

## Study of Effect of Pesticides on Total Bilirubin and Direct Bilirubin Levels in Blood of Workers of Pesticide Formulation & Packing Plants in Pakistan: Multiplexing with ALP, ALT and AST

MUHAMMAD FAHIMUL-HAQ<sup>1\*</sup>, SADIA MAHMOOD<sup>1</sup>, NAKHSHAB CHOUDHRY<sup>2</sup>, TAZEEM SHAHBAZ<sup>3</sup>, SHAMA AKRAM<sup>1</sup>, RIFFAT YASMIN<sup>4</sup>

### ABSTRACT

**Aim:** To evaluate the Impact of pesticides on T Bilirubin and D Bilirubin, ALP, ALT and AST among workers of pesticide formulation & packing plants in Pakistan

**Methods:** One hundred workers in the age group of 20-50 years working at different pesticide formulation and packing units for at least one year were included in this study.

One hundred healthy workers with no history of exposure to pesticides with matching age, sex and social status were taken as control. Biochemical tests T Bilirubin and D Bilirubin were carried out employing chemistry autoanalyzer, Dimension RXL (Dade Behring),

**Results:** In current study, mean T Bilirubin value for 100 control subjects was 0.51 mg/dl. Regarding 100 pesticide industrial workers mean T Bilirubin value was 0.68 mg/dl. On the other hand serum of 100 control subjects indicated mean D Bilirubin value of 0.20 mg/dl. Regarding mean D Bilirubin value for 100 pesticide industrial workers was 0.24 mg/dl. These results revealed that all the hundred pesticide industrial workers had normal T Bilirubin and D Bilirubin levels but on the higher side as compared to control subjects. P value was significant.

**Conclusion:** All pesticide industrial workers had normal T Bilirubin and D Bilirubin levels but on the higher side as compared to control subjects. Future studies must converge on a deeper analysis of the oxidative stress induced misrepresentation of protein network at cellular level.

**Keywords:** Pesticide industrial workers, T Bilirubin and D Bilirubin

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### INTRODUCTION

Data obtained through high throughput technologies is deepening our understanding about the fact that pesticides have a major impact at cellular level. Pesticides induced oxidative stress severely compromises cellular activities. DNA damage, genomic instability and misrepresentation of signaling cascades are some of the major targets of oxidative stress. Moreover, there are some other considerable damages noted in the cell lines and animal model studies. Mounting evidence suggests that oxidative stress damages membrane of erythrocytes.

It was reported that under normal conditions in human adult  $1.2 \times 10^8$  erythrocytes are destroyed per hour<sup>1</sup>. Thus in one day, 70kg adult human turnover approximately 6gms of hemoglobin. During disintegration of hemoglobin, the globin (protein part) may be reutilized as such or in the form of its constituents amino acids while the iron of heme is

stored in the body tissues in the form of ferritin and hemosidrin. However, the iron-free porphyrin skeleton is also degraded mainly from its one end of the methane bridges and a green pigment biliverdin is formed. This is then reduced to yellow pigment to bilirubin which is carried by the plasma albumin to the liver for eventual excretion.

It is estimated that one gram of hemoglobin yields 35 mg of bilirubin. The daily bilirubin formation in human adults is about 250-350mg. So due to hazardous effects of pesticides on human health, the developed countries are preferring the use of hormonal pesticides and phytopesticides instead of using synthetic pesticides.

In Pakistan there is a tremendous use of pesticides without any proper monitoring. In accordance with this assumption, a research group evaluated, side effects of improper use of pesticides on farmers in Gadap (rural area) Karachi. Results indicated that ALP, ALT, AST and total bilirubin levels were considerably raised in the samples of the subjects as compared to the controls Azmi et al (2005)<sup>2</sup>. It is becoming increasingly apparent high level of ALT and AST in the blood of occupational workers chronically exposed to organophosphate pesticides was observed Misra et al (1985)<sup>3</sup>, Kamal

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<sup>1</sup>Department of Biochemistry Rashid Latif Medical College 35 Km Ferozepur Road, <sup>2</sup>Department of Biochemistry KEMU, <sup>3</sup>Department of Community Medicine Rashid Latif Medical College 35 Km Ferozepur Road, <sup>4</sup>Department of Biochemistry, FPGMI, Sheikh Zayed, Lahore, Pakistan. Correspondence to Dr. Muhammad Fahimul Haq Email: fahimul-haq@hotmail.com

et al (1990)<sup>4</sup>. It is intriguing to note that wide ranging pesticides severely disrupt the enzymatic levels in persons exposed to the pesticides Goel et. al. (2000)<sup>5</sup> Mani et al (2000)<sup>6</sup>, Altuntas et al (2002)<sup>7</sup>, Desi et al (1996)<sup>8</sup>, Ranjbar et. al. (2002)<sup>9</sup>, Dahamana et al. (2004)<sup>10</sup>.

**MATERIALS AND METHODS**

In this cross-sectional study one hundred workers in the age group of 20-50 years working at different pesticide formulation and packing units for at least one year were included in this study. One hundred healthy persons with same age and sex with no history of exposure to pesticides were taken as control.

Individuals, working at pesticide industry and controls, who were known diabetic, hypertensive, smokers, obese, having previous history of jaundice or positive cases of hepatitis B or C were excluded from the study.

Blood samples (6-8 ml) were collected from individuals of both groups under current study. Blood samples were drawn and allowed to clot for 20-30 minutes to obtain serum. All the blood samples were brought to the laboratory and sera were immediately separated by centrifugation at 3000 rpm for five minutes and were transferred to eppendorf tubes and kept frozen at -2 to 4°C for later analysis.

Biochemical tests Total Bilirubin (TBIL), Direct Bilirubin (DBIL), were carried out employing chemistry autoanalyzer, Dimension RXL (Dade Behring). Screening for Hapatitis B and C virus was done by immunochromato- graphic technique. This study was in collaboration with ALP, ALT, and AST,

Results of, T Bilirubin, D Bilirubin, were expressed as mean ± SD. Data were analyzed according to the unpaired students t test. ANOVA technique was applied for the comparison of three groups i.e., control group and workers of pesticides

industry having normal and abnormal LTF's Level. A p value of less than 0.05 was considered statistically significant.

**RESULTS**

In the present work blood samples of 200 workers were taken; 100 workers from other than pesticides industry called as control group and 100 pesticides industrial workers. LFTs (ALP, ALT, AST, GGT, T Bilirubin, D Bilirubin) were performed on Auto Analyzer Dimension RXL, Dade Behring. Screening for Hepatitis B and C virus was done by immunochromatographic technique to confirm that all the samples included in current study were negative for Hepatitis B and C virus. Data of 100 pesticides industrial workers showed that LFTs (ALP,ALT,AST) of 80 workers out of 100 were within the normal range whereas 20 workers had abnormally raised level of LFTs (ALP, ALT,AST). Regarding **Total Bilirubin(TBIL) and Direct Bilirubin(DBIL) it was observed that both are with in normal range, however on the upper side as compared to control group.**

**Total Bilirubin(TBIL):** Normal range of total bilirubin in serum is 0 to 1.2 mg/dl. In Current study, mean total bilirubin for 100 control subjects was 0.5 mg/dl (Table 1). Regarding 100 pesticide industry workers, mean Total Bilirubin value was 0.68 mg/dl with SD 0.22 and 95% confidence interval 0.24 to 1.12. Total bilirubin values ranged between 0.4 and 1 mg/dl. Eighty out of 100 pesticides industrial workers had mean T.Bilirubin 0.63 mg/dl (Table 2). Regarding rest of 20 workers mean Total Bilirubin value was computed as 0.85 mg/dl with SD 0.17(Table 2) and it was as high as 1 mg/dl and the lowest was 0.5 mg/dl i.e within normal range, however on the upper side as compared to control and normal pesticide industrial workers. P value was significant (Table 3 & 4).

Table 1: Means, Standard Deviations, 95% Confidence Interval of Total Bilirubin (TBIL), Direct Bilirubin (DBIL) for Control Subjects and Pesticide Industrial Workers having normal and abnormal LFT's jointly

LFT's	Control Subjects (n=100)			Pesticide Industrial Workers (n=100)		
	Mean	SD	C .I 95%	Mean	SD	C. I 95%
T. Bili (mg/dl)	0.51	0.10	0.489-0.530	0.68	0.22	0.24-1.12
D.Bili (mg/dl)	0.200	0.07	0.184-0.215	0.24	0.07	0.11-0.38

Table 2: Means, standard deviations, 95% confidence Interval of Total Bilirubin (TBIL), Direct Bilirubin (DBIL) for Control Subjects and Pesticide Industrial Workers having normal and abnormal LFT's separately

LFT's	Control Subjects (n=100)			Pesticide Industrial Workers with normal LFT's (n=80)			Pesticide Industrial Workers with abnormal LFT's (n=20)		
	Mean	SD	C .I 95%	Mean	SD	C. I 95%	MEAN	SD	C. I 95%
T.Bili (mg/dl)	0.51	0.10	0.489-0.530	0.63	0.213	0.591-0.686	0.850	0.170	0.770-0.929
D.Bili (mg/dl)	0.200	0.07	0.184-0.215	0.242	0.070	.776-0.258	0.265	0.049	0.242-0.288

Table 3: Means, Standard Deviations, ANOVA of Total Bilirubin ( TBIL), Direct Bilirubin (DBIL) for Control Subjects and Pesticide Industrial Workers having normal and abnormal LFT's separately

LFT's	Control Subjects (n=100)		Pesticide Industrial Workers with normal LFT's Levels (n=80)		Pesticide Industrial Workers with abnormal LFT's levels (n=20)		ANOVA
	Mean	SD	Mean	SD	Mean	SD	F(2,197)
T.Bili (mg/dl)	0.51	0.10	0.63	0.213	0.850	0.170	41.300***
D.Bili (mg/dl)	0.200	0.07	0.242	0.070	0.265	0.049	11.437***

\*\*\*p&lt;0.001

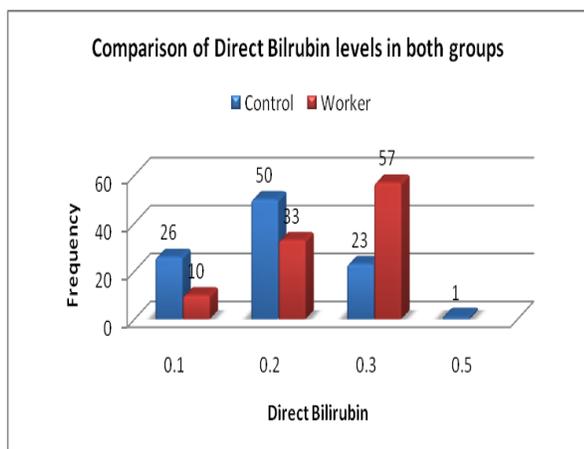
Table 4: Means, Standard Deviations, t-test of Total Bilirubin ( TBIL), Direct Bilirubin (DBIL) for Control Subjects and Pesticide Industrial Workers having normal and abnormal LFT's jointly

	Control Subjects (n=100)		Pesticide Industrial Workers (n=100)		t-values
	Mean	SD	Mean	SD	
T.Bili(mg/dl)	0.51	0.10	0.68	0.22	7.0***
D.Bili(mg/dl)	0.200	0.07	0.24	0.07	4.6***

\*\*\*p&lt;0.001

Table 5: Comparison between Bilirubin levels of control &amp; workers

Bilirubin Range	Control		Workers	
	T.Bili (NV 0-1.2mg/dl)	D.Bill (NV 0-0.4)	T.Bili (NV 0-1.2mg/dl)	D.Bill (NV 0-0.4)
0-0.2	0	76	3	43
0.3-0.4	24	23	22	57
0.5-0.6	76	1	6	0
0.7-0.9	0	0	61	0
1.0-1.1	0	0	8	0



Normal range of Direct Bilirubin in serum is 0 to 0.4 mg/dl. In current study, analysis of serum of 100 control subjects indicated mean Direct Bilirubin value 0.20 mg/dl (Table 1). Mean Direct Bilirubin value for 100 pesticide industrial workers was 0.24 mg/dl with SD 0.07 and 95% confidence interval 0.11 to 0.38. Out of aforesaid 100 workers, 80 workers had mean T.Bili value 0.24 mg/dl with SD 0.04 (Table 2). Regarding rest of 20 workers mean Direct Bilirubin value was computed as 0.26 mg/dl (Table 2). P value was found significant (Table 3 & 4).

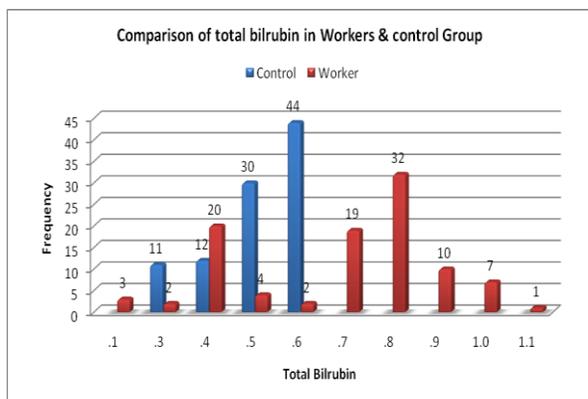
Among control group, 26% D. bilirubin was 0.1mg/dl, in 50 % direct bilirubin level was 0.2mg/dl, in 23 % it was 0.3mg/dl and in only 1% bilirubin was

0.5 mg/dl. Whereas in exposed/ worker group, 10%'s bilirubin level was 0.1mg/dl, in 33% it was 0.2mg/dl and in 57% bilirubin level was 0.3mg/dl.

Pesticides industrial worker's (with normal LFT's level) Direct bilirubin level was statistically significantly higher than control group (p-value <0.001). Whereas Pesticides industrial worker's (with abnormal LFT's levels) total bilirubin level was statistically significantly higher than control (p-value <0.001) but it was not statistically different to Pesticides industrial worker's bilirubin (with normal LFT's Level) (p-value 0.424).

In control group, the Total bilirubin levels range was 0.3-0.6. Whereas, in worker group the range was 0.1-1.1 and more than 69% worker's bilirubin was above 0.6mg/dl.

Pesticides industrial worker's (with normal LFT's level) Total bilirubin level was statistically significantly higher than control group (p-value <0.001). Pesticides industrial worker's (with abnormal LFT's levels) total bilirubin level was statistically significantly higher than control as well as Pesticides industrial worker's bilirubin (with normal LFT's Level) (p-value <0.001).



## DISCUSSION

In present study blood samples of 100 pesticide industry workers having exposure to pesticides during formulation, filling and packing were selected after observing the inclusion and exclusion criteria. Similarly 100 subjects from other than pesticide industry were also selected as control. It was observed that all control groups had LFT's not only within normal limits but also averagely on the lower side of normal levels. Out of 100 pesticide industry workers, blood samples of 80 workers showed normal level of LFT's, however, slightly on the higher side except GGT which was slightly on the lower side as compared to control subjects. Other 20 pesticide industry workers had raised levels of ALP, ALT and AST whereas GGT level was slightly on the lower side but within normal range and the other tests i.e., T. Bilirubin, D. Bilirubin were within normal range but close to upper limits of normal levels.

T. Bilirubin and D. Bilirubin in both groups were not only within the normal range but were also comparatively close to upper normal limit in pesticide industrial workers. High Billirubin level after exposure to pesticides has also been reported by other researchers<sup>11,12,13,14,15,16</sup>. It might be attributed to prolonged exposure to pesticides which disturbed the normal red blood cell metabolism, affecting the hepatic dysfunction and increased the level of bilirubin in the blood thereby causing hyperbilirubinemia which might be due to production of more bilirubin than the normal liver can excrete.

## CONCLUSION

Pesticide industry workers were affected more as compared to control subjects due to continuous exposure of pesticides. Upper normal levels of Total Bilirubin (TBIL) and Direct Bilirubin (DBIL) were observed in serum of pesticide industrial workers.

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