Macrothink Institute™

Journal of Biology and Life Science
ISSN 2157-6076
2014, Vol. 5, No. 2

Link between Chronic Pesticides Exposure and Reproductive Problems in Male Farmers

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Received: April 24, 2014 Accepted: May 4, 2014

doi:10.5296/jbls.v5i2.5501 URL: http://dx.doi.org/10.5296/jbls.v5i2.5501

Abstract

The demand for potent and new pesticides increases day by day because of pest resistant and more food production. The objective of this study was to find out the effects of chronic pesticides exposure on the reproductive health of poor and reckless farmers.A 5 mL blood was drawn from 80 men (40 farmers, 40 controls) and was analyze for serum total testosterone using Biocheck (USA) kit. A detail questionnaire was designed about socio-demographic, occupational activities and clinical characteristics.In farmer group the Mean±SEM of total serum testosterone was 546±10 ng/dl and in control was 664±17 ng/dl. Total serum testosterone was significantly lowered (*P*< 0.0001) in farmers at 95% confidence interval as compared to control group. Reproductive health problems like infertility, erectile dysfunction, ejaculatory problems, decreased libido, absent morning and nocturnal erection and general health problems like asthma, rheumatoid arthritis, hypertension, Parkinson and diabetes mellitus were more common in farmers.

Pesticides negatively affect the physiology of endocrine system. It reduces the serum total testosterone concentration in farmers significantly.This low testosterone produces negative effects on the reproductive as well as general health.

Keywords: Libido, Infertility, Nocturnal.

1. Introduction

In today's world pesticides are one of the most important contaminant actively involved in environmental contamination. These chemicals are toxic to vectors of diseases and pest. Pesticides are marketed as herbicides, insecticides and fungicides. The demands for new and potent pesticides are increasing day by day because of pest resistance and more food production to fulfill the need of world growing populations. Only in European Union 140,000 tons of pesticides are used annually for agriculture purpose, such sort of extensive application raised the questions about hazards of pesticides to human health, including reproductive. On one side pesticides helped the human by controlling infectious diseases and increasing the yield of agriculture products. But on the other hand their extensive long term use can harm the health. Pesticides can disturb the physiology of reproductive, endocrine, renal, cardiovascular, respiratory, nervous and immune system causing Alzheimer, multiple sclerosis, diabetes, Parkinson, renal and cardiovascular problems (Abdollahi*et al.*, 2004;Ramazzini, 2009; De Souza *et al.*, 2011;Mostafalou and Abdollahi, 2012).

Most of the pesticides cause reproductive toxicities by interfering with the physiology of endocrine system (Figa-Talamanca *et al.*, 2001; Cocco, 2002; Tiemann, 2008). Pesticides like,endosulfans, dieldrin, DDT and aldrin, the herbicides atrazine and the fungicide vinclozoline are considered as endocrine disruptor chemicals (PAN, 2009).Both male and female reproductive systems are negatively affected by pesticides exposure (Kumar, 2004; Shojaei and Abdollahi, 2012). Chronic pesticides exposure can causes decreased fertility in both sexes, high rate of miscarriage, altered sex ratio and has also antiandrogenic effects (Frazier, 2007).Pesticides exposure causes hormonal imbalance that eventually leads to infertility and sterility. Pesticides like, carbamates, pyrethroids, organophosphatases, Thio-and dithiocarbamates, chlorphenoxy acids and chlormethylphosporic acids reduces testosterone concentrations in male after acute exposure during exposure season (Evamarie*et al.*, 1999).A pesticide that changes the physiology of endocrine system by acting as hormone agonists/antagonists or changing endogenous steroidal hormonal level may negatively affect the developmental, biochemical and behavioral functions that are very important for reproductive success (Gerald *et al.*, 1997). Pesticides are endocrine disrupting chemicals (EDCs) that interfere with the synthesis, transport, metabolism and elimination of hormone there by, changing the concentration of natural hormone (Gore, 2010).Up to now a totals of 101 pesticides have listed to be endocrine disruptors (EDs) by the pesticides action network UK (PAN, 2009).

In Pakistan especially in district Dir (lower) the awareness about pesticides hazardous effects is lacking in farmers either due to illiteracy or poverty. These farmers never use masks, gloves, eye glasses and other protective coverings during fumigations. They even never wash their hands after fumigationand take their breakfast or meal in their fields. During fumigation, all the clothes and body of farmers are washed by pesticides. They are also addicted to snuff which during fumigation are contaminated by pesticides. For the sake of high yields with low efforts they uses excessive and multiple pesticides. These farmers are exposed to pesticides from generation to generation. The present study is design to analyze the ill effects of pesticides on the male reproductive health of farmers.

2. Material and methods

2.1 Pesticides Used in Study Population

Most commonly used pesticides are Mancozeb, cymoxanil, metalaxyl, cypermethrin, tebuconazole, nitonpiram, hexythiazox,chlorothalonil, metiram, pyraclostrobin, dimethomorph, levofenoron, pyrimethanil, imidacloprid, thiophanate methyl, bromoxynil, MCPA, pendimethaline, nicosulfuron, atrazine and endosulfon (now banned).

2.2 Inform Consent

Before starting the study a written informed consent was signed by each participant.

2.3 Study Population

We selected the agriculture community of village Tazagram, district Dir lower, Khyber pakhtunkhwa, Pakistan for study. This area was selected because it is surrounded by agriculture fields whose main products include vegetables (tomatoes, onions, spinach, cauliflowers, cabbage, garlic, brinjals, ladyfingers)crops (maize, rice, wheat, tobaccos) as well as fruits (oranges, grapes, apricots, pears). It is situated at 695 meters above sea level. The climate is temperate so, agricultural production is possible all year round.We selected 80 men randomly from the community. They were divided into two groups i.e. control and exposed. Each group contained 40 participants. The exposed group participants were farmers by profession while control group participants were engage in other professions. Their age ranges from 29 to 50 years and were divided into two age groups i.e. 29 -40 and 41-50 respectively. Each participant was interviewed directly regarding his socio-demographic characteristics, alcohol and smoking habit, occupational activities and clinical characteristics.

2.4 Inclusion Criteria

Farmers directly exposed to pesticides for at least 20 years.

2.5 Exclusion Criteria

No history of chronic illness, chemotherapy and radiotherapy.

2.6 Blood Sampling

Samples were collected during the heavy spraying season (March to July).5 mL of the morning blood sample was collected from the antecubital vein of each participant by a phlebotomist aseptically. Immediately blood was transferred into Vacutainer tube containing no additives. At room temperature blood was allowed to clot and then centrifuged at 3200 rpm for 15 minutes at 4 C^0. The resulting serum samples were then stored at -20 ℃ for later analysis.

2.7 Assay

For the measurement of serum total testosterone, testosterone enzyme immunoassay test kit, Bio-check USA was used according to the manufacturer protocol and procedures.

2.8 Data Analysis

Analysis of data was done by using Graph Pad Prism (www.graphpad.com), *version 6.03 (Graph Pad Software Inc*., San Diego, CA, USA*).* Student un-paired *t* test was used for the comparison of serum total testosterone in both groups. The results were represented through Mean ±SEM. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1 Direct Fumigation Effects

The farmers did not use protective coverings like, masks, gloves, dresses etc. so are directly exposed to pesticides. The effects like, headache, nausea, dizziness, cough, blurring of vision, throat, skin, nose and eye irritation were common in farmers during and after fumigations.

3.2 Selected Characteristics of the Study Population

The characteristics of the study population are presented in Table 1

Table 1. Characteristics of the study population

Age Group (Years)	Exposure time to pesticides (years)	Fumigation frequency (per week)	Protective clothing	Hand wash	Addiction (%)	General health problems	Reproductive health problems
Control 29-40 (n=20)				yes	05 snuff 6 cigarette	01 DM*	absent

41-50 (n=20)				yes	11 snuff 6 cigarette	02 HT*	absent
Farmer 29-40 (n=20)	≥ 20 yrs.	≥ 7/week	absent	no	8 snuff 9 cigarette	04 HT 02 A* 01 RA*	02 infertility 04 DL* 03 EP* 04 AMN*
41-50 (n=20)	≥ 20 yrs.	≥ 7/week	absent	no	13 snuff 5 cigarette	06 HT 03 DM 02 P*	02 infertility 04 DL 05 EP

DL*= Decreased Libido, EP*= Erection Problems, AMN*= Absent Morning and Nocturnal erection.

DM*= Diabetes Mellitus, HT*= Hypertension, A*= Asthma, RA*= Rheumatoid Arthritis, P*= Parkinson,

3.3 Analysis of total serum testosterone concentration in study population

Total serum testosterone concentrations in both groups are summarized in Table 2. A significant reduction ($P < 0.0001$) in total serum testosterone concentration was found in farmers as compared to control group at 95% confidence interval.

Table 2. Total serum testosterone concentration in study population

Parameters	Control Mean ±SEM	Farmer Mean ±SEM	95% C.I*	*P* value
Total serum testosterone concentration	664 ±17 (ng/dl)	546 ±10 (ng/dl)	-158 to -78.61	< 0.0001

CI*= Confidence Interval

4. Discussion

Our main finding in this study were low serum total testosterone, reproductive and general health problems, that were more pronounce in farmers as compared to control group. In this community the most common symptoms related to pesticides reported were nausea, headache, dizziness, blurring of vision, throat, skin, nose and eye irritations that were in agreements with other reports link with direct exposure to Organophosphates (OP) and carbamates (CB) pesticides (Catanoet *al.,* 2008).Total serum testosterone and estradiol level decreases in peoples exposed to OP (Padungtod*et al.*, 1998; Straube*et al.*, 1999). Experimentally this is confirmed that, OP exposure alters the metabolism of testosterone and estradiol (Butler and Murray 1993; Murray and Butler 1995) thus supporting our results.OPaffects the reproductive functions primarily via decreasing brain acetylcholinesterase (AChE) activity and secondarily via gonads. This decrease in AChE leads to increase the concentration of acetylcholine, gamma-aminobutyric acid (GABA), epinephrine, norepinephrine, 5-hydoxytryptamine and dopamine concentration (Glisson*et al.,* 1974; Gupta *et al.,* 1984). Increased level of GABA then inhibit the release of gonadotropin releasing hormone (GnRH) in the median eminence, which is responsible for the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. These LH and FSH are then involved in steriodogenesis and gametogenesis (Mitsushima*et al.*, 1994; Terasawa and Fernandez, 2001). Like nicotine and cocaine, pesticides increases dopamine concentration that negatively regulate GnRH secretion suppressing the reproductive functions(Kuhar*et al.*, 1991; Corrigall*et al.*, 1992; Murphy *et al.*, 1998; Di Chiara, 2000; Watkins *et al.*, 2000). OP and carbamates alter pituitary adrenal axes (Clement 1985; Kokka*et al.*, 1987). Acephate andmetamidophos stimulate the secretion of hypothalamic corticotropin-releasing hormone,which in turn stimulate adrenocorticotropic hormone (ACTH) and so cortisol(Spassova*et al.*, 2000). Receptor of cortisol are located on GnRH neurons, RFRP-3(mammalian ortholog of GnIH) and on gonadotrops. High cortisol level inhibits the secretion of GnRH and so LH and testosterone while positively regulate RFRP-3 secretion, thus suppressing reproductive system (Kirby *et al.*, 2009). OP and CB affect the pituitary-thyroidal axes (Clement, 1985)reducing the level of serum fT4 (free thyroxin) in farmers (Khan *et al.*, 2013) by increasing T4 metabolism through induction of of hepatic UDP-glucuronosyltranferase enzymes (Hill *et al.*, 1989)and to displacement of T4 from plasma binding-proteins, altering hepatic androgen metabolism (Van *et al.*, 1991).The pesticides like imidacloprid (IMI) and acetamiprid (ACE) bind to insect nicotinic acetylcholine receptor in central nervous systems and kill them (Tomizawa and Casida, 2005). But in vivo studies have found that neonicotinoid pesticides including IMI and ACE affect the reproductive organs of mammals causing retardation of testicular development, damage to spermatogenesis, decrease in sperm quality and change of ovary morphology (Kapoor*et al.*, 2010; Kapoor*et al.*, 2011; Bal*et al.*, 2012_a ; Bal*et al.*, 2012_b). OP exposure can result paternal fertility problems. OPisgenotoxic to animal sperm because it is a very potent phosphorylating agent. It binds to DNA and protamines, altering the chromatin structure that results DNA to become more susceptible to induced denaturation in situ (Evenson*et al.*, 2002; Sergerie*et al.*, 2005;Pina-Guzman *et al.*, 2005;Boe-Hansen *et al.*, 2006;). In Chinese pesticides factory workers the effects of OP exposure on their reproductive system was done. It was found that OP exposure increases LH and FSH but decreases serum total testosterone (Padungtod*et al.*,1998).In adult men OP and CB metabolites reduces the serum level of LH and total testosterone (Meeker *et al.*, 2006).In Djutitsa (West Cameroon) a significant reduction in serum total testosterone and androstenedionewere found in farmers exposed to agro pesticides (Manfo*et al.*, 2010). A lot of wildlife species have been effected by endocrine disrupting chemicals(EDCs) showing alteration in penis/gonopodium development, decreased testosterone level, alters spermatogenesis, impaired reproduction, reduce hatchability and/ or viability of offspring, aberrant sexual behavior and affect sex determination, differentiation of the gonads and timing of sexual maturation(Oberdörster and Cheek, 2001; IPCS, 2002; Stoker *et al.*, 2003;Jobling*et al.*, 2006; Milnes*et al.*, 2006; Crain *et al.*, 2007; Orlando and Guillette, 2007;Guillette and Edwards, 2008;Stoker *et al.*,2008). Exposure toendosulfan (END) and atrazine (ATZ) during the embryonic life of *Caiman latirostis*altered histoarchitecture of the testis as well as the balance between proliferation and apoptosis of hatchlings' testicular cells. In adult male exposed to END, a decreased testosterone level was observed (Florencia*et al.*, 2009). Impaired testicular functions were also suggested for factory workers exposed to chlordecone (Anderson *et al.*, 1976). Delayed spermatogenesis, decreased sperm counts, altered sperm motility and a higher incidence of intersex were observed in fishes exposed to

END (Joblinget *al.*, 2002; Aravindakshanet *al.*, 2004; de Montgolfier *et al.*, 2008). Some studies have found high testosterone level (Sarkar*et al.*, 2000) or no significant change in the testosterone level (Rogelio *et al.*, 2005) of model animals as well as human exposed to pesticides. This may be due to the agrochemicals use, severity and/or length of exposure, agricultural practices and protection equipment pattern of use (Rogelio *et al.*, 2005).

5. Conclusion

In summary pesticides exposure negatively affects the endocrine system directly or indirectly, resulting into serious consequences. In district Dir there is a need to educate the farmers about the appropriate use of pesticides, implement a formal worker protection program for agricultural workers and preventive health monitoring for early detection of pesticide exposure.

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