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Metabolomics: An Aid in Cyanide Toxicity

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Abstract:

Any chemical can be harmful when taken in large quantity or in certain conditions. Toxicity is the magnitude to which an organism can be damaged or harmed by a particular substance. For the forensic toxicology, the toxicity mechanism provides perception as to how a physical or chemical substance can cause death or incapacitation. To know the working of such toxic compounds, metabolomics plays a major role. Metabolomics is the field of "omics" which deals with the analysis of small molecules/ metabolites within the living cells, tissues or organisms. It is influenced by genetic and environmental factors providing in depth analysis of altered metabolic pathways that are targeted by harmful chemicals in forensic toxicology. Acute toxicity may harm an organism in short term exposure. A true poison like arsenic and cyanide is lethal even if it is consumed in minute amounts. Cyanide refers to a chemical containing carbon- nitrogen bond (C-N bond) having negatively charged ion. It causes arrest of aerobic metabolism in living beings as CN attaches to the iron atom in Cytochrome C Oxidase [CCO] in mitochondria of cells. Its exposure most often occurs through consumption or inhalation. Metabolomics helps in the understanding of cyanide pathway in ETC and the concentration of cyanide can be detected in the human by separation/ detection techniques like HPLC and GC-MS chromatographic techniques. In this context, Cyanide toxicity is explained with the help of a case study. The metabolite concentrations, physical characteristics, phenotypic changes and the postmortem appearance are reviewed in detail.

Keywords: *Metabolomics, Forensic toxicology, Metabolite, Phenotype, Chromatography.*

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Introduction

The degree of toxicity of any substance relies on how much enters our body and the period of its existence or shelf life of that toxic compound. A true poison like Arsenic, Cyanide is considered to be lethal even if the consumption is miniscule. Whereas intoxicant like Carbon monoxide and Alcohol is proved lethal only if taken in large amounts. Detection of such small metabolites which can be a possible cause for the toxicity is done with the help of metabolomics **(Manchester and Anand, 2017).**

Analysis of small-molecule metabolite profiles or data related to the diseases is called Metabolomics. It mainly relies on the cellular process analysis whose chemical traces are left behind which gives an accurate analysis of metabolic pathways that might be altered by the presence of harmful chemicals **(Fiehn, 2002).** The metabolome indicates the entire group of metabolites in each and every cell or tissue or organ or organism that is considered to be the cellular processes' end products. It requires the utilization of advanced analytical technologies to recognize and assess the quantity of cellular metabolites, along with the extraction of relevant statistics such that the data/details can be analysed and applied in a practical manner.

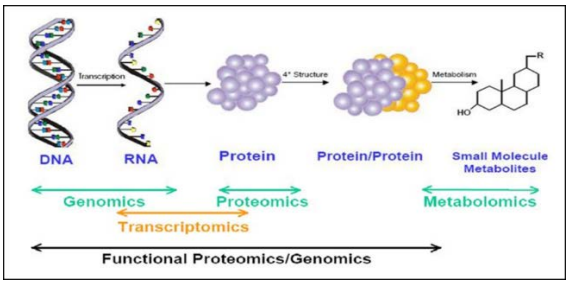

Figure No. 1: Overview on Metabolomics

Metabolomics is important in diagnosing such small metabolites for the following reasons:

1. Almost 95% of all diagnostic clinical assays test for small molecules.

2. Most of the known small drugs (around 89%).

3. Half of most drugs are derived from already present metabolites.

4. 30% of known genetic disorders arise due to the metabolism of small molecules.

5. Cofactors and signalling molecules of proteins in large numbers are small molecules.

6. Since metabolome is mostly related to a constituent of the genome of an organism, its environment and its physical conditions, metabolomics provides a unique option to look at genotype-phenotype and genotype-envirotype relationships **(Robertson *et al.*, 2011).**

With the futuristic analytical technologies, Metabolomics is emerging as a robust tool in discovering novel drug targets, predicting drug responses, identification of diagnostic diseases biomarkers, interpretation of drug action mechanisms, illustration of the pathological mechanisms, and facilitating treatment of patients precisely. Moreover, large-scale screening of drugs or chemical compounds can be achieved through *in vitro* metabolomics approaches **(Nicholson *et al.*, 2002).**

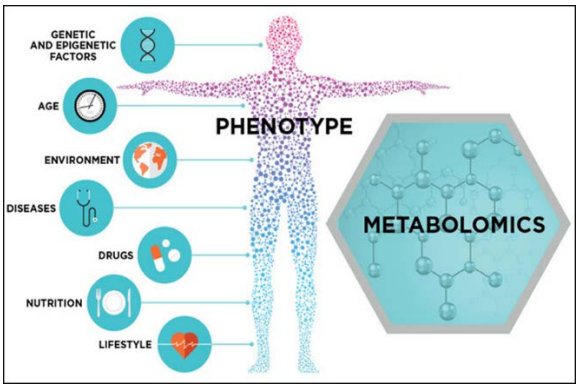

Figure No. 2: Factors influencing Metabolomics

The main factors that alter the phenotype of the individual include: genetic factors, age of the individual, environmental stress and conditions' effects on that individual, drugs taken and its effects in the host resulting in the change of the phenotype, diseases that he/she has conquered in their lifetime, the nutrition they take and the lifestyle of that individual affects the changes in the phenotype and hence can be detected the pathway of these factors with the analysis of metabolomics. Metabolomics has a direct correlation with abnormalities being caused.

Several methods can be utilized for the separation of metabolites in the analytes according to the retention time of the analytes. This serves as information regarding its identity: Gas chromatography (GC), High-Pressure Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), Ultra Performance Liquid Chromatography (UPLC), Ion Chromatography (IC), and Fourier Transform.

Some of the detection techniques of metabolites include Nuclear Magnetic Resonance Spectroscopy, Mass Spectroscopy (MS) and their combination of techniques such as GC-MS, HPLC-MS.

Metabolomics in Cyanide Toxicity

The term "cyanide" refers to any chemical containing a carbon-nitrogen (CN) bond and it is an ion that is negatively charged. Few derivatives of cyanide are Hydrogen cyanide (gas form), Potassium cyanide and Sodium cyanide (solid form). The lethal form of cyanide is its vapour form (HCN) (**Baskin *et al.*, 2001)**. The major sources of cyanide are cyanide salts, apple seeds, almond seeds, apricot, wild cherries. Exposure of our body to Cyanide is through inhalation or ingestion, but liquid cyanide is absorbed easily by the skin or eyes. Once absorbed, the cyanide enters the circulatory system and it spreads swiftly to all organs and tissues in the body which causes the aerobic metabolism to stop.

Mechanism

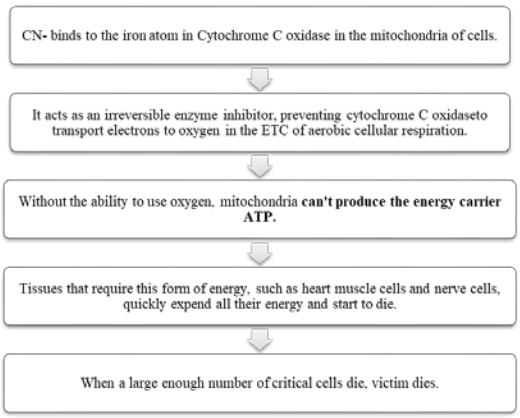

Figure No. 3: Workflow of Mechanism of Cyanide Toxicity

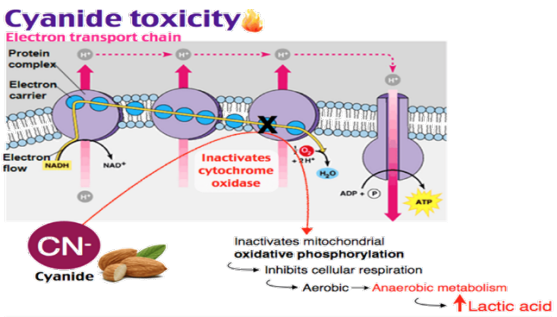

Figure No. 4: Mechanism of Cyanide Poisoning

Signs and Symptoms: Dizziness & Headache, Nausea & Vomiting, Low blood pressure, Hypoxia – results in disorder in the functioning of all cells, Loss of consciousness, rapid breathing associated with respiratory failure.

An oral toxic dose of HCN is 60-90 mg, whereas for NaCN or KCN is 300 mg.

Case study of Cyanide Poisoning: In a metal chrome plating shop, a 41-year-old patient had collapsed for an undetermined time duration. The condition of the patient was found to be asystolic when the Emergency medical services had arrived and they started advanced cardiac life support protocol. Intubation was done on the field. Norepinephrine and dopamine were given to the patient to increase his blood pressure. The patient received an antidote for cyanide toxicity which consisted of sodium nitrite [$NaNO_2$] 300 mg, sodium thiosulfate [$Na_2\ S_2O_3$] 12.5 grams due to his work environment after 14 minutes of arrival at the emergency department **(Meillier and Heller, 2015).**

Samples were taken: Blood and urine; Detection: HPLC & GC-MS Chromatographic techniques.

Signs and Symptoms: These crucial signs were observed: Temperature - 93.2°F, pulse 120 beats/minute, Respiratory rate - 16 breaths/minute, Pulse oxygenation - 95% on ventilation and Blood pressure - 115/81 mmHg.

On performing the initial physical examinations, relevant positives included no response with coarse or rough breath sounds on the ventilator. Gag reflex was not observed and the patient had nonreactive 4 mm pupils. The laboratory tests showcased these results: Sodium 145 mEq/L, Potassium 5.4 mEq/L, Chloride 107 mEq/L, Bicarbonate 13 mEq/L, Blood urea nitrogen 8 mg/dL, Creatinine 1.91 mg/dL, White blood cell count was 12.9 mg/dL, Haemoglobin 13.2 mg/dL, Platelet count 106/mm^3, Arterial blood gas included pH 6.67, Carbon dioxide 86 mmHg and Oxygen partial pressure 157 mmHg, AST 151 u/L, ALT 53 u/L and Alkaline Phosphatase 74 u/L.

Central nervous system or neuromuscular medications were not administered which resulted in the brain death protocol. Maintained systolic blood pressure greater than 100 mmHg was observed and the body temperature was within the normal limits. The Apnea Test failed and reflexes were limited when two of the neurological examinations were performed. The patient was revealed dead.

Medical Forensic Examination for Cyanide Poisoning: The stomach, brain, liver, blood and muscles all appear to be in bright red colour, Ciliatary

loss of tonsils, Cyanosis of face and lips, foam out of the mouth and bruised corpse **(Meillier and Heller, 2015).**

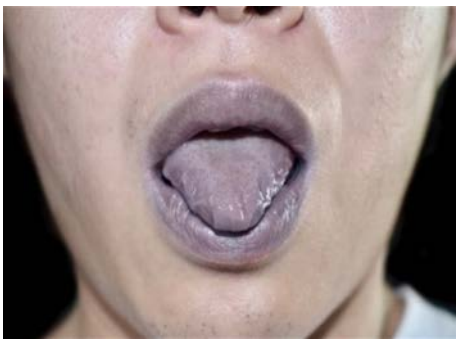

Figure No. 5: Cyanosed Face and Lips

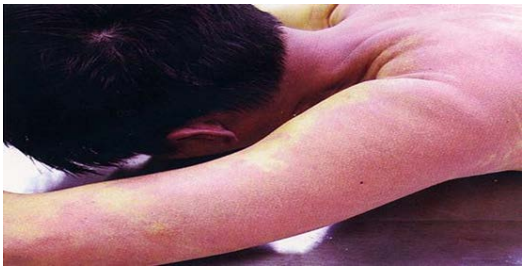

Figure No. 6: Red bruises all over the body

Figure No. 7: Dilated Pupils

Analysis

Cyanide is analysed/detected through HPLC & GC-MS Chromatographic techniques. To avoid Cyanide degradation, biological samples have to be prepared so that cyanide can be diagnosed as soon as possible. At neutral pH, cyanide can volatilize to hydrogen cyanide or it is decomposed in the presence of oxidizing agents. Dionex II H cartridge (1cc) was used to transfer the sample solutions as it removes high concentrations of alkaline earth metals, cationic metals and alkali and by using a syringe filter, the sample was filtered before the sample injection process. These samples were analysed instantly. Until the test was done the samples were preserved in polypropylene tubes, at a temperature of −15°C by protecting it from light.

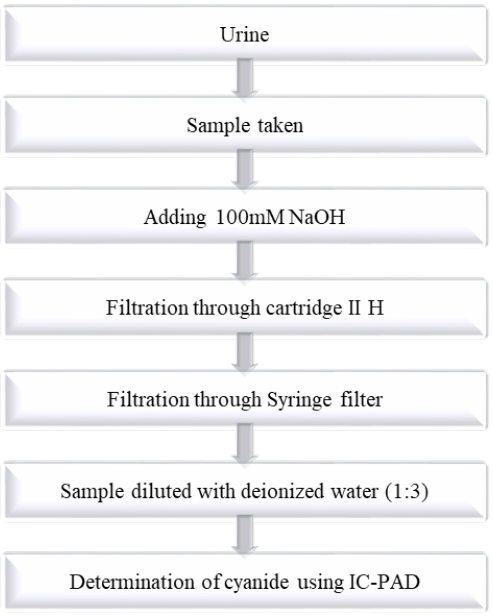

Figure No. 8: Method of biological sample preparation

Based on the signal-to-noise ratio numerical values of the samples, the limit of detection (LOD) is found out that contains minimum analyte concentrations. After determining the LOD, the peak area is plotted against the standard concentration to assess the linearity of calibration curves and it is expressed or given by the correlation coefficient (R^2) **(Jaszczak *et al.*, 2017).**

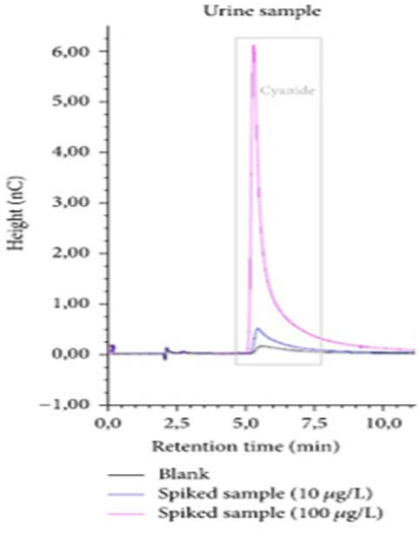

Figure No. 9: Chromatogram result of the Sample

Table 1: Parameters of the IC-PAD method for the determination of cyanide ion.

Sample	Urine
Linear range [μg/L]	1-10 1-100
Curve pattern	y = 0.019x-0.034 y = 0.169x-0.304
R	0.983 0.992
SD	0.001 0.003
CV (%)	0.42 1.63
Recovery (%)	62 80
LOD [μg/L]	1.8

Result & Discussion

There were no significant changes that were noted down during the retention time of cyanide ions in the analysis of the properly prepared biological samples, which indicates that there was good laboratory practice followed with reliable instrumentation. Upon analyzing the calibration curves, it was shown to be linear at a concentration range of 1 to 100 μg/L for urine with a correlation coefficient of 0.992 (Table 1 and figure 9). The average recovery result was found to be high in urine (around 80%) and recoveries range between 80 and 120% are generally acceptable. Since there were no duplex peaks observed even though there was an addition of a known concentration of cyanide, it supported the presence of the cyanide.

Conclusion

Though there are many procedures to test for cyanide content, but when compared to other methods employed for determining cyanide ions, the developed biological (urine, blood, saliva) sample material preparation procedure is simple and it is minimal time-consuming. In addition, it is possible to use a low amount of sample (approximately around 1 mL), which is the main advantage in relation to biological materials samples, as it is hard to obtain in huge quantities.

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