



## Ethanol levels in legally autopsied subjects: Analytical approach and epidemiological relevance in a prospective study in the touristic region of the Canary Islands (Spain)



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### ABSTRACT

The aim of this study was to analyze the presence of alcohol in individuals (137) that were subjected to a mandatory medico-legal autopsy in the Institute of Legal Medicine of Las Palmas (Canary Islands, Spain) during 2015. Blood and vitreous humor samples (232) were analyzed by gas chromatography. 46.0% of the individuals were positive for alcohol, being half of them tourists. Blood alcohol concentrations (BAC) was higher in men over 60 years ( $p = 0.041$ ). 10.2% of the series died in a traffic accident, and victims had the highest proportion of positives to alcohol (64.3%) and the highest BAC (2.56 g/L,  $p = 0.048$ ). The BAC:VHAC (alcohol in vitreous humor) ratio was higher among victims of traffic crashes ( $p = 0.036$ ), suggesting a short elapsed interval between the alcohol intake and the fatal accident. The results of this study indicate that ethanol is still heavily involved in non-natural deaths.

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### 1. Introduction

Ethanol is a psychoactive substance with addictive properties. The World Health Organization (WHO) has estimated that there were 2100 million regular consumers of alcohol in the world, which would mean that about 43% of the world's adult population consumes alcohol.<sup>1</sup> Furthermore, it was estimated that there were 3.3 million deaths directly attributable to alcohol, representing 5.9% of all deaths that year worldwide.<sup>2</sup> Alcohol consumption is considered

a very important risk factor for traffic accidents and for other causes of accidents, and is frequently involved in deaths classified as violent by its accidental, suicidal, or homicidal origin.<sup>3</sup> For all this reasons, alcohol usually tops the list of psychoactive substances found in postmortem toxicological analysis.<sup>4</sup>

The percentage of alcohol consumers varies widely by region, from as low as 9.8% in Asian countries,<sup>1</sup> to close to 90% of the adult population in European countries.<sup>5</sup> Within Europe, it has been estimated that alcohol consumption ranges from 66% in the countries of Eastern Europe to 88.2% in the countries of Western Europe.<sup>1</sup> In the case of Spain, the data for the period 1997–2007 indicated that about 60% of the population over 15 years consumed alcohol regularly.<sup>6</sup> The statistics indicate that in subsequent years this figure increased to almost 65% of the population between 15 and 64 years old.<sup>7</sup> It is interesting to note the change in trend from the year 2007, which is the year of the beginning of the economic crisis that still affects this country. In that sense, the economic crisis is one of the reasons proposed to explain the increase in alcohol consumption in the last years. It is well known that psychological

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distress caused by unemployment and reduced income may increase drinking problem.<sup>8</sup> Other proposed cause have to do with the apparent increase in alcohol consumption in adolescence, associated with the widespread fashion in Spain during the last decade of consuming large amounts of alcohol on the public road. In fact, it has been recently reported that 41.6% of boys aged between 14 and 18 y.o. consume alcohol in the street on weekends.<sup>9</sup> Finally, the increase in alcohol consumption in Spain could be influenced by the fact that the tourism sector has grown steadily, reaching the highest position in this sector worldwide according to the World Economic Forum World.<sup>10</sup> This is relevant because in periods of leisure (holidays), alcohol consumption increases,<sup>11</sup> and it is also remarkable that workers employed in the tourist sector also show a higher prevalence of alcohol consumption.<sup>12</sup> In Spain, tourism employs more than half million people.

The Spanish archipelago of the Canary Islands meets several of the above cited features. On one side, it is one of the Spanish regions hardest hit by the crisis, with a per capita income well below the national average,<sup>13</sup> an unemployment rate higher than the national average (23.70 vs. 31.08%, in mainland Spain and in the Canary archipelago, respectively), and a youth unemployment rate above 50%.<sup>14</sup> On the other hand, the Canary Islands is a very important touristic region in the European Union (EU), receiving around 12 million tourists per year,<sup>15</sup> and this activity generated in 2014 35.9% of the employment in the region (almost 275,000 jobs).<sup>16</sup> Despite these circumstances, epidemiological studies suggest that the prevalence of alcohol consumption in this region is lower than the national average (55.6% in the Canary Islands vs. 64.4% in mainland Spain for people aged 15–64 years).<sup>7</sup> However, these data may not accurately reflect the level of alcohol consumption in the Archipelago since it only considers the population permanently residing in the islands, but does not take into account the huge “floating” population, mainly tourists, that continuously visit the Canary Islands. In this sense, it is worthy to mention that a strong relationship between tourism and recreational use of alcohol exists.<sup>11,17</sup>

The aim of this work was to prospectively evaluate the prevalence and relevance of ethanol consumption in a highly touristic region. For this purpose, we measured ethanol levels in all the victims died in violent deaths which were autopsied at the Institute of Legal Medicine of Las Palmas, based on the premise that, despite current laws and educational interventions, alcohol intake continues to be a relevant factor commonly involved in the violent deaths in our region, specifically in traffic crashes.

## 2. Materials and methods

The Canary Islands are located 1600 km away from southwest Spain, in the Atlantic Ocean, and hardly 100 km from the nearest point of the North African coast (southwest of Morocco). The economy of the Canary Islands is based fundamentally on tourism and to a much lesser extent on farming, livestock production, and fishing. The archipelago has a resident population of about 2 million inhabitants and a visitant population close to 12 million people each year (mainly tourists). The archipelago is divided into two provinces: Las Palmas and Santa Cruz de Tenerife. The Institute of Legal Medicine of Las Palmas serves a population of 1.1 million inhabitants from the province of Las Palmas.<sup>18</sup>

The universe selected represented 100% of victims ( $n = 137$ ) of violent deaths that were subjected to medico-legal autopsy at the Institute of Legal Medicine of Las Palmas during 2015. A quantitative determination of ethyl alcohol was performed in blood and vitreous humor samples ( $n = 232$ ). The sociodemographic characteristics of the victims were obtained from the forensic reports and were studied in relation to the results of toxicological analyses. The

data collected included age, gender, geographical origin (resident vs. non-resident), type of sample, cause of death, date of autopsy and concentration of alcohol. The cases represent all the medico-legal autopsies in the province of Las Palmas in 2015.

Samples were analyzed in a Trace-Focus GC headspace gas chromatography (GC-HS) equipped with a flame ionization detector (FID) (Thermo Fisher Inc., Waltham, MA, USA). This method is of choice in forensic sciences for determination of ethanol and other volatiles in body fluids.<sup>19–21</sup> Samples were diluted 1:50 in a solution of NaCl 8% to a final volume of 5 mL. Tert-Butanol was used as internal standard.<sup>22</sup> The mixture was kept airtight in 10 mL glass vials with crimped-on rubber septum (Thermo Fisher Inc.). The vials were heated to 85 °C for 10 min, and a total of 1 mL of vapor was automatically injected with a TriPlus automated sampler (Thermo Fisher Inc.) equipped with a 2.5 mL gas-tight syringe (Hamilton, Reno, NE, USA) in split mode with a split ratio of 5.

Gas chromatography separation was performed on a J&W HP-Blood Alcohol column of 7.5 m length, 20  $\mu$ m film thickness, and 0.32 mm ID (Agilent Technologies, Santa Clara, CA, USA). Helium (99.999% pure) was used as carrier gas. The separation run was completed in 6.80 min in a gradient temperature program as follows: 120 °C x 1 min, a ramping of 25°C/min till 165 °C and a final step of 165 °C x 4 min.

The peak areas were quantified with the Chrom-Card data system (Thermo Fisher Inc.) using a commercial standard curve of 5 points from 0.05 to 3 g/L of ethanol in duplicate. In each experiment, one negative and two positive controls were included in duplicate. A variation coefficient of  $\leq 10\%$  and an accuracy of  $\geq 95\%$  were required in all analyses.

Standard descriptive statistics were calculated for the levels of ethanol in all samples. The distribution of ethanol deviates significantly from normality. Hence, nonparametric tests were applied. Spearman's correlation coefficients were used to calculate the associations between ethanol concentrations in blood and vitreous humor. Differences between categorical variables were assessed by  $\chi^2$  test. A value of  $p < 0.05$  (two-tailed) was considered to be statistically significant. Database management and statistical analyses were carried out with the PASW v 17.0 software (SPSS Inc., Chicago, IL).

## 3. Results and discussion

A total of 139 autopsied subjects involved in violent deaths were included in our study. Blood was available in 29 subjects, vitreous humor was available in 13 subjects and blood and vitreous humor were both available in 95 subjects. Two subjects were excluded from the series because only gastric content samples were taken and data about alcohol level were not available. Thus, the series was formed by 137 subjects.

Information on gender and age were not available in some cases, with a missing percentage of 0.7% for age (1 case corresponding to a neonate) and 7.3% (10 cases) for gender. Cause of death was available in all cases. These percentages of missing data are similar to those reported by other authors in similar studies.<sup>23,24</sup> Finally, the series was formed by 96 men (70.1%) and 40 women (29.2%), and showed a mean age of  $50.7 \pm 14.8$  years old (median = 51, range = 0–82 years). There were no differences in age distribution according to gender ( $p = 0.843$ , data not shown).

A total of 63 cases were positive for alcohol in blood and/or vitreous humor (46.0% of the series). It has drawn our attention that half of the subjects which were positive for alcohol, were tourists (Table 1), reflecting the fact that there is a large floating population on the islands whose alcohol consumption might be relevant.<sup>11,17</sup> The proportion of ethanol positive determinations was higher among men (50.0 vs. 37.5%), although this difference was not

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