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Beyond the Fields: Unlocking the Predictive Power of Neutrophil-to-Lymphocyte Ratio and Hepatic Markers in Chronic Pesticide Toxicity

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Abstract

Objectives: To investigate predictive role of N/L ratio coupled with hepatic markers in agricultural workers that are chronically exposed to pesticides.
Methodology: This case control study was carried out at Al Tibri Medical College, Karachi. Data was collected using purposive sampling from agricultural workers between ages 18-75 years working for minimum of 2 years in agricultural farms of Gadap town, Sindh, Pakistan involved in spraying pesticides were included. Duration of study was six months from July 2024 to December 2024. Liver function tests and neutrophil to lymphocyte ratio were analyzed and compared between cases and controls through SPSS by applying independent t-test between them keeping $p<0.05$ as statistically significant.
Results: From total of 386 workers included, 193 were included in exposed and non-exposed group. The mean N/L ratio in the exposed group was significantly higher (3.96 ± 1.41) compared to the non-exposed group (1.78 ± 0.86, $p<0.001$). In addition, exposed participants had significantly elevated AST and ALP levels compared to non-exposed participants ($p<0.001$ for both), while ALT and GGT did not differ significantly. Hematological indices including hemoglobin, RBC count, hematocrit, MCH, MCHC, and platelet counts were significantly lower in the exposed group.
Conclusion: Chronic pesticide exposure is associated with increased inflammatory and hepatic stress, as evidenced by raised N/L ratio and hepatic markers dysfunction.
Keywords: Liver Function Test, Organophosphorus, Pesticides, Neutrophil-to-Lymphocyte Ratio

Introduction

For agricultural workers, exposure to pesticides is a substantial occupational hazard. Persistent systemic inflammation along and liver dysfunction are caused for chronic exposure to pesticides.[1] Neutrophil to lymphocyte (N/L) ratio which can be easily derived from routine blood count is being recognized increasingly as an accessible, simple and easy to calculate inflammatory marker. It has been associated to multiple chronic disorders and might tend to offer predictive value in both occupational and environmental exposure that includes toxicity to pesticides.[2]

Pesticides which include insecticides, herbicides, rodenticides and fungicides are mostly utilized for controlling pests and pest-induced disorders, especially in public health and agriculture.[3] Occupational pesticide exposure in the field of agriculture takes place when preparing (loading and mixing) and then during application of pesticide whilst spraying.[4] The exposure of pesticides among the workers and handlers becomes much higher than the exposure to general population.[5]

In the context of constant and higher than normal exposure to pesticide, it presents a significant potential health hazard to not only human but also environmental health.[6] Since chronic exposure to occupational hazards such as pesticides constantly at low doses is a challenge to diagnosis due to transient, non-specific symptoms which are often overlooked.[7] Depending upon the toxicological and physicochemical profiles of used pesticide, the clinical signs and symptoms vary in occurrence and severity.[8] Parameters important in such cases include the profile of exposed pesticide, its duration and mode of application, frequency of application and the use or disuse of personal protective equipment (PPE).[9]

Authorship Contribution: [1,3] Conceived and planned the idea of the study, data drafting the work or revising it critically for important intellectual content, final approval of the version to be published, [3-6]data entry, literature review, [2,4]Active participation in active methodology

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Some epidemiological researchers have investigated long term exposure of pesticides and their various adverse health effects such as respiratory, neurological, endocrinal, cancerous and other ill effects, however even after extensive research, clear associations and linkages are not yet established.[10]

The liver, being a primary site for xenobiotic metabolism, is highly susceptible to pesticide-induced injury. Enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) reflect hepatic function and cellular injury.[11, 12]

Worldwide, the most commonly used pesticide includes organophosphorus (OP) are used for their broad spectrum, increased toxicity towards pests and highly effective mechanism of action.[13, 14] Despite widespread pesticide use in Pakistan, few studies have examined the combined predictive role of the N/L ratio and hepatic markers in agricultural workers. The study aims to investigate predictive role of N/L ratio coupled with hepatic markers in agricultural workers that are chronically exposed to pesticides.

Methodology

This was a case control study carried out at Al Tibri Medical College, Karachi from July 2024 to December 2024. Post ethical approval from Ethics Review Committee of Al Tibri Medical College Karachi, data collection was started. The data was collected using purposive sampling from agricultural workers between ages 18-75 years working for minimum of 2 years in agricultural farms of Gadap town, Sindh, Pakistan involved in spraying pesticides were included. Through open epi online software for calculation of sample size, keeping 95 % confidence level, the sample size came out to be 196.[15] Sample size was calculated using OpenEpi software. Assuming a finite population of 1,000,000, an expected outcome frequency of 15%, a precision of 5%, and a 95% confidence level, with a design effect of 1, the minimum required sample size was estimated to be 196 participants.

After ethical approval and informed consent from workers, data collection started. Workers were selected using purposive sampling technique. Included workers belonged to Gadap town malir, Sindh, Pakistan. Agriculture workers exposed to pesticides chronically was included in the exposed group. Age and demographic related individuals residing in the same district were added to non-exposed group. From 392 participants six refused to participate in the research so the sample size reduced to 386.

386 participants with 193 in each group were included. For laboratory testing, 10 ml venous blood was collected using aseptic measures. Yellow top (containing gel) and purple top (containing EDTA) vacutainers were labeled and collected from each participant and anonymously recorded for confidentiality. Each specimen was shifted to laboratory on dry ice. Centrifuge was done to separate serum at 2000 rpm for 10 minutes then stored appropriately at <20 C within an hour after collection. Samples of whole blood were stored at 4 C and their analysis was done within 2 hours of collection. For LFTs, Cobas c111 chemical analyzer, Germany was utilized.

SPSS version 23.0 was used for analysis of data. Demographical and laboratory data was reported as frequency and percentage for categorical variables while for continuous variables, mean and standard deviation were recorded. For comparison of various blood cells indices and liver function parameters, independent t-test was applied between exposed and non-exposed group while chi-square was applied for categorical variables while keeping p-value of <0.05 as significant statistically.

Results

Table I shows baseline demographics of participants (n=386), comparing the exposed (n=193) and non-exposed (n=193) groups. Mean age of exposed was 38.73 ± 10.75 years, while non-exposed was 38.58 ± 9.45 years. The mean weight was 65 ± 14.45 kg in the exposed group and 64.78 ± 13.76 kg in non-exposed. Mean height was 171 ± 5.67 cm in exposed and 170 ± 8.98 cm in non-exposed. Mean BMI of exposed was 21.56 ± 5.67 kg/m², compared to 20.96 ± 4.59 kg/m² in non-exposed. Smoking was reported by 22 (11.39%) of the exposed participants and 15 (7.77%) of the non-exposed participants. The mean duration of pesticide exposure in the exposed group was 11.23 ± 8.68 years.

Table II compares hematological and liver function parameters between exposed and non-exposed. Mean hemoglobin level was significantly lower in exposed (12.21 ± 1.89 g/dL) compared to non-exposed (14.92 ± 1.34 g/dL, p<0.001). Similarly, red blood cell count was significantly lower in exposed (4.23 ± 0.77 mm³) compared to non-exposed (4.63 ± 1.66 mm³, p<0.001). The hematocrit level was also lower in exposed (40.13 ± 0.45%) than in non-exposed (44.34 ± 2.57%, p=0.01). Mean corpuscular hemoglobin (MCH) was 26.44 ± 1.84 pg in exposed and 29.81 ± 3.89 pg in non-exposed (p=0.01), while MCHC was significantly reduced in exposed (28.35 ± 1.97%) compared to non-exposed (34.42 ± 1.73%, p<0.001). White blood cell (WBC) count was markedly elevated in exposed (10.34 ± 3.22 mm³) compared to non-exposed (6.12 ± 1.72 mm³, p<0.001).

Table I: Baseline demographics of participants included in the study. (n=386)

Demographics	Exposure Group (n=193)	Non-exposure Group (n=193)
Mean age (years)	38.73 ± 10.75	38.58 ± 9.45
Mean Weight (kg)	65 ± 14.45	64.78 ± 13.76
Mean Height (cm)	171 ± 5.67	170 ± 8.98
Mean BMI (kg/m^2)	21.56 ± 5.67	20.96 ± 4.59
Smoking status (%)	22 (11.39 %)	15 (7.77 %)
Mean Duration of exposure to pesticide (years)	11.23 ± 8.68	-

The mean N/L ratio in the exposed group was significantly higher (3.96 ± 1.41) compared to the non-exposed group (1.78 ± 0.86), with a statistically significant difference (p < 0.001). Platelet counts were significantly lower in exposed (150.84 ± 117.15 mm³) than in non-exposed (251.64 ± 51.92 mm³, p=0.01).

Regarding direct bilirubin (0.19 ± 0.10 mg/dL vs. 0.21 ± 0.12 mg/dL, p=0.61) or total bilirubin (0.41 ± 0.34 mg/dL vs. 0.40 ± 0.26 mg/dL, p=0.07), insignificant differences were reported between the groups. However, ALP levels were significantly elevated in exposed (155 ± 9.84 U/L) compared to non-exposed (56.72 ± 17.91 U/L, p<0.001). GGT levels did not show a significant difference (18.91 ± 26.47 U/L vs. 16.62 ± 18.53 U/L, p=0.65). ALT levels were similar in both groups (18.72 ± 9.49 U/L vs. 18.7 ± 10.81 U/L, p=0.22). AST levels were higher in exposed (68.34 ± 8.82 U/L) compared to non-exposed (27.49 ± 9.88 U/L, p<0.001).

Discussion

This research supports the findings of impaired Neutrophil-to-lymphocyte (N/L) ratio and impaired liver function tests among individuals exposed to organophosphorus pesticides. Majority of workers in our study were males and although similar frequencies were

Table II: Comparison between hematological and liver function test between exposed and non-exposed group. (n=386)

Variables	Exposed (n=193)	Non-Exposed (n=193)	P-value
Hemoglobin (g/dl)	12.21 ± 1.89	14.92 ± 1.34	<0.001*
Red Blood Cell Count (mm^3)	4.23 ± 0.77	4.63 ± 1.66	<0.001*
Hematocrit (%)	40.13 ± 0.45	44.34 ± 2.57	0.01*
MCH (pg)	26.44 ± 1.84	29.81 ± 3.89	0.01*
MCHC (%)	28.35 ± 1.97	34.42 ± 1.73	<0.001*
White Blood Cell Count (mm^3)	10.34 ± 3.22	6.12 ± 1.72	<0.001*
Neutrophil-to-Lymphocyte Ratio	3.96 ± 1.41	1.78 ± 0.86	<0.001*
Platelets (mm^3)	150.84 ± 117.15	251.64 ± 51.92	0.01*
Direct Bilirubin mg/dl	0.19 ± 0.10	0.21 ± 0.12	0.61
Total Bilirubin mg/dl	0.41 ± 0.34	0.40 ± 0.26	0.07
ALP U/L	155 ± 9.84	56.72 ± 17.91	<0.001*
GGT U/L	18.91 ± 26.47	16.62 ± 18.53	0.65
ALT U/L	18.72 ± 9.49	18.7 ± 10.81	0.22
AST U/L	68.34 ± 8.82	27.49 ± 9.88	<0.001*

observed in another study from Nigeria wherein 94 % of agricultural workers exposed to pesticides showed impaired liver functions.[16] This was probably due to the cultural norm in Pakistan and other under developed countries where the agricultural fields are worked upon only by males whilst females are busy in household works.[17] Furthermore, the technique by which pesticide is sprayed is via knapsack sprayer which is carried on the back-containing mixture of pesticide of around 10 liters and is manually operated, therefore requiring substantial physical power.[18] The non-exposed group included only male participants to make demographical-comparison near-to-equal. N/L ratio and liver dysfunction have been used for assessing risk of pesticide induced toxicity among agricultural and other occupational workers until recently.[19, 20] Samareh A et al., in their study on the farmworker's exposure to pesticides and its effect on oxidative markers observed that in 74 farmworkers, levels of OP were significantly higher than controls (non-farmworkers). In addition, the study also reported that activity of acetylcholine esterase and total antioxidant capacity in farmworkers were substantially reduced than in non-farmworkers (controls).[19] Similar findings were reported in our research as well. In another study by Forté CA et al., assessed the exposure of pesticides on agricultural workers and reported that farmworkers showed substantially lowered red blood cell and white cell count, monocytes, serum calcium and acetylcholinesterase activity as well. The changes in their blood profile were significantly and potentially linked with occupational exposure to pesticides, similarly to the findings of the current study.[20]

A cross-sectional study compared 99 insecticide-exposed factory workers with 107 non-exposed workers to assess hematological changes. The exposed group showed significantly higher rates of thrombocytosis, elevated white blood cell and platelet counts, increased red blood cell anisocytosis, and higher neutrophil-to-lymphocyte ratios. They also reported more headaches, itchy skin, cough, and sleep disorders. The findings suggest that chronic occupational insecticide exposure can alter hematological parameters within the normal range, and that complete blood count (CBC) is a practical tool for monitoring the health of exposed workers.[21] Similar reports were observed in another research as well.[22]

Together, both N/L ratio and hepatic parameters provide a cost-effective, easily obtainable, and clinically relevant biomarker set for early detection and risk stratification in populations at risk for pesticide toxicity. Incorporating routine monitoring of N/L ratio alongside hepatic function tests into occupational health programs could facilitate timely intervention, potentially reducing the long-term burden of pesticide-related morbidity.

Conclusion

Chronic pesticide exposure in agricultural workers is associated with significant systemic inflammatory and hepatic changes. The elevated neutrophil-to-lymphocyte (N/L) ratio observed in exposed individuals reflects an ongoing inflammatory response, while alterations in hepatic markers particularly elevated AST and ALP—indicate early hepatocellular stress and possible toxic injury.

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