



Butyrylcholinesterase as Biomarker of Occupational Exposure Among Female Cotton Workers

Aima Iram Batool^{1*}, Syeda Humaira Jabeen¹, Naima Huma Naveed², Fayyaz Ur Rehman³, Iram Inayat¹, Fareeha Idrees¹, Asma Noreen¹, Hakim Bibi¹

Abstract

Objectives: Lack of safety measures and unawareness about the harmful effects associated with pesticide exposure among cotton picking females contribute to poor health quality in this group of workers. The activity of butyrylcholinesterase (BChE) acts as potential biomarker of pesticide exposure among these females. Our study aimed to determine the relationship between decreasing activity of BChE level in serum with pesticide exposure in female cotton pickers that can lead towards health impairments.

Materials and Methods: The BChE level in serum samples of 50 female cotton pickers were compared to that of 50 controls using the *t* test. A questionnaire was prepared and asked from each female worker which was related to precautionary measures, work history and personal information.

Results: Serum BChE activity in exposed females was (6194.80 U/L) as compared to non-exposed females (7209.77 U/L). Our studies confirm and extend the association between reduced BChE activity and pesticide exposure. BChE activity could be used as an assessment tool for pesticide poisoning among exposed workers. In regard to precautionary measures none of the females used gloves or eye glasses. Face mask was used by only 10% of the females for protection. 30% of the females did not take bath or wash their hands and face after picking, and almost all females were found to eat and drink during picking.

Conclusion: Pesticide exposure along with no safety measures decreased the BChE activity among exposed female workers that could be responsible for deranging the physiological responses of their body.

Keywords: Acetylcholinesterase, Butyrylcholinesterase, Female, Pesticides

Introduction

Millions of tons of cotton are hand-picked by women and girls every year in cotton growing belts of Pakistan particularly starting in October and extending to the end of January. Female labors of all age groups are engaged in cotton picking with the youngest being 6-8 years old. An elated percentage of cotton pickers belong to countrified areas, generally the poorer strata of population with an average of 8 hours working and 50-100 kg of cotton picked per day.

In addition to low payoff, longer employed hours and steely job, these females are also exposed to pesticides sprayed heavily on cotton crop (1). Fear of pests such as whitefly and American bollworm, prompt farmers to use pesticides even during the cotton picking period. Relating to health risks, out of 2.6 million working females (cotton pickers), annually 2.2 million females get sick due to exposure to pesticides used on cotton fields. In Pakistan female cotton pickers are more prone to pesticides exposure because 95% do not have/use any of the precautionary measures (2). Furthermore cuts and skin rashes during work make those females more susceptible to pesticides hazards by absorption via dermal route.

Picking cotton is common during pregnancy and breast feeding which poses additional risk to the health

of the women and their children (3). The most common health problems which majority of the female cotton pickers suffer include headache (80%), tingling in body parts (66.70%), sweating (63.30%), muscular weakness (73.30%) and skin allergies (53.30%).

Observing butyrylcholinesterase (BChE) and hematologic parameters of an agriculture worker can be an effective indicator for checking health hazards associated with pesticides exposure (4). BChE inhibition was proved as a good bio-indicator to determine pesticide poisoning by the World Health Organization (WHO). With its protective role against pesticides, BChE hydrolyzed the active ingredient of these agents making them unable to inhibit acetylcholinesterase (AChE) (5). Several studies have verified the correlation between pesticide exposure and BChE inhibition (6-9). Serum BChE is a sensitive enzyme for measuring pesticides poisoning, shows various level of inhibition, 20-50%, 80-90% and over 90% relatives to mild, moderate and sever poisoning respectively.

Hemoglobin (Hb) level of a pesticide exposed person decreases sufficiently (10). Concentration of blood hemoglobin reduces in females due to pesticide exposure (11). The low level of Hb may be due to binding of organophosphorus pesticides with iron followed by a lack of incorporation of iron in hemoglobin (12).



This study aimed to determine the level of BChE in serum of female cotton pickers and establishing a correlation between serum BChE and pesticide exposure as they vary inversely with each other.

Materials and Methods

Two stations namely Multan, and Mianwali were selected for this study. Samples were collected randomly from Khaniwal (Multan) and Dohaba (Mianwali) which have long history of cotton production and identified as extensive cotton growing areas of Pakistan.

Study Design and Target Population

A total of 100 females were recruited for study after getting written consent from them. All the females had similar socio-economical status and there was no major difference in their diet and living conditions. These females were categorized into exposed and non-exposed groups. Each category was composed of 50 individuals. The non-exposed group consisted of those females which never indulged in agricultural work or were never directly exposed to pesticides. They were only involved in household chores. The exposed group was also composed of 50 females from Multan and Mianwali areas, 25 females from each. These females were involved in cotton picking for more than 2 years and spend 8 hours in the fields, daily. The study was carried out from December to mid of January.

Blood Sampling

Blood samples were collected from each subject under study. Eight milliliters blood sample was collected by using sterilized disposable syringes from sub-clavian vein of all volunteers. Serum was separated within an one hour of sample collection; each centrifuge tube had

5 ml of clotted blood which was placed in centrifuge machine and centrifuged at 10000 rpm for separation of serum. Serum samples were stored at -20°C . Serum samples were analyzed for BChE enzyme activity for exposed and non-exposed population.

Principle

Cholinesterase hydrolyses butyrylthiocholine into thiocholine and butyric acid. Thiocholine further reduces yellow potassium hexacyanoferrate (III) to colorless potassium hexacyanoferrate. The decrease of absorbance is measured at 405 nm. This test was performed on Merck micro lab 200.

Material

Reagents (component and concentration):

R1: pyrophosphate pH 7.6, 95 mmol/L

Potassium hexacyanoferrate (III), 2.5 mmol/L

R2: butyrylthiocholine, 75 mmol.

Assay Procedure

Measured quantity of 5 mL of R1 and 20 mL of R2 were mixed together to prepare the working reagent. Out of the working reagent, 1250 μL was taken and mixed with 20 μL

of each sample. Without any delay this prepared sample was ingested in Merck micro lab 200 and after 5 minutes reading was recorded.

Statistical analysis

Statistical analysis of data was done by XLSTAT and SPSS. Statistical differences were examined by using analysis of variance (ANOVA) and student *t* test.

Results

Table 1 shows the percentage of precautionary measures taken by female cotton pickers during picking. None of the females used gloves or eye glasses during cotton picking. Only 10% of the females used face mask to protect themselves. Thirty percent of females did not take bath or wash their hands and face after picking. Majority of the female workers ate and drank during picking (100%).

Regarding the physical status of cotton pickers, 34% of females were pregnant while 74% were on practice of feeding babies.

Table 2 shows BChE level which was significantly lower in female cotton pickers as allegorized to females in control group; BChE activity was 6194.80 U/L and 7209.77 U/L, respectively. Highly significant statistical difference in value of Hb was present between exposed and non-exposed females ($t=5.38$, $P=0.00$). Statistically no significant difference was shown in comparison of work duration between cotton-pickers and non-exposed group. Both groups had same work duration with ($P>0.05$). Number of abortions was greater in cotton pickers than non-exposed females, but statistically no significant difference was seen according to numbers of abortions. In cotton picker average weight was 44.10 kg which was much higher in non-exposed group (51.93 kg).

Table 2 shows ($P<0.05$) a significant statistical difference among female cotton pickers and non-exposed groups related to height ($t=2.75$, $P=0.05$). Non-significant difference was present between cotton-pickers and non-exposed females according to number of children and abortion.

Discussion

Pakistan is an agriculture based country but due to lack of knowledge, careless attitude, and rough practice in han-

Table 1. Precautionary Measure Taken by Cotton Pickers Females During Picking and Their Physical Status

Precautions	Number	Percent
Use gloves	0	0
Use mask	5	10
Use eye glasses	0	0
Cover body	50	100
Wear shoes	15	34
Eat and drink	50	100
Wash hands and face after picking	35	70
Pregnant	15	34
Breast feeding	37	74

Table 2. Comparative Analysis Between Cotton Picker and Non-exposed Females

	Group	Mean	SD	SE	t	P
Age	Cotton picker	35.80	14.27	2.61	4.28**	0.00
	Non-exposed	23.57	6.41	1.17		
Butyrylcholinesterase	Cotton picker	6194.80	1422.38	259.69	-2.55*	0.013
	Non-exposed	7209.77	1649.01	301.07		
Hb	Cotton picker	10.11	1.81	0.33	-5.38**	0.000
	Non-exposed	12.18	1.08	0.20		
Number Of Children	Cotton picker	4.19	1.89	0.41	2.66*	0.014
	Non-exposed	2.00	1.26	0.52		
Number Of Abortion	Cotton picker	1.90	0.88	0.28	1.40	0.192
	Non-exposed	1.00	0.00	0.00		
Height	Cotton picker	4.99	0.19	0.04	-2.75**	0.008
	Non-exposed	5.15	0.26	0.05		
Weight	Cotton picker	44.10	8.89	1.62	-3.688*	0.001
	Non-exposed	51.93	7.56	1.38		

** Highly significant; *Significant.

dling of pesticides, farmers face serious health problem to (13). Present investigation was carried out to monitor the health problem among female cotton pickers. Yousaf et al (14) investigated symptoms of pesticide exposure female agriculture workers of Punjab, Pakistan. Data was collected from 50 female cotton pickers by using well-prepared interviewing schedule. Majority of females agreed that they were always facing problems such as stomach disease, breathing problem, diarrhea and swelling of hands and other parts of body during or after picking cotton.

The most economical blood test for checking the hazardous effect of pesticides in agricultural workers exposed to organophosphorus pesticides is serum cholinesterase especially BChE. Its inhibition is taken as a biomarker for exposure. BChE is highly reactive with pesticides; it protects AChE against pesticides. Therefore, serum BChE activity measurement is the most sensitive way to detect pesticide exposure. BChE is recommended as a biomarker for exposure to organophosphorus pesticides even outside the spraying season (15).

This study showed highly significant decrease in BChE activity in cotton pickers as allegorized to non-exposed group. The observed significant decrease of BChE activity was in accordance with findings of other investigators. Jintana et al (16) suggested that measuring cholinesterase activity could be an admirable biomarker for assessing pesticide exposure and health effect in exposed population. Results of his study showed that there were statistically decrease in BChE activity during high exposure period related to low exposure period. BChE in normal group was higher than in exposed group population. Farahat et al (17) organized the studies to seek out the effect of chlorpyrifos – a widely used organophosphates pesticide – on BChE activity, among agriculture worker. It was established that BChE activity decreased gradually by end of the spraying season. BChE activity remained inhibited even after 8 to 10 days the application of pesticides had

ended. The exposure to pesticides was stated as a causative factor for the changes in hemoglobin levels in an earlier study (18). Hb level was also found significantly lower among pesticide exposed females. Among cotton pickers Hb level was observed as 10.11±1.81 g/dL while the same in non-exposed females was noticed as 12.18±1.08 g/dL.

Conclusion

The our study affirms and extends the connection between reduced BChE manifestation and pesticides exposure. So, BChE manifestation could be used as a tool for pesticides poisoning among exposed female workers.

Ethical Issues

The ethical committee of University of Sargodha approved the study.

Conflict of Interests

The authors declare no conflicts of interests.

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