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BChE(Butryl cholinesterase) as Biomarker of Occupational Exposure among female cotton workers.

Abstract:
Pesticides commonly sprayed on cottoncrops,during cotton picking season inhibits BChE activity. In this study, butyrylcholinesterase level in serum samples of 50 cotton picker females were compared to 50 controls using the T-test. Significant decrease in serum BChE level was found allegorized to non- exposed females of same areas. Serum butyrylcholinesterase activity in exposed females was (6194.80 U/L) as compared to non-exposed females (7209.77U/L). Our studies confirm and extend the association between reduced BChE activity and pesticides exposure. So, BChE activity could be used as an assessment tool for pesticides poisoning among exposed workers.
Key words: BChE, pesticides, cotton picker, females

Introduction:
A millions of tons of cotton are hand-picked by women and girls every year in cotton growing belts of Pakistan's Punjab and Sindh provinces, which contribute about 2 percent to the GDP [1]. The cotton-picking season starts in the middle of October and extends to the end of January. Feminine labors of all age groups are engaged in cotton picking play from the age of 6-8 years. An elated percentage of cotton pickers belong to countrified areas, generally the poorer strata of population on average they spend 8 hours per day and pick 50-100 kg of cotton. Besides too low payoff, longer employed hours and steely job these females are also exposed to pesticides sprayed heavily on cotton crop [2]. Fear of pests such as White fly and American Bollworm prompt farmers to use pesticides even during the cotton picking period. Health hazards to women cotton pickers showed that out of 2.6 million females who pick cotton annually 2.2 million females got sick due to their exposure to pesticides used on cotton field. In Pakistan females cotton pickers are at greater risk of pesticides because 95 percent are not taking any precautionary measures [3]. Furthermore cuts and skin rashes that cotton pickers get during their work make them more susceptible to pesticides hazards by making absorption via skin more easy .Picking cotton is common during pregnancy and breast feeding which poses additional risk to women and their children health [4].Most common health impairment with which majority of cotton pickers females Suffering were headache (80%), tingling in body parts (66.70%),sweating (63.30%),muscular weakness (73.30%) and skin allergies (53.30%). Observing BChE and hematologic parameter of agriculture worker can be useful indicator for checking health hazards associated with pesticides exposure [5]. WHO, [6]also approved BChEinhibition as good bio-indicator to determine pesticide poisoning . BChE has important protective role against pesticides exposure. BChE hydrolyzed the active ingredient of pesticides making them unable to inhibit AchE. In this way BChEguards against pesticide toxicity [7]. Several studies have verified correlation between pesticide exposure and BChE inhibition [8-11]. Serum BChE is sensitive enzyme for measuring pesticides poisoning, shows various level of inhibition, 20-50% 8090% and over 90% relatives to mild, moderate and sever poisoning respectively. Hb level of pesticide exposed person decrease sufficiently [12]. Concentration of blood hemoglobin reduce in females due to pesticide exposure [13] .The low level of Hb may be resulted from to binding of organophosphorus pesticides on iron followed by a lack of incorporation of iron in hemoglobin [14]. Hb is a metalloprotein which is present in RBC and bind oxygen and carbon dioxide alternatively and transport it to all cells of body. The hemoglobin test is an important blood test and it can be used as indicator for certain kinds of blood disorders.

Methods:
Study setting: Aim of this study was to evaluate the harmful effect of pesticides on female cotton picker. Two stations viz; Multan, and Mianwali were selected for study. Samples were collected randomly from Khaniwal(Multan) and dohaba (Mianwali) which have long history of cotton production ever identified as extensive cotton growing areas of Pakistan.

Study design and target population: 100 females were recruited for study after getting written consent from them. All the females were have same social economical status, there is no major difference in their diet as well as in living conditions. These females were categorized into exposed and non-exposed group. Each category was composed on 50 individuals Non-Exposed group was consisted on those females which never remain indulge in agricultural work or never directly exposed to

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pesticides. The non -exposed females were involved in household chores. Exposed group female: This group was also composed of 50 females from Multan and Mianwali area 25 females from each. These females were entangled in cotton picking work for more than 2 years and spend 8 hours in field daily. The study was carried out from December to mid of January.

Blood sampling:
Blood samples were collected from each subject under study.8ml blood samples were collected by using sterilized disposable syringes from sub-clavian vein of all volunteers. Serum was separated within the one hour of sample collection each centrifuge tube having 5ml of clotted blood was placed in centrifuge machine and these samples were centrifuged at 10,000 rpm for separation of serum. Serum samples were stored at -20°C. Serum samples were analyzed for activity of butyrylcholinesterase enzyme for exposed and non exposed population.

Principle:
Cholinesterase hydrolyses butrylthiocholine into Thiocholine and butyric acid by cholinesterase. Thiocholine further reduces yellow potassium hexacyanoferrate (111) to colorless potassium hexacynanoferrate (11). The decrease of absorbance is measured at 405 nm.
This test was performed on Merck micro lab 200.

Material:
Regeants (component and concentration):
R1: pyrophosphate pH 7.6 95mmol/L
Potassium hexacyanopferrate (111) 2.5mmol/L
R2: Butrylthiocholine 75mmol

Assay procedure:
Measured quantity of 5 ml of R1 and 20ml of R2 were mixed together to prepare the working reagent .out of working reagent 1250µl was taken and mixed with 20µl of each sample. Without delayed this prepared sample is ingested in Merck micro lab 200 and after five 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 percentage of precautionary measures taken by cotton pickers females during picking. None of the females were using gloves or eye glasses during cotton picking. Only 10% females were using face mask to protect themselves. 30% of females do not take bath or wash their hand and face after picking. Majority of females eat and drink during picking (100%).

Table 1 precautionary measure taken by cotton pickers females during picking.

precautions	frequency	percentage
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%

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Table: 2 physical status of female cotton pickers

status	frequency	Percentage
pregnancy	15	34%
Breast feeding	37	74%

Table (2) shows the physical status of cotton pickers 34% of females were pregnant while 74% of females were on practice of feeding babies.

Table 3: Comparative analysis between cotton picker and non-exposed females.

	Group	Mean	SD	SE	t-value	Prob
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^{NS}	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		

Table 3 shows butyrylcholinesterase level was significantly lower in cotton pickers females as allegorized to normal group females that were not involved in cotton picking..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 work duration comparison between cotton-pickers and no exposed group. Both groups had same work duration with (P>0.05). No of abortion was greater in cotton pickers than non-expoed females, but statistically no significant difference was seen according to numbers of abortions.In cotton picker average weight was 44.10 kg that was much higher in non-exposed group (51.93 kg). Table 3 shows (P<0.05).significant statistical difference was present among 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 numbers of children and abortion.

Discussion:

Pakistan is an agriculture based country but due to lack of knowledge, careless attitude, and rough practice in handling of pesticides constitute serious health problem to farmers [15]. Present investigation, study was carried out to monitor the health problem among female cotton pickers, associated with inhibition of butyrylcholinesterase activity due to pesticides exposures. Yousaf and his coworkers [16] investigated symptoms of pesticide exposed in females' agriculture worker of Punjab, Pakistan. Data was collected from 50 females' cotton picker by using well-prepared interviewing schedule. Majority of females agreed that they were always facing problem of stomach disease, breathing problem, diarrhea and feel swelling inflammation on hands other parts of body during or after picking cotton.

The most economical blood test for the checking the pesticides hazard in agricultural workers who are 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 sacrificially protects AChEagainst pesticides. Therefore, serum BChE activity

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measurement is the most sensitive way to detect pesticide exposure. BChE is recommended as a biomarker for exposure to OP pesticides even outside the spraying season [17]. This study showed highly significant decrease in BChE activity in cotton pickers as allegorized to nonexposed group. The observed significant decrease of BChE activity was in accordance with findings of other investigators. Jintana et al.,[18]suggested that measuring cholinesterase activity could be an admirable bio-marker 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 prieod. While BChE in normal group was higher than in exposed group population.Fayssal et al., [19,] organized the studies to seek out the effect of chloropyrifos (CPF) a widely used organophosphorous pesticides, 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 eight to ten 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 [20]. Hemoglobin level was also found significantly lower among pesticides exposed females. Among cotton pickers Hb level was observed as 10.11±1.81 while same in non-exposed females was noticed as 12.18±1.08.

Conclusion:

Our studies affirm and extend 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.

List of abbreviation:

Hb	hemoglobin
AChE	Acetylcholinesterase
BChE	butyrylcholinesterase
OP	organophosphorus
CPF	chloropyrifos
RBC	Red blood cells

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