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Effect of Blood sugar, temperature and time on blood alcohol levels in Autopsy blood samples

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Abstract

The effects of blood sugar, temperature and time on blood alcohol concentration in 60 autopsy blood samples were studied. Femoral blood samples were collected which were divided into twp groups (A and B). Group A samples were stored at –20 ^{0}C and Group B samples were stored at room temperature for 60 days. The mean blood sugar of all the samples was 275.81$\pm$ 26.3 mg%. The mean baseline Blood alcohol concentration (BAC) was 39$\pm$ 5mg%. Samples stored at –20 ^{0} C showed insignificant rise in BAC i.e. 44.6 $\pm$ 4mg% and 52.75 $\pm$6mg% after 15 and 60 days respectively. Group B showed significant rise in BAC i.e. 84.5 $\pm$ 3 mg% and 118.6 $\pm$ 5mg% after 15 and 60 days respectively. Samples with higher blood sugar levels i.e. > 140mg% had higher BAC in both Group A and B, as compared to blood sugar levels < 140mg%.

Key Words: *Alcohol; Postmortem generation; Blood sugar; Temperature; Time*

Introduction

Since ancient times, consumption of alcohol has been documented. Ethyl alcohol is the psychoactive ingredient in alcoholic beverages and its consumption is commonly associated with accidents and crime in the society. In the practice of forensic medicine it is essential to know whether the person was under the influence of alcohol at the time of death or not. One of the most vexing problems faced is whether the measured blood alcohol concentration (BAC) indicates antemortem consumption or postmortem ethanol formation by action of microorganisms.

Several factors (temperature, duration, preservative, humidity, aerobic/anaerobic conditions, blood glucose levels etc) have been identified, which results in endogenous alcohol generation or degradation in autopsy blood samples. Due to controversy in the literature on postmortem endogenous alcohol generation, it is imperative to establish the relation between endogenous alcohol generations with time. This aspect has utmost medicolegal importance as falsely elevated BAC above the acceptable levels i.e. 0.150 %, is attached with severe penalties in both criminal and civil proceedings[1]. To prove this relationship the present study was undertaken.

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Material and Methods

The present study was conducted in the department of Forensic Medicine and department of Pharmacology, Lady Hardinge Medical College, New Delhi. The material consists of 60 autopsy blood samples taken from cadavers that came for postmortem examination. Venous blood samples (60ml) was collected from a femoral vein following all aseptic precautions in two sterile glass bottles (labeled A and B) with airtight aluminum screw caps. No preservative was added in the blood samples. All the samples were estimated immediately for blood sugar and baseline blood alcohol levels. The samples labeled 'A' were stored in the deep freezer at -20^0 C and the samples labeled 'B' were stored at room temperature for 60 days. The BAC was estimated using the enzymatic method of alcohol estimation. Randox alcohol estimation kit was used in RA 50 semiautoanalyzer. The BAC was also measured on day 15 and day 60. All autopsies irrespective of the cause and time of death were included in the study, where as autopsy cases with history of antemortem alcohol consumption were excluded. Student's't' test was used to analyze the data. Level of $P< 0.005$ was considered significant.

Results

Mean blood sugar level of all the blood samples was 275.81$\pm$ 26.36 mg% and mean baseline BAC was 39 $\pm$ 0.5 mg/100ml. For blood samples stored at -20^0 C, BAC levels increased to 44.6 $\pm$ 0.4mg% and 52.75 $\pm$ 0.6mg% at day 15 and day 60 respectively. This increase in BAC at day 15 and 60 was statistically insignificant. On the other hand, in the samples stored at room temperature showed significant generation of blood alcohol. On day 15, BAC increased significantly to 84.5 $\pm$ 0.3 mg% ($P< 0.001$), further BAC was increased to 118.6 $\pm$ 0.5 mg% ($P< 0.001$) at day 60. Increase in BAC between day 15 and 60 was significant ($P<0.001$). (Table 1)

Out of 60 blood samples, in 21 instances baseline blood alcohol level was zero but later blood alcohol generated was comparable to other blood samples at day 15 and 60. In 5 instances, blood alcohol remained 0.0 mg% even after 60 days. On the contrary, in two instances baseline BAC level was very high i.e. 165mg% and 250mg%. In blood sample with BAC 165mg%, the BAC reduced to 75mg% and 76mg% at day 15 and 60 respectively. In another instance, BAC reduced from 250mg%

Table 1: Showing BAC of all the samples stored at -20^0 C temperature (group A) and room temperature (group B).

	Parameters BAC (Mean $\pm$ Standard Error)		
Group	Baseline	15 day	60 day
A	39.05 $\pm$ 5	44.61 $\pm$ 4	52.75 $\pm$ 6.8
B	39.05 $\pm$ 5	84.55 $\pm$ 3.7	118.6 $\pm$ 5

* - $P < 0.05$; * * - $P < 0.01$; * * * - $P < 0.001$
0 - $P < 0.05$; * * - $P < 0.01$; * * * - $P < 0.001$
* - Comparison of 0 day BAC with 15 and 60 days within the group
0 – Comparison of 15 and 60 days BAC between group A and group B

to 70mg% and 1.00mg% at day 15 and 60 respectively. In 14 instances BAC reduced from the baseline levels after 60 days.

In the group B, the generation or increase in mean BAC after 15 and 60 days was statistically significant. (Table 1) Out of 60 samples 53 samples showed ethanol generation after 15 and 60 days. There were 26 instances in which BAC was generated from 0 baseline level. Only 2 instances showed loss of baseline blood alcohol level after storage. In blood sample with BAC 165mg%, BAC reduced to 76mg% and 85mg% after 15 and 60 days respectively. In another instance, BAC reduced from 250mg% to 170mg% and 190mg% after 15 and 60 days respectively. It was observed in one instance in-group A, that there was no change in baseline BAC after 60 days of storage (70 mg %) on day 0, 15 and 60.

In both the groups (-20^0 C and room temperature) all cases were sub grouped into Blood Sugar Level < 140mg % and Blood Sugar > 140mg%.(Table 2 and 3).In group A (-20 ^{0}C), in samples with blood sugar level <140mg% (mean blood sugar 92.73 $\pm$ 4.19 mg%) showed increased blood alcohol generation after 15 days and 60 days but the increase was not statistically significant (P<0.001) in both the cases. Similarly, in samples with blood sugar levels >140mg% showed significant increase in alcohol generation than samples with blood sugar level <140mg% (Table 2 and 3).

Discussion

In our present study the mean BAC was 39 $\pm$ 5mg% which increased marginally after 15 days and 60 days when stored at -20^0 C. But increase was significant (P<0.001) after 15 and 60 days when

Table 2: Showing Mean BAC at -20^{0}C and its relation with Blood sugar levels (<140mg% and >140 mg %)

Blood Sugar Range (mg %)	Mean blood sugar (mg %)	n	Mean BAC (mg/100ml)		
			B/L	15 day	60 day
<140	92.73 $\pm$ 4.19	23	39.6 $\pm$ 8	36.6 $\pm$ 6	46.9 $\pm$ 6
>140	389.6 $\pm$ 30.1	37	38.7 $\pm$ 7.9	49.75 $\pm$ 4.7	54.1 $\pm$ 4.1

Table 3: Showing Mean BAC at room temperature and its relation with Blood sugar levels (<140mg% and >140mg %)

Blood Sugar Range (mg %)	Mean blood sugar (mg %)	n	Mean BAC (mg/100ml)	(mg/100ml)	
			B/L	15 day	60 day
<140	92.73 + 4.19	23	38.6 + 8	74.4 + 5	107 + 9
>140	389.6 + 30.1	37	38.7 + 7.9	90.8 + 4.8	125.5 + 5

samples were stored at room temperature (25- 35^0 C). Nicloux (1935)[2] reported that there is postmortem generation of ethanol in putrefying bodies. He also found postmortem generation of ethanol in ox blood samples stored at different temperature and time (9.1mg/100ml after 4 days at 20- 22^0 C; 5.5mg/100ml after 8 days at 15-18^0 C; 3.7mg/100ml after 13 days at 3^0 C). Redetzki (1952) also reported a similar ethanol formation of 35mg/100ml after 24 days of storage at room temperature[3]. Many authors have reported ethanol formation at a faster rate when stored at room temperature (Pluenkhahn[4,5],Redetzki[3], Schwerd andGarhammer[6], Paulus and Janitzki[7], christopoulus[8]). Pluenkhahn (1968) reported a mean BAC of 70mg/100ml after 2- 9 days of storage at 20-25^0 C[5]. Friemuth (1951) found a maximum BAC of 210mg/100g after 13 days of storage at 20-26^0 C[9]. Gonzales (1954) found a maximum BAC of 4mg/100ml in samples stored for 7-47 days in refrigerator[10].

Higher temperature not only increased alcohol generation but also enhanced the rate of disappearance of ethanol from samples. In 2 instances in our study, higher baseline BAC (250mg % and 165mg %) reduced after 15 and 60 days of storage at both temperature. Nicloux (1935) also studied the rate of disappearance of ethanol in mice injected with ethanol immediately before death. He found that ethanol disappeared at a rate that depended on the temperature of storage. The rate was slowest at the lowest temperature, taking more than 100 days to reach zero in mice at 3^0 C and about 25 days in mice stored at 20-22^0 C[2].

In our study, in 15 days significant amount of alcohol was generated in blood samples stored at room temperature. Other workers have reported generation of alcohol at 2-4 days (Pluenkhahn[5], Nicloux[2]) and 8-14 days (Friemuth[9], Gonzales[10], Schwerd and Garhammer[6], Paulus and Janitzki[7]).

In the present study there were 26 instances in which BAC was generated from zero baseline level when stored at room temperature. Similarly, Christopoulus (1973) found an increase in BAC from zero baseline level up to 31- 205mg/100ml after 40-60 days of storage at room temperature[8].

Storage at -20^0 C lead to insignificant generation of alcohol after 60 days. Likewise, Gonzales (1954) reported insignificant alcohol generation at 7 and 47 days in refrigerated samples[10]. Suggesting that the BAC is more reliable if blood samples are stored at-20^0 C and that blood samples stored at -20^0 C, may be analyzed within 60 days for alcohol estimation. If samples are stored at room temperature, the samples should be analyzed as early as possible, preferably within 48 hours as it has been reported that at 20-25 ^{0}C, ethanol is generated within 2-3 days[2,5].

In the present study, the blood samples were analyzed at 15 and 60 days. There have been studies which have shown alcohol generation as early as 1-2 days of storage[5], therefore future studies are necessary to find out the exact time of beginning of alcohol generation in stored blood samples.

Like temperature, blood sugar also appeared to influence ethanol generation in stored blood samples. In our study, samples with blood sugar level <140mg% had lesser ethanol generation after 15 and 60 days as compared to the samples with blood sugar level >140mg%. Pluenkhahn (1968) added glucose to the blood samples to a final blood sugar level of 450mg/100ml of blood. This lead to increases of more than 50mg/100ml in 8 instances those were associated with high blood sugar level (250-760mg100ml)[5]. Similarly, Iribe et al (1974) also reported more ethanol generation after incubation at 27^0 C for 6-10 days when glucose was added to it, than with no glucose added. Iribe et al have reported that ethanol generation is proportional to the glucose level[11]. Similar trend of blood sugar level and ethanol generation is seen in our study.

It is advisable to test the baseline blood sugar level before estimating the BAC, as higher blood sugar levels are associated with higher ethanol levels. Other factor, which influences ethanol generation, is contamination of the blood samples. This aspect was not studied in the present work, but it is documented that contamination of body fluids with species of proteus, e.coli and candida albicans etc. increases BAC[12]. Therefore, blood samples should be collected and stored in sterile conditions and if possible any prevalent infections should be ruled out.

Since the present study has shown various factors which influence the postmortem BAC, proper collection, packaging, transmission, storage, analysis and strict observance of the legal chain of custody is required to guarantee the reliability and integrity of the blood alcohol values on which the forensic expert relies.

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