Research Article

Assessing Haemato-Biochemical, and Genotoxic Effects of Pesticide Exposure on Rural Inhabitants in South Punjab, Pakistan: A Biomonitoring Study

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Abstract | Blood samples (n=100) were collected from the people in district Bahawalnagar, Punjab who were exposed to pesticides, a total of 5ml blood was collected in both EDTA tubes and serum vials. The purpose was to study the hematological, biochemical, and genotoxic potential in rural inhabitants exposed to pesticides. Samples were collected across different age groups and compared with those from 100 unexposed individuals. Demographic characteristics of the pesticide-exposed workers in the district were also observed. Hematological and biochemical parameters, including WBCs, RBCs, hemoglobin, HCT, MCHC, MCH, lymphocytes, MCV, neutrophils, monocytes, eosinophils, potassium, sodium, calcium, and phosphorus, as well as AST, CPK, LDH, glucose, urea, creatinine, bilirubin, ALT, and ALP, were analyzed. Multiple comparisons were made with different diseases, revealing variations between pesticide-exposed and unexposed individuals. The comet assay method was utilized to observe damaged DNA in pesticide-exposed individuals and compare its prevalence across different age groups. The findings indicated an increase in the rate of DNA damage with age. Highly damaged cells exhibited a greater tail length of DNA compared to slightly damaged, damaged, and undamaged cells.

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Introduction

Pesticides serve specific purposes or target particular species, but they also produce harmful effects on non-targeted organisms, including animals and humans (Hernández *et al*., 2011). Pesticides are supposed to be less effective for human beings rather than targeted species but not as the case as considered because these are much toxic for human health even in low quantity (Tsatsakis *et al*., 2009; Zeliger, 2011). It also causes toxic effects by using free radical method that can also be detected with measurements of oxidative degeneration of lipids in body. During developmental period pesticides exposure cause major damage not only in development but also leave its effects in later life especially effecting the brain and hormonal system (London *et al*., 2012). Pesticides effects can be ensured by observing the chemical changes in life before its exposure in adverse conditions (Patil *et al*., 2003). Diseases in human beings as well as crops losses are mostly caused by harmful pests (Kazmi *et al*., 2023), which are being reduced by using pesticides, but its usage is on such large scale that pests are getting immunity against the pesticide day by day. To avoid this trouble, a large number of pesticides have been introduced into the market, which are classified as fungicide, insecticide, herbicides etc. (Speck-Planche *et al*., 2012). Uncontrollable way of using pesticides has caused a serious damage to our environment regarding human health and other existing all life on this planet (Agrawal *et al*., 2010), because it spreads toxicity in natural flora and fauna (Rashid *et al*., 2010). Low quantity of insecticides containing chlorinated hydrocarbons when added to animal feeds remains in their tissues. Chlorinated hydrocarbons (Lipophilic) are amassed in fat particles of the body because they are fat soluble (Mansouri *et al*., 2017; Reichelt-Brushett, 2023). Workers that work with pesticides have to face the chemical mixing, equipment loading and cleaning and getting rid of waste bottles. Workers regularly exposed to the pesticides have skin diseases rate higher than respiratory diseases (Jallow *et al*., 2017; Febriana *et al*., 2023). Second source of exposure is entering the field after treating it with pesticides because their particles remain in the field even after treatment for a short time (Widowati *et al*., 2022). The exposure is higher at the time of direct handling as compared to second entry (Damalas and Koutroubas).

Pesticides have produced large positive impact on man life by increasing the agricultural products and disease control but on the other hand, their overuse has also destroyed the man health greatly (Al-Saeed *et al*., 2023). They affect various organs and systems, such as the reproductive, renal, cardiovascular, immune, and respiratory systems, particularly in individuals with extensive pesticide exposure, leading to chronic diseases such as Parkinson's, aging-related conditions, Alzheimer's, and kidney diseases (Abdollahi *et al*., 2004; De Souza *et al*., 2011; Mostafalou and Abdollahi, 2012). Study for pesticides shows that steroid hormones like estrogens and androgens affects as reduced with several chemicals, environmental pollutants, drugs and pesticides like methoxychlor and DDT have anti-androgenic effect (Aziz *et al*., 2022).

Pesticides used on a large scale includes organochlorines (OC), organophosphates (OP), and carbamates (CB). The action of OB and CB inhibits the activity of the acetylcholinesterase enzyme (Kwong, 2002). The poisoning effect of CB is short-term compared to OP. Organochlorines, which are readily lipid-soluble, neurotoxic, chemically stable, and have endless half-lives in the environment, are known to cause continuous lethal effects by opening sodium gates in neurons, leading to decreased KB absorption and reduced Ca, K, Na-ATPase. Pesticides which cause 50% poisoning are organophosphates and also used as dangerous nerve gas (Soltaninejad *et al*., 2007; Karami-Mohajeri and Abdollahi, 2011; Jayaraj *et al*., 2016). The current study was therefore devised to detect hematological and biochemical alterations in rural inhabitants exposed to pesticides and to monitor genotoxic potential using single-cell gel electrophoresis in blood samples from rural pesticide-exposed workers.

Materials and Methods

Collection of samples

Survey was done for collection of blood samples of pesticides exposed workers from district Bahawalnagar, Punjab, Pakistan. The total 5ml blood was collected of which 2.5ml added in EDTA tubes for the study of DNA damage, hematology and biochemical analysis and 2.5ml in Eppendorf tubes for the study of serum. The total 100 samples were collected included 50 of pesticides exposed workers and 50 of shopkeepers working with pesticides. The workers of different age groups were selected ranging from 15 to 70 years. The samples were collected and handled with great care. Additionally, a survey was conducted on 100 pesticide-exposed and 100 unexposed individuals to gather information on various diseases, including diabetes, flu, fever, hepatitis, skin allergy, throat infection, uric acid, sneezing, nasal allergy, kidney problems, eye irritation, chest tightness, blood pressure, and cardiac problems.

Hematological analysis

For hematological analysis, blood samples were inspected to study different parameters, including white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), platelets (PLT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), lymphocytes (LYM), hematocrit (HCT), monocytes and eosinophils.

Serum analysis

Different parameters of freshly collected blood have been analyzed with available kits in the market with the use of spectrophotometer. The blood centrifugation was done at first for 10-15 minutes at 1600 rpm for the separation of serum and plasma and collected the serum in Eppendorf tubes. The parameters selected for analysis are bilirubin, alanine aminotransferase (ALT), Urea, creatinine, lactate dehydrogenase (LDH), aspartate aminotransferase (AST) Alkaline Phosphatase (ALP), creatinine phosphokinase (CPK), sodium (Na), phosphorus (P), potassium (K), triglycerides, calcium (Ca) and cholesterol. Alkaline Comet assay was performed with protocols described by Singh *et al.* (1988) with small changes.

Statistical analysis

The data collected in this study underwent statistical analysis using SPSS 15 software. Results are presented as means for hematological and serum analyses, comparing exposed and unexposed workers through multiple comparisons.

Results and Discussion

Clinical abnormalities

The survey conducted in this study revealed a higher prevalence of diseases among pesticide-exposed workers compared to those who were not exposed. Table 1 illustrates the percentages of clinical abnormalities observed in both groups, highlighting significantly higher values in pesticide-exposed individuals across various conditions such as throat infection, nasal allergy, skin allergy, eye irritation, and others. Previous research, as noted by Srivirojana *et al.* (2005), has indicated a substantial prevalence of pesticide exposure among agricultural workers in Pakistan, particularly in crops like cotton and sugarcane. This exposure has led to a significant number of health impacts on the affected workers, motivating the current investigation and the implementation of safety measures (Aroonvilairat *et al.*, 2015).

Table 1: *Percentage of clinical damage within exposed and unexposed rural dwellers of pesticides.*

Parameters	Exposed	Unexposed
Diabetes	09%	07%
Throat infection	46%	42%
Blood pressure	22%	20%
Cardiac problems	21%	17%
Cough	78%	70%
Kidney problems	11%	08%
Hepatitis	11%	09%
Skin allergy	38%	31%
Asthma	52%	48%
Fever	35%	30%

The study examined demographic and socioeconomic factors, including age, education level, marital status, work experience with pesticides, and training received. Additional factors such as monthly household income, social class, household size, and number of children were also considered. These parameters are detailed in the accompanying Table 2. The age range of pesticide users in this study varied from 18 to 59 years, with exposure durations ranging from 3 to 35 years, consistent with findings reported by Chuisseu *et al.* (2015). The study further confirmed that the observed clinical abnormalities in pesticide-exposed individuals were likely attributable to pesticide exposure compromising their immune systems (Henneberger *et al.*, 2014).

Blood parameters

Results of blood parameters expressed in Table 4 as results revealed significant hematological changes in pesticide-exposed workers compared to unexposed individuals. Specifically, hemoglobin level, WBCs, RBCs, HCT, MCV, MCH, and MCHC were markedly decreased in pesticide-exposed workers with clinical abnormalities, such as blood pressure, skin allergy, cardiac problems, and diabetes, as detailed in Table 3. Conversely, lymphocyte and monocyte counts were significantly higher in pesticide-exposed workers compared to their unexposed counterparts. This finding aligns with similar research where decreased levels of total leukocytes and monocytes were also reported (Aroonvilairat *et al*., 2015). Another study investigating the health impacts of pesticide exposure during peak agricultural periods documented a significant decrease in monocytes and platelets, coupled with an increase in total leukocytes (Saraiva, 2009). Interestingly, contrasting results were observed in another study, which noted an increase in total leukocytes, platelets, and monocytes among pesticide-exposed individuals (Wafa *et al*., 2013). These findings collectively suggest that pesticides may induce changes in leukocytic activity and potentially

Table 2: *Demographic appearances of pesticide exposed rural inhabitants in surroundings of Bahawalnagar district.*

Characteristics	No. (%)
Age	
≤ 25	26%
26- 36	24%
37-46	30%
≥ 47	20%
Highest level of education	
None	82%
1-7th grade	15%
8th- 10th	3%
Marital status	
Married	72%
Uumarried	28%
Number of years working in pesticides	
≥1	15%
2-10	75%
≤11	10%
Received information or training about pesticides	
Received	1%
Not received	99%
Monthly household income	
≤5000	55%
6000-15000	35%
≥16000	10%
Social classes	
Upper	1%
Middle	15%
Poor	84%
Number of households members	
≤3	30%
4-6	60%
≥6	10%
Number of children in household	
None	29%
≥1	71%

activate immune defense mechanisms (Cattelan et al., 2018). These observations emphasize the complex impact of pesticide exposure on blood parameters and suggest potential health consequences that require further investigation. Understanding these hematological alterations is crucial for developing effective strategies to mitigate the health risks associated with pesticide use in agricultural settings.

Biochemical parameters

In our study, pesticide-exposed workers showed high levels of phosphorus and potassium, but low levels of sodium and calcium. While Serum parameters such as glucose, urea, creatinine, bilirubin, ALT, and ALP in exposed individuals, indicative of greater prevalence of kidney problems, asthma, and skin allergies (Table 4). The group that was exposed to pesticides showed lower mean total cholesterol in relation to the group that does not use, a result consistent with a similar study involving exposed agricultural populations (García-García *et al*., 2016; Cattelan *et al*., 2018). However, other studies (Remor *et al*., 2009; Wafa *et al*., 2013; Adad *et al*., 2015) presented divergent results for this laboratory data. The effects of pesticides differ according to their toxicological classification (Karami-Mohajer and Abdollahi, 2011) , present different mechanisms of action and exert distinct toxicological effects. In the early stages of exposure, carbohydrates are used to provide energy and address stressful conditions, and later lipids and proteins serve as the main energy sources. This may explain the divergent results among various studies involving agricultural populations, given that research encompasses several classes and mixtures of pesticides and different occupational exposure conditions. All liver determinations were within reference values for both groups, but alkaline phosphatase and albumin concentrations were significantly lower in the pesticide-using group. Similar findings were reported in other studies (Aroonvilairat *et al*., 2015), suggesting that while other liver enzymes are within reference values, reduced alkaline phosphatase and albumin levels may indicate alterations in hepatic function without hepatocellular necrosis (García-García *et al*., 2016; Cattelan *et al*., 2018). These findings highlight the need for further research to understand the mechanisms behind these biochemical changes and to develop strategies to mitigate the health risks of pesticide exposure, considering the effects of different pesticide classes and occupational exposure conditions.

Table 3: *Mean values of hematological parameters in various diseases of pesticide exposed and unexposed individuals.*

Diseases		Hematological parameters										
		Hemoglobin	RBC	WBC	Hematocrit	MCV	MCH	MCHC	Lymphocytes	Neutrophils	Monocytes	Eosinophils
Asthma	Exposed	13.36	4.97	7.67	41.93	51.36	26.80	20.14	36.34	51.86	4.80	3.00
	Unexposed	15.21	5.91	6.00	42.24	78.60	24.10	29.80	30.00	52.80	5.06	3.90
Blood pressure	Exposed	13.40	4.79	8.00	41.76	86.96	27.90	20.35	35.78	52.60	5.20	3.00
	Unexposed	14.56	4.56	5.90	42.34	84.90	27.00	31.50	31.00	54.50	3.10	3.67
Cardiac problem	Exposed	13.92	5.18	6.95	43.63	52.50	16.68	20.24	40.42	51.48	3.80	3.60
	Unexposed	14.76	4.81	6.12	40.18	85.00	26.20	32.30	34.00	53.60	4.07	4.05
Cough	Exposed	14.06	5.14	8.98	43.97	86.04	27.50	20.16	35.78	56.12	4.20	3.40
	Unexposed	15.00	5.02	6.12	42.58	80.90	30.00	30.10	31.00	53.70	2.81	3.08
Diabetes	Exposed	13.22	5.04	7.87	41.87	51.40	16.10	19.96	39.42	53.62	4.20	2.60
	Unexposed	14.24	5.00	7.38	43.01	81.60	24.90	32.60	40.00	55.40	5.63	3.15
Fever	Exposed	14.02	5.31	8.64	44.27	83.52	26.36	19.86	34.30	58.60	4.00	3.60
	Unexposed	15.05	5.89	6.20	42.46	80.40	26.90	30.80	37.00	54.70	5.00	4.15
Hepatitis	Exposed	13.48	5.03	8.03	42.63	52.82	16.56	19.96	36.24	57.34	3.20	3.40
	Unexposed	14.98	4.76	7.05	41.56	82.80	28.50	32.60	30.00	57.90	3.90	4.56
Kidney problem	Exposed	13.40	4.79	8.00	41.76	86.93	27.93	20.30	37.22	52.60	5.24	3.00
	Unexposed	15.01	5.78	6.91	40.17	81.90	27.60	30.90	35.00	52.20	4.44	2.89
Skin allergy	Exposed	14.08	4.91	9.10	44.72	90.94	28.62	7.900	38.48	52.60	4.40	3.40
	Unexposed	14.75	5.26	7-21	43.71	84.30	25.90	34.40	33.00	56.10	2.38	4.21
Throat infection	Exposed	13.66	5.07	8.69	42.53	53.62	17.82	19.92	36.34	53.58	4.40	3.80
	Unexposed	14.79	5.34	5.71	42.47	81.80	27.20	32.40	30.00	55.60	3.40	4.00

Table 4: *Mean values of serum parameters in different diseases of pesticide exposed and unexposed individuals.*

Diseases		Biochemical parameters																	
		Glu	Urea	Creat.	Bili.	ALT	ALP	Chol.	Trig.	HDL-C	LDL-C	VLDL	AST	CPK	LDH	Na	K	Ca	P
Blood pressure	Exposed	144.2	30.60	0.92	0.68	23.60	101.8	147.8	137.2	39.20	117.6	27.60	34.20	106.2	402.0	134.6	4.06	8.68	3.68
	Unexposed	130.0	31.20	0.86	1.02	34.40	253.8	123.0	139.0	38.50	133.0	29.90	40.00	140.0	675.9	139.1	4.91	9.55	3.30
Diabetes	Exposed	128.0	33.40	0.68	0.78	27.80	115.4	168.0	191.8	38.20	136.8	26.60	23.40	125.8	413.6	135.2	3.96	9.38	3.76
	Unexposed	124.0	34.20	0.89	1.10	33.60	261.0	175.0	150.0	37.40	136.0	28.50	31.00	170.0	674.9	131.9	3.04	8.89	3.00
Cardiac problem	Exposed	122.8	33.60	0.64	0.70	22.80	105.6	184.2	166.6	38.40	119.8	27.60	28.20	117.2	355.2	135.6	3.76	9.38	3.38
	Unexposed	132.0	30.50	0.85	1.05	35.90	230.5	176.0	149.0	36.10	142.0	29.90	37.00	190.0	671.9	135.4	3.33	8.33	2.80
Throat infection	Exposed	136.2	32.00	0.86	0.76	29.60	97.00	160.0	165.4	36.20	131.2	32.60	32.80	155.2	418.4	136.1	4.10	8.98	3.84
	Unexposed	120.0	30.60	0.88	1.04	34.40	234.6	198.0	150.0	35.40	150.0	28.40	36.00	78.00	671.2	137.1	3.51	9.24	2.70
Kidney problem	Exposed	144.2	30.60	0.92	0.68	23.60	101.8	147.8	137.2	39.20	117.6	27.60	34.20	106.2	402.0	134.6	4.06	8.68	3.68
	Unexposed	127.0	33.70	0.86	1.04	32.00	244.7	180.0	141.0	35.70	149.0	30.80	39.00	155.0	666.3	136.2	4.10	8.76	3.20
Hepatitis	Exposed	128.2	36.80	0.72	0.66	28.20	123.4	202.6	172.0	37.40	122.8	31.60	30.80	105.6	393.5	137.0	4.04	9.56	3.72
	Unexposed	139.0	30.70	0.88	1.01	35.90	230.1	143.0	140.0	34.80	138.0	28.70	36.00	100.0	672.5	134.0	3.29	9.02	2.90
Asthma	Exposed	134.2	37.20	0.74	0.92	34.00	139.6	172.2	135.4	36.60	119.8	30.00	34.20	112.0	468.8	134.8	4.04	9.72	3.78
	Unexposed	124.0	29.30	0.87	1.02	32.80	254.4	187.0	147.0	36.70	148.0	33.90	32.00	110.0	676.9	135.5	4.00	8.41	3.80
Skin allergy	Exposed	141.4	32.60	0.88	0.66	34.60	117.4	181.2	182.0	43.60	120.4	31.40	33.60	104.0	443.2	134.0	3.70	9.38	3.94
	Unexposed	120.0	29.80	0.86	1.01	30.10	262.3	165.0	144.0	38.90	140.0	29.50	35.00	98.00	670.1	132.3	3.86	9.10	3.10
Fever	Exposed	134.4	31.60	0.80	0.78	34.40	106.0	183.6	191.2	37.40	134.8	26.00	30.60	122.4	444.2	136.4	3.84	8.76	3.34
	Unexposed	129.0	31.80	0.87	1.00	30.40	246.6	169.0	143.0	35.00	141.0	30.00	32.00	166.0	670.8	132.6	3.98	9.99	3.10
Cough	Exposed	142.4	28.00	0.92	0.76	34.60	97.20	163.8	175.8	41.00	137.2	24.80	29.80	129.2	432.4	133.4	3.74	8.40	3.44
	Unexposed	135.0	31.60	0.89	1.09	35.20	232.0	150.0	140.0	35.20	145.0	30.10	38.00	125.0	669.0	138.6	3.09	8.23	3.00

Genotoxicity

In the results and discussion section, the study reveals significant differences in DNA damage between pesticide-exposed and unexposed individuals across various age groups. The comet assay categorizes DNA damage into highly damaged, slightly damaged, and undamaged cells. Among pesticide-exposed workers, there is a notable increase in the proportion of damaged DNA cells with advancing age, contrasting with unexposed individuals who show a higher prevalence of undamaged DNA.

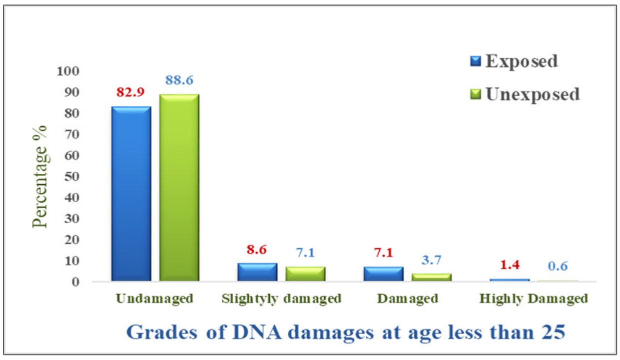


Figure 1: *Ratio of DNA damaged at age less than 25 in comparison to both exposed and unexposed inhabitants.*

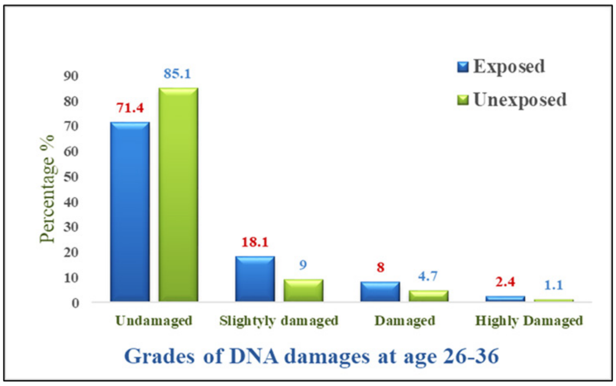


Figure 2: *Ratio of DNA damaged at age less than 26-36 in comparison to both exposed and unexposed inhabitants.*

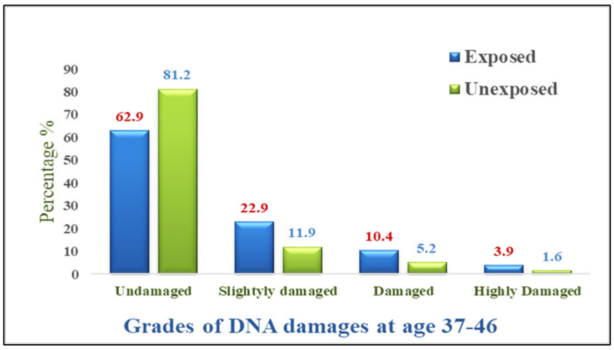


Figure 3: *Ratio of DNA damaged at age less than 37-46 in comparison to both exposed and unexposed inhabitants.*

Specifically, highly damaged cells, characterized by greater tail lengths in the comet assay, are predominantly observed in pesticide-exposed workers, whereas unexposed individuals exhibit predominantly undamaged cells. These findings are detailed in Table 5 and Figures 1, 2, 3, 4, 5, illustrating the varying extents of DNA damage observed. This study's comet assay results underscore significant DNA damage among pesticide-exposed workers, with damage severity correlating with increasing age. The prevalence of highly damaged cells with elongated tails further supports the notion that pesticide exposure contributes to genetic alterations. This aligns with previous research by Cook and Brazell (1975), reinforcing the heightened genetic risk faced by agricultural workers exposed to pesticides.

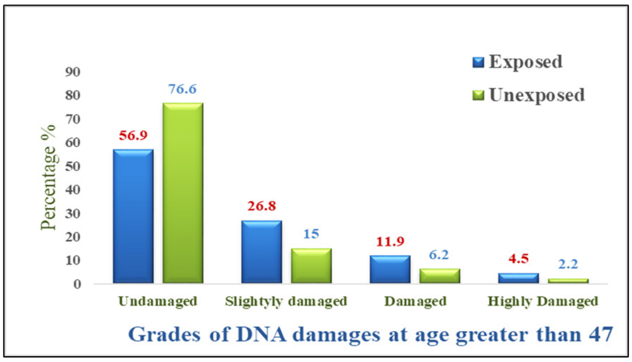
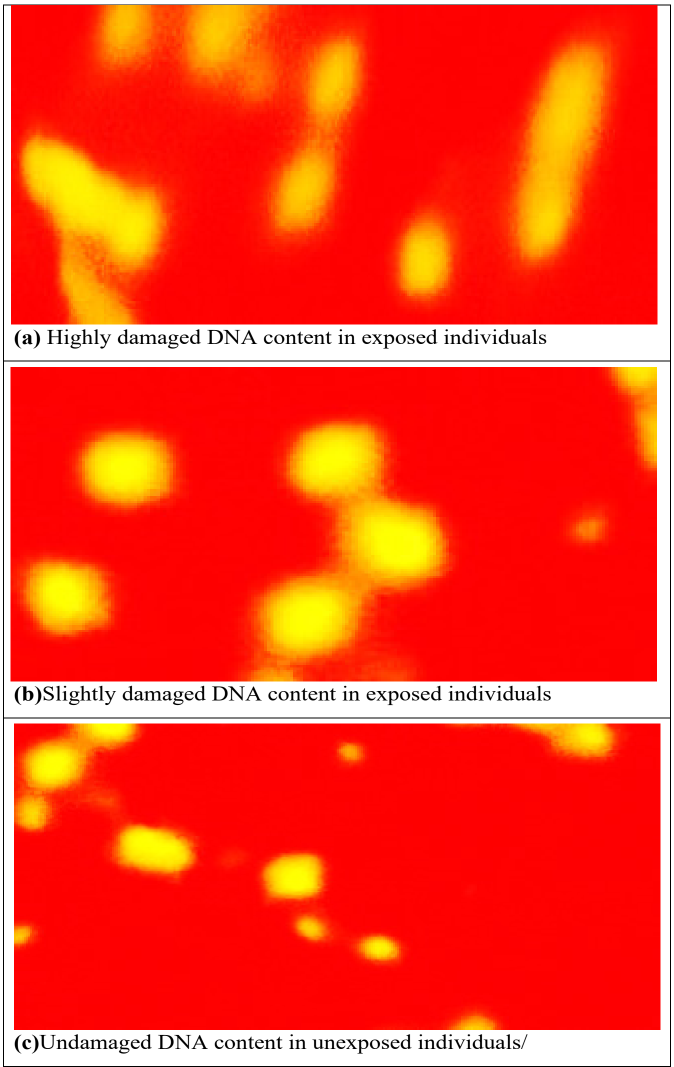


Figure 4: *Ratio of DNA damaged at age greater than 47 in comparison to both exposed and unexposed inhabitants.*

Table 5: *Pesticides exposed and unexposed individuals showing different levels of DNA damaged with reference to age groups.*

Age groups (years)	Grades of DNA damages							
	Undam-aged		Slightly damaged		Damaged		Highly damaged	
	No.	%	No.	%	No.	%	No.	%
Less than 20								
Exposed	2900	82.9	300	8.6	250	7.1	50	1.4
Unexposed	3100	88.6	250	7.1	130	3.7	20	0.6
20-29								
Exposed	2500	71.4	635	18.1	280	8	85	2.4
Unexposed	2980	85.1	315	09	165	4.7	40	1.1
30-39								
Exposed	2200	62.9	800	22.9	365	10.4	135	3.9
Unexposed	2844	81.2	417	11.9	182	5.2	57	1.6
40-49								
Exposed	1990	56.9	937	26.8	415	11.9	158	4.5
Unexposed	2680	76.6	526	15.0	217	6.2	77	2.2
Greater than 50								
Exposed	1540	44	1060	30.3	615	17.6	285	8.1
Unexposed	2330	66.6	772	22.0	308	8.8	90	2.6



(a) Highly damaged DNA content in exposed individuals

(b)Slightly damaged DNA content in exposed individuals

(c)Undamaged DNA content in unexposed individuals/

Figure 5: *Comet assay results depicting the damaging level of DNA content in exposed and unexposed individuals (a-c).*

Genetic biomonitoring serves as an essential tool in assessing the potential carcinogenic risks associated with pesticide exposure. While all populations face some risk from pesticide exposure, workers directly involved in pesticide production and agricultural application are particularly vulnerable to its genetic effects (Sailaja *et al.*, 2006). This underscores the importance of continuous genotoxic monitoring in these populations to mitigate long-term health impacts. The comet assay's utility in evaluating DNA damage in lymphocytes of farmworkers exposed to pesticide mixtures has been demonstrated in previous studies (Garaj-Vrhovac and Zeljezic, 2000; Muniz *et al.*, 2008). Consistently, our findings corroborate increased DNA damage in farmworkers, indicating exposure to genotoxic components of pesticides.

Conclusions and Recommendations

It is concluded that pesticides have potential for causing clinical and hematological abnormalities plus serum biochemical alterations and genotoxic effects in pesticides exposed workers. There is a requirement to form training institutes that teach the farmers about safety measures while using pesticides. Non-targeted organisms such as local inhabitants are found to get affected by the application of pesticides. Engineering measures should be taken to get rid of these chemicals and aware the occupational workers about the hazardous impact of these pesticides. There is also needed to start a treatment program to treat the pesticide exposed workers who indicate clinical abnormalities in their bodies.

Acknowledgements

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Novelty Statement

This research presents a pioneering comprehensive investigation into the effects of pesticides in a structured and systematic manner, providing valuable insights to the rural inhabitants.

Author's Contribution

Abdul Ghaffar: Supervision, conceptualization, formal analysis.
Ayesha Maqsood: Executed sampling and data collection and laboratory work.
Riaz Hussain: Formal analysis, data curation.
Ghulam Abbasand Rabia Tahir: Writing review and editing.
Habiba Jamil: Data curation.
Fozia Afzaland Ahrar Khan: Edited and formatted the manuscript.
Muhammad Ahmad Chishti Shahnaz, Aliya Noreen: Helped data curation and proofreading of the MS.
Kashfa Akram and Maria Niaz: Helped in sampling, data collection, and laboratory work.

Conflict of interest
The authors have declared no conflict of interest.

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