnosed in adults due to its mild symptoms.

Animal studies have demonstrated that organophosphorus pesticides change brain neurotransmitters, which finally change function of thyroid gland (21, 22). However, there are few studies on the relationship between non-persistent pesticides exposure and thyroid function in humans. There is no study considering thyroid disorders in pesticide sprayers of Mazandaran Province, in which agriculture is common. In northern regions, the effect of pesticide absorption is higher due to high temperature and humidity. Nevertheless, due to the large particle size, it is not possible for pesticide to reach alveolar space. Therefore, their absorption by lungs is prevented. In contrast, skin is the important path of absorption because of lipophilic nature of the pesticides. However, humid environmental conditions lead to dilated skin vessels and lack of protective cover would complicate the problem. Participant in this study declared that they do not use suitable covers while spraying in hot seasons due to humidity.

As can be evident in this study, T_4/T_3 ratio in the spraying people was insignificantly more than control ones. Considering T_4/T_3 ratio, it can be stated that likely disorder may be peripheral conversion of T_4 to T_3 , which occurred in spraying people. Therefore, changes in the TSH, T_3 and T_4 levels may result of stimulation of the thyroid hormone synthesis or changes in peripheral conversion of T_4 . Based on present study, T_4 to T_3 ratio in the spraying people was more than that in the control ones, which can be due to less conversion of T_4 to T_3 . Anyway; the people studied in this work seemed to be healthy. The results of this study suggested that exposure to organophosphorus and organochlorine pesticides, which are the most widely used pesticides, may changes serum level of TSH, total T_4 and total T_3 in human in the long-term. In another study conducted in Mexico, the relationship between urine concentration of dialkyl phosphate and TSH and thyroid hormone level was examined among farmers (21-23). Other studies have demonstrated that greenhouse workers absorb much of the pesticides during handling of pesticide. Thus, the rate and amount of chemical exposure is very important. In this study, some sprayers had longer work experience than others. In these people, TSH level was more than 4.5 mIU/l, which indicative of hypothyroidism. Considering different studies, it can be stated that inhibition of TSH and TRH synthesis through negative feedback can be affected by exposure to pesticides. This issue maybe the possible cause of high TSH level in the sprayers despite their normal T_4 level.

What is evident is that minimizing of the exposure to pesticides and considering the safety comment can minimize the influence of these pesticides.

Animal studies have shown that exposure to pesticides such as dichlorodiphenyldichloroethane (DDT), amitrol and thiocarbamate related compounds resulted in decreased free and total T_3 and T_4 along with the increased of TSH level (21, 22, 24-26).

Both lindane and chlordane are organochlorine insecticides, which have been reported to affect the level of thyroid hormones in human (7, 8). It has been showed that increased chlordane level in breast milk is related to higher level of congenital hypothyroidism¹⁷. Malathion is an organochlorine insecticide, which influences thyroid hormone level in freshwater fish and tadpoles (12, 13).

Experiments have shown that maneb/mancozeb causes hypothyroidism in rabbits (14). Working people who were exposed to ethylenethiourea demonstrated low level of total T_4 significantly (27). In other studies on people exposed to ethylene bis dithiocarbamate without protection, TSH level increased but T_4 levels did not show any significant difference compared with control group (15).

Paraquat is one of the most widely used herbicides, which is generally used for controlling the broad leave grass. There is also a relationship between paraquat herbicide and hypothyroidism. Analysis of the people intoxicated with paraquat showed a measurable source of paraquat in their thyroid glands, which was higher in women compared with men (15).

Considering the present study and other studies, there is growing concern about public health due to long term exposure to even low level of organophosphorus and organochlorine compounds. Therefore, providing of information on the risk of exposure to toxic chemicals used in agriculture would be helpful in preventing and developing of complications.

Conclusions:

All kinds of pesticides are used around the world as insecticide and pesticide against the variety of agricultural crops in all seasons. Therefore, it is valuable to perform this study for investigating of the effect of pesticides on endocrine system and thyroid function. Based on this study, it is concluded that pesticide spraying without considering safety issues could result in hypothyroidism. Finally, it is suggested that farmers be trained about appropriate use of pesticides to prevent their side effects. It is also advised a thorough planning to use food supplements in order to protect people against harmful factors of workplaces, despite of intensified physical protective instrument, suitable cover at the time of exposure to pesticides.

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