ORIGINAL ARTICLE

# Assessment of Pesticide Exposure in Female Cotton Pickers of District Jamshoro, Sindh

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# ABSTRACT

**Background:** The Butyrylcholinesterase (BChE) is widely known chemical biomarkers to identify pesticide exposure. The presence of cholinesterase in the serum of female cotton pickers indicate the exposure to organophosphate and carbamate pesticides. Assessment of the cholinesterase levels in agricultural workers provides the most important indicator for the initiation of medical treatment.

**Objectives:** The present study was designed to compare the activity of serum BChE among female cotton pickers (exposed group) and non-cotton pickers (non-exposed group).

**Methodology:** The level of serum BChE of 100 female cotton pickers was compared with that of 100 non-cotton picker females. A self-designed questionnaire was used for the collection of primary data after a review of related studies. The R computer program (a language and environment for statistical computing and graphics) was used for the analysis of primary data.

**Results:** The level of BChE was recorded, and the means for the two groups were compared. The exposed mean was 5975.90  $\pm$  541.85U/L, and the non-exposed mean was 6981.76  $\pm$  782.92U/L and the difference was found to be statistically significant (p < 0.001). The result of the current study confirmed the negative association between decreased serum BChE and pesticide exposure. It was also found that 76% of female cotton pickers did not wash their hands before eating during the cotton picking. Similarly, 81% of pickers did not use gloves for their safety.

**Conclusion:** The inappropriate use of Personal Protective Equipment (PPEs) and the lack of awareness regarding pesticides' adverse the impact on female cotton pickers' health are significant factors of pesticide exposure.

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# INTRODUCTION

Pesticides are commonly used to kill almost every crop's harmful pest<sup>1</sup>. It was estimated that all over the world, around 43% of the agriculture labor force is women<sup>2</sup>. In Sub-Sharan Africa, 80% of women workers belonged to the agricultural sector compared to Tanzania, where 70% of

women were involved in the horticultural sector<sup>3</sup>. In developing countries such as Pakistan, women play a vital role in the development of a nation, and their share is 67% in forestry, fishing, and agriculture<sup>4,5</sup>.

It has been gauged that 15,000 chemicals and 35,000 chemical formulations have been prepared and used in the agricultural sector since 1945<sup>6</sup>. Although chemical pesticides are a significant controlling mechanism over pests in the agricultural sector, they are also a threat to the environment and living organisms<sup>7</sup>. The cotton crop is more prone to pest attacks; therefore, due to a lack of knowledge and a lack of awareness regarding Integrated Pest Management Techniques (IPM), Pakistani farmers widely use pesticides to control cotton crop pests. This intensive use of pesticides leads to pesticide exposure of female cotton pickers during the cotton harvesting, as well as to agricultural workers.

Pesticides impose two types of hazards (Acute and Chronic) on human health. The acute hazard results in headache, fatigue, rashes, feelings of weakness, vomiting, nausea, impaired vision, excessive sweating, panic attacks, tremors, cramps, skin discomfort, feelings of weakness, chronic bronchitis, and dizziness, along with coma and death in severe cases<sup>8,9</sup>. Usually, acute poisoning is a result of accidental or intentional exposure to a high dose of pesticide. Exposure to pesticides even in small quantities for longer intervals leads to acute illness. A study into the hazards of pesticide exposure is directly linked with different respiratory problems, i.e., wheeze, cough, allergy, and Organic Dust Toxic Syndrome (ODTS) among non-smokers<sup>10</sup>.

The shared statistics may vary among other less developed countries, especially in Pakistan, due to underreporting, lack of awareness, inaccessibility of health facilities, misdiagnosis and unavailability of the data management system, and non-availability of strict regulations for using or handling of pesticides<sup>11-13</sup>.

In developing countries like Pakistan, it is always challenging to assess occupational pesticide exposure of female cotton pickers. Therefore, it is the easiest way to estimate the extent of exposure through biological monitoring. Thus, the most common test for this purpose is the quantification of Butyrylcholinesterase (BChE) activity due to its inhabitation by Carbamate (CM) and Organophosphorus (OF) pesticides<sup>14-16</sup>. Both (pesticide exposure and level of BChE) are inversely related to each other<sup>17</sup>. The statistical results of a research study showed that 80% of pesticides are used on cotton crops and the

remainder on sugarcane, fruits, paddy, and vegetables in Pakistan<sup>12</sup>.

Women are lynching excel in traditional Sindhi families, and their sickness leads to crisis and chances of social disintegration. Therefore, the present research aimed to determine the level of BChE in the serum of female cotton pickers in District Jamshoro, Sindh, to evaluate the correlation between the level of BChE and pesticide exposure. Variables, such as the knowledge of female cotton pickers regarding symptoms of exposure, use of PPEs during the cotton picking, socioeconomic background of pickers, and health-related issues of cotton pickers, were documented. It is a pioneer study on female cotton pickers in district Jamshoro, and there is an urgent need for such studies on a mass level in developing countries such as Pakistan. This data may be used to better understand the extent of pesticide exposure among female cotton pickers.

## MATERIALS AND METHODS

The ongoing study examined the lethal effects of frequently used pesticides on the female cotton pickers' health in District Jamshoro. It is primarily a chemical biomarker study based on female cotton pickers' health concerns.

One district of Sindh-Pakistan; Jamshoro was selected for the study. The district consists of the Indus plain's hilly and plain (cultivated) areas. Jamshoro district is spread over 11,517 square kilometers and is administratively subdivided into 4 talukas (Kotri, Sehwan Sharif, Thana Bulla Khan, and Manjhand). Agriculture is the district's primary income source involving cotton, rice, sugarcane, maize, baira, wheat, barley, and pulses. Five villages of District Jamshoro (Bhutto, Lashari, Sobho Hajana, Sobho Lashari & Umeed Ali), taluka Manjand, were selected randomly from 1<sup>st</sup> October 2019 to 30<sup>th</sup> November 2019. An attempt was made to select respondents from the same socioeconomic and demographics. After the selection, respondents were segregated into two groups: exposed and non-exposed. Both groups contained 100 respondents. Thus, face-to-face interviews of 200 females (female cotton pickers-100 & non-cotton pickers-100) were conducted to complete a structured questionnaire. This can be regarded as an expensive method for data collection, but it provided the rich and complex information required, along with the highest response rate. The

questionnaire was developed after the review of the literature and was pre-tested before the collection of data. The respondents were selected through a purposive sampling technique, and written consent was obtained from participants after sharing the aims and objectives of the study in the local language (Sindhi). Participation was voluntary, and all the respondents were informed of their right to withdraw their consent at any time. Analyses have been performed using the computer program R<sup>18</sup>.

#### **Sample Collection**

In addition to the face-to-face interview, 3ml of blood was collected from all participants (200) through venipuncture to determine Butyrylcholinesterase. The blood samples were preserved in specialized glass tubes without any coagulant. Each tube was assigned a unique number for identification for confidentiality. The serum was obtained from blood and stored at -20°C for analysis.

### Working Principle of BChE

Serum BChE is a plasma cholinesterase, a serine hydrolase in all mammals, and can be detected in the liver and plasma<sup>19-21</sup>. BChE is the front defense line against toxic agents/compounds reaching the bloodstream. Thus, the exposure to pesticides, especially (organophosphate) or nerve agents, can be assessed by measuring the decreased level of BChE<sup>22</sup>. There are various methods of detecting the pesticide residue, including mass spectrometer (MS)<sup>23</sup>, high-performance liquid chromatography (HPLC)<sup>24</sup>, colorimetric assay<sup>25</sup>, and liquid chromatography (LC)<sup>26</sup> and biosensors<sup>27</sup>.

Cholinesterase hydrolyses butyryl thiocholine into thiocholine and butyric acid. Further, thiocholine decreases yellow potassium hexacyanoferrate (R+) to colorless potassium hexacyanoferrate. The decrease of absorbance was measured at 405nm on Merck Micro Lab-300. The said method is already used in a study on female cotton workers in Pakistan with the help of Merck micro lab 200<sup>17</sup>.

### **Analysis Procedure**

The modified spectrophotometric method<sup>28</sup> was adopted to determine AChE activity based on the rate of hydrolysis of acetylthiocholine by AChE. The yellow color liberated by the reaction of Thiocholine with DTNB (5,5' dithio-2-

nitrobenzoic acid) was quantified spectrophotometrically at  $\lambda$ max 412nm. The variation in Optical Density (OD)/min for four minutes is directly proportional to AChE activity that is determined by the following formula:

#### AChE activity (U) = $\Delta A \times 132.35$

Where,  $\Delta A$  is the mean absorbance.

Considering that 1 kU.L<sup>-1</sup> = 1000 U.L<sup>-1</sup>, AChE activity was expressed as  $U.L^{-1, 25, 29}$ .

A commercial laboratory kit (BChE A8K7P8) was applied to measure the serum BChE level. The change in the intensity of the yellow-colored compound (hexacyanoferrate-III) to colorless (hexacyanoferrate-II) was monitored spectrophotometrically at λmax 412nm.

#### Inclusion and Exclusion Criterion

Only married female cotton pickers having more than 3 years of cotton-picking experience and 100 married females from the same area who were not involved in cotton picking (as a control group) were interviewed. All the respondents were thoroughly briefed regarding the aims and objectives of the research. They were informed of their right to withdraw from the study without giving any reason.

## RESULTS

The percentage of adopted precautionary measures by female cotton pickers during the picking and the health status of female cotton pickers is indicated in Table 1. Only 26% of females washed their hands before eating during cotton picking or after cotton picking. The data shows that 51% of cotton pickers take a bath before engaging in domestic work. Only 23% cover their heads, and 20% cover their faces or use a mask during cotton picking. Only 16% of females cover their feet, and 19% use gloves for their protection. Out of all, 51% of respondents were lactating mothers, and 15% of respondents were pregnant. The level of BChE was recorded, and the means for the two groups were compared. The exposed mean was 5975.90 ± 541.85U/L, and the non-exposed mean was  $6981.76 \pm 782.92U/L$ , and the difference was found to be statistically significant (p < 0.001).

# Table 1. Precautionary Measures Taken by Female Cotton Pickers during the Picking of Cotton and Their Health Status.

Precautions	Percentage
Wash hands during or after picking before eating anything?	26%
Taking a bath before involving in domestic work	51%
Cover head	23%
Use mask/cover face	20%
Use gloves/cover hands	19%
used closed shoes/ covered feet	16%
Lactating Mothers	49%
Pregnancy	15%

## Table 2. Summaries of Continuous Variables by Exposure Group.

		Mean difference N - E						
Variable	E = Exposed (n = 100)		N = Non-exposed (n = 100)		Standard	95 % confidence limits		P-value
	Mean	SD (IQR)	Mean	SD (IQR)	Error	Lower	Upper	
	<median></median>	[Range]	<median></median>	[Range]				
Age of	38.06	8.360	35.95	9.460	-2.110	-4.579	0.338	0.095
respondent	<40.00>	(30 to 45)	<36.50>	(28 to 45)	(1.254)			
(years)	n = 100	[20 to 55]	n = 100	[19 to 55]				
Height	149.44	7.740	155.33	7.380	5.889	3.765	7.915	< 0.001
(cm)	<149.35>	(143.3 to 155.4)	<152.40>	(152.4 to 161.5)	(1.0593)			
	n = 100	[134.1 to 176.8]	n = 100	[125.3 to 167.6]				
Weight (kg)	52.22	12.390	54.82	7.190	2.600	-0.199	5.341	0.075
	<50.00>	(43.0 to 58.0)	<53.00>	(50.0 to 59.0)	(1.4151)			
	n = 100	[30.0 to 91.0]	n = 100	[39.0 to 78.0]				

Standard deviations are based on within-group data (not on pooled estimates).

Standard errors of differences between means and confidence limits have been obtained using 9999 bootstrap samples.

P-values for the comparison of the group means have been obtained using 10000 permutation samples.

### Table 3. Number of Abortions by Exposure group.

	Exposure Groups				Overall	
Presence of Abortion	Exposed		Non-exposed		Overall	
	Count	%	Count	%	Count	%
None	43	43.0	89	89.0	132	66.0
1 or 2	57	57.0	11	11.0	68	34.0
Overall	100	100	100	100	200	100

Fisher's Exact test P-value for association between presence of abortion and exposure group is p < 0.001.

The quantitative variables have been summarized by the exposure group, using numerical statistical summaries and graphical displays. The means for the two exposure groups have also been compared. It has been found that the

difference between the means for the groups of the heights of the respondents (non-exposed – exposed) is 5.889cm (95% CL 3.765 to 7.915), and this difference is statistically significant (p < 0.001) (Table **2**). The differences between

the group mean for age and weight have not been found to be statistically significant.

Abortion counts have been tabulated and compared by the exposure group (Table 3). The difference between the percentages of mothers having any abortions for the two groups (exposed – non-exposed) is 46% (95% CL: 33.6 to 56.4), and the difference is statistically significant p < 0.001).

## DISCUSSION

Various studies suggest that cotton pickers and agriculture workers are prone to pesticide exposures, even though they are not directly involved in the use of pesticides<sup>30-32</sup>.

The serum BChE activity as a biological indicator of pesticide exposure was evaluated in both exposed and non-exposed groups of females<sup>33, 34</sup>. Serum BchE is a wellrecognized and reliable indicator of pesticide exposure<sup>35,</sup> <sup>36</sup>. Decreased levels of (BChE) in the exposed group compared to the non-exposed group in this study confirm the adverse impacts of pesticide exposure in female cotton pickers. Various research studies propounded the decrease in cholinesterase levels due to exposure to a pesticide in the human population<sup>36-41</sup> and strengthened the ongoing research findings. The primary cause of pesticide exposure among female cotton pickers is the lack of, or less use of Personal Protective Equipment's (PPEs) due to the unavailability of guidelines for cotton pickers from the provincial as well as federal government in Pakistan. The study revealed that 15% of pregnant women were involved in cotton picking, and only 26% pickers were washing their hands before eating. The results suggest that the risk of pesticide toxicity is directly linked to the extent of exposure, inappropriate use of PPEs, and exposure to pesticide residue on cotton crops.

The misuse of pesticides on the cotton crop is a severe threat to the environment and farmworkers of the cotton crop. In Pakistan, cotton picking is a role of women; therefore, they are more prone to pesticide exposure. They can be exposed to pesticides during picking through the dislodging of pesticide dust from the boll stem, and leave settled and fluffy bolls on their naked parts of the body, i.e., arms, hands, face, or through inhalation<sup>42</sup>. In Jamshoro, women are involved in cotton picking for 2-3 months. Therefore, continuous and prolonged pesticide exposure through different entry points (eyes, nose, skin, and mouth)

poses greater health-related issues. Most female cotton pickers belong to needy families, and they are forced to work even in pregnancy to cope with financial upheavals without knowing the consequences of pesticide exposure on their bodies and the fetus.

It was estimated that approximately 200,000 people out of 1-5 million cases around the world die every year due to pesticides among agricultural workers, and the majority of exposed people belonged to developing countries<sup>43, 44</sup>.

Policymakers, non-governmental organizations, and civil societies must provide training and technical support along with PPE for female cotton pickers to protect them from hazardous pesticide residue.

## CONCLUSION

Lack of awareness, illiteracy, careless attitudes, traditional cotton picking methods, and the unavailability of PPEs are the leading causes of pesticide exposure among female cotton pickers. The result of this study suggests that the BChE biomarker helps study the adverse effects of pesticides on female cotton pickers. Moreover, the results may be generalized to other crops due to the increasing involvement of females in different phases, from sowing to picking.

# ETHICAL APPROVAL

Ethical approval for the study was obtained from the Research & Education Development Department of Rural Education & Economic Development Society (REEDS), Pakistan.

## **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

## FUNDING SOURCE

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# LIST OF ABBREVIATIONS

IPM	Integrated Pest Management
ODTS	Organic Dust Toxic Syndrome
PPEs	Personal Protective Equipment's
Serum BChE	Serum Butyrylcholinesterase

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