

ARTICLE

Alterations in Serum Thyroid and Reproductive Hormone Levels in Occupationally Exposed Pesticides Sprayers



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Competing interests

The authors have declared that no competing interests exist.

Abstract

Background: Pesticides contain endocrine disrupting chemicals that have potential to perturb the normal thyroid and reproductive hormone axis. This study was designed to estimate the variations in serum tri-iodothyronine (T3), tetra-iodothyronine or thyroxine (T4), thyroid stimulating hormone, (TSH) and testosterone levels in occupationally exposed pesticide sprayers in comparison to controls.

Methods: For this purpose, blood samples of pesticide sprayers (n=50) were collected from District Layyah, South Punjab, Pakistan. Whereas, samples (n=25) of healthy control subjects were obtained from University of the Punjab, Lahore. Levels of T3, T4, TSH, and testosterone in the serum were estimated by the ELISA technique. The data obtained was analyzed by applying Un-paired Student "t" test at significance level of $P \leq 0.05$ using Graph pad prism version 6.0 software.

Results: Pronounced ($P \leq 0.0001$) decrease of both T3 and T4, whereas, a significant increase in TSH ($P \leq 0.005$) level was evidenced in pesticide sprayers as compared to controls. Moreover, a significant decrease ($P \leq 0.001$) was noted in the level of testosterone in pesticide sprayers as compared to controls.

Conclusion: Pesticides are a potent source of inducing imbalances in T3, T4, TSH, and testosterone secretion. Pesticide sprayers are, therefore, recommended to use prophylactic measures like wearing face masks and opt for safer protocols in order to avoid direct hazardous exposure to endocrine disrupting chemicals.

Keywords: Pesticides, T3, T4, TSH, Testosterone

Introduction

Utilization of pesticides at agricultural sites and their associated environmental as well as health hazards are widespread phenomena throughout the world (Gunstone et al., 2021). These pesticides pose serious threats to normal human metabolism. Due to rapidly growing population in Pakistan, the demand for food is always increasing. To cope up with the elevated food demand, farmers have to use a variety of pesticides to deal with pests. As Pakistan is agro-based country, great significance lies in the use of pesticides in the management of different crops. While using these chemicals, there is always a chance of exposure to these substances (Khan et al., 2020).

Pesticides are used to prevent, control, or destroy pests (El-Magd et al., 2011). Pests are the organisms that cause harm to plants, which may be used as food by humans (Quraishi et al., 2015). The minimal knowledge of pesticide usage, improper observation of prophylactic measures during spraying and application of pesticides, insufficient safety warnings on the packaging and poor understanding of the health risks are major factors in manifesting health abnormalities in the individuals dealing with its application in fields (Arafa et al., 2013).

Pesticides are classified as persistent and non-persistent. Persistent pesticides have long half-lives as compared to non-persistent and are thus more toxic (Roberts & Reigart, 2013). Persistent pesti-

cides are banned all over the world for many years. Non-persistent pesticides are now most commonly used for pest control in the agricultural sector (Mnif et al., 2011).

Pesticides contain endocrine disrupting chemicals (EDCs) with a potential to disrupt the hormonal functions. The body misidentifies EDCs as naturally occurring hormones, resulting in hormonal imbalance in the body by imitating hormones, interfering with hormone synthesis, modifying the development of hormone receptors, or their binding to receptors (Kumar et al., 2020).

Thyroid hormones (THs) include tri-iodothyronine (T3) and thyroxine or tetra-iodothyronine (T4). THs increase the body's metabolic rate (Guyton & Hall, 2006). The hypothalamic–pituitary–thyroid axis (HPT-axis) regulates the synthesis of THs. Thyrotropin releasing hormone (TRH) from hypothalamus regulates the secretion of thyrotropin or thyroid stimulating hormone (TSH) from the anterior pituitary gland. TSH in turn regulates the secretion of THs by the thyroid gland (Sellitti & Suzuki, 2014).

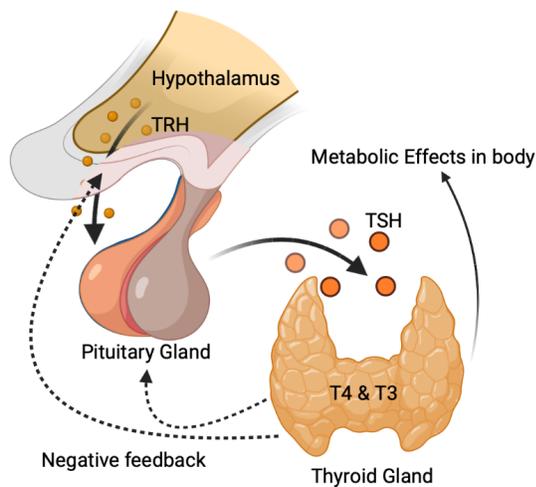


Figure 1: Regulation of thyroid hormones secretion by the hypothalamus and anterior pituitary Thyrotropin releasing hormone (TRH) from hypothalamus regulates the secretion of thyrotropin or thyroid stimulating hormone (TSH) from the pituitary gland. (Adapted from King, 2005).

Increased TSH levels with suppressed THs levels represent hypothyroidism, whereas low TSH levels with elevated levels of THs indicate hyperthyroidism. Sub-clinical hypo and hyperthyroidism is marked by normal levels of THs with high and low level of TSH, respectively (Koulouri & Gurnell, 2013). Clinical hypo and hyperthyroidism are characterized by abnormal levels of TSH and THs (Requena et al., 2019).

In Pakistan, the prevalence of clinical hyper and hypothyroidism is documented to be 5.1% and 4.1%, respectively. While, the incidence of subclinical hyper and hypothyroidism is reported to be 5.8% and 5.4%, respectively (Reza et al., 2013). Hypo- and hyperthyroidism (clinical or sub clinical) has been reported higher in females than males (Iqbal et al., 2019). The dysregulation of THs cause particularly thyroid diseases and many other adverse health effects, such as cardiovascular disorders, bone diseases, neuro-developmental disorders and cancer (Boas et al., 2012). Several reports showed an increased

prevalence of hypothyroidism, hyperthyroidism and thyroid cancer in many countries. Exposure to pesticides is one of the major causes of dysregulation of THs and TSH (McLeod & Cooper, 2012; Pellegriti et al., 2013).

Testosterone is produced by the testes in males. It is a steroid sex hormone that regulates fertility. It has an important role in producing sperms and red blood cells, distributing fats, balancing water and electrolytes, maintaining muscles strength and mass (Rastrelli et al., 2021). In the adult male, gonadotropins control the process of sperm production i.e., spermatogenesis. Gonadotropins include follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are produced by the anterior pituitary gland. When LH receptors are activated, they stimulate the production of testosterone by Leydig cells in testes (Ishibashi et al., 2018). Level of testosterone in male semen represents efficiency of Leydig cells (Abdallah et al., 2017).

Pesticides directly affect Sertoli cells, leydig cells, and germ cells, resulting in an imbalance of reproductive hormone levels i.e., testosterone (Ishibashi et al., 2018). This decline in testosterone level leads to a decline in sperm concentration and motility in the semen, thus affecting fertility. It also damages sperm DNA, causing developmental disorders in offsprings. When testosterone levels are below normal, many symptoms occur, including decreased sex drive, anxiety, mood swings, and weight gain (Abdallah et al., 2017).

The present study was aimed to examine the health hazards i.e., variation in thyroid and reproductive hormone levels, manifested in pesticide sprayers due to their exposure to chemicals present in pesticides.

Materials and Methods

The Institutional ethical review committee of Institute of Zoology, University of the Punjab, Lahore, granted approval of this study. A total of 75 male subjects were recruited for the phlebotomy. Among them, 50 subjects were pesticide sprayers recruited from District Layyah, South Punjab, Pakistan. While, 25 healthy control individuals were engaged from the Institute of Zoology, University of the Punjab, Lahore.

The sprayers were exposed to fifteen organophosphate pesticides, including Abamectin, Emamectin, Radar, Mega Mos, Gengwei, Imidacloprid, Paraquat, Bermoxynil, Voltar Super, Talstar, Polytrin C, Acephate, Tilt, Nativo, and Excel. These pesticides were applied mainly in granular form. Average exposure time of each sprayer to these pesticides was 6 to 7 years, while, day time exposure was 3 to 4 hours.

A registered health technician was engaged in the process of blood sampling. As the investigation centered on human subjects, therefore, complete precautionary measures were observed. After pricking each individual, 5 mL blood sample was dispensed in serum tubes. All blood samples were centrifuged for 5 minutes at 3000 rpm. From each tube, serum was pipetted out and divided into two equal aliquots.

Demographic parameters of the concerned subjects were recorded on a comprehensive proforma also used for informed consent from each of study participants. Inclusion criteria for pesticide sprayers include an active lifestyle and 5 to 7 years of exposure to pesticides. Body mass index (BMI) and blood

pressure (mmHg) of each participant were also noted. Subjects with any positive history of thyroid ailments, reproductive hormone dysfunction, obesity, diabetes mellitus, hypertension, cardiovascular complications, and hepatitis were excluded from this investigation.

The concentrations of T4, TSH and Testosterone in control and pesticide sprayer serum determined by commercially available kit of Bioactive Diagnostic (Germany) using the ELISA technique. The concentration of T3 was estimated by the Perkin Elmer (USA) ELISA Kit. The working principle of used kits was based on sandwich ELISA method. Assessment of all hormonal parameters was performed on a BioTek microplate reader in Physiology/Endocrinology Laboratory, Institute of Zoology, University of the Punjab, Lahore.

Statistical analysis

Overall biochemical comparisons of the results between control and pesticide sprayers were carried out by unpaired student “t” test using GraphPad prism version 6.0. software.

Results

The Tri-iodothyronine (T3):

The average level of T3 was found to be 1.48 ± 0.06 ng/mL in control and 0.87 ± 0.04 ng/mL in sprayers group. Whereas, the comparison of pesticide sprayers vs controls demonstrated a significant decrease ($P \leq 0.0001$) of 52% in the level of T3 in sprayers as compared to controls (Figure 2A, Table 1).

Thyroxine (T4):

The average level of T4 was found to be 5.13 ± 0.26 μ g/mL in control and 2.99 ± 0.16 μ g/mL in sprayers group. Whereas, the significant decrease ($P \leq 0.0001$) of 53% was evidenced in the

level of T4 in pesticide sprayers as compared to controls (Figure 2B, Table 1).

Thyroid stimulating hormone (TSH):

The average level of TSH was found to be 0.71 ± 0.06 μ IU/mL in control and 1.05 ± 0.07 μ IU/mL in sprayers group. Whereas the comparison of controls vs pesticide sprayers depicted a significant elevation ($P \leq 0.005$) of 39% in the level of TSH in sprayers as compared to healthy subjects (Figure 2C, Table 1).

Testosterone:

The average level of Testosterone was found to be 8.04 ± 0.22 ng/mL in control and 4.91 ± 0.14 ng/mL in sprayers group. Whereas, significant decrease ($P \leq 0.001$) of 39% was observed in the level of testosterone in pesticide sprayers as compared to controls (Figure 2D, Table 1).

Table 1: Comparison of thyroid and reproductive profile in controls and pesticide sprayers. Values are shown as Mean \pm SEM.

Parameters	Mean \pm SEM		P value	Percentage Difference
	Control N=25	Sprayers N=50		
T3 (ng/mL)	1.48 \pm 0.06	0.87 \pm 0.04	≤ 0.0001 ***	52.40↓
T4 (μ g/dL)	5.13 \pm 0.26	2.99 \pm 0.16	≤ 0.0001 ***	53↓
TSH (μ IU/mL)	0.71 \pm 0.06	1.05 \pm 0.07	≤ 0.005 **	39↑
Testosterone (ng/mL)	8.04 \pm 0.22	4.91 \pm 0.14	≤ 0.001 ***	39↓

↓ Decrease; ↑ Increase; ***, ** Significance at $P \leq 0.001$ and $P \leq 0.01$; ng/mL: nanogram per milliliter; μ g/dL: microgram per deciliter; μ IU/mL: micro international unit per millilitre

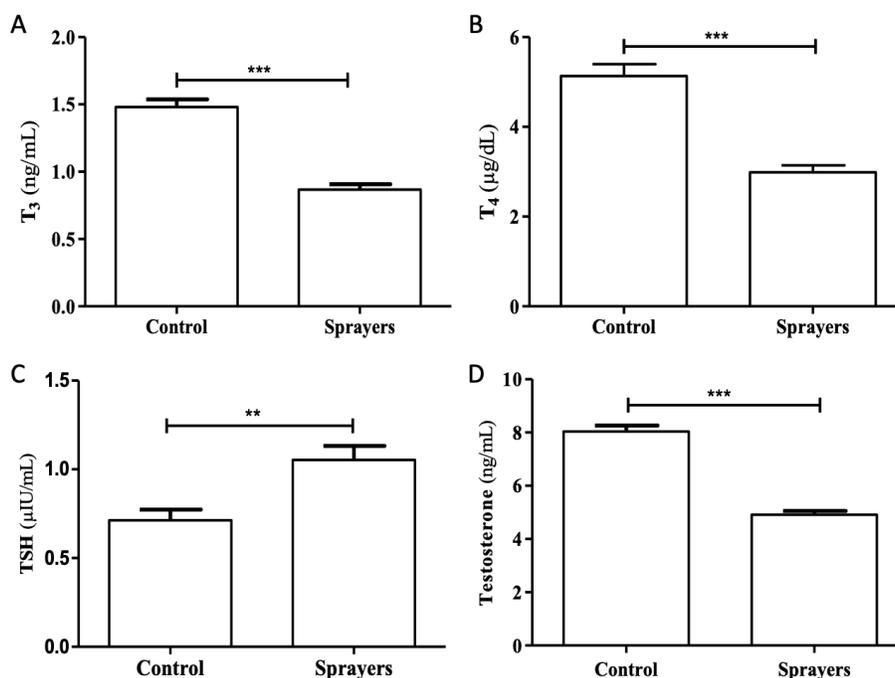


Figure 2: Serum levels of T3, T4, TSH and Testosterone (A-D) in pesticide sprayers compared with the Control. Values are Mean \pm SEM. *** Significance at $P \leq 0.001$, ** Significance at $P \leq 0.01$.

Overall, thyroid and its regulatory hormone assessment prognosticate hypothyroid condition in sprayers as compared to healthy controls. While, evaluation of testosterone predict hypogonadism in pesticide sprayers as compared to healthy controls.

Discussion

Our findings have shown a significant increase in TSH level and a prominent reduction in T3 and T4 level in pesticide sprayers as compared to controls. An increased serum level of TSH and a decreased serum level of T3 and T4 indicate hypothyroidism. The symptoms of hypothyroidism include sluggishness, drowsiness, increased sleep, muscular fatigue, decreased heart rate, slow hair growth, scaly skin, a harsh voice, and myxedema in severe cases (Guyton & Hall, 2006).

Higher exposure to pesticides might be responsible for the decrease in T4 serum level and increase in TSH serum level (Rathore et al., 2002). McClain (1992) and Kohn et al. (1996) have reported that chemicals in pesticides may result in hypothyroidism through a variety of intra and extra thyroidal mechanisms. Moreover, in the investigation of Requena et al. (2019), it was documented that the chance of having thyroid ailments was markedly greater in regions with more usage of pesticide. Additionally, it was estimated that people living in these areas have a 49% greater risk for hypothyroidism as compared to other diseases.

According to Requena et al. (2019), pesticide exposure impairs T4 serum levels, which subsequently increase the secretion of TSH from the anterior pituitary to compensate for the deficiency of THs. This condition might lead to goiter. Iodide uptake by the thyroid gland can be inhibited by many chemicals present in pesticides that block or compete with the sodium iodide symporter (NIS). This reduced iodide uptake results in decreased THs production and a subsequent increase in the production of TSH (Rousset et al., 2015).

Inhibition of deiodinase-1 (DIO1) and deiodinase-2 (DIO2) results in reduced, biologically active T3 formation in peripheral tissues followed by a compensatory stimulation of TSH secretion by the pituitary gland (Hernández et al., 2020).

Thyroperoxidase is the follicular cell enzyme that incorporates iodide into thyroglobulin, thus making it an important enzyme for T3 and T4 synthesis. Inhibition of thyroperoxidase by the action of pesticides can be a reason for the decrease in levels of T3 and T4 which may increase TSH levels by a negative feedback mechanism, resulting in hypothyroidism.

Binding of these pesticides to the thyroid hormone receptors leads to changes in the expression of genes that leads to the expression of constitutive androstane receptor (CAR)/pregnane-X receptor (PXR) in hepatic parenchyma. This is followed by the production of hepatic enzymes glucuronosyltransferase and sulfotransferase which are responsible for the hepatic metabolism and biliary excretion of thyroid hormones (Crofton, 2008; Lerro et al., 2018; Mughal et al., 2018). Some substances in pesticides have structural similarities with thyroid hormones. They may bind to thyroid receptors in place of THs, thus altering levels of THs and TSH (Meeker et al., 2007).

A trend of reduced testosterone levels in pesticide sprayers is present in the investigation. It is a uniform fact that deficiency

in the circulating thyroid hormone levels affects all body mechanisms that deviate growth hormone, glucocorticoids, corticotrophin, and gonadal functions. Generally, testosterone concentrations are reduced in hypothyroid conditions (Meikle, 2004). A decrease in testosterone levels represents hypogonadism (Surampudi & Swerdloff, 2017). Symptoms of hypogonadism include insomnia, mood swings, depression, fatigue, insulin resistance, obesity, loss of hair, metabolic disturbances, and infertility (Petraakis et al., 2017).

It is worth mentioning here that pesticides also have the ability to induce apoptosis in Leydig cells, thereby, decreasing overall production of testosterone. Pesticides also induce the increased production of hypothalamic corticotrophin releasing hormone, which stimulates the release of cortisol and ACTH which subsequently hinder GnRH and hence, testosterone and LH level decline (Spasova et al., 2000).

Conclusion:

It is pertinent to say that laborers that are actively involved in spraying different pesticides are predisposed to different health hazards. Presumably, long term exposure to pesticides causes reproductive and thyroid malfunctions. Hence, the government should actively participate in reducing pesticides exposure by improvising necessary precautionary measures such as the use of protective masks and gloves.

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