Original Article

Estimation of Alcohol in Preserved Blood Samples

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Blood samples containing alcohol from 30 adult male volunteers were stored under different conditions and blood alcohol concentration (BAC) determined by Kozelka-Hine method. There was a significant reduction in BAC on storage. When samples were stored at 4 deg C for 72 hours, there was a loss of 11.63 mg% due to diffusion of alcohol from blood into the air in the bottle. Storage at room temperature for 72 hours resulted in a loss of 23.41 mg% due to diffusion as well as temperature dependent alcohol oxidation. Storage at room temperature without preservative for 24 hours, followed by addition of sodium fluoride and storage at 4 deg C for 72 hours resulted in a loss of 34.67 mg%, due to delayed addition of sodium fluoride and greater duration of storage. In stored samples, a near control value could be extrapolated by using a set of prediction equations formulated in this study by correlation and regression statistics.

Keywords : *Alcohol Estimation, Fatal Aircrash Investigation, Preserved Blood Samples.*

The prime object of investigating into the human factors in aircraft accidents is to obtain evidence regarding the cause, sequence and effect of the accident through examination of the crew and passengers. Several studies have shown that alcohol is an important factor for human error in aircraft accidents[1-6].

Before implicating alcohol as being a causative factor leading to an accident, one must consider the variables which might affect the alcohol concentration in the blood. These variables include death - post mortem interval, putrefaction, contamination of samples, use of preservative and conditions of storage and despatch. In our country since all histopathological/toxicological studies in fatal aircraft accidents are done at IAM, some delay in analysis from the time of collection of sample is inevitable. This delay is likely to affect the alcohol concentration. Since it is desirable to know the blood alcohol concentration (BAC) at the time of the accident, this study was undertaken to assess the effect of storage on BAC. An attempt was also made to extrapolate the BAC determined in the sample tested to that existing at the time of collection of samples.

Material and Methods

30 healthy male volunteers were the subjects of this study. All were moderate social drinkers. Each subject consumed 120 ml of 75 deg proof rum in 30 mts. After 60 mts (to ensure complete absorption and maximum BAC), blood samples were drawn and put into 4 vials. Different storage conditions were devised to simulate some of the frequently observed conditions in an aircraft accident scenario.

Sample I : Preserved in sodium fluoride and tested immediately. This represents the actual BAC.

Sample II : Preserved in sodium fluoride at 4 deg for 72 hours. This represents the situation where the autopsy is done early and the sample collected, ideally stored and sent to IAM for analysis.

Sample III :Preserved at room temperature (RT) for 72 hours. This re-presents a situation where the autopsy is done early and the sample collected but could not be transported in a refrigerated condition.

Sample IV Preserved at RT for 24 hours. The sodium fluoride added and preserved at 4°C for further 72 hours. This was devised to simulate a condition where autopsy was delayed by 24 hrs but subsequently the blood sample was collected and stored ideally as prescribed in IAP 4305.

In all the blood samples, alcohol was estimated by the Kozelka-I line method[7].

Results and Discussion

Correlation between samples I and II (n=29).

The mean of the actual BAC (Sample I) was 92.69 mg% (SD=18.34) and that of the ideally stored sample (Sample II) was 81.06 mg% (SD=18.42). The mean difference between these samples was 11.63 mg% and was statistically significant ($p<0.05$). There was a linear correlation between the two samples ($r=0.93$; $p<0.05$). This fall in alcohol level is attributable to the diffusion of alcohol from the blood into the airspace in the container. This decrease is consistent with the findings of Harger[3]. Smalldon and Brown[6] have shown that the extent of alcohol loss is proportional to the volume of airspace in container.

In such a situation, the value of the actual BAC (Sample I) can be estimated with a high degree of confidence by using the regression equation (Sample I in mg% = (0.92 x Sample II) + 17.46).

Correlation between samples I and III (n=29).

The mean BAC of the sample stored at ambient temperature (Sample III) was 69.52 mg% (SD=20.24) and that of the actual BAC (Sample I) was 92.93 mg% (SD=18.26). The mean difference between these samples was 23.41 mg% and was statistically significant ($p<0.05$). There was a linear correlation between the two samples ($r=0.93$; $p<0.05$). Apart from diffusion into the airspace above, this further fall in BAC can be explained on the basis of temperature dependent ethanol oxidation. Brown et al[1] have shown that even in the presence of sodium fluoride, the rate of alcohol oxidation rises wth increasing temperature.

In such a situation, the value of the actual BAC (Sample I) can be calculated with a high degree of confidence by using the regression equation (Sample I in mg% = (0.83 x Sample III)+ 34.88).

Correlation between samples I and IV (n=30).

The mean BAC of the sample which attempts to simulate a delayed autopsy followed by ideal storage (Sample IV) was 57.96 mg% (SD=16.67) and that of the actual BAC was 92.63 mg% (SD=18.26). The mean difference between these samples was 34.67 mg% and was statistically significant ($p<0.05$). There was a linear correlation between the two samples ($r=0.85$; $p<0.05$). This further fall in the alcohol concentration is because of storage for 24 hrs without sodium fluoride. Brown et al[1] found that the period of storage without fluoride is more important than the time with fluoride. They also found that the fluoride - temperature interaction is important because fluoride is more essential for preservation at room temperature than under refrigeration.

In such a situation, the value of the actual BAC (Sample I) can be calculated with high degree of confidence by using the regression equation (Sample I in mg% = (0.92 x Sample IV) + 39.18).

Conclusion

To obtain a realistic value of BAC at the time of accident/time of collecting the sample, the samples should be obtained as early as possible, sodium fluoride added immediately and stored at 4°C. There should be minimum air space above the stored blood sample in the container. In stored samples, a near control value can be extrapolated by using a set of regression equations formulated in this study by correlation and regression analysis.

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