Psychiatric comorbidity and plasma levels of 2-acyl-glycerols in outpatient treatment alcohol users. Analysis of gender differences

Comorbilidad psiquiátrica y valores plasmáticos de 2-acilgliceroles en consumidores de alcohol en tratamiento ambulatorio. Análisis de las diferencias de género

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Abstract

Alcohol addiction is associated with high psychiatric comorbidity. Objective stratification of patients is necessary to optimize care and improve prognosis. The present study is designed to gain insights into this challenge by addressing the following objectives: a) to estimate the prevalence of psychiatric comorbidities in a sample of outpatients seeking treatment for alcohol use disorder, b) to describe the existence of gender differences and c) to validate 2-acyl-glycerols as biomarkers of alcohol use disorder and/or psychiatric comorbidity. One hundred and sixty-two patients were recruited and evaluated with the semi-structured interview PRISM. The presence of psychopathology was associated with a greater number of criteria for alcohol abuse and dependence according to DSM-IV-TR. We found gender differences in psychiatric comorbidity, e.g., mood disorder, as well as in comorbid substance use disorders. The prevalence of lifetime psychiatric comorbidity was 68.5%, with mood disorders the most frequent (37%), followed by attention deficit disorder (24.7%) and anxiety disorders (17.9%). Substance-induced disorders were more frequent in mood and psychotic disorders, whereas the primary disorders were more prevalent in patients with comorbid anxiety disorders. We found that 2-acyl-glycerols were significantly decreased in comorbid anxiety disorders in alcohol dependent patients in the last year, which makes them a potential biomarker for this psychopathological condition.

Keywords: Psychiatric co-morbidity; Addiction; Alcohol; Outpatient; Gender; 2-acyl-glycerols.

Resumen

La adicción al alcohol se asocia con una elevada comorbilidad psiquiátrica que complica el tratamiento, siendo necesaria una fenotipación clínica objetiva de estos pacientes para optimizar la atención y mejorar el pronóstico. El presente estudio aborda este problema mediante los siguientes objetivos: a) estimar la prevalencia y tipos de comorbilidad psiquiátrica de una muestra de pacientes que buscan tratamiento por uso de alcohol, b) describir las diferencias de género en su presentación y c) analizar los valores plasmáticos de 2-acilgliceroles (incluyendo el endocannabinoide 2-araquidonilglicerol), estudiando su posible valor como biomarcador de alcoholismo y/o comorbilidad psiquiátrica. Para ello se reclutaron 162 pacientes evaluados con la entrevista semiestructurada PRISM, para evaluar la presencia de comorbilidad y su carácter primario o inducido. Los resultados obtenidos indican que la presencia de psicopatología se asoció a un mayor número de criterios de abuso y dependencia de alcohol Se encontraron diferencias de género tanto en la comorbilidad psiquiátrica, especialmente en trastornos del estado de ánimo. La prevalencia de comorbilidad psiquiátrica encontrada a lo largo de la vida fue del 68,5%, destacando los trastornos del estado ánimo (37%), y seguidos por el trastorno por déficit de atención (24,7%, monitorizado específicamente por la entrevista WURS) y los trastornos de ansiedad (17,9%). Entre los trastornos del estado de ánimo y psicóticos fueron más frecuentes los inducidos, mientras que en los trastornos de ansiedad los primarios fueron más prevalentes. Además, se encontraron concentraciones disminuidas significativamente de 2-acilgliceroles en pacientes con trastornos de ansiedad comórbidos diagnosticados en el último año.

Palabras clave: Comorbilidad psiquiátrica; Adicción; Alcohol; Ambulatorio; Género; 2-acilgliceroles.

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Alcohol is a psychoactive substance with addictive properties, the consumption of which can have powerful economic, social and health impacts on the individuals who consume it. The harmful use of this substance is an etiological factor in mortality at a global level, with 5.9% of deaths up to 2012 found to have a link to alcohol (World Health Organization, 2014). Although consumption trends have stabilised in Spain over the last 10 years, levels of alcohol use are still high. A 4.9% rate of problematic alcohol use is observed among the 15-65 year-old population, with 4.5% presenting high-risk consumption [1,600,000 people, 1,300,000 men and 300,000 women]. The average age of alcohol consumption onset is 16.7 years and alcohol is linked to a greater prevalence of the consumption of other drugs, since alcohol is present in 90% of polydrug consumption patterns (EDADES, 2013)

Given the frequency of alcohol use disorders (AUD), the association with other medical complications presents a challenge to health systems. Among these, psychiatric comorbidity in alcohol addiction, i.e. the co-existence in one person of AUD alongside another disorder distinct from the addiction, constitutes a serious health problem (Goldsmith, 1999) which demands a differential approach. Patients with psychiatric comorbidity are a risk group from a clinical and social perspective. They access hospital services more frequently (Ruffles, 2009), have higher suicide rates (Fiedler et al., 2012) and respond less well to treatment than patients with only AUD (Karila et al., 2012). Furthermore, from a social point of view, they provide a greater source of conflict at occupational, judicial and social inclusion levels (Karila et al., 2014).

AUD patients are 2 to 4 times more likely to suffer a depressive disorder during their lifetime than those who are not alcohol dependent (Hasin et al., 2005, 2007; Kessler et al., 1997; Ross, 1995). The National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) in North America reports rates of 39.5% in AUD populations compared to 14.8% in the general population, as well as a high psychopathological comorbidity of AUD with disorders of mood (MD), anxiety (AD) and personality (PD) (Grant et al., 2004). With regard to psychotic disorders, these are found within the general population at a rate of 0.4%, while among the AUD population this rises to 4.0% (Engelhard et al., 2015).

These data from North America are matched by research results in Europe. In Copenhague, AUD prevalence was 7,6% and half of them had a psychopathological comorbidity: 24% PD, 16,8% MD and 16,6% another SUD (Flensborg-Madsen et al., 2009). The prevalence of AUD in our country in 2010 was 2.3% in men and 1.3% among both sexes (WHO, 2014). Disorders associated with alcohol use, such as intoxication or withdrawal symptoms, impair the majority of social relations (Blanco et al., 2015). Patients with high severity of alcohol dependence disorder are very prone to emotional and anxiety disorders (Blanco et al., 2015). In one study carried out among hospitalised patients in Madrid, for example, 24.9% presented substance abuse, with the majority of them (78.1%) also suffering from AUD (Rodriguez-Jimenez et al., 2008).

The profile of patients needing treatment for AUD is different from that of patients with substance use disorders (SUD), given that the typical withdrawal symptoms of AUD have an effect on the search for and motivation to enter a treatment programme (Blanco et al., 2015). For all these reasons, subjects with AUD have a greater degree of comorbidity with other mental disorders than the general population, and this comorbidity leads to more severe forms of AUD, greater disability and a higher likelihood that they will seek treatment at mental health centres, which reinforces the need for accurate detection of AUD in patients attending these.

Both SUD psychiatric comorbidity as well as the presence of non-substance related psychiatric comorbidity are factors which contribute to the worsening of alcohol abuse to the point of dependence. In terms of AUD comorbidity and gender differences, it is known that women start drinking later and develop dependence more quickly than men (Keyes et al., 2010). Among AUD patients, men have a greater prevalence of personality disorders, while women are more frequently affected by other psychiatric disorders (Ávila Escribano et al., 2007). Given the gender differences we believe it is necessary to establish programmes and strategies to control the excessive consumption of alcohol and its growing use among the female population, while also describing the similarities and differences in the patterns of use and psychiatric comorbidity that might be found.

We can see that there are very few studies dealing with the phenomenon of psychiatric comorbidity in the AUD population in Spain. The present study may therefore serve as a reference in extending this field of knowledge, especially in the hospital outpatient context.

One of the basic problems of clinical practice in treating addictions is the lack of objective biological tests to determine the level of consumption, the severity of SUD, toxicity and the response to treatment of this type of patient. These objective tests, or biomarkers, are fundamental to the diagnosis, stratification, prognosis and treatment approach with regard to addictive disorders. One way of searching for biomarkers is to explore compartments of cell signalling in connection with the changing responses to addictive substances, as has been observed in patients who consume cocaine (Araos et al., 2014; Pavón et al., 2013; Pedraz et al., 2015). The present study is focused on signal molecules called acylglycerols, ethers formed from fatty acids. The best known molecules of this type include 2-araquidonil-glycerol (2-AG) and 2-linoleoyl-glycerol (2-LG). The 2-AG is the principal endogenous endocannabinoid and its activity is linked to the preference for alcohol as well as the development of

tolerance, as many studies have borne out in preclinical models (Basavarajappa et al., 2005; Caillés et al., 2007; Malinen et al., 2009; Serrano et al., 2012). These molecules are present in the central nervous system and more abundant than other lipid transmitters such as the N-acyl-ethanolamines, with which they share their endogenous endocannabinoid profile (Piomelli, 2003).

In sum, this study has been designed to assess the prevalence of psychiatric comorbidity among alcohol use disorder patients under outpatient treatment. We describe gender differences and determine the plasma levels of N-acyl-ethanolamines and their value as biomarkers.

Method

Study design and target population

In 1994, a specific programme was set up in Madrid's 12 de Octubre hospital with the aim of providing an integrated response for AUD patients in the shape of a mixed medical-psychiatric unit, bringing together patients from the psychiatry and internal and digestive medicine departments. This unit has proceeded to recruit patients to carry out an observational descriptive transversal study in order to determine the prevalence of psychiatric comorbidities among subjects seeking treatment at the hospital, and to obtain plasma samples for the validation of the 2-acylglycerols as biomarkers.

The study sample consisted of 262 participants divided into two groups. The first group included abstinent patients undergoing outpatient treatment for alcoholism in the programme for addictive behaviour disorders at the 12 de Octubre hospital in Madrid after being diagnosed with AUD. The second group was a control group of patients without any previous diagnosis of illegal substance abuse and/or dependence, and with no history of concomitant psychiatric diagnosis (both according to DSM-IV-TR diagnostic criteria). The clinical assessment study included 162 patients taking part in the above mentioned programme, of which 133 consented to provide a biological sample, and 100 control patients matched for age, sex and body mass index.

To calculate the sample size, the work of the Pérez-Gálvez team (Pérez-Gálvez et al., 2008) was used as a reference point. Here, psychiatric comorbidity in AUD patients is approximately 70%. In order to obtain an accuracy of 8% in the estimation of proportion with a normal asymptotic confidence interval of 95% bilaterally, assuming the proportion is 70%, a sample of approximately 160 subjects would be necessary. Given the aims of the study, a pragmatic attitude was taken in the selection of subjects for the sample. For this purpose, non-restrictive criteria were used in order to maximise the representativeness of the selected sample and the extrapolation of the study's results. Consecutive and non-random sampling was applied on patients as they arrived at the hospital outpatients unit after checking that they met the selection criteria.

These criteria were: being a patient on the programme and under treatment for AUD, with at least 30 days' abstinence, willing to participate and giving informed consent. The criteria for inclusion in the control group was not having a record of substance abuse or dependence, concomitant psychiatric comorbidity, and a signed letter of informed consent.

Reasons for exclusion from both study and control groups were the presence of cognitive disorders which would hamper the application of diagnostic assessment instruments, and the patient's refusal to participate in the study.

The ethical aspects of the study were approved by the ethics committee of the Clinical Research Department of the 12 de Octubre hospital in Madrid. All patients were informed and only those signing the letter of informed consent were approved by the committee. The study was part of the "Medical consequences of alcoholism" programme of the Carlos III Health Institute's Addictive Disorders Network (Red de Trastornos Adictivos).

The assessment process was carried out by a general health psychologist with special training and qualified in psychopathological assessment.

Procedure

The programme's psychiatrists and nurses collaborated in informing the patients during consultations and group therapy sessions about the existence of the study in the hospital, referring them to the research team if they met the inclusion criteria and agreed to take part. After arranging an appointment with the patient, once the informed consent letter had been signed, clinical assessment was carried out in the Oupatient Activity Centre (Centro de Actividades Ambulatorias) of Madrid's 12 de Octubre university hospital.

Psychopathological assessment was carried out in a single morning during an individual consultation lasting between one and two hours. The interviews took place between October 2013 and March 2015, and on completion of each interview the results were registered in the database designed for the purposes of this study.

Measurement instruments

PRISM. The PRISM diagnostic interview (*Psychiatric Research Interview for Substance and Mental Diseases*) is the first instrument to evaluate psychiatric and substance related disorders. It is a semi-structured clinical interview designed to solve the problems of diagnosing patients with level of alcohol and/or substance consumption, assessing the subject's consumption history in the first module and generating an abuse and dependence diagnosis covering both the previous year and before. In addition, it evaluates 20 Axis I disorders and the two most prevalent Axis II disorders in this population: borderline personality disorder and antiso-

cial personality disorder. The diagnoses focus on two time frames, with the interview assessing the current disorders, i.e. those present during the last year on the one hand, and those existing before the last year on the other. In general terms, the subject's lifetime diagnostic prevalence would include all diagnoses made previously in both periods, i.e. the most recent and those made earlier.

One of the most important festures of this instrument is that it makes it possible to differentiate between substance-induced disorders and the expected symptoms of the effects of intoxication and withdrawal. The PRISM criterion for whether a psychiatric disorder is substance induced is that it must occur within the context of pathological use of the substance, in either of the following two situations: a) chronic intoxication, i.e. substance use on four days or more per week over the course of a month; b) bingeing over three consecutive days. To differentiate the induced psychiatric symptoms from those expected during intoxication or withdrawal, a sudden change in consumption patterns needs to be detected (Hasin et al., 1996; Torrens et al., 2004).

This interview has good test-retest reliability, validity and inter rater reliability, with the Kappa coefficient ranging from 0.66 to 1.00 (Morguello et al., 2006).

WURS. As a result of the special interest surrounding the link between AUD and attention deficit and hyperactivity disorder (ADHD), it was decided to test for the the prevalence of ADHD using a further instrument, the WURS (*Wender-Utah Rating Scale*). This test is used for the restrospective assessment ADHD in adult patients. It is a self-administered questionnaire with 61 items, from which 25 were selected for their capacity to differentiate adult patients with a childhood history of ADHD from other populations such as patients with depression or in control groups. The WURS has shown internal reliability and stability over time in various studies. It includes questions regarding mood, relationship problems with family members, colleagues and figures of authority, as well as medical, school and academic problems. The Cronbach coefficient for the subscale was 0.94. The cut-off point of 32 optimised sensitivity (91.5%) and specificity (90.8%). The positive and negative predictive values were 81% and 96% respectively (Rodríguez-Jiménez et al., 2001).

CIDI. For the psychiatric assessment of the participants in the control group, the Spanish version of the Composite International Diagnostic Interview (CIDI) (Robins et al., 1988) was applied. This structured interview has a section which assesses 22 diagnoses of different disorders in a screening for mood, anxiety and substance use disorders, early-onset disorders in childhood and others (such as personality and psychotic disorders) (Kessler and Ustün, 2004). In addition, for the assessment of substance use disorders in the control group, the history of substance use section of the PRISM interview was applied.

Processing blood samples and obtaining plasma samples

Peripheral blood samples were obtained from the 233 patients (133 in abstinence and 100 control subjects) in the morning, 8-12 hours after last eating or drinking and on the same day as the interview. Samples of 10ml of peripheral blood were taken using BD vacutainer® tubes with K2 EDTA by nursing staff participating on the project. To obtain the plasma, the samples were centrifuged at room temperature at 2200 xG for 15 minutes. Tests were immediately carried out on each sample to eliminate the presence of infectious diseases (AIDS-HIV, hepatitis B and C). Each plasma sample was registered and and recorded individually and those containing any kind of infection were rejected in accordance with safety protocols. Finally, all samples were frozen at -80°C for later analysis. The time between extraction and freezing at no point exceeded 30 munites.

Analysis of 2-acyglycerol plasma concentrations

The plasma samples were processed following standard techniques after organic extraction of plasma lipids (Pavón et al., 2013). Chromatic separation was carried out using a Zorbax 80 Å StableBond C8 column (2.1 x 100 mm, 1.8 µm particle size) maintained at 40° C, with a mobile phase of 0.4 ml/min flow. The composition of the mobile phase was A) 0.1% (v/v).

formic acid in water; B) 0.1% (v/v) formic acid in acetonitrile. The initial conditions were 40% B. The gradient was increased linearly to 100% B over 4 min, maintained at 100% B for 4 min, and returned to the initial conditions for a further 5.5 min, with a total run time of 13.5 min. The tandem quadrupole mass spectrometer operated on the positive electrospray mode. Desolvation gas temperature of 350°C and a gas flow rate of 10 l/min were used. The pressure of the nebulizer was set at 40 psi and the capillary voltage at 4,000 V. The fragmentor was set at 135 V and 20 ms for all analytes. The monitoring mode for multiple reactions was used for the analysis with the following precursors to product ion transitions: m / z 379.2 / 287 for 2-AG, m / z 384.3 / 287 for 2-AG-d5 and m / z 355.2 / 263 for 2-LG. An external calibration curve of 6 points in the mobile phase (10:90, A: B) was used with the addition of 0.8 at 50 ng of acylglycerols for quantification as has been described by our group in a previous study (Pavón et al., 2013).

Description of the study

The descriptive study aimed to research: the existence of gender differences in the socio-demographic characteristics and alcohol consumption patterns; the prevalence of psychiatric comorbidity among the participating patients with AUD and SUD (abuse or dependence); the frequency of psychiatric comorbidity among non-SUD patients; and finally, the analysis of 2-acyl-glycerol plasma levels in the population in comparison to the control population, while also

Table 1. *Socio-demographic variables by sex in a population of alcohol users under treatment.*

Variables		Total	Men	Women	Value p
Number of patients [N(%)]		162 (100)	122 (75,3)	40 (24,7)	-
Age [mean (SD)]		49,3 (8,1)	49,1 (8,5)	49,9 (6,9)	0,575 [1]
Body mass index [mean (SD)]		26,2 (4,5)	26,8 (4,5)	24,1 (3,9)	**‹0,001** [1]
Marital status [N(%)]	Single	47 (29,0)	34 (27,9)	13 (32,5)	**‹0,05** [2]
	Married	60 (37,0)	50 (41,0)	10 (25,0)	
	Separated/Divorced	51 (31,5)	37 (30,3)	14 (35,0)	
	Widow(er)	4 (2,5)	1 (0,8)	3 (7,5)	
Educational level [N(%)]	No school	4 (2,5)	3 (2,5)	1 (2,5)	0,200 [2]
	Primary	53 (32,7)	45 (36,9)	8 (20,0)	
	Secondary	83 (51,2)	60 (49,2)	23 (57,5)	
	University	22 (13,6)	14 (11,5)	8 (20,0)	
Work situation [N(%)]	Employed	46 (28,4)	36 (29,5)	10 (25,0)	0,466 [2]
	On sick leave	27 (16,7)	23 (18,9)	4 (10,0)	
	Unemplyed/Invalidity	68 (42,0)	49 (40,2)	19 (47,5)	
	Retired	17 (10,5)	14 (11,5)	3 (7,5)	
	Domestic	4 (2,5)	-	4 (10,0)	
Criminal record [N(%)]	Yes	40 (24,7)	37 (30,3)	3 (7,5)	**‹0,01** [2]
	No	122 (75,3)	85 (69,7)	37 (92,5)	
Other medical problems [N(%)]	No	89 (54,9)	65 (53,3)	24 (60,0)	0,215 [2]
	Endocrine	8 (4,9)	4 (3,3)	4 (10,0)	
	Circulatory	23 (14,2)	20 (16,4)	3 (7,5)	
	Digestive	33 (20,4)	27 (22,1)	6 (15,0)	
	Nervous system	9 (5,6)	6 (4,9)	3 (7,5)	
Patient referred by [N(%)]	Psychiatry	120 (74,1)	86 (70,5)	34 (85,0)	0,192 [2]
	Internal/digestive Medicine	35 (21,6)	4 (3,3)	1 (2,5)	
	Mental Health Centre	5 (3,1)	30 (24,6)	5 (12,5)	
	Other	2 (1,2)	2 (0,16)	-	
Initial psychiatric treatment [N(%)]	‹1 year	38 (23,5)	29 (23,8)	9 (22,5)	0,167 [2]
	1-5 years	46 (28,4)	36 (29,5)	10 (25,0)	
	5-10 years	16 (9,9)	15 (12,3)	1 (2,5)	
	›10 years	62 (38,3)	42 (34,4)	20 (50,0)	
Psychiatric medication [N(%)]	Yes	123 (75,9)	86 (70,5)	37 (92,5)	**‹0,01** [2]
	No	39 (24,1)	36 (29,5)	3 (7,5)	
Family history of addiction [N(%)]	Yes	100 (61,7)	45 (36,9)	17 (42,5)	0,576 [2]
	No	62 (38,3)	77 (63,1)	23 (57,5)	
Smoker [N(%)]	Yes	111 (68,5)	80 (65,6)	31 (77,5)	0,218 [2]
	Ex-fumador	26 (16,0)	23 (18,9)	3 (7,5)	
	No	25 (15,4)	19 (15,6)	6 (15,0)	
Substance use disorder (SUD) [N(%)]	Only AUD	98 (60,4)	68 (55,7)	30 (75,0)	**‹0,05** [2]
	AUD + other SUDs	64 (39,6)	54 (44,3)	10 (25,0)	
Other psychiatric comorbidity disorder* [N(%)]	Yes	111 (68,5)	78 (63,9)	33 (82,5)	**‹0,05** [2]
	No	51 (31,5)	44 (36,1)	7 (17,5)	

Note. Abbreviations: N=number of subjects; SD=standard deviation; AUD=alcohol use disorder; SUD= substance use disorder.
Value p is the level of significance calculated by: (1) Student's t test, and (2) Fisher's exact test or chi-square.
(*) Substance use disorders are excluded (SUD)

establishing the links that exist with the psychiatric comorbidity of the participating patients.

Statistical analysis

The data was expressed in numerical terms, percentage of subjects [n (%)], means and standard deviations (SD). The differences between categorical variables were determined using Fisher's exact test or the Chi-square test (χ^2), while continuous variables were measured with different statistical approaches depending on the number of comparisons and the distributions involved. For the two-group comparisons the Student's t-test was used for continuous variables with normal distributions and the Mann-Whitney test was applied as a non-parametric test. For comparisons of three groups or more, the Kruskal-Wallis test was used, with Dunn's post-test being used for non-parametric analyses. Normal distribu-

tions were tested for using the D'Agostino and Pearson tests for general normality. The lower *p*-value of 0.05 was considered statistically significant.

The statistical programmes SPSS version 19.0 (SPSS Inc., an IBM Company) and version 5.04 of Graph-Pad Prism (GraphPad Software, San Diego, CA, USA) were used for statistical analyses.

Results

Socio-demographic characteristics and gender differences

Table 1 shows the results of the socio-demographic analysis.

With regard to gender differences in the socio-demographic variables, we can say that 75.3% of the sample was made up of men, and 24.7% were women, without differences in terms of age [mean: 49.3 years (SD=8.1)]. The body mass index, however, did display very significant differences (*p<0.01) between sexes, being higher in women [mean: 24.1 (SD =3.9)] than men [mean: 26.8 (SD=4.5)]. Other very significantly different gender differences (*p<0.01) were found in criminal records and psychiatric medication. Men have more criminal records, while women take more psychiatric medication. Other significant differences (*p<0.05) were found in marital status, with 41% of men and 25% women being married, and substance use disorders (SUD), with 25% of women having AUD comorbidity as against 44.3% of men. Finally, there were significant differences (*p<0.05) in psychiatric comorbidity with other disorders unrelated to substance use, with 82.5% of women presenting such comorbidity compared to 63.9% of men in this variable.

Alcohol consumption patterns in terms of sex and psychiatric comorbidity

Table 2 shows the variables related to acohol consumption patterns, gender differences and psychiatric comorbidity with disorders not related to substance use.

Highly significant differences (*p<0.01) were found in the drinking onset age variable. There are very significant differences (*p<0.01) between the two comorbidity groups, with women starting to drink alcohol later [mean age 18.2 years (SD=3.2)] than men [mean age 15.92 (SD=3.6)]. There were also significant differences (*p<0.05) in the onset age for problematic drinking between sexes and psychiatric comorbidity. Here, there were also significant specific differences (*p<0.05) between men with psychiatric comorbidity [mean age 25.73 years (SD=9.8)] and women with psychiatric comorbidity [mean age 32.5 years (SD=12.9)]. These data tell us that women start drinking later than men and also begin problematic drinking later.

Highly significant differences (*p<0.001) are also found in number of years of problematic drinking, where we also see significant differences (*p<0.05) between the two psy-

Table 2. *Alcohol consumption patterns by sex and diagnosis of lifetime psychiatric comorbidity in a population of drinkers under treatment.*

Alcohol consumption patterns	**Total** N=162	**Men** N=122		**Women** N=40		**Value p**
		Without psychiatric comorbidity*	**With psychiatric comorbidity***	**Without psychiatric comorbidity***	**With psychiatric comorbidity***	
Onset age of alcohol use [mean (SD)]	16,8 (4,2)	17,30 (5,4)	15,92 (3,6)	16,2 (2,4)	18,2 (3,2) bb	‹0,01
Onset age of problematic alcohol use [mean (SD)]	28,5 (11,5)	29,32 (11,9)	25,73 (9,8)	34,4 (13,3)	32,5 (12,9) b	‹0,05
Duration of problematic consumption (years) [mean (SD)]	14,9 (7,8)	17,2 (9,1)	15,3 (6,9)	12,0 (8,2)	11,2 (6,3) b	‹0,01
Previous periods of abstinence ** [mean (SD)]	1,2 (1,1)	1,0 (0,9)	1,3 (1,2)	1,4 (1,3)	0,9 (1,0)	0,345
Duration of last period of abstinence (months) [mean (SD)])]	11,4 (17,5)	6,4 (7,2)	13,0 (20,0)	9,6 (11,7)	12,0 (19,6)	0,584
Addiciton criteria met in episode of maximum severity [mean (SD)]	7,1 (2,2)	6,4 (1,8)	8,0 (2,3) aaa	6,1 (1,1)	6,2 (1,9) bbb	‹0,001

Note. Abbreviations: N=number of subjects; SD=standard deviation
Value p is the level of significance calculated on the basis of the Kruskal-Wallis analysis of variance by ranks
([aaa]) p‹0.001 compared to the group "Men without comorbidity"; ([b, bb, bbb]) p‹0.05, p‹0.01 and p‹0.001 compared to the group"Men with comorbidity". Calculated with Dunn's post test.
(*) Substance use disorders are excluded (SUD)
(**) minimum of 6 months abstinence

Table 3. *Diagnoses of alcohol use and other substance use disorder (DSM-IV-TR) by sex and diagnosis of lifetime psychiatric comorbidityin a population of alcohol users under treatment* .

Variable		Total N=162	Men N=122		Women N=40		Value p Gender comorbidity
			Without psychiatric comorbidity*	With psychiatric comorbidity*	Without psychiatric comorbidity*	With psychiatric comorbidity*	
Alcohol [N (%)]	Abuse and/or Dependence	162 (100)	44 (36,1)	78 (63,9)	7 (17,5)	33 (82,5)	-
Cocaine [N (%)]	Abuse and/or Dependence	48 (29,6)	6 (14,3)	36 (85,7)	-	6 (100)	‹0,001
Cannabis [N (%)]	Abuse and/or Dependence	22 (13,6)	2 (11,1)	16 (88,9)	-	4 (100)	‹0,05
Sedatives [N (%)]	Abuse and/or Dependence	6 (3,7)	1 (25,0)	3 (75,0)	-	2 (100)	0,543
Other stimulants** [N (%)]	Abuse and/or Dependence	7 (4,3)	2 (33,3)	4 (66,7)	-	1 (100)	0,627

Note. Abbreviations: N=number of subjects; SD=standard deviation.
Value p is the level of significance calculated by Fisher's exact/chi-square test with patients grouped by sex and psychiatric comorbidity
(*) Excluding substance use disorders (SUD)
(**) Meta-amphetamines and derivatives.

Table 4. *Description of lifetime psychiatric comorbidity (DSM-IV-TR) in a population of alcohol users under treatment.*

Variable		Total N=162	Men N=122	Women N=40	Value p
Psychiatric comorbidity* [N (%)]	Some psychiatric disorder	111 (68,5)	78 (63,9)	33 (82,5)	‹0,05
	MD	74 (45,7)	47 (38,5)	27 (67,5)	‹0,05
	AD	32 (19,8)	23 (18,9)	9 (22,5)	0,650
	Psychotic disorders	15 (9,3)	10 (8,2)	5 (12,5)	0,529
	ED	2 (1,2)	-	2 (5,0)	0,060
	Personality disorders	39 (24,1)	28 (22,9)	11 (25,0)	0,670
	ADHD	46 (28,4)	37 (30,3)	9 (22,5)	0,421
Mood disorders (MD) [N (%)]	Primary Induced Primary+Induced	30 (18,5) 32 (19,8) 12 (7,4)	19 (15,6) 22 (18,0) 6 (4,9)	11 (27,5) 10 (25,0) 6 (15,0)	0,516
Anxiety disorders (AD) [N (%)]	Primary Induced Primary+Induced	19 (11,7) 11 (6,8) 2 (1,2)	12 (9,8) 9 (7,4) 2 (1,6)	7 (17,5) 2 (5,0) -	0,422
Psychotic disorders [N (%)]	Primary Induced Primary+Induced	5 (3,1) 9 (5,6) 1 (0,6)	4 (3,3) 6 (4,9) -	1 (2,5) 3 (7,5) 1 (2,5)	1,000
Eating disorders (ED) [N (%)]	Anorexia Bulimia	- 2 (1,2)	- -	- 2 (5,0)	0,060
Childhood conduct disorder (CCD) [N (%)]		14 (8,6)	13 (10,7)	1 (2,5)	0,192
Personality disorders [N (%)]	Antisocial disorder Bordeline personality disorder	7 (4,3) 24 (14,8)	7 (5,7) 15 (12,3)	- 9 (22,5)	0,152
Attention deficit and hyperactivity disorder (ADHD)** [N (%)]		24 (14,8)	19 (15,6)	5 (12,5)	0,799

Note. Abbreviations: N=number of subjects; SD=standard deviation.
Value p is the level of significance calculated by Fisher's exact/chi-square test with patients grouped by sex.
(*) Axis I=Clinical disorders [substance use disorders are excluded (SUD)]; Axis II=Personality disorders.
(**)ADHD diagnosis (WURS).

Table 5. *Description of lifetime psychiatric comorbidity (DSM-IV-TR) in a population of alcohol users under treatment, by sex and diagnosis of alcohol use and other substance use disorders.*

Variables	Total	Men N=122		Women N=40		Value p
		AUD + other SUD	AUD	AUD + other SUD	AUD	
Number of patients [N (%)]	162 (100)	53 (43,4)	69 (56,6)	10 (25,0)	30 (75,0)	‹0,05
Psychiatric comorbidity * [N (%)]	111 (68,5)	46 (37,7)	32 (26,2)	10 (25,0)	23 (57,5)	‹0,01
Psychiatric comorbidity (≥ two diagnoses) * [N (%)]	63 (38,9)	31 (25,4)	14 (11,5)	8 (20,0)	10 (25,0)	0,087
Mood disorders (MD) [N (%)]	74 (45,7)	24 (19,7)	23(18,9)	8 (20,0)	19 (47,5)	‹0,001
Anxiety disorders (AD) [N (%)]	32 (19,8)	15 (12,3)	8 (6,5)	2 (5,0)	7 (17,5)	‹0,05
Psychotic disorders [N (%)]	15 (9,3)	5 (4,1)	5 (4,1)	2 (5,0)	3 (7,5)	1,000
Eating disorders (ED) [N (%)]	2 (1,2)	-	-	2 (5,0)	-	-
Antisocial personalilty disorder [N (%)]	11 (6,8)	9 (7,4)	1 (0,8)	1 (2,5)	-	-
Borderline personality disorder [N (%)]	28 (17,3)	13 (10,6)	5 (4,1)	7 (17,5)	3 (7,5)	1,000
Attention deficit hyperactivity disorder (ADHD) ** [N (%)]	46 (28,4)	28 (22,9)	9 (7,4)	4 (10)	5 (12,5)	0,106

Note. Abbreviations: N=number of subjects; TUA=trastornos por uso de alcohol; TUS=trastornos por uso de otras sustancias.
Value p is the level of significance calculated by Fisher's exact/chi-square test with patients grouped by sex and AUD and SUD diagnosis
(*) Axis I= Clinical disorders [substance use disorders are excluded (SUD)]; Axis II=Personality disorders
(**)ADHD diagnosis (WURS)

chiatric comorbidity groups with non-substance related disorders. Men with other psychiatric comorbidity are affected for longer by the condition [mean 15.3 years (SD=6.9)] than women [mean 11.2 years (SD=6.3)] before seeking treatment for AUD. Finally, there are very significant differences (*p<0.001) in the number addiction criteria in the most severe episode. Specifically, we found very significant differences (*p<0.001) between the group of men with psychiatric comorbidity and those without comorbidity in terms of the number of criteria in the severest episode. The differences are also highly significant (*p<0.001) with regard to gender when comorbidity is present, with men meeting more criteria [mean 8.0 criteria (SD=2.3)] than women when comparing the two psychiatric comorbidity groups.

In addition, gender differences are reported when severe physical dependence symptoms are present, convulsions and/or *delirium tremens*, with a prevalence of 7.4% among men alone, irrespective of the presence of non-AUD psychiatric comorbidity or not.

SUD psychiatric comorbidity. Substance abuse and dependence

Table 3 shows the SUD comorbidity of the population under scrutiny in relation to psychiatric comorbidity and gender. Significant differences can be seen (*p<0.05) in cocaine SUD linked to gender, with men being affected more. Very significant differences (*p<0.001) were found in the prevalence of comorbid disorders in patients with cocaine SUD compared to those without cocaine SUD. Significant differences (*p<0.05) were also discovered in relation to cannabis consumption, with a high prevalence of cannabis SUD in other psychiatric comorbidity disorders, but without gender differences. Patients with AUD and cannabis and cocaine SUD comorbidity are more likely to be comorbid with other, non-substance related disorders than those patients without SUD.

We must point out that with regard to the remaining other substances studied, no statistically significant differences were found between groups throughout patients' lives.

Psychiatric comorbidity of disorders not related to substance use

Table 4 shows the different diagnoses, excluding the disorders related to the use of substances presented by the patients and gathered with the assessment instruments (PRISM and WURS), differentiated by gender. We found statistically significant differences (*p<0.05) in the prevalence of psychiatric disorders, with 82.5% of women as opposed to 63.9% of men diagnosed with non-addictive mental disorders. Very significant differences (*p<0.01) are also present in the prevalence of MD among women (67.5%) versus men (38.5%). No significant gender diffences were found in the other disorders.

Gender differences in SUD psychiatric comorbidity.

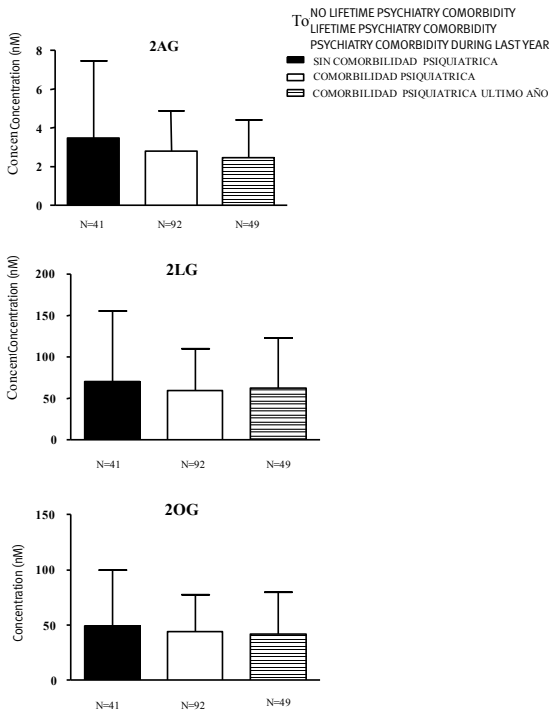
This section (see Table 5) will describe in detail the distribution of psychiatric disorders across the differences arising from gender and the type of SUD diagnosed. It can be said that when differentiating according to substance, psychiatric comorbidity varies among those who have just one (AUD) compared to those who have a SUD as well as an AUD. In the case of women with SUD comorbidity, we can see that 100% are also affected by other psychiatric disorders during their lifetime, most frequent among them MD and borderline personality disorder. Men without SUD comorbidity (AUD only) are less likely to suffer from other psychiatric disorders if we compare them to those who have SUD comorbidity, and although these differences are not noticeable in MD diagnoses, they are very pronounced in personality disorders.

It is worth looking at the attention deficit and hyperactivity disorder, given its close link to depressant drugs and its high prevalence in SUD (Daigre et al., 2013; Polanczyk et al., 2014), and also in AUD, as is also the case in our patients (Ponce et al., 2000).

Plasma levels of 2-acyl-glycerols

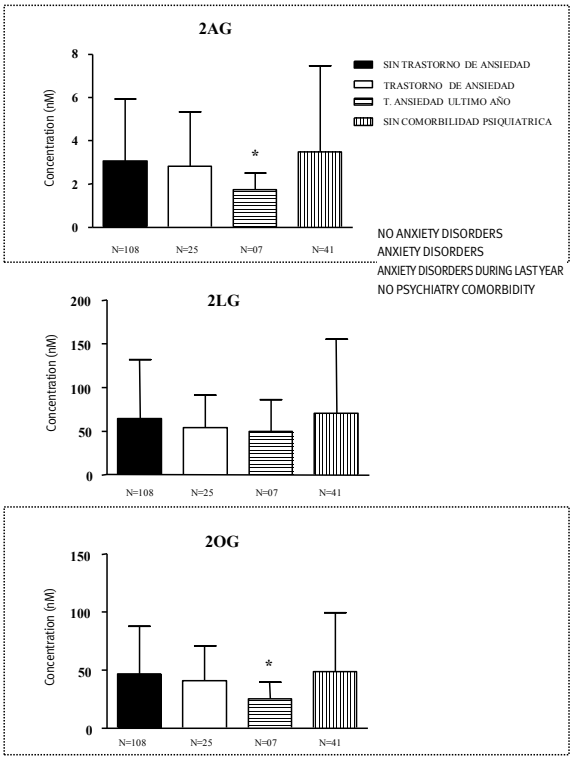
Plasma levels of 2-acyl-glycerols are lower in patients with psychiatric comorbidity (excluding SUDs), but there are no significant differences between the groups with regard to psychiatric comorbidity. We have made a distinction in time frames among patients with psychiatric comorbidity (lifetime versus last year) and a comparison with patients without psychiatric comorbidity (Figure 1).

The 2-AG and 2-OG plasma levels are significantly lower in (*p<0.05) in patients diagnosed with anxiety disorders in



(#) Substance use disorders (SUD) were excluded.

Figure 1. Plasma levels of 2-acy-glycerol (2-AG, 2-linoleil glycerol (2-LG) and 2-oleoylglycerol (2-OG) in alcohol dependence patients grouped by psychiatric comorbidity (#).



(*) p<0.05 indicate significant differences from no anxiety disorders group.

Figure 2. Plasma levels of 2-acy-glycerol (2-AG, 2-linoleil glycerol (2-LG) and 2-oleoylglycerol (2-OG) in alcohol dependence patients grouped by anxiety disorders (DSM-IV-TR)].

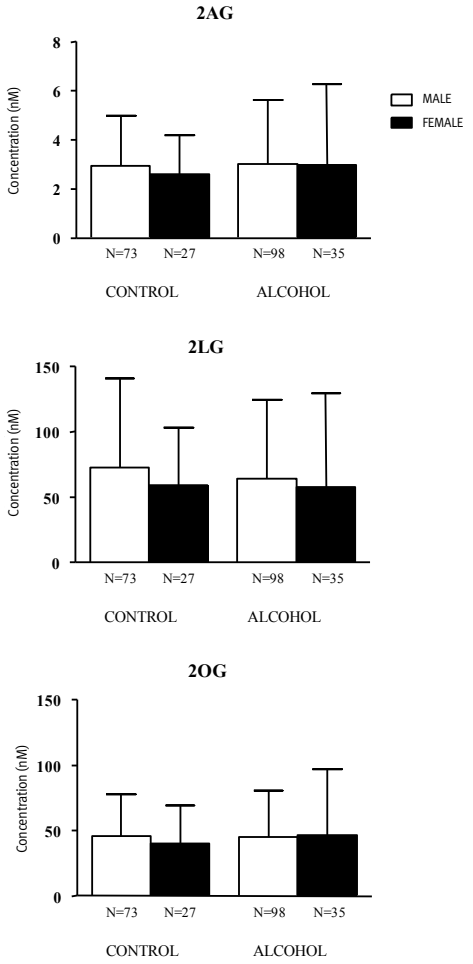


Figure 3. Plasma levels of 2-acy-glycerol (2-AG, 2-linoleil glycerol (2-LG) and 2-oleoylglycerol (2-OG) in alcohol dependence patients and control subjects grouped by sex.

the last year when comparing their levels to patients having anxiety disorders throughout their lives, those free of anxiety disorders and patients without psychiatric comorbidity (Figure 2).

Levels of 2-acyl-glycerols show no significant gender differences when comparing the sample of AUD patients with a control group (Figure 3).

Discussion

The results of the present study indicate that patients needing treatment for alcohol use disorders are more frequently affected by psychiatric comorbidity, and in some cases, especially in connection with anxiety disorders, this is reflected in altered 2-acyl-glycerol levels. The prevalence of lifetime psychiatric comorbidity was found to be high (68.5%) in this study, in line with results of some studies carried out on different types of AUD and other pathologies, which reported 60-70% psychiatric comorbidity (Pérez-Gálvez et al., 2008; Driessen et al., 1998). The outpatient profile is mainly masculine, of a low-middle educational background, with a mean age of 49.27 years and a high level of unemployment. Cases with a diagnosis of severe mental disorder have been excluded from this population, but not organic pathologies. These characteristics may lead to a series of biases which need to be taken into account when comparing our data with other AUD populations.

There are important gender differences within AUD, most notably that although women start drinking later than men, they take less time to develop dependence symptoms and meet fewer severity criteria than men. These data match those found in the bibliography, where women have intense drinking patterns, with a faster development from onset to dependence. This pattern could be the reason why they are more likely to suffer concomintant medical problems in comparison to men with AUD (Ávila Escribano et al., 2007; Keyes et al., 2010). Gender differences are also found in disorders not related to substance use, where men are more likely to suffer personality disorders. Antisocial personality disorder, for example, is diagnosed three times more frequently among men than women (Alegría et al., 2013). Women, on the other hand, have a greater likelihood of being affected by depressive disorders (Ávila Escribano et al., 2007). The lack of social support among women and the interpersonal problems in family contexts are risk factors and possible causes of the gender differences in non-SUD psychiatric comorbidity (Alegría et al., 2013).

In the analysis of the population with respect to SUD comorbidity, we found that polydrug use may be related to psychiatric comorbidity, especially when AUD is accompanied by other SUDs involving cocaine and cannabis. It is known that cocaine SUD is linked to a high prevalence of psychiatric comorbidity (Araos et al., 2014), and the literature also reports high polydrug use in patients receiving treatment, mostly young men who have never been married, with a low onset age and psychiatric comorbidity (Blanco et al., 2015). An analysis of psychiatric comorbidity in our sample reveals firstly that primary and substance-induced mood disorders are equally frequent over a lifetime. We know that depression beginning before the onset of SUD reduces the probability of remission from dependence, as does severe induced depression (Samet et al., 2013). Some studies claim that the severity of SUD is a good predictor of depressive disorders arising during patient follow-up (Boschloo et al., 2012), and this is makes sense in our sample when we see the connection between the presence of psychiatric comorbidity and the severity of AUD addiction symptoms. Secondly, with repect to anxiety disorders (AD), primary were more prevalent than induced disorders in terms of lifetime diagnoses. Thirdly, psychotic disorders (PD) in our sample are mostly induced, although they are less far frequent than those found in other populations with substance use disorders, where the prevalence was high (15.5%) (Araos et al., 2014). Although primary mood and anxiety disorders are

generally more frequent than substance-induced disorders (Torrens et al., 2011), our results are in line with those of other studies carried out with PRISM for mood and psychotic disorders in cocaine SUD populations, where induced disorders were more common than primary disorders, and anxiety disorders were more frequently primary than induced (Araos et al., 2014; Vergara-Moragues et al., 2012). Finally, with regard to personality disorders, our population has a 6.8% prevalence of antisocial personality disorders and a 17.4% rate of borderline personality disorder. These figures are lower than those found in other SUD populations involving substances such as cocaine (Araos et al., 2014). There is some evidence suggesting that personality disorders are more frequently associated with other SUDs because the consumption of the substances involved increases behavioural problems, clinical severity and social difficulties (Salom et al., 2014).

With regard to ADHD, this is diagnosed in 7% of children with approximately 4% continuing to be affected as adults (Kessler et al., 2006). In our population an above average prevalence of this disorder was detected, and this result may have been influenced by the use of a specific measurement instrument in the diagnosis. Nevertheless, symptoms in adults may give rise to errors such as, for example, restlessness being interpreted as anxiety or distractibility as lack of interest or motivation (Quintero et al., 2013). Having SUD comorbidity increases the psychiatric comorbidity of the sample (Tómasson y Vaglum, 1995). In our sample, when we eliminate other SUDs in AUD patients, the prevalence of other psychiatric comorbidity is reduced (from 82.5% to 57.5% among women, and from 63.9% to 26.2% in men). Mood and anxiety disorders are the most prevalent among AUD patients. The frequency of personality disorders also went down because antisocial disorder among men without SUD comorbidity fell to 0.8% and borderline personality disorder in women dropped to 7.5%. Our figures are in line with those obtainaed in studies of similar populations (only AUD) in Spain, where antisocial disorder appears in around 2% of cases and borderline personality in about 6% (Fernández-Montalvo et al., 2006). These patients have both internal and external behavioural problems in comparison to those only suffering AUD (Salom et al., 2014).

The results regarding psychiatric comorbidity described above support the need to find objective biological evidence which could serve as specific biomarkers for AUD subgroups with specific psychiatric comorbidities psiquiátricas. In this regard, our research contributes a description of the 2-acyl-glycerol plasma values in these patients for the first time. Our data suggests that 2-AG and 2-OG are affected by anxiety disorders diagnosed during the last year. Endocannabinoid signaling has been involved in anxiety modulation and emotional response (Navarro et al., 1997). An increase in the liberation of endocannabinoids is associated with the anxiolytic effect, which has driven the development of many pharmaceuticals, especially those linked to the blockade of endocannabinoid degrading enzymes FAAH and MAGL (Gaetani et al., 2003; Kinsey et al., 2011). We have found studies linking the deregulation of acylglycerols with difficulties in adapting to stressful and adverse stimuli, with an increase in stress, anxiety and fear responses (Guggenhuber et al., 2015; Jenniches et al., 2015). Our further findings of a relationship between changes in 2-acyl-glycerols and the diagnoses of anxiety disorders places our research closer to potential stress and anxiety therapy targets.

Among the limitations of our study we have to point out that the retrospective assessment of clinical and withdrawal symptomatology was not ideal, and the same must be said of the abstention periods as reported by the patients. In addition, sample size was small from a statistical point of view, although adequate from a clinical perspective, and the percentage of women included in the study was also modest.

Continuing research in this area of phenotypic characterization should be carried out with larger and more representative samples. A longitudinal study which would enable testing for psychopathological symptoms and addiction severity while increasing the female population meeting the inclusion criteria would be welcome. In this way, more accurate comparisons can be made between the sexes and more specialised treatments can be designed. Furthermore, there is a need to incorporate and increase the samples of the different biomarkers to diagnose consumption, severity and comorbidity in an effort to improve prognoses and optimise treatments adapted to the needs of each type of patient.

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Conflict of interests

None.

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