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VARIOUS ANALYTICAL METHODS USED FOR DETERMINATION OF CARBON MONOXIDE IN POST-MORTEM BLOOD - A REVIEW.

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Abstract— Carbon monoxide is a colorless, odorless, tasteless poisonous gas produced by the incomplete combustion of gasoline, propane, charcoal, and other fuels. There are various chemicals, like methylene chloride paint stripper, which are also responsible for the production of CO. If the levels of CO rise above 5% in the human body, CO poisoning can easily occur due to the formation of a compound called carboxyhemoglobin, which leads to symptoms like anoxia, cell damage, unconsciousness, headache, seizures, and death.

There are various techniques to cure CO poisoning in live cases like providing various concentration of oxygen to the victim but, the major question that we raised in this review paper was what are the techniques which are used to detect the level of CO poisoning in post mortem blood to which in the research papers we went through the answers were TBCO determination, spectrophotometric determination of COHb, gas chromatography with reduction gas detector, Gas Chromatography coupled with FID detector.

Keywords— Carbon Monoxide, Carboxyhaemoglobin, Spectrophotometric determination, postmortem blood, Gas Chromatography. Hypoxia.

I. INTRODUCTION

Carbon monoxide is an odorless, tasteless, colorless, poisonous gas that causes a shortage of oxygen in the blood (hypoxia). It is sometimes called the 'silent killer'. CO is obtained from burning fuels, running cars, cigarette smoke, partially burnt wood, blocked chimneys, etc. Cases of Carbon

monoxide poisoning are generally seen in the winter season, as some people burn wood and place in the room for warmth, and sometimes close the door. As the CO gas is inhaled by nasal passage it travels to lungs where it reaches the bronchioles where the exchange of gases take place and, the CO reaches the blood stream and due to its high affinity

towards Hb which leads to formation of a strong bond between HB and CO which forms a complex known as Carboxyhaemoglobin which results to inhibition of oxygen transport leading to toxicity by lowering down the percent of oxygen in blood which leads to problem in breathing, hypoxia, headache, seizures, etc.[1] [4] COHb blood appears cherry red in color, due to which the person intoxicated with CO shows reddish lips, cheeks, and also other body parts. The symptoms of CO poisoning vary according to the percent of CO in the blood. There are no symptoms until the percent of CO is more than 10%. CO not only binds to Hb protein but also with other proteins such as myoglobin, cytochromes, guanyl cyclase which leads to cardiac malfunction, impairment of cellular respiration and cerebral vasodilation, respectively[2]. CO does not show similar effects in everybody, people with comorbidity are most susceptible to this gas whereas people living in polluted areas have a tolerance level of 15%. In case of pregnant women are also affected severely and more faster than a person who smokes as smokers may have a tolerance level of 5-10%. Diagnosis of CO is misinterpreted because of nonspecific symptoms that appear on the body. Misdiagnosis of CO poisoning leads to other problems/disturbance in the body which may lead to severe health issues. Most general method for treating CO poisoning is giving oxygen of different concentrations to the patient. Though the exact timing for which the oxygen is provided is not known yet but usually it is given till the level of CO in blood reduces to less than 5%. Various concentrations of that are provided are Normobaric 100% Oxygen, Hyperbaric 100% oxygen, 28% oxygen etc. The medics are known to provide 28% oxygen which is effective but not more than higher concentrations. [1]

Even after presence of a cure many deaths occur in a year due to CO poisoning as it goes undetectable due to which determining the fact if the death is due to carbon monoxide or not has become necessary. Possible medico-legal findings in CO poisoning cases on external examination are post mortem staining, discolouration of the skin, mucosal membrane, nail plates into reddish- cherry colour. The



internal examination findings might show the appearance of cherry- red colour on the visceral organs such as spleen, liver, heart, lungs [9].

Carboxyhaemoglobin is the biomarker for detection of CO poisoning. Percentage of carbon monoxide in body can be measured by expired breath, blood sample or any other tissue sample such as hepatic tissues, lungs and etc. CO in blood can be diagnosed by various analytical technique such as spectrophotometric determination, Gas chromatography coupled with reduction gas detector, Automated headspace gas chromatography with methaniser and FID.

The standard analytical method used for determination of percentage of COHb is spectrophotometric analysis which determines the amount of hemoglobin that has been combined to CO. In this technique, Sundin and Larsson procedure was used to liberate the CO for the intoxicated blood. CO was liberated by using sulphuric acid, a detergent that causes hemolysis i.e., saponin. Solution for the same is prepared by adding 7.5 g of saponin in 473ml of water and 27ml of sulphuric acid. Two solutions are prepared (sample solution and standard 100% intoxicated sample is prepared). 400 microliter of blood is transfused into 20ml of head space vial which is to be closed with silicon septum and magnetic cap then a syringe needle having nitrogen is used to remove the sample and 800 microliter liberated sample was added with the help of syringe needle.

The percentage COHb is calculated by the following formula $CO [Hb] \% = \frac{\text{area of original sample}}{\text{area of intoxicated sample}} \times 100$ [3]

The other analytical technique for CO poisoning detection is spectrophotometric determination in which COHb act as biomarker. The instrument used in this technique is carbonmonoxideoximeters which determines the level of COHb, O₂Hb, MetHb in blood. It is suitable only when the level of COHb is very high in blood. The sample is prepared using a dye known as methylene blue cardiac dye or sulfhemoglobin dye which may interfere with the result by overestimating the amount of COHb in blood. The sample prepared is analyzed under the visible spectrum of spectrophotometer having wavelength (390-440) nm. [5]

Gas chromatography with reduction gas detector is the other technique which is used to detect CO if present even in very low percentage. In this the intoxicated blood is treated with potassium Ferro cyanide which oxidizes the Hb to MetHb and CO is liberated which is the inserted into a gas chromatograph. In GC, CO reacts with mercuric oxide and forms mercury vapors which are detected spectrophotometrically at a wavelength of 254nm. The results are directly proportional to the amount of gas means that the more the amount of gas in the blood the higher will be the value of absorbance. [5]

The other technique used for the detection of carbon monoxide poisoning is Gas Chromatography – Flame Ionizer Detector. In this technique, the intoxicated sample is mixed to liberate CO from the sample. The solution is

prepared by injecting 400 microliter of sample with HS syringe heated at the temperature of 50 °C by applying split injector followed by gas chromatography. It is performed in a silica column which is 50m long having diameter of 0.53mm and molecule sieve 5A. The gas used is helium with a flow of 12ml/min. A nickel catalyst was added to the column, which is a methaniser that converts CO to methane and is then further detected by FID at 320 degree C. [2]

II. CONCLUSION

CO is a gas which is responsible for lowering the level of oxygen in human body due to formation of compound called carboxyhaemoglobin. Since this gas is odorless, has no color or taste it goes undetectable normally due to which CO poisoning occurs in the person inhaling it. It has been seen that the poisoning starts to show its symptoms only when it rises above 5% and has different symptoms in different people. Even after presence of treatment many people die due to carbon monoxide because of its undetectability. For determining the cause of death there are methods like :- spectrophotometric determination, Gas chromatography coupled with reduction gas detector, Automated headspace gas chromatography with methanizer and FID which are used to detect CO in post mortem blood. Also, awareness should be spread among the people regarding carbon monoxide poisoning as it can save many innocent lives. [8]

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