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Effects of Occupational Exposure to Pesticides on Male Sex Hormones

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Abstract

Pesticides have been extensively studied for their toxic hazards. The effect of exposure to pesticides has been studied among occupationally exposed workers. This study aimed to determine the role of occupational exposure to pesticides on male reproductive hormones. Follicle Stimulating Hormone (FSH), Luteinizing hormone (LH), and testosterone of 51 pesticide sprayers occupationally exposed to pesticides and 50 controls were estimated. Results revealed a significant increase in FSH concentration among the workers compared to controls. While there was no significant difference in the concentrations of LH and testosterone between the two groups. In conclusion: occupational exposure to pesticides may result in a significant increase of FSH, but not affecting LH and testosterone in the studied pesticide sprayers, and that could be attributed to their use of non-persistent pesticides and the resting durations between the peaks of their occupational exposures.

Keywords: Pesticide sprayers; Follicle Stimulating Hormone; Luteinizing hormone; Testosterone.

Introduction

Pesticides are chemicals used to control agricultural pests and their correct application is the most accepted and effective for maximum production and quality of crops (**Martinez and Gomez, 2007; Mansour, 2008).** During the last decades, the use of pesticides has increased steadily in developing countries to increase food production and control of vector-borne diseases. There are more than 65,000 chemicals that are classified as pesticides (**Mansour, 2008**). Because large amounts of these chemicals are released into the environment daily and many of them affect non-target organisms, that has resulted in negative side effects on human health and the environment **(Konradsen et al.2003; Hamilton et al.2004).**

World Health Organization classified pesticides in various ways such as, by their target, chemical nature, physical state, and mode of action as shown in figure (1). With the banning of most Organochlorines (OCs) pesticides, other types of compounds came into widespread use. The largest group of insecticides currently used globally includes Organophosphates (OPs), which have both agricultural and residential uses **(Weiss et al., 2004)**, and they are readily available commercially for domestic and industrial purposes **(Aardema et al., 2008)**. OPs are the generic term that includes all insecticides containing phosphorus. OPs were considered as a major cause of morbidity and mortality in the third world countries**,** as a result of poor regulation, monitoring, and even availability of technology and infrastructure **(Jaga and Dharmani, 2003).**

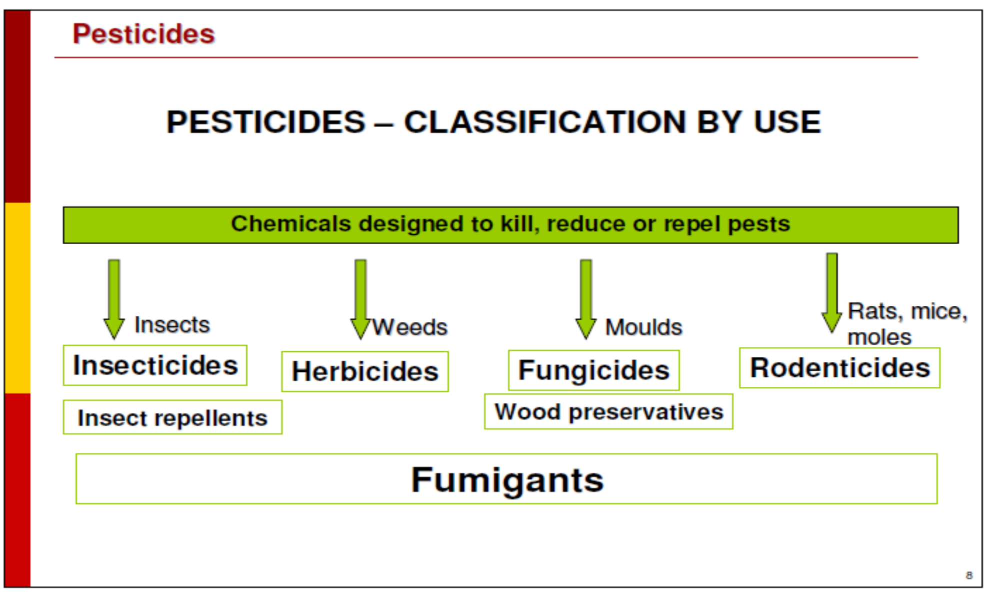

Figure (1): Pesticides classifications by use ***(WHO. 2008)***

World Health Organization report shows that every year about three million deaths occur worldwide related to OPs poisoning **(WHO, 2008)**. There is also toxicological evidence that repeated low-level exposure to OPs may affect neurodevelopment **(Gbaruko et al., 2009)**, immune system (**Galloway and Handy, 2003; Li, 2007),** and reproduction **(Peiris-John and Wickremasinghe, 2008; Fattahi et al., 2009).** There is a possibility that the Acephate compound; an OP insecticide, could affect the reproductive health of humans and wildlife in their natural habitats **(Adachi et al. 2008)**.

The hormonal balance of male reproductive hormones is an important factor in maintaining fertility and regulating the reproductive process **(Kegley et al, 2010)**. Sex hormones are divided into three classes: androgens (mainly testosterone), estrogens (mainly 17 beta-estradiol in the ovarian cycle), and progesterone **(Bhatia et al., 2014).** The pituitary gland produces follicle-stimulating hormone (FSH), which stimulates spermatogenesis, and luteinizing hormone (LH), which stimulates androgen production by interstitial cells **(Grover et al., 2005).** There have been rising concern in many developed countries about the adverse effects of pesticides on the human reproductive system, ranging from female and male sub-fertility to abortion, stillbirths, birth defects, and malformations **(Peiris-John & Wickremasinghe, 2008).** The aim of this study is the determination the effects of occupational exposure to pesticides on male reproductive hormones.

Methodology

Subjects:

A cross-section comparative study was conducted. The study included two groups; 51 agriculture pesticide sprayers for more than 5 years, and 50 control subjects not occupationally exposed to pesticides and matched in age, special habits, and socioeconomic status.

Questionnaire:

All enrolled participants were fulfilling personal, environmental, and occupational questionnaires during an individual interview. The personal questionnaire included age, gender, and smoking habits. An environmental questionnaire is designed to exclude from the workers those exposed to huge amounts of pesticides from sources other than occupations, and to exclude from the control group those exposed to a huge amount of pesticides in their environment or through their work. The occupational questionnaire contained a type of the pesticides used mostly, the way of exposure, the duration of exposure in years and days every year, using personal protective equipment and safety measures, and way of application.

Moreover, the biomarkers of exposure (acetylcholinesterase: AChE and butyrylcholinesterase: BuChE) of the pesticides sprayers were significantly lower than that of their controls (AChE: 327.4±217.6 and 556.9±374.6, and for BuChE: 616.7±561.1 and 3700.2±1050.9 respectively), as proved in the previous study done on the same selected groups **(Noshy et al., 2017)**.

Laboratory investigation

About 3 ml of the venous blood samples were collected from all the included workers and controls to determine levels of testosterone, LH, and FSH in the serum. All samples were stored at -70 °C until an assessment of the biomarkers.

Male sex hormones;

- Testosterone was estimated by an ELISA kit (**Chen *et al*., 1991**).

- LH was estimated by ELISA kit (**Knobil., 1980**).

- FSH was estimated by ELISA kit (**Uotila *et al*., 1981**).

Statistical Methods:

Statistical analysis of the collected data and the laboratory results were performed with SPSS version 18. Comparisons between the exposed workers and their controls were done through an Independent t-test, and Pearson correlation coefficients were used to test the association between different parametric variables. Mann-Whitney U test was used in comparisons of non-parametric variables. P-value ≤ 0.05 was considered to be significant. Tabulation and figures were illustrated using the excel program.

Results

- **Socio-demographic criteria of the study groups**

Analysis of questionnaires revealed that All the examined subjects in this study were males. There was no significant difference in the age between the workers and the controls (35.4±11.8 and 33.4±7.5 years respectively). About 35.3% of the workers and 32% of the controls were smokers without a significant difference between the two groups. Workers included in the present study were exposed to pesticides during their working days for more than 5 years (9.8±3.5 years), and inform of 141.5±80.6 days/ year.

Table (1) revealed that the workers were exposed to pesticides during their working days through inhalation, skin contact, and accidental ingestion (94.1 %, 100%,52.9% respectively. Ingestion exposure was accidental from contaminating their work through their meals during working hours, but not to level to have symptoms of acute poisoning. Their exposure to pesticides during different work tasks as Storage, Transportation, Mixing, and Application. During daily work, less than 50% of the pesticide workers wear personal protective equipment (PPE), while, 62% of them wear special clothes as shown in.

Table1: Characteristics data of workers population

Characteristic of workers population	No. (51)	(%)
Route of exposure		
- inhalation	**48**	94.1
- skin contact	**51**	100
- ingestion*	**27**	**52.9**
Working tasks		
- Storage	**29**	**56.9**
- Transportation	**33**	**64.7**
- Mixing	**35**	**68.6**
- Application	**30**	**58.8**
Using (PPE).		
- Special cloths	**31**	**61.5**
- Boots	**18**	**34.6**
-Musks	**3**	**5.8**
-Gloves	**0**	**0.0**
Dealing Way of (PPE).		
- re-use	**47**	**92.3**
- Washing after use	**44**	**86.5**
- Washing with family clothes	**12**	**23.1**
Wrong habits		
-Eating during work	**27**	**52.9**
-Smoking during work	**18**	**35.0**
-Not change their clothes	**31**	**61.5**
-Using an empty container for storage of food	**8**	**15.6**

- **Biochemical analysis**

In this study, table (2) showed that there was a significant increase in FSH concentration in workers compared to controls. On the other hand, LH and testosterone concentrations showed no significant difference between the workers and their control.

Table 2: Comparison between the biomarkers of exposure and the concentration of male sex hormones in pesticide workers and their controls

Group Statistics	Control (50) Mean ±SD	Workers (51) Mean ±SD	*P*-value*
FSH (mIU/ml)	**3.9±1.02**	**11.3±2.7**	**P<0.001**
LH (mIU/ml)	**9.6±6.3**	**10.8±5.2**	**0.099**
Testosterone (ng/ml)	**2.7±2.1**	**3.4±3.1**	**0.946**

*P-value was calculated using independent Mann-Whitney U test

Table (3) showed that there were no significant correlations between studied male hormones and the duration of exposure, for both the number of days per year or the number of years.

Table (3) Correlation between the duration of exposure and the concentration of male sex hormones among the exposed workers

	Duration of exposure (days/year)		Duration of exposure (years)	
	r	P-value	r	P-value
FSH (mIU/ml)	0.2	0.19	0.1	0.25
LH (mIU/ml)	0.1	0.23	0.1	0.22
Testosterone (ng/ml)	0.1	0.57	0.2	0.17

Discussion

The primary routes by which pesticides enter the body are ingestion in food, soil, or water; inhalation, contact through the skin, and eyes. The exposure of pesticides mainly occurs during the mixing and loading of the equipment and the spraying of insecticides with improper handling **(Azmi et al., 2006**). In this study, it was observed that the workers exposed to pesticides during their working days through inhalation were (94.1%), skin contact was (100%). Their exposure to pesticides during different work tasks was (56.9%) storage, (64.7%) transportation, (68.6%) mixing, (58.8%) application.

The farmers who use pesticides have only a little or no access to information about proper use or the precautions needed when handling pesticides. Therefore, they often do not use even the simplest hygienic and protective measures **(Maroni et al., 2006).** This work showed that during daily work, less than 50% of the pesticide workers wear some protective equipment (PPE), while, 62% of them wear special clothes during their working day. These results were in agreement with **(Yassin et al., 2002)** they reported that rare use of PPE was reported in several studies among Palestinian farmers and among other farmers in developing **(Del Prado-Lu.,2007; Corriols et al., 2009)** and developed countries **(Perry et al.,2002; MacFarlane et al., 2008).**

This work showed that the wrong habits of workers, which increase the risk of exposure to pesticides as smoking at work were (35%), eating during work was (52.9%), not change their clothes (61.5), and using an empty container of pesticides for storage of food (15.6). These findings were in agreement with other studies in developing countries that have the same habits, showing that, farmers reported smoking while applying pesticides and also having a meal in the field. This increases their risk of exposure to pesticides, (**Salameh et al.,**

2004; Ergonen et al., 2005; Recena et al., 2006).

Pesticides may act like hormones in the endocrine system and disrupt the function of the natural endogenous hormones, when doing so they are often called endocrine-disrupting chemicals (EDC) **(Diamanti-Kandarakis et al., 2009).** Exogenous compounds that alter the normal functioning of the endocrine system are called EDCs, based on several in vitro and in vivo studies, among them are several pesticides **(Andersen et al., 2008).** Several pesticides were proved to have adverse effects on male reproductive hormones experimentally and epidemiologically **(Slimani et al., 2011).**

Many studies have denoted that AChE and BuChE activities have been used as primary biomarkers of pesticide exposure, especially in the field of occupational exposures **(Noshy et al., 2017; Saad-Hussein et al., under reviewing)**, as well as in medical emergencies in cases of clinical and accidental poisoning from OPs and/or carbamates (CB) exposure **(Simoniello et al., 2010; Ueyama et al., 2010).** The main mechanism of action of OP and CB compounds is inhibition of AChE or BuChE to different degrees **(Costa et al., 2005)**, depending on the intensity and duration of exposures **(Araoud et al., 2011)**. This inhibition; especially of AChE, will lead to an increase in acetylcholine, and this increase may lead to inhibition of the release of gonadotrophin-releasing hormone, that inhibits the release of LH and FSH **(Terasawa and Fernandez, 2001).** In the present study, occupational exposure to pesticides leads to significant elevation in the serum levels of FSH in the exposed workers compared to their controls, but this elevation did not affect significantly the level of testosterone. This could be attributed that there was no significant elevation in the LH levels in the exposed workers compared to their controls. This was against what was proved in previous studies, this could be attributed to the situation of the studied sprays work only 141.5±80.6 days per year, even they are working for more than 5 years. These resting days result in the excretion of the high levels of pesticides from the body and to the recovery of their AChE and BuChE levels to do their proper work, as OPs are non-persistent pesticides, and do not accumulate in the body over a long period.

In conclusion: occupational exposure to pesticides may result in a significant increase of FSH, but not affecting LH and testosterone in the studied pesticide sprayers, and that could be attributed to their use of non-persistent pesticides and the resting durations between the peaks of their occupational exposures.

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