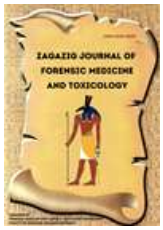

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Original article

Leucocytic Count and Serum Acetylcholinesterase Level as Predictors of Acute Organophosphate Poisoning: A descriptive cross-sectional study

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Abstract

Background: The extensive utilization of organophosphate compounds (OPCs) poses a significant health concern, necessitating the identification of an appropriate biomarker. **Aim of the study:** To improve patient outcomes in cases of organophosphate poisoning by utilizing leucocytic count as a prognostic marker. **Patients and Methods:** A descriptive cross-sectional study was conducted involving 88 cases of acute organophosphate poisoning (AOPP) admitted to the Poison Control Center Ain Shams University Hospitals (PCC-ASUHs) from August 2021 to February 2022. The diagnosis was based on the patient's history, clinical manifestations of AOPP, low acetylcholine esterase enzyme (AChE) levels, and total leucocyte count. Patients were categorized into mild, moderate, and severe groups according to the Peradeniya Organophosphorus poisoning (POP) score. The prognostic value of leucocytic count measured at admission was evaluated to predict the severity and outcomes of AOPP. **Results:** There was a significant association between leucocytic count and severity of poisoning. Leucocytic count values demonstrated a sensitivity of 60% and specificity of 50% for counts exceeding 11,000, 40% sensitivity and 56% specificity for counts above 12,000, and 15% sensitivity with 92% specificity for counts greater than 20,000 in predicting mortality in AOPP. However, no significant difference was observed in the mean leucocytic count between survivors and non-survivors. **Conclusion:** The leucocytic count serves as a readily obtainable marker parameter. The ability to differentiate between patients with and without severe poisoning suggests its potential effectiveness, in conjunction with clinical signs, for evaluating AOPP in facilities that do not have AChE measurement.

I. Background

Pesticides are used extensively utilized in agriculture due to their role in eliminating pests that damage crops and improving productivity. These chemicals pose significant and long-term risks to human health and the environment (Rani et al., 2021).According to the World Health Organization (WHO), there are 3 million cases of pesticide poisoning annually, leading to more than 250,000 deaths. Most of them are from developing countries (Boedeker et al., 2020). The major classes of pesticides are insecticides, herbicides, fungicides, rodenticides, and fumigants. The most commonly used pesticides are OPCs (Akashe et al., 2018). Organophosphorus compounds cause irreversible inhibition of acetylcholinesterase enzyme (AChE), leading to the accumulation of acetylcholine (Ach) and subsequent

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nicotinic and muscarinic manifestations (Slavica et al., 2018). Diagnosis depends on the history of ingestion or inhalation of organophosphorus compounds, the characteristic clinical presentation of acute organophosphate poisoning, and decreased AChE levels in the blood (Chaudhary et al., 2019). Rapid diagnosis and early intervention are critical for improving patient outcomes and reducing morbidity and mortality associated with AOPP (Karakus et al., 2014).

The POP scale is an effective and straightforward scoring system. It includes six clinical parameters summarized in table (1) including pupil size, respiratory rate, heart rate, fasciculation, level of consciousness, and seizure. Each parameter is evaluated on a three-point scale ranging from 0 to 2. It is acquired at the initial presentation prior to any medical intervention. The classification of poisoning severity was categorized as mild (score 0-3), moderate (score 4-7) or severe (score 8-11) (Raveendra et al., 2020). Therefore, identifying a novel prognostic marker for the management of AOPP is essential. The complete blood cell count (CBC) is a frequently utilized laboratory test, including red blood cells, white blood cells (WBCs), and platelets (PLTs).

Few studies have examined the clinical relevance of the CBC test in diagnosing and prognosticating AOPP; nonetheless, AOPP may adversely affect hematological parameters (Tang et al., 2018). Several previous studies indicated that the leukocyte count of non-survivors upon admission was significantly elevated (Abdel Baseer et al., 2021). AOPP leads to a significant inflammatory response due to oxidative stress, as OPCs cause an overproduction of free radicals and hinder the body's antioxidant mechanisms. This results in lipid peroxidation of cellular membranes, leading to cellular damage and increased chemokine production, which in turn causes leukocytosis and neutrophilia (Elhosary & Abdelbar, 2018). This increase in leukocyte count correlates with the severity of the inflammatory response. Upon exposure to OPCs, an acute immune response characterized by a series of reactions may occur. In the acute phase, the initial response involves releasing white blood cells from reservoirs such as the spleen and lungs, followed by their migration to injured tissues, which are crucial for the immune response (Corsini et al., 2013).The study aimed to improve the outcome of organophosphate poisoning patients using leucocytic count as a prognostic marker in organophosphate poisoning cases.

II. Patients and methods

II.1 Study design:

A descriptive cross-sectional, hospital-based study was carried out on 88 AOPP patients admitted to Poison Control Center Ain Shams University Hospitals from August 2021 to February 2022. The diagnosis was based on the history of exposure and clinical manifestations associated with OPC poisoning.

Exclusion criteria comprised individuals under 18 years of age, those receiving pre-hospital treatment, cases of co-ingestion of other substances, discharge against medical advice, transfer to another facility, and the presence of comorbidities such as severe heart disease, heart failure, kidney disease, diabetes, or trauma.

Patients are suspected to have a high leukocyte count due to current infection, inflammation, or tumor presence. Patients were classified into mild, moderate, and severe groups based on the POP score.

The prognostic value of leucocytic count measured upon admission to the emergency department was assessed for its ability to predict severity and outcomes in patients with organophosphate poisoning.

II.2 Laboratory analysis:

A 5 ml blood sample was collected under strict aseptic conditions upon admission, following the initial resuscitation and stabilization of the patient, for biochemical analysis of arterial blood gases (pH, PaCO2, and HCO3), complete blood count (including WBCs), and serum AChE level.

Management and hospital disposition were conducted according to the patient's condition and established protocols at PCC-ASUHs. The results included complete recovery, recovery with complications (such as mechanical ventilation-acquired pneumonia, shock, and intermediate syndrome), or death.

II.3 Statistical analysis:

The data were revised, coded, and tabulated for statistical analysis using the statistical program Statistical Package for Social Sciences (SPSS 20.0). Data were presented, and an appropriate analysis was conducted based on the type of data collected for each parameter. Continuous data were presented as mean ± SD, while categorical variables were expressed as percentages.

Student t-tests were used to assess the statistical significance of the difference between the two study groups for quantitative data (Comparison between survivors and non-survivors of OPP patients). However, the One-Way ANOVA test was used to assess the statistical significance of the difference between more than two quantitative variables; the Post hoc test was used to determine the significant pair(s) after ANOVA was found significant.

Receiver Operating Characteristic (ROC) curve analysis was done to evaluate the validity of white blood cell (WBC) count in predicting mortality in organophosphorus poisoning, determining sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) at different WBC cutoff points, with the area under the curve (AUC) reflecting the overall predictive accuracy.

II.4 Ethical considerations:

Approval for performing the study was obtained from the ethical committee of scientific research of faculty of Medicine Suez Canal University (Reference number: #4650). The administrative approval of Ain shams University was obtained. An informed consent was taken from patients or their relatives. Confidentiality of information was preserved, and the collected data was used only for the purpose of the study.

III. Results

The mean age of the patients was (27.06 years ±12.845) with a range from 18 to 64 years. A majority of patients (62.5%) fell within the age range of 18 to 30 years. Approximately 85.2% of the patients resided in urban areas. In terms of occupation, the majority of patients (68.2%) were students. The predominant method of poisoning was an intentional pattern, accounting for 86.4% of cases. The delay between exposure and the pursuit of medical advice varied from 1 to 24 hours, with a mean ± SD of 5.19 ± 5.997 hours. The baseline characteristics of the patients, including POP score, AChE, WBCs, and outcomes, are presented in Table (2).

Regarding AChE, there was a statistically significant relationship between AChE level and mortality, as shown in Table (3). In addition, a statistically significant difference in mean AChE levels was observed among patient groups with varying grades of poisoning based on POP scores, indicating a strong inverse relationship between AChE levels and the severity of poisoning, as presented in Table (4).

The increased leucocytic count demonstrated a significant relation with the severity of poisoning, evidenced by a statistically significant difference among patient groups categorized by varying degrees of organophosphorus poisoning, as illustrated in Table (4). No statistically significant difference exists in the mean leucocytic count between survivors and non-survivors, as indicated in Table (3). Post hoc analysis revealed significant differences in WBC counts between the severe and moderate groups ($p = 0.016$), as well as between the severe and mild groups ($p < 0.001$), Similarly, AChE levels were significantly different between the severity groups. Post hoc comparisons showed that the severe group had the lowest AChE levels (624.28 ± 115.83), which were significantly lower than those observed in the moderate (1548.02 ± 298.99) and mild groups (2447.78 ± 249.58), with p-values < 0.001 for both comparisons (severe vs. moderate and severe vs. mild), as presented in Table (4). Of the patients, 73.8% exhibited leukocytosis upon admission. It demonstrated a sensitivity of 60%, specificity of 50%, and a negative predictive value of 55.5% for leukocyte counts exceeding 11,000, sensitivity of 40%, specificity of 56%, and a negative predictive value of 48.2% for counts above 12,000; and a sensitivity of 15%, specificity of 92%, and a negative predictive value of 51.9% for counts surpassing 20,000 regarding mortality prediction in cases of OPI poisoning, as shown in Table (5).

Table (1): Peradeniya Organophosphorus Poisoning (POP) scale (Manar et al., 2017)

Parameters	0	1	2
Pupil size	≥2 mm	<2 mm	Pinpoint
Respiratory rate	<20/min	≥20/min	≥20/min with central Cyanosis
Heart rate	>60/min	41-60/min	<40/min
Fasciculation	None	Present. generalized/ Continuous	Both generalized and continuous
Level of Consciousness	Conscious and Rationale	Impaired response to verbal commands	No response to verbal commands
Seizure	Absent	Present	-
Grade	Mild (0-3)	Moderate (4-7)	Severe (8-11)

Table (2): Baseline characteristics of the patients (n=88)

Variables		Total
Age (years)	mean± SD	27.06±12.845
	Range	(18-64)
Age groups (n, %)	(18-30)	55(62.5)
	(30-45)	24(27.3)
	(45-65)	9(10.2)
Sex (n, %)	Male	23 (26.13)
	Female	65(73.86)
Residence (n, %)	Urban	75(85.2)
	Rural	13(14.8)
Occupation (n, %)	Student	60(68.2)
	Unemployed	20(22.7)
	Farmer	8(9.1)
Manner of poisoning (n, %)	Intentional(suicidal)	76(86.4)
	Un-intentional	12(13.6)
POP score	mean± SD	5.60±1.816
	Range	(3-9)
POP score (n, %)	Mild (0-3)	9(10.2)
	Moderate (4-7)	61(69.3)
	Severe (8-11)	18(20.5)
AChE	Mean± SD	1451.09±566.39
	Range	(200-3000)
WBCs	Mean± SD	11.100±4.8626
	Range	(5.5-28.2)
Duration of delay(hours)	Mean± SD	5.19±5.997
	Range	(1-24)
Outcome (n, %)	Spontaneous recovery	60(68.2)
	Mechanical ventilation acquired pneumonia	12(13.6)
	Shock	9(10.2)
	Intermediate syndrome	7(8.0)
Mortality (n, %)	survivors	68(77.3)
	non-survivors	20(22.7)

n: total number, %: percentage, AChE: acetylcholine esterase enzyme, POP: Peradeniya Organophosphorus poisoning, WBCs: white blood cells, SD: standard deviation

Table (3): Comparison between survivors and non-survivors regarding AChE level and WBCs.

Variables	Non-survivors (n=20)	Survivors (n=68)	Test-value	p-value
AChE (mean± SD)	1058.75±521.43	1566.49±529.14	t=-3.784	**<0.001***
WBCs (mean± SD)	13.805±5.1073	12.184±4.7645	t=1.31	**0.192**

n: total number, AChE: acetylcholine esterase enzyme, WBCs: white blood cells, SD: standard deviation, Statistical analysis by independent sample t-test, *Significant (p-value ≤ 0.05)

Table (4): Comparison between groups of patients with different grades of organophosphorus poisoning regarding WBCs and AChE level.

Variables	Mild (n=9)	Moderate (n=61)	Severe (n=18)	Test value	p-value
WBCs (mean± SD)	10.60±1.59	12.01±4.90	15.33± 4.84	4.362	≤ 0.05* <0.001**
ACHE (mean± SD)	2447.78 ±249.58	1548.02± 298.99	624.28± 115.83	152.26	<0.001# <0.001**

n: total number, AChE: acetylcholine esterase enzyme, WBCs: white blood cells, SD: standard deviation
Statistical analysis by One way ANOVA test followed by post-hoc test,
*Significant at p-value ≤ 0.05(comparison between sever and moderate) **highly Significant at p-value ≤ 0.001(comparison between sever and mild) # highly Significant at p-value ≤ 0.001(comparison between sever and moderate)

Table (5): Validity of WBCs to predict the mortality in organophosphorus poisoning.

Indicators	AUC	Cutoff point	Sensitivity	Specificity	PPV	NPV
WBCs	0.617	>11,000	60%	50%	54.5%	55.5%
		>12,000	40%	56%	47.6%	48.2%
		>20,000	15%	92%	65.2%	51.9%

WBCs: white blood cells, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve for the Receiver Operating Characteristic (ROC) curve analysis

IV. Discussion

Acute poisoning is a significant contributor to morbidity and mortality globally. It is estimated that annually, 200,000 deaths occur due to poisoning, with the incidence in developing countries having doubled over ten years starting in 1990, according to the WHO (Muley et al., 2014). Conversely, poisoning by OP compounds is highly preventable and completely treatable if well-managed (Raveendra et al., 2020). In clinical practice, levels serve as a well-known marker for organophosphorus poisoning in humans; however, this marker is often unavailable in resource-limited areas. Recognizing potent prognostic biomarkers in the clinical management of AOPP patients is essential for predicting outcomes. The leucocytic count is a commonly utilized parameter that is readily accessible and efficient to measure. A few studies have examined the clinical significance of this in diagnosing human poisoning (Kumar et al., 2018).

This descriptive, cross-sectional, hospital-based study was conducted on 88 patients admitted to Poison Control Center Ain Shams University Hospitals with AOP. Participants were selected according to the predetermined inclusion and exclusion criteria during the period from August 2021 to February 2022. This work aimed to improve the outcome of OP poisoning patients using leucocytic count as a prognostic marker in organophosphate poisoning cases. Additionally, leucocytic counts and AChE levels were assessed and compared as early predictors of OPC poisoning.

The diagnosis was based on a history of OPC exposure and an initial assessment of clinical manifestations using the POP score. The patients were categorized into groups based on the severity of poisoning: mild, moderate, and severe. Laboratory parameters, including AChE levels and

a complete blood count with WBCs, were obtained, and patient outcomes were recorded.

Leukocytosis is a common lab finding in organophosphate poisoning patients. Numerous studies have demonstrated that an increase in leukocyte count correlates with the severity of poisoning. Furthermore, prompt diagnosis and early intervention are essential for reducing morbidity and mortality associated with AOPP. Therefore, identifying a simple, rapid, and accessible prognostic marker is critical for improving outcomes (Sapkota et al., 2018). The increase in WBCs is correlated with the extent of the inflammatory reaction. Exposure to OPCs can trigger an acute immune response characterized by a series of reactions resulting from oxidative stress (Corsini et al., 2013).

The current study demonstrates a significant association between elevated leucocytic count and the severity of poisoning. A statistically significant difference exists among patient groups with varying grades of poisoning, with the highest mean observed in the severe poisoning group (15.33±4.84). This finding aligns with (Kumar et al. 2018), who indicated that leukocytic count upon admission serves as a prognostic marker for the severity of AOPP, effectively distinguishing between patients with and without severe poisoning. Additionally, AChE exhibited a significant inverse correlation with poisoning severity, as evidenced by a statistically significant difference in the mean AChE levels among patient groups categorized by varying degrees of poisoning, with the highest mean observed in the group with mild poisoning (2447.78±249.58). This aligns with research by (Manar et al. 2017), which indicated a negative correlation between AChE levels and poisoning severity. The study found that AChE activity significantly decreased as the severity, measured by the POP score at admission, increased. A study by (Kumar et al. 2017) found no correlation between the severity of poisoning and AChE levels.

Regarding mortality, levels measured upon admission averaged (1451.09±566.39). A statistically significant difference was observed in the mean AChE levels between survivors and non-survivors, indicating a strong reverse correlation between AChE levels and mortality. These findings align with those of (Chaudhary et al. 2013), who reported a significant correlation between mortality rates and AChE levels. A study by (Oreby and El-Madah 2017) indicated a significant decrease in serum cholinesterase levels upon admission in both surviving and non-surviving patients, suggesting it is not a reliable predictor of mortality. In the present study, 73.8% of patients exhibited leukocytosis upon admission. It demonstrated a sensitivity of 60%, a specificity of 50%, and a negative predictive value of 55.5% for leukocyte counts exceeding 11,000. For counts greater than 12,000, the sensitivity was 40%, specificity 56%, and negative predictive value 48.2%. When counts surpassed 20,000, sensitivity dropped to 15%, specificity increased to 92%, and the negative predictive value was 51.9% concerning mortality prediction in patients with OPP. The findings align with a study by (Kumar et al. 2018), which indicated that leukocytosis upon admission exhibited a sensitivity of 60%, specificity of 76%, and a negative predictive value of 85% for counts exceeding 12,000; 30% sensitivity, 95% specificity, and an 80% negative predictive value for counts above 15,000; and 10% sensitivity, 98% specificity, and a 76% negative predictive value for counts greater than 20,000 in predicting mortality in patients with OPP poisoning. There is no statistically significant difference in mean leucocytic count between survivors and non-survivors, indicating that it cannot serve as a predictor of mortality. This aligns with findings from a study by (Abdel Baseer et al. 2021), which indicated that while non-survivor patients exhibited a higher leukocytic count, there was no statistically significant correlation between leukocytic count, patient outcomes, and mortality. A study by (Tang et al. 2018) demonstrated a statistically significant difference between the survivors and non-survivors groups regarding mean leucocytic count.

According to the outcome, out of 88 patients, (77.3%) of patients have survived, while only (22.7%) of patients have died. This aligns with the findings of (Sapkota et al. 2018) results, where the majority of cases resulted in survival. A statistically significant relationship was observed between the severity of organophosphorus poisoning and mortality. All cases of mild poisoning survived, while 76.5% of patients with moderate poisoning and 10.3% of patients with severe poisoning (7 out of 18) survived, from a total of 61 and 18 patients, respectively. The findings are consistent with the results of a study

conducted by (Kamath and Gautam 2021), who reported that all patients except one with mild-grade poisoning were improved. The mortality rate for moderate-grade poisoning was 5.4%, while for severe poisoning, it was 52.8%. This association was statistically significant ($P < 0.0001$). Furthermore, a statistically significant relationship was observed between the severity of organophosphorus poisoning and various outcomes. All patients with mild poisoning achieved recovery, while a majority of those with moderate poisoning also recovered (77.0%), with some developing mechanical ventilation-acquired pneumonia (13.1%). In cases of severe poisoning, patients predominantly experienced shock (27.8%) and intermediate syndrome (27.8%), with subsequent occurrences of mechanical ventilation-acquired pneumonia (22.2%) and recovery (22.2%). This coincides with a study conducted by (Malaviya et al., 2023) who reported that grades of POP scoring significantly correlated with various outcomes. In contrast, a study conducted by (Twayana et al. 2019) showed that the correlation between grades of POP score with different outcomes had no statistical significance.

V. Conclusion

The leucocytic count is a valid, rapid, and readily accessible measure, even within primary healthcare facilities. However, it should not be used alone. The measurement, in conjunction with other parameters, such as signs and symptoms of toxicity, may significantly contribute to predicting the severity and outcome in patients with acute organophosphate poisoning.

VI. Recommendations

The measurement of leucocytic count may facilitate the early assessment of acute organophosphate poisoning. The early assessment of acute organophosphate poisoning cases utilizing the POP score facilitates prompt intervention, thus averting complications and improving patient outcomes. Healthcare authorities worldwide should limit the use of organophosphorus compounds to specific applications and employ highly hazardous substances judiciously. Researchers need to identify innovative and safer compounds that can act as alternatives to OPCs, thereby enhancing the protection of individuals and communities. Further studies on a larger scale are needed to validate our results.

VII. Limitations of the study

The study's limitations include the absence of chronic and long-term exposure measurements. In addition, the small sample size restricts the applicability and generalizability of the findings to a broader population in the context of an evidence-based practice.

Declarations

Funding: This work was self-funded by authors.

Data availability statement: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest: The authors declared that there was no conflict of interest.

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