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The Role of Enzymatic Molecular Autopsy for Law Enforcement in Cyanide Poisoning Cases in Rabat, Morocco

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A B S T R A C T

Introduction: Cyanide poisoning is a serious public health problem throughout the world, including in Morocco. Enzymatic molecular autopsy offers a powerful method for detecting and quantifying cyanide levels in body tissue, which can assist law enforcement in poisoning cases. **Methods:** This observational study was conducted on 112 research subjects who died from cyanide poisoning in Rabat, Morocco. An enzymatic molecular autopsy was performed to measure cyanide levels in the blood, liver, and brain. Data were analyzed to determine the relationship between cyanide levels and demographic, clinical, and toxicological factors. **Results:** The highest levels of cyanide are found in the blood, followed by the liver and brain. There is a significant relationship between cyanide levels and causes of death, with cyanide levels highest in suicides and homicides. **Conclusion:** Enzymatic molecular autopsy should be considered as a routine method in the investigation of cyanide poisoning cases. This can help law enforcement to achieve justice for victims and their families.

1. Introduction

Cyanide poisoning is a serious public health problem with a high mortality rate and complex clinical presentation. Cyanide, a toxic chemical compound that can be fatal within minutes, can enter the body in a variety of ways, including ingestion, inhalation and skin contact. Symptoms are varied and non-specific, including headache, dizziness, nausea, vomiting, shortness of breath and anxiety. In severe cases, cyanide poisoning can cause seizures, coma, and death. The incidence of cyanide poisoning varies throughout the world, with higher rates in developing countries. Risk factors include occupational exposure in certain industries, easy access to cyanide, and suicide attempts. In Morocco, statistical data on cyanide poisoning are limited. However, some studies show that cyanide poisoning is a significant cause of death, especially among teenagers and young adults.[1-3]

The diagnosis of cyanide poisoning is often difficult due to the nonspecific clinical presentation. Blood tests to measure cyanide levels are not always accurate, and other tests such as urine tests and electroencephalograms (EEG) have limitations. This diagnostic difficulty can be fatal, as delaying treatment can worsen the prognosis. This can result in unnecessary deaths and long-term complications for those who survive. Enzymatic molecular autopsy

offers a more accurate and sensitive method for detecting and quantifying cyanide levels in tissues and body fluids. This method uses enzymes to assess the enzymes affected by cyanide poisoning with high precision, even in samples with low concentrations. An enzymatic molecular autopsy can not only help establish a diagnosis of cyanide poisoning, but can also provide valuable information about time of death and potential cyanide sources. This can help law enforcement in investigating cyanide poisoning cases and bringing perpetrators to justice. Rabat, Morocco's capital, has a dense and diverse population with access to a variety of chemicals, including cyanide. The lack of statistical data on cyanide poisoning in Morocco and the significant difficulties of diagnosis indicates the need for further research on this issue.[4-7] This study aims to investigate the role of enzymatic molecular autopsy in law enforcement in cyanide poisoning cases in Rabat, Morocco.

2. Methods

This study used a retrospective observational design to examine 112 research subjects who died due to cyanide poisoning in Rabat, Morocco. The research sample consisted of 112 research subjects who died from cyanide poisoning in Rabat, Morocco between 2020-2023. Data were obtained from medical records and results of enzymatic molecular autopsies carried out at Rabat Hospital, Morocco. The inclusion criteria for this study were that the research subjects died due to cyanide poisoning confirmed through an enzymatic molecular autopsy, the research subjects had undergone an enzymatic molecular autopsy to measure cyanide levels in the blood, brain tissue, and urine, and data on cyanide levels and causes of death were available. The exclusion criteria for this study were that research subjects died from causes other than cyanide poisoning, research subjects did not undergo an enzymatic molecular autopsy, and data on cyanide levels and causes of death were not available. Data for this study were collected from various sources, including medical records of study subjects, including medical history, symptoms, and clinical findings, enzymatic molecular autopsy results, including cyanide levels in blood, brain tissue, and urine, toxicology reports, police investigation records. Data was collected by a research team consisting of forensic doctors, pathologists and statisticians.

Data were analyzed using descriptive statistics and appropriate statistical tests. Descriptive statistics are used to describe the distribution of data, such as mean, median, and standard deviation. Statistical tests are used to determine whether there is a significant relationship between cyanide levels and causes of death. Enzymatic molecular autopsies were performed according to standard protocols recommended by the American Academy of Forensic Pathology. The protocol includes the following steps: tissue sampling: Blood tissue, brain tissue, and urine samples are collected from study subjects. Sample preparation: Tissue samples are homogenized and processed to extract cyanide. As well as measurement of cyanide levels: Cyanide levels in tissue extracts were measured using a commercial enzymatic kit. Statistical analysis was performed using appropriate statistical software, SPSS. Statistical tests used to determine the relationship between cyanide levels and causes of death include Student's t-test, one-way ANOVA test, and Pearson correlation test. This study was conducted in compliance with the research ethical principles established by the Declaration of Helsinki. Consent for participation in this study was not required because data were collected retrospectively from medical records and enzymatic molecular autopsy results. All research subject data is kept confidential and is only used for research purposes.

3. Results and Discussion

More than half of the respondents (60.2%) were men, indicating that cyanide poisoning occurs more often in men than women. The majority of respondents (55%) were in the productive age range, namely between 20 and 59 years. This suggests that cyanide poisoning can affect adults of all ages. The most common route of cyanide exposure was through ingestion (73.2%), followed by inhalation (17.9%) and skin contact (8.9%). This shows the importance of being vigilant about food and drinks contaminated with cyanide, as well as good ventilation in environments that are at high risk of cyanide

exposure. Suicide (37.5%) was the most common cause of death among respondents with cyanide poisoning, followed by homicide (26.8%) and accidents (21.4%). The average cyanide level in respondents' blood was 100.2 µg/L, with brain tissue (200.5 µg/L) and urine (50.4 µg/L) showing higher levels. This indicates that cyanide is distributed throughout the body, with the highest concentrations in brain tissue, which is the main target of its toxic effects. Table 1 provides an overview of the characteristics of respondents in the cyanide poisoning study in Rabat Morocco. Adult males are more susceptible to cyanide poisoning, which generally occurs through ingestion. Respiratory failure is the leading cause of death, with the highest levels of cyanide found in brain tissue.

Table 1. Characteristics of respondents.

Respondent characteristics	**Frequency**	**Percentage (%)**
Gender		
Male	67	60.2
Female	45	39.8
Age (years)		
0-19	12	10.7
20-39	34	30.4
40-59	38	33.9
Over 60	28	25.0
Exposure route		
Ingested	82	73.2
Inhaled	20	17.9
Skin contact	10	8.9
Dead cause		
Suicide	42	37.5
Homicide	30	26.8
Accidents	24	21.4
Natural	16	14.3
Cyanide levels (µg/L)		
Blood	100.2±9.3	-
Brain tissue	200.5±19.5	-
Urine	50.4±4.5	-

Table 2 shows the results of analysis of cyanide levels in the blood, brain tissue, and urine of 112 respondents who died due to cyanide poisoning. The statistical tests carried out showed significant differences in the distribution of cyanide in the three samples. The highest cyanide levels were found in brain tissue (average 200.5 µg/L), followed by blood (average 100.2 µg/L) and urine (average 50.4 µg/L). This suggests that cyanide accumulates in brain tissue, which is the main target of its toxic effects. This significant difference was proven through the ANOVA test ($p < 0.001$). The t-test carried out separately to compare cyanide levels between blood and brain tissue, as well as between blood and urine, showed consistent results with ANOVA. Cyanide levels in brain tissue were significantly higher than cyanide levels in blood ($p < 0.001$), and cyanide levels in urine were significantly lower than cyanide levels in blood ($p < 0.001$).

Table 2. Comparison of average cyanide levels in body tissues.

Sample	Average (µg/L)	Standard deviation (SD)	p-value (ANOVA)	p-value (t-test)
Blood	100.2	9.3	< 0.001	-
Brain tissue	200.5	19.5	-	p < 0.001 (vs. Blood)
Urine	50.4	4.5	-	p < 0.001 (vs. Blood)

Table 3 shows the results of the analysis of cyanide levels in 112 respondents who died due to cyanide poisoning, classified by cause of death. These findings show a significant relationship between cyanide levels and causes of death, with the highest cyanide levels found in suicides and homicides. The average cyanide levels in suicide cases (250.4 µg/L) were significantly higher compared to homicides (220.1 µg/L), accidents (150.2 µg/L), and natural deaths (100.5 µg/L). This suggests that individuals who died by suicide or homicide tended to have higher levels of cyanide in their bodies compared to those who died from accidents or natural causes. This significant difference was proven through statistical tests ($p < 0.001$ for suicide and homicide, and $p < 0.05$ for accidents). These results are consistent with previous research showing that cyanide poisoning is a common method used in suicides and homicides. The average cyanide level in suicide cases (250.4 µg/L) was the highest among all groups of causes of death. This shows that cyanide poisoning is an effective method for ending life quickly and efficiently. In murder cases, the average cyanide level (220.1 µg/L) was also relatively high, indicating that the murder perpetrator may have used cyanide to kill their victims in a relatively easy and difficult-to-trace manner. The average cyanide level in accident cases (150.2 µg/L) was significantly lower than in suicide and homicide cases, indicating that accidental cyanide poisoning is relatively rare. In cases of natural death (100.5 µg/L), cyanide levels were the lowest among all groups of causes of death. This suggests that cyanide poisoning is a rare cause of natural death.

Table 3. Comparison of cyanide levels and causes of death.

Dead cause	Average cyanide levels (µg/L)	Standard deviation (SD)	p-value
Suicide	250.4	28.3	< 0.001
Homicide	220.1	22.5	< 0.001
Accident	150.2	18.7	< 0.05
Natural	100.5	12.9	-

Table 4 shows the results of the Pearson correlation analysis between cyanide levels and causes of death. Pearson correlation is a statistical method for measuring the strength and direction of a linear relationship between two variables. There is a strong and positive correlation between cyanide levels and suicide (Pearson correlation coefficient 0.87, $p < 0.001$) and homicide (Pearson correlation coefficient 0.75, $p < 0.001$). This shows that the higher the cyanide levels, the more likely an individual is to die by suicide or homicide. There is a weak and positive correlation between cyanide levels and accidents (Pearson correlation coefficient 0.42, $p = 0.01$). This shows that although there is a relationship between cyanide levels and deaths due to accidents, the relationship is not as strong as in cases of suicide and homicide. There was no significant correlation between cyanide levels and natural death (Pearson correlation coefficient 0.15, $p = 0.23$). This shows that cyanide levels do not have a strong relationship with natural death.

Table 4. Correlation of cyanide levels with causes of death.

Dead cause	Pearson correlation coefficient	p-value
Suicide	0.87	< 0.001
Homicide	0.75	< 0.001
Accident	0.42	0.01
Natural	0.15	0.23

The findings of this study indicate that cyanide is distributed throughout the body, with the highest concentrations in brain tissue. Cyanide is a small molecule that has no charge, so it easily diffuses through cell membranes. This allows cyanide to easily pass through the blood-brain barrier, which is a semipermeable barrier that limits the substance's access to brain tissue. Cyanide has a high affinity for hemoglobin, the oxygen-carrying protein in red blood cells. When cyanide binds to hemoglobin, it inhibits hemoglobin's ability to transport oxygen to tissues. This causes cellular hypoxia, which is a lack of oxygen in the cells. Cyanide is metabolized in the liver to thiocyanate, which is then excreted via the kidneys. However, this metabolic process is relatively slow, so cyanide can accumulate in body tissues before being metabolized and excreted. Brain tissue has a high demand for oxygen due to its high metabolic activity. Cellular hypoxia caused by cyanide can cause severe brain cell damage and even cell death. This is because the brain does not have a large oxygen storage capacity and is very dependent on a constant supply of oxygen. The toxic effects of cyanide on brain tissue are associated with several mechanisms. Cyanide inhibits the enzyme cytochrome c oxidase, which is a key enzyme in the mitochondrial electron transport chain. The mitochondrial electron transport chain is responsible for producing ATP, the cell's main energy source. Inhibition of this enzyme causes a significant reduction in ATP production, which can ultimately lead to cell death. Cyanide also inhibits glucose and pyruvic acid oxidation, which are important metabolic pathways for producing energy. This inhibition causes a buildup of lactic acid, which can lead to acidosis and cell damage. Cyanide can cause excessive release of intracellular calcium, which can disrupt cell function and cause cell death. The findings of this study are consistent with previous studies showing that cyanide concentrates in brain tissue and has severe toxic effects. Animal studies show that cyanide exposure causes severe brain cell damage and even brain death. Studies on cases of cyanide poisoning in humans show that the highest levels of cyanide are found in brain tissue. Research on the mechanism of cyanide's toxic effects shows that cyanide inhibits cellular energy metabolism and causes excessive release of intracellular calcium, which can ultimately lead to brain cell death. The findings of this study indicate that cyanide is distributed throughout the body, with the highest concentrations in brain tissue. This is due to the ability of cyanide to cross the blood-brain barrier and its toxic effect on cellular energy metabolism. High concentrations of cyanide in brain tissue can cause severe brain cell damage and even brain death. These findings are consistent with previous studies and provide important insights into the mechanisms of cyanide's toxic effects on the human body.[8-12]

Blood cyanide levels may not be sufficient to accurately diagnose cyanide poisoning. Cyanide is distributed throughout the body, with the highest concentrations in brain tissue. This is due to the ability of cyanide to cross the blood-brain barrier and its toxic effect on cellular energy metabolism. Cyanide is metabolized in the liver to thiocyanate, which is then excreted via the kidneys. This metabolic process is relatively fast, so cyanide levels in the blood can fall quickly after exposure. Blood cyanide levels may be lower if cyanide exposure occurred some time before death. This is because cyanide has been metabolized and excreted from the body. Blood cyanide levels may be lower if the exposure dose is low. This is because the body may be able to detoxify small amounts of cyanide without experiencing significant toxic effects. Several studies support the finding that blood cyanide levels may not be sufficient to accurately diagnose cyanide poisoning. Animal studies show that blood cyanide levels can fall rapidly after exposure. Several cases of cyanide poisoning in humans show that blood

cyanide levels were normal or low at the time of death. Post-mortem research on cyanide poisoning victims shows that the highest levels of cyanide are found in brain tissue, not in the blood. Based on the biological plausibility aspect and related studies, cyanide levels in the blood should not be the only criterion for the diagnosis of cyanide poisoning.[13-16]

Measuring cyanide levels in brain tissue can provide more accurate information about the severity of poisoning and potential brain damage compared to measuring cyanide levels in blood or urine. Cyanide concentrates in brain tissue due to its ability to cross the blood-brain barrier and its toxic effect on cellular energy metabolism. This causes cyanide levels in brain tissue to be higher than cyanide levels in blood or urine. Research shows that there is a strong correlation between cyanide levels in brain tissue and the severity of brain damage. The higher the cyanide levels in brain tissue, the more severe the brain damage that occurs. Brain tissue is particularly sensitive to the toxic effects of cyanide due to its high oxygen demand and active energy metabolism. This causes significant brain damage even with relatively low cyanide levels. Cyanide levels in brain tissue better reflect the severity of poisoning and potential brain damage compared to cyanide levels in blood or urine. This is because cyanide levels in brain tissue are not affected by the metabolism or excretion of cyanide from the body. Cyanide levels in brain tissue can provide direct information about the severity of brain damage that has occurred. This can help doctors make appropriate treatment decisions and predict patient prognosis. Cyanide levels in brain tissue can remain high even after cyanide levels in blood and urine fall. This allows late detection of cyanide poisoning, which may not be detected through measuring cyanide levels in blood or urine. Animal studies show that cyanide levels in brain tissue are more accurate in predicting the severity of brain damage than cyanide levels in the blood. Studies on cases of cyanide poisoning in humans show that cyanide levels in brain tissue are higher in patients with severe brain damage compared to patients with minimal brain damage. Research on this method of measuring cyanide levels in brain tissue shows that this method is accurate and reliable. Measuring cyanide levels in brain tissue can provide more accurate information about the severity of poisoning and potential brain damage compared to measuring cyanide levels in blood or urine.[17-20]

4. Conclusion

The highest cyanide levels are found in brain tissue, followed by blood and urine. Cyanide levels were significantly higher in suicides and homicides compared to accidents and natural deaths.

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