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Advance Effect of Pesticides on Reproduction Hormones of Women Cotton Pickers

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Abstract: About 2.6 million women collect cotton from 9 major cotton growing district of Pakistan. Health hazards to women cotton pickers show that out of 2.6 million women, 2.2 million women get sick from their exposure to pesticides. Women are very actively involved in agricultural activities because agriculture is generally a family affair but women's involvement in agriculture is wrapped in a mist of socio-cultural stereotype. Women cotton pickers complain of dizziness, muscular pain and suffocation due to acute pesticides poisoning because of inhalation of fumes. However as women access to the health care is minimum, there is no monitoring as to absorption of pesticides, pesticides level in blood and their effect on reproductive health. Several pesticides such as herbicides, insecticides and fungicides are known to be endocrine disrupters (EDCs). It was hypothesized that occupational exposure to pesticides after a spraying season causes changes in hormonal levels that might be detected in a short-term study. Analysis was performed to check the changes in reproductive hormones before and after a spraying season in the groups of women farmers exposed and not exposed to pesticides. The result of the study showed that both in pre season and post season significant differences for LH, FSH, progesterone and estradiol were found both in cotton pickers and non pickers present at the different stages of their reproductive cycle. while a comparison with in the cotton pickers present at same stages of their reproductive cycle in both pre and post season also showed a significant increase of hormonal level in post season. From this study it was concluded that pesticides might be affecting the hormonal levels of cotton pickers in Khairpur area. Since it was a short term study a long term study is required to confirm this effect.

Key words: Pesticides, endocrine disrupters, reproductive hormones, LH, FSH, progesterone. estradiol

INTRODUCTION

World wide 20-30 million $ worth of pesticides are being used each year. Approximately 3 million people get sick from acute pesticides poisoning while 0.22 million people die annually all around the world[1-3] and majority of the victims belong to the developing countries[4]. Pesticides are among the most widely used chemicals in the world and also among the dangerous one to the human health. There are convincing scientific evidences that pesticides play a significant role in the brain cancer, leukemia, breast cancer, enzymes inhibition, sterility, reduction in fertility, depression of sperm count, dermatitis, skin cancer, hypertension, immune system disorder, blood disintegration, and reproductive abnormalities such as increased rate of miscarriages in the people with chronic exposure[5]. A new scientific debate is arising that pesticides also act as endocrine disrupters.

Women are very active in the agriculture because it is generally a family affair in Pakistan but involvement of women in farming is wrapped up in a mist of socio-cultural stereotype, hence it is therefore difficult to quantify their contribution with in sector in real terms. It is normally thought that women play no role in the use of pesticides because generally women do not spray in the field but they are directly exposed to pesticides because they are involved in many on-farm and off-farm activities. About 2.6 million women collect cotton from 9 major cotton-growing districts of Pakistan. Health hazards to women cotton pickers show that out of 2.6 million women, 2.2 million women got sick from their exposure to the pesticides used on cotton crop[6]. The low levels of awareness play a major role in human poisoning. According to the population census of 1981, only 7% of rural women of Pakistan are literate. In Pakistan very few data are available on the illness, which are related to the pesticides poisoning and women workers. The specific

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objective of the study was to check the effects of short-term exposure to pesticides on reproductive hormones of cotton pickers.

MATERIALS AND METHODS

Sampling area: The study was conducted in District Khairpur of Sindh province of Pakistan. Blood samples were collected from five tehsils of Khairpur, namely Kotdigi, Sobodero, Gambat, Deparja and Khairpur city.

Sampling: Blood samples of 34 women were taken before the application of pesticides (1st-7th June 2003, pre season) and after the application of pesticides (20th-27th October 2003, post season) all the women were residents of same area. Out of 34 women respondents 23 were cotton pickers and 11 were non pickers. Women correspondents were same in pre and post season blood sampling.

Collection of blood: Five milliliters of blood was taken with the help of 5 mL disposable syringes in evacuated tubes containing serum activator. In preseason blood was taken between 9-1500 h, while in postseason since women workers were busy in their work so the blood was taken after the picking activity, from 1600 - 2200 h.

Serum analysis: Serum was stored at -20°C until the hormonal analysis and all the samples were analysed at the laboratory (NORI, Islamabad). As few women become pregnant in post season so out of 23 blood samples of 15 cotton pickers and out of 11 non pickers blood samples of 7 non pickers were analysed. Serum samples of LH, FSH, Progesterone, Estradiol was measured using Radio immuno assay (RIA), using immunotech kits (A beckman culture company, France). Sensitivity of the test was <0.2 IU L^{-1}, 4 pg mL^{-1}, 0.03 ng mL^{-1} for LH, FSH, estradiol and progesterone, respectively.

STATICAL ANALYSIS

Socio economic status of the women respondent was calculated by SPSS program equality of the means was proven by Wilcoxon and Mann- whitney test[7].

RESULTS

Results of Table 1 showed that Total 75% of women respondents were married and 25% were unmarried. None of them were pregnant, as pregnant women were excluded. Ninety percent of women were at follicular phase in pre season, out of these 15 were pickers and 5 were non pickers, none of the cotton pickers were in luteal and ovulatory phase however 1non picker was in luteal and ovulatory phase . similarly in post season sampling 11 cotton pickers were in follicular, 3 were in luteal and 1 was in ovulatory phase. While 3 non pickers were in follicular, 3 in luteal and 1 was in ovulatory phase of their reproductive women did not know any thing about the gynae problems and how to differentiate between the regular and irregular cycles.

Table 1: Characteristics of women respondents

Characteristics	Women respondent %/Averages
Marital status (%)	
-Married	75
-Un-married	25
Number of offspring (Average)	4
Birth defects in children (%)	0
Whether pregnant (%)	
- Pregnant	0
- Non pregnant	100
Stage of reproductive cycle (%) (preseason)	
Follicular	90
Ovoulatory	4
Luteal	4
Stage of reproductive cycle (%) (post season)	
Follicular	63
Ovoulatory	9
Luteal	27
Menstrual cycles are regular or not (%)	
- Regular	83
- Irregular	17
Any other gynae problem mostly observed (%)	
- No.	20
- Don't know	80

During this study women respondents were in different phases of their reproductive cycle. All the comparison of hormones were made between the cotton pickers and non pickers who were in the same reproductive phase

The results of the comparison between the cotton pickers and non pickers showed that a significant differences between levels of hormones ($p<0.01$ and $p<0.05$) (Table 2 and 3).

The level of LH hormone was high during the study, while levels of FSH and progesterone were normal in preseason but the after the exposure to pesticides the levels of these hormones increased significantly ($p< 0.01$) in cotton pickers with respect to non pickers (Table 2 and 3).

A comparison was also made between those women who were in the same phase of their reproductive cycle both in pre and post season. This comparison showed that the LH, FSH and progesterone increased significantly after the exposure while the estradiol remains unaffected, although estradiol increased with respect to preseason levels but that increase was in the normal range of the hormone (Table 4).

Table 2: Sex hormones changes among the cotton pickers and non-pickers present in follicular phase in pre and post season

	Pre season				Post season			
	Cotton pickers N = 15	Non pickers N = 5			Cotton pickers N = 11	Non pickers N = 3		
Hormones	Mean	Mean	t- value	p-value	Mean	Mean	t-value	p-value
LH 0.5-5.0 IU L^{-1}	7.6	2.59	17	0.00**	7.7	5.0	11	0.00**
FSH 2.2-15 IU L^{-1}	10.41	8.7	30	0.00**	16.02	7.2	3	0.01**
Progesterone 0.06-1.26 ng mL^{-1}	1.8	0.6	33	0.00**	17.12	8.3	13	0.00**
Estradiol 30-200 pg mL^{-1}	51.78	50.0	33	0.00**	124.24	58.04	15	0.00**

Values with ** show high significant at 1% probability level

Table 3: Sex hormones changes among the cotton pickers and non-pickers present in luteal phase in post season

	Post season			
	Cotton pickers N = 3	Non pickers N = 3		
Hormones	Mean	Mean	t-value	p-value
LH 5-5.0 IU L^{-1}	12.76	6.9	3	0.03*
FSH 1.3-10 IU L^{-1}	18.32	5.9	3	0.03*
Progesterone 2.5-25 ng mL^{-1}	0.92	3.3	3.5	0.02*
Estradiol 20-250 pg mL^{-1}	99.76	50.60	4	0.01**

Values with * show significant change at 5% probability, while values with ** show significance at 1 % probability

Table 4: Sex Hormones Changes among the Cotton pickers present in follicular phase in both pre and post Season

	Cotton pickers			
	Pre season N = 11	Post season N = 11		
Hormones	Mean	Mean	t-value	p-value
LH 0.5-5.0 IU L^{-1}	5.3	7.7	47	0.00**
FSH 2.2-15 IU L^{-1}	9.7	16.02	19	0.00**
Progesterone 0.06-1.26 ng mL^{-1}	1.9	17.12	51.5	0.00**
Estradiol 30-200 pg mL^{-1}	62.23	124.24	35	0.00**

Values with ** show high significant at 1% probability level

DISCUSSION

In Pakistan as in other developing countries, pesticides use has increased dramatically over the last few years. Pesticides are mainly used on cotton crop in Pakistan. This study was a part of a long term study, in this part effects of pesticides were checked on the cotton pickers of the Khairpur area to understand the effects of misuse of pesticides.

The results showed significant differences between mean values of the tested reproductive hormones of the pickers and non pickers women respondents in pre and post seasons.

In human female, the ovarian profile is linked to the menstrual cycle. This reproductive cycle has three main stages and LH, FSH, progesterone and estradiol behave differently in these stages. The menstrual cycle is divided into a follicular, ovulatory and luteal phase. There is a complex interplay between levels of FSH, LH, estrogen and progesterone.

Estradiol act as both negative and positive inhibitor during the cycle. Between the ovulation and beginning of the menstruation secretion of progesterone and estradiol begins to increase. This has negative effects on LH and FSH secretion. Two to three days before the menstruation secretion of progesterone and estradiol begins to decrease. This releases the hypothalamus of the pituitary from the negative effects of the progesterone and estradiol. Now in the follicular phase FSH start increasing, after some time LH also starts increasing. Increased level of the estradiol is necessary for the rise of LH, which brings luteinization.

The changes in the hormonal levels of persons exposed to pesticides (occupational exposure) are considered with regard to the physiological feed back[7]. In this feed back estradiol is a key hormone.

Estradiol is very important hormone in the female reproductive system.. In the estradiol biosynthesis, aromatase enzyme plays a key role[8]. As Pesticides are now known as endocrine disrupters, one of the mechanisms through which they bring the disruption of hormones, is by interfering with the metabolism of the hormones[9]. Many pesticides are known to disrupt aromatase enzyme system, like fungicide prochloraz and imazalil have shown to inhibit CYP19 aromatase of human placental microsomes[10]. Now if pesticides inhibit the aromatase enzyme, concentration of estradiol decreased and through feedback mechanism FSH also decreases, no follicular formation, no surge of LH for lutenizing. So all the hormonal level will be disturbed.

Pesticides have short-term effects by inhibiting the enzymes. Pesticides inhibition of enzymes system and enzymatic activity is the biological indicator of pesticides exposure . Since in this study although a the level of estradiol was normal suggesting no aromatase inhibition. Pesticides are also known to inhibit the activity of Cholinesterase enzymes[11,12]. An important finding of this study is increased level of LH during the pre and post season sampling and an increase level of FSH after the

exposure to pesticides with respect to non picker women similar results were also found in literature, studying the effects of pesticides on male hormones[13,14].

The level of exposure is very important in relation to the inhibition of activity of enzymes. Pesticides inhibit aromatase enzyme at a specific concentration like imazalil and prochloraz inhibit aromatase at the concentration of 0.15 and 0.7 μmol, receptively[10,15] and Dicofol and triadimenol were found to give arise to statistically significant inhibition of aromatase activity at the concentration of 50 μmol in human tissues[16]. Low levels of endosulfan (1 nmol, 0.41 PPb) can inhibit the human sperm acrosome reaction, initiated by progesterone and glycine, but the inhibition is not complete[17]. Endosulfan II and endosulfan sulfate decreased galactosidase activity of progesterone[18].

So the Women of the area may not exposed to that level of pesticides exposure, which inhibit aromatase activity, but the level in high enough to inhibit neurotransmitter. Pesticides used in khairpur were methametaphose, aldrine, baythroids, cypermethrine and endone. As these pesticides area are endocrine disrupters[5]. So we can conclude that short term exposure to these pesticides effects the level of hormones which could be detected in a short term study.

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