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Endogenous Ethanol Production Levels in Saudi Arabia Residents

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Abstract

The word "endogenous" means produced or originating from within the body, so endogenous ethanol therefore implies a spontaneous auto regulation of ethanol through various human metabolic processes. In the current research, endogenous ethanol concentrations in blood were determined by sensitive headspace gas chromatography/mass Spectrophotometry in 1400 residents of Saudi Arabia. The subjects were from 14 nationalities, of both sexes and of different age groups. There was no significance difference in blood ethanol concentration between nationalities or between sexes within and between nationalities. The data was extracted and the overall mean ± SD, minimum, maximum, 5% percentile and 95% percentile were 0.14, ± 0.35, 0.00, 1.53 , 0.00, 1.20 mg/dl respectively. The values of blood ethanol concentration as reported in this study indicate they are far too low to have any forensic significance.

Keywords: Legal alcohol level; Endogenous ethanol production; Auto brewing; Saudi Arabia

Introduction

The concentration of ethanol in blood constitutes important evidence for prosecuting drunk drivers and other forms of crimes. For various reasons, the reliability of the results of forensic alcohol analysis is often challenged by defense. One such argument for produced acquittal concerns the notion that alcohol could be produced naturally in the body, hence the term "auto brewery" syndrome [1].

The legal limits of blood ethanol level vary from country to country and although it is usually in the range of 30-80 mg/dl, there is a tendency to lower the limit in certain areas. In some professions, e.g., pilots, there is a zero tolerance for blood alcohol level. Also, countries like Saudi Arabia (SA) need to adopt a similar rule for zero tolerance for blood alcohol if a person, for any reason, is suspected of being under the effect of alcohol [2].

However, endogenous ethanol concentrations are found in the venous blood in human. It is assumed that micro-flora in the gastrointestinal tract is the source of these alcohols (auto brewing), as well as from intermediary metabolism, [1] and amount that formed subsequently oxidized in the liver by the alcoholic dehydrogenase enzyme [2].

The term 'auto brewery syndrome' has been introduced to describe patients who become repeatedly inebriated after the ingestion of food with a high carbohydrate content in the presence of abnormal yeast and bacterial intestinal proliferation. Furthermore, in some patients with unexplained, but otherwise common multi-organ symptoms, mild increased blood ethanol concentrations were found and intestinal fermentation by Candida was hypothesized. Alcohol fermentation occurs in fungal species, such as Candida, that have metabolic pathways for converting pyruvic acid to ethanol. This process involves the decarboxylation of pyruvic acid to acetaldehyde under aerobic conditions, followed by reduction of acetaldehyde to ethanol. Normally, the liver is able to detoxify the metabolites of yeast fermentation, but with abnormal Gastrointestinal Fermentation Syndrome, sufficient ethanol accumulate in the blood to cause feeling of intoxication "auto-brewery syndrome" [3]

Clearly, from jurisdictions adopting zero tolerance for blood alcohol, normal values of blood alcohol levels have to be established from a large sample number of the population keeping in mind individual variability and analytical errors [4].

The Aim of the Work

The objective of the current study was to establish the normal ethanol blood levels in residents of Saudi Arabia in attempt to advice on legal limits.

Materials and Methods

Study subjects

One thousand four hundred heparinized blood samples (5 ml) were provided by the Dammam Fitness Center- Pre-Employment Unit. Most of the subjects attended the center for a medical examination as a prerequisite for a job appointment. Another group attended the Dammam poison control center- Outpatient clinic for renewal job allowance certificate where a drug of abuse tests investigation are mandatory. The subjects were of 14 nationalities, of both sexes (Table 1) and different age groups (mean ± SD: 29.8 ± 9.5 years) (Table 2). These were compared to the group of people from which blood samples were received by forensic science laboratory. The time from blood sampling to analysis of ethanol took no longer than five hours.

Analysis of blood alcohol

Blood ethanol analysis was performed by static headspace analysis. The normal limits for blood ethanol is low, thus, a three – point calibration curve covering the blood concentration range 0.01 mg/dl to 10 mg/dl was constructed. Ethanol standards, quality control samples and internal standards (n-propanol, 10 mg/dl) were prepared in distilled water from HPLC grade solvents. A resolution mixture of ethanol, n-propanol, acetaldehyde, methanol and acetone were

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Nationality	Blood Alcohol Level (mg/dl)					
	No.	Minimum	Maximum	Mean ± SD	5% Percentile	95% Percentile
Saudi	1064	0.00	1.53	0.14 ± 0.36	0.00	1.20
Indian	190	0.00	1.48	0.14 ± 0.32	0.00	0.98
Filibino	48	0.00	1.46	0.16 ± 0.41	0.00	1.25
Egyptian	23	0.00	1.20	0.07 ± 0.26	0.00	0.32
Jordan	15	0.00	1.23	0.18 ± 0.41	0.00	1.20
Pakstanian	17	0.00	1.10	0.14 ± 0.32	0.00	1.10
Benali	19	0.00	0.90	0.05 ± 0.21	0.00	0.90
Sudanese	8	0.00	0.55	0.08 ± 0.19	0.00	0.55
American	2	0.00	0.00	0.00	0.00	0.00
Sri Lanka	7	0.00	0.00	0.00	0.00	0.00
Iraq	1	0.00	0.00	0.00	0.00	0.00
Syrian	1	0.00	0.00	0.00	0.00	0.00
Cuba	4	0.00	0.00	0.00	0.00	0.00
Bahrin	1	0.00	0.00	0.00	0.00	0.00

Table 1: Concentrations of endogenous ethanol in whole blood of healthy subjects under uncontrolled conditions except for alcohol abstinence based on nationality.

Age-group (years)	Male No. (%)	Female No. (%)	Total No. (%)
Group A (<20 years)	126 (9.0%)	12 (0.9%)	138 (9.9%)
Group B (20-29 years)	694 (49.6%)	94 (6.6%)	788 (56.2%)
Group C (30-39 years)	211 (15.1%)	38 (2.7%)	249 (17.8%)
Group D (40-49 years)	125 (8.9%)	23 (1.6%)	148 (10.6%)
Group E (50-59 years)	52 (3.7%)	9 (0.6%)	61 (4.4%)
Group F (≥ 60 years)	13 (0.9%)	3 (0.2%)	16 (1.1%)
ALL GROUPS	**1221 (87.2%)**	**179 (12.8%)**	**1400 (100.0%)**
Mean ± SD 29.8 ± 9.5 Yrs	**Min.** 17 Yrs	**Max.**70 Yrs	

Table 2: Sample distribution across age-groups and sexes.

	Temperature
Initial temp	45ºC
Max Temp [ºC]	350ºC
Ram 1 Initial temp	90ºC
Inlet Temperature	230ºC
MS Transfer Line	250ºC
Ion source 220 [ºC]	220ºC
Syringe temperature	60ºC
	Time
Hold Time	00.00 minutes
Prep Run Timeout	99.00 minutes
Equilibrium Time	00.50 minutes
Ram 1 Hold Time	01.00 minutes
Prep Run Timeout	99.00 minutes
Equilibrium Time	00.50 minutes
Gas Saver Time	03.00 minutes
	Volume
Sample Volume	01.00 ml

Table 3: Headspace parameters.

prepared in distilled water from HPLC grade solvents at a concentration of 100 mg/dl each.

Headspace procedure

The samples were placed in 20 ml headspace vials by adding 1.0 ml of whole blood samples, standards or quality control samples and 1 ml of internal standard. The samples were sealed using crimp top vial caps with septa and were placed in the headspace rack (Table 3).

Gas Chromatography Analysis

The quantitative analysis of ethanol and its separation from other low-molecular volatiles was done with a TRACE™ Ultra Gas Chromatograph.

Capillary gas chromatography/mass spectrometry analysis (GC/MS)

The quantitative analysis of ethanol and its separation from other low-molecular volatiles was done with a TRACE™ Ultra Gas Chromatograph. Injections were made in the split mode. The column temperature program was isothermal at 90ºC. Helium was used as the carrier gas. The injector and transfer line temperatures were 230ºC and the split ratio was 10:1 with each sample lot, a calibration curve, a blank (distilled water) and quality control samples were run. The resolution mixture was first run in a scan mode in the mass range of m/z 20 to m/z 120 to identify the compounds and subsequently the mixture was run in a SIM mode for the samples. The methods were linear in the concentration range used.

Statistical analysis

A latest SPSS statistical package, Version 21 was employed to evaluate the data statistically. The data was put as number, percentage, minimum, maximum, mean ± Standard Deviation of means (Mean ±SD), and 5% - 95% percentile.

Results

The presence method gave good separation of ethanol and n-propanol from acetaldehyde, acetone, acetone and methanol which are potential interferents in ethanol analysis.

There was no significance difference in blood ethanol concentration between nationalities, between sexes within nationalities or between nationalities. The data was therefore pooled and the overall mean ± SD, minimum, maximum, 5% percentile and 95% percentile were 0.14 ± 0.35, 0.00, 1.53, 0.00 and 1.20 mg/dl respectively.

The percentage of sample with blood ethanol concentration of zero mg/dl, between less than 0.5 mg/dl, 0.5 to 1 mg/dl and more than 1 mg/dl were 78.8%, 08.4%, 4.4% and 8.4% respectively (Table 4). The concentration of blood ethanol was not correlated with age (r2= 0,004, p= 0.98).

Discussion

The occurrence of small amounts of ethanol in the blood in the

Blood Alcohol Level (mg/dl)	Minimum	Maximum	Mean ± SD	No. (%)
Group I ND (Zero level)	0.00	0.00	0.00	1104 (78.8%)
Group II ND (<0.50 mg/dl)	0.02	0.45	0.18 ± 0.15	118 (8.4%)
Group III (0.50-1.00 mg/dl)	0.50	0.98	0.70 ± 0.17	61 (4.4%)
Group IV(>1.00 mg/dl)	1.01	1.53	1.26 ± 0.16	117 (8.4%)
ALL GROUPS	**0.00**	**1.53**	**0.14 ± 0.35**	**1400 (100%)**

Table 4: Concentrations of endogenous ethanol in whole blood of healthy subjects under uncontrolled conditions.

blood and tissue from normal human subjects has interested scientists for more than a century [5]. The present method has proved to be quick, efficient and specific, due to use of mass spectrum for detection and by using one quantitative ion and two qualifier ions including the molecular ion of the compound. Qualifier ions have to lie within the time window of the compound and satisfy ± 20% relative abundance ratio relative to the quantitative ion.

The mean ± SD blood alcohol concentration reported in this study is low (0.14 ± 0.35 mg/dl) and agrees with earlier reports [4-9] which support the presence of endogenous ethanol levels. One survey report after 1958, which measured the endogenous ethanol in whole blood, plasma, serum, urine and breath. The mean blood ethanol concentrations in all reports were less than 1 mg/dl except for one which reported a value of 3.9 mg/dl [10,11]. It should be noted, however, that the sample size in those reports was small and ranged from 5 to 170. The maximum blood ethanol concentration reported in this study was 1.53 and 1.41 mg/dl in males and females, respectively. It is possible that those values were obtained from persons who take medications which contain alcohol. Further evidence of continuous endogenous production of ethanol in human was presented by the study of Sarkola and Eriksson [7] who used 4-methyl pyrazole, a specific competitive inhibitor increase in ethanol levels over time after taken 4-methylpyrazole compared with a placebo. Another significant elevation in ethanol was also observed after intake of lingberry juice containing about 40 g of carbohydrates. They concluded that in human there is a persistence endogenous production as well as clearance of ethanol and that it is, at least in part, metabolized by alcohol dehydrogenase. The effect of juice in increasing endogenous levels is well documented and suggested that it is produced by intestinal micro flora [2,6,7]. The variability of blood alcohol levels seen in this study might be due to the different diets of subjects, which contain different amounts of carbohydrates.

Some researches indicated that the normal production of alcohol might result in blood levels, which are defensible in court [12-14]. However, the result of those study and from others [4-9] indicate that the current legal limit of blood alcohol in many countries are too large to be blood ethanol concentration resulting from carbohydrates fermentation in gut would occur if its production rate exceeds the metabolism of ethanol of 6-8 g/h by alcohol dehydrogenase [15]. As in Saudi Arabia, where the legal limit of blood ethanol concentration is too high 30 mg/dl. This however, is unlikely as the enzyme alcohol dehydrogenase; with a km value of 5-10 mg/dl is efficient in metabolizing ethanol at low concentration [15]. Furthermore, unmetabolised ethanol would further be diluted with body water which is 50-69% of body weight. Exception might be expected in persons with abnormal proliferation of Candida in the gut and with gut stagnation [12]. The values of blood ethanol reported in this study and those reported by others indicates that they are far too low to have any forensic significance.

Conclusion and Recommendation

In Saudi Arabia the current cut-off point of positive blood ethanol level for Medico-legal issues was 30 mg/dl that put the huge high level from current discovered mean endogenous ethanol blood level 0.16 mg/dl and the maximum ethanol level 1.53 mg/dl results. So, the great difference range from detected endogenous ethanol production and the cut-off point of positive ethanol level related to variables medico-legal issues is unaccepted in Saudi Arabia and Zero level of alcohol should be considered. Finally, zero tolerance for blood alcohol should be applicable in Saudi Arabia because the unexplained very high cut-off level for blood alcohol level that is released from endogenous ethanol production become completely omitted with the results from the current research.

Consent

Informed consent was obtained from each of the studied case.

Conflict of Interests

The authors declare that they have no competing interests.

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Ethical Approval

Approval for this study was granted by the Dammam Poison Control Center-Ethical Research Committee, 2-135/2014.

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