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Research Article

Peripheral Blood Lymphocytes and Blood Tests are Modified According to the Alcohol Consumption Pattern in Young People

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Abstract

The most socially accepted addictive substance worldwide is alcohol. Indeed, regardless of sex and/or world region, individuals aged 15 to 29 years consistently presented the highest proportion of alcohol-related deaths. However, the effect of alcohol consumption on biochemical parameters and the number of immune cell in peripheral blood in young people is currently unknown.

Aims: The present study analyzed the effects of the alcohol consumption pattern on biochemical tests and blood lymphocyte profile.

Results: There were changes in the hazardous and harmful (HH) group in relation to alcohol consumption/occasion, biochemical tests including red cell counts and lymphocyte profile in the absence of clinical evidence of disease. The AUDIT score correlated directly with alcohol consumed per occasion, total craving, age, liver enzymes (GGT, AST), and NK and NKT cell levels. A dependent subgroup had a higher count of NKT cells and a lower count of B cells than the control group. These outcomes mirror similar results published in regard to alcoholic liver disease. Also, the numbers of these cells are one of the first changes that follow alcohol consumption. Therefore new research on young people with alcohol use disorders is needed.

Conclusion: Higher drinking per occasion or binge drinking induced biochemical and cellular alterations the first of which was the increase in the Neutrophil to Lymphocyte ratio and NKT cells in young adults. This finding can be a useful tool to help design new detection strategies and treatments or the prevention of excessive alcohol consumption, thus avoiding the development of alcoholic liver disease and burden of disease among young subjects.

ABBREVIATIONS

GISAH: Global Information System on Alcohol and Health; ALD: Alcoholic Liver Disease; UNAM: Universidad Nacional Autonoma de Mexico; BMI: Body Mass Index; AUDIT: Alcohol Use Disorders Identification Test; WHO-CIDI: World Health Organization's Composite International Diagnostic Interview; ICD-10: International Classification of Diseases; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders; MACS: Multidimensional Alcohol Craving Scale; CT group: Nondrinking group; HH group: Hazardous and Harmful drinking group; A: Alcohol abuse subjects; D: Alcohol Dependence subjects; H: Hazardous Drinking Subgroup; ALT: Alanine amino Transferase; AST: Aspartate amino Transferase; GGT: Gamma-Glutamy Transferase; HBsAg: Hepatitis B surface Antigen; EDTA: Ethylene Diamine Tetraacetic Acid; mL: Milliliter; µL: Microliter; Hb: Hemoglobin; Ht: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; NAFLD: Nonalcoholic Fatty Liver Disease; NLR: Neutrophil to Lymphocyte Ratio; T Cell: Lymphocyte T; T -Cell CD4+: Helper Lymphocytes; T-Cell CD8+: Cytotoxic T-cell; NK cells: Natural Killer cells; NKT cells: Natural Killer T cells; Kg/m^2: Kilogram per square meter; g/dl: Grams per deciliter; %: Percentage; μ^3: Cubic Microns; mm^3: Cubic Millimeters; U/L: Units per liter; %: Percentage; SD: Standard Deviation; NS: Not Significant.

INTRODUCTION

The Global Information System on Alcohol and Health (GISAH) reports that the harmful/excessive use of alcohol results in the death of 2.5 million people annually. In 2005, the worldwide total consumption was 6.13 liters of pure alcohol per person aged 15 years and older. The highest adult per capita alcohol intake was observed in the developed countries [1].

Indeed, regardless of sex and/or world region, individuals aged 15 to 29 years consistently presented the highest proportion of alcohol-related deaths [2]. In addition to these long-term risks, alcohol abuse in childhood and adolescence is associated with an increased risk of developing alcohol dependence [3,4], along with acute and chronic alcoholic liver diseases (ALD) [2,5].

In Mexico, alcohol use disorders are a growing public health concern, because they lead to diseases associated with excessive alcohol consumption. They are also associated with a reduced healthy lifespan [6,7]. Young Mexicans (15 to 29 years old) exhibited harmful and hazardous alcohol consumption per occasion (binge drinking), getting drunk [7,8]. This age group reported that their preferred drink was beer (45.6%), followed by other liquors (29.3%) [7].

Several studies have been performed to elucidate the differences between immune-phenotype and function of peripheral blood cells. Laso *et al.,* (2010) demonstrated that patients with chronic alcohol consumption and active ethanol intake, but without liver disease, had an increase in T lymphocytes CD4+, T lymphocytes CD8+, and NK cells, whereas subjects with alcoholic liver cirrhosis exhibited a reduction in the number and cytotoxic activity of NK cells [9].

Naude *et al*., (2011) evaluated the peripheral blood lymphocytes of adolescents who had alcohol use disorders. They concluded that dependent subjects with binge drinking had a higher risk of lymphopenia and increase in the susceptibility to infections due to decreased immune system capacities, when compared with controls [10].

The effects of moderate discontinuous ethanol feeding on peripubertal male rats support the hypothesis that this type of consumption may be more harmful for the immune system than continuous use, because it induces greater stress on immune cells [11].

Existing studies tend to be epidemiological, focusing on chronic drinking in general population samples. However, comparatively fewer studies have examined the relationship between alcohol consumption and peripheral blood parameters in young participants [12].

Most human studies have been performed on persons between 30 and 60 years of age with chronic and excessive alcohol consumption. For young people between the ages of 18 and 29 years, alcohol abuse is mostly episodic, similar to binge drinking (more than 5 standard alcohol drinks per occasion). Despite the fact that the relationship between alcohol use disorders in young adults (crime, traffic accidents, educational issues)and the potential dependence on alcohol is well known [7,13,14], the difference between use and abuse of alcohol intake is not clear, especially in youths. This difference is defined by the amount, frequency, and pattern of consumption.

There are no reports of similar studies on young adults, even though it is the group with the highest rate of binge drinking. They represent a vulnerable group for developing dependence, because the brain is undergoing structural maturation and finally, this group of subjects may also develop chronic diseases [15]. The effect of alcohol consumption on biochemical parameters and the number of immune cells in peripheral blood is currently unknown in young consumers.

The objective of the present study was to assess biochemical parameters, levels of craving, and the lymphocytic profile in young persons, in accordance with their alcohol consumption pattern.

MATERIALS AND METHODS

Subjects

The subjects (n=252) included in this cross-sectional study were college students from the Universidad Nacional Autonoma de México (UNAM) in Mexico City, recruited from 2008 to 2009. Participants were Mexican and of mixed race (mainly mestizos). Evaluation procedures included a detailed physical examination with anthropometry that included body mass index (BMI). The subjects were placed in 3 groups: normal weight (19.9-24.9 Kg/m^2), overweight (25-29.9 Kg/m^2), and obesity (>30 Kg/m^2). We used the Alcohol Use Disorders Identification Test (AUDIT) to examine the prevalence of hazardous and harmful drinking during the previous year. This self-report instrument includes 10 items that examine the frequency and intensity of drinking [16]. Alcohol dependence and abuse were assessed with the Spanish version of the World Health Organization's Composite International Diagnostic Interview (WHO-CIDI 3.0). It is a completely structured and extended interview based on the International Classification of Diseases (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. It has been validated with the standardized clinical assessments of the World Mental Health Services-WHO [17].

We also used the Multidimensional Alcohol Craving Scale (MACS) [18] containing 12 items that are divided into two factors: 1) desire to drink and 2) behavioral dis inhibition (lack of resistance). The score is from 10 to 50 and from 2 to 10, respectively, and the global score is from 12 to 60. Its interpretation is shown in Table 1. Participants were assigned to one of the two groups according to their AUDIT scores: a nondrinking group (CT) or a hazardous and harmful drinking group (HH). In accordance with the WHO-CIDI 3.0, the HH group was subdivided into: the alcohol abuse (A) or alcohol dependence subgroup (D), and subjects that did not meet these criteria were designated as the hazardous drinking group (H). In the control group the dose of alcohol intake was <12 g/day or, alternatively, it consisted of individuals that never consumed alcohol.

The pattern and amounts of ethanol intake and smoking habits were assessed through specifically designed questionnaires. A standard drink (each providing 13 g of ethanol) was defined as one beer or a wine cooler (340 mL), one glass of wine (150 mL), or a 45mL drink of liquor (i.e. tequila, brandy) [7,13].

This study was approved by the Institutional Review Board of the UNAM. All participants provided written informed consent, and the study was carried out according to the provisions of the Declaration of Helsinki.

Laboratory Tests

Biomedical testing consisted of hematological analyses and biochemical tests that were performed with automated systems (Vitros 250, Johnson & Johnson, New Jersey, USA and HMX-AL Hematology Analyzer Beckman Coulter California, USA). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) values in normal limits were 21-72, 17-59, and 8-78 U/L, respectively. Hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus were also negative.

Immunological Assays

In the subsample of 170 subjects (107 control subjects and 63 drinkers), peripheral blood collection and sample preparation EDTA venous blood samples (5 mL) for the immunological assays were collected via venipuncture in the morning and delivered to the laboratory within 2 hours. The following parameters were measured for each participant: total T-cells (CD3+), CD4+ T cells (T-helper) and CD8+ T cells (T-cytotoxic), CD4+:CD8+ ratio, Natural Killer cells (CD3-CD16-CD56+), NKT cells (CD3+ CD16-CD56+), and B lymphocytes (CD19+).

The fresh blood received within the laboratory was processed for flow cytometric analyses that use multicolor staining and single platform technology (Becton Dickinson, San Jose, CA, USA). Well-mixed blood (50 µL) was incubated with the mixture of monoclonal antibodies defining discreet T cell subsets. The commercial antibody mixture (Becton Dickinson, San Jose, CA) contained antibodies reacting to the following lymphocyte subsets: CD45+, CD3+, CD4+, CD8+, CD16+56+, and CD19+. A lyse/no-wash method was used and samples were analyzed on a FACS CANTO flow cytometer using Diva software. Results were expressed as the percentage (%) and absolute counts of positive cells.

Statistical Analysis

Values were expressed as mean ± standard deviation (SD). Data were assessed using the Kolmogorov-Smirnov Z test to examine the distribution type; if the data did not exhibit a normal distribution, they were logarithmically transformed prior to analysis. One-way ANOVA plus orthogonal contrasts were used to determine the differences between all groups and the covariance analysis (ANCOVA) were used to assess the interactions between variables. Correlations were calculated with the Spearman's rank correlation, as required. The analyses were carried out with the Windows SPSS 15.0 statistical software (SPSS Inc, Chicago, IL). Differences were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

The main characteristics of the study population are presented according to the study subgroups (Table 2). Of the 252 participants, 146 subjects were in the CT group and 106 in the HH group; 68% of the HH group and 43%of the CT group were male students. The following parameters rendered higher mean values in the HH group than in the CT group: age (22 vs 21 years old, $p<0.001$), alcohol consumption/occasion (108±88g vs 31±26g or drinks/occasion: 7.7±6 vs 2±1.9, $p<0.001$), and alcohol consumption/day (21±29 g vs 2±4 g, $p<0.001$). The total craving score was moderate for the HH group and mild for the CT group (Table 2). We also analyzed the blood cell counts and liver enzyme levels according to alcohol consumption and sex and the results are shown in three categories:

1. Differences in the two groups by sex: in the control and HH groups, there were higher levels (counts) of leukocytes and platelets and lower levels of erythrocytes, hemoglobin (Hb), and hematocrit (Ht) in women than in men. Liver enzyme values (AST, ALT, and GGT) were also lower in women than in men ($p<0.001$); only AST showed a tendency to increase in male consumers, compared with the male controls (Table 2).

2. Differences in white blood cells in the control group by sex: there was a higher level of monocytes and lymphocytes ($p<0.03$) and a lower percentage of neutrophils ($p=0.005$) in men than in women (Table 2).

3. Differences by alcohol consumption: there were higher hemoglobin and hematocrit values in female drinkers, when compared with female non-drinkers; the number of neutrophils was higher and the lymphocyte percentage was lower in the HH men versus the control men ($p<0.005$). The mean corpuscular volume (MCV) values were higher in female and male drinkers than in female and male non-drinkers (Table 2).

When the study population was further classified according to BMI and sex, the AST, ALT, and GGT enzyme levels were higher in men with obesity (Figure 1 A-C). Furthermore, when we studied the interaction between alcohol consumption and BMI, hepatic enzymes rose in the participants who had higher BMI and alcohol consumption (Figure 1 D-F). The subgroups from normal to high BMI differed from one another in an additive fashion, with the highest values occurring in participants with drinking combined with obesity, especially in men.

In the lymphocytic profile, we found differences between the HH and CT groups in the total number of CD45+ cells (1.0±0.5 vs. 0.5±0.3) and NKT cells (0.08±0.06 vs. 0.06±0.05, $p=0.022$), both being higher in the HH group (Table 3-A). The total count of NKT cells ($p=0.02$) was greater in the abuse subgroup and the B cell average ($p=0.03$) was lower in the dependence subgroup than in the CT group (Table 3-B). When comparing NKT cell values in different alcohol consumption patterns and sex, the values were higher in the hazardous drinking and alcohol abuse men than in the male non-drinkers ($p<0.04$). In other lymphocyte subpopulations no differences were observed between the subgroups of the HH group (Table 3 A-B).

We found that the AUDIT score was directly related to alcohol consumption per occasion ($r_s= 0.726$, $p<0.001$), total craving ($r_s=0.721$, $p<0.001$), age ($r_s=0.216$, $p=0.001$), BMI ($r_s= 0.199$, $p=0.004$), high hemoglobin levels ($r_s=0.225$, $p=0.001$), GGT ($r_s=0.181$, $p= 0.007$), AST ($r_s= 0.186$, $p=0.006$), and the percentage of CD45+ ($r_s= 0.244$, $p=0.002$), NK ($r_s=0.190$, $p=0.015$), and NKT cells ($r_s=0.211$, $p=0.007$), i.e. higher AUDIT scores correlated with higher values of these parameters. According by sex; we found

Table 1: Interpretation of craving levels.

	Absent	Mild	Moderate	Intense
Desire to drink	10	11-17	18-34	>34
Uninhibited behavior	2	3	4-7	>7
Total Craving	12	13-22	23-40	>40

The MACS is a self-report designed to assess levels of alcohol craving. It is composed of two factors which have shown good to excellent internal consistency: desire to drink and behavioral disinhibition (lack of resistance). MACS validation was based on an observational study. The scales used as standards were an Analogic Visual Scale for alcohol craving and the Obsessive –Compulsive drinking Scale. Data taken from Guardia-Serecigni *et al.,* 2004.

Table 2: Demographic, clinical and biochemical characteristics according to alcohol consumption patterns and sex.

	Control		Drinkers (All)		Hazardous		Abuse		Dependence		*p* value
	F	M	F	M	F	M	F	M	F	M	
Gender n (%)	83 (57)	63 (43)	34 (32)	72 (68)	9 (8)	20 (19)	4 (4)	19 (18)	21 (20)	33 (31)	NS
Age (years)	20.5±2.0	21.2±2.9	21.2±2.5	22.4±3	23.2±3.5	22±2.5	20.5±1.9	22±2.4	20.5±1.6	23±3.5	0.05+,‗,†
BMI(Kg/m²)	23.1±3.7	24.1±3.3	24.4±4.0	24.6±3.4	24.2±4.0	24.6±3.0	20.5±1.9	23.9±3.7	23.9±3.5	25.0±1.6	NS
Drinks per occasion (grams)	30±27	32±26	107±81	109±92	90±51	97±54	122±139	118±82	111±81	110±114	<0.001*,+, ‖,†,□,◊
AUDIT	2.4±1.9	3.0±2.1	13.7±4.5	14.7±5.4	11.8±2.4	15.3±4.3	13.7±1.8	11.0±2.3	14.5±5.3	16.8±6.2	<0.001*,+,‗ ‖,‡,†,□,◊
Uninhibited behavior	3.9±2.1	4.4±2.1	5.2±1.8	5.5±4.2	4.8±1.1	5.1±1.3	4.5±0.5	5.1±1.9	5.5±2.1	6.0±6.3	<0.001*,□ <0.05‗,‖,◊
Desire to drink	13±4.5	15±6.3	26±9.8	25±9.2	28±8.9	30±9.9	22±11.1	25±6.9	27±10.1	24±10.6	0.028+ <0.001*,‗,‖, ‡,†,□,◊
Total Craving	17±5.4	19±6.9	31±10.2	30±11.4	28±8.9	30±9.9	27±11.6	30±7.6	33±10.4	30±14.6	0.039+ <0.001*,‗,‖, ‡,†,□,◊
Hb (g/dl)	14.6±1.5	17.2±1.5	15±1.1	17.3±1.0	14.6±1.4	17.4±0.9	14.7±0.1	17.5±1.0	15.3±1.0	17.2±1.0	0.040□
Ht (%)	43±5.1	50±5.1	45±3.3	51±3.1	43±4.4	51±2.6	44±0.8	52±3.2	46±2.9	51±3.3	0.021* 0.007□
MCV (μ³)	88.9±4.9	89.1±4.9	91±3.7	91±3.2	92±3.0	91±2.7	91±4.5	91±3.2	91±3.9	91±3.6	0.005*,+ 0.033‗,□
Leukocytes (miles/mm³)	7.7±2.3	6.3±1.4	7.6±1.7	6.4±1.6	6.7±1.2	6.2±1.8	10.2±1.1	6.5±1.4	7.5±1.5	6.4±1.5	0.010‡
Neutrophils (%)	59.6±10.0	54±11.5	61±8.4	60±8.7	60±10.2	59±10.6	60.5±9.1	61±7.9	61±7.9	60±8	0.002+ 0.016† <0.001◊
Monocytes (%)	5.1±2.1	7.5±4.9	5.6±2.1	6.4±2.7	5.2±2.1	6.3±3.5	6.2±1.5	6.1±2.3	5.7±2.2	6.7±2.3	NS
Lymphocytes (%)	32±8.5	34.7±8.5	30.6±6.4	30.4±7.6	30.3±6.5	31.7±9.1	31.2±8.5	29.6±7.3	30.6±6.3	30.1±6.9	0.005+ 0.02†,◊
NLR	1.84±0.5	1.56±0.7	1.99±0.6	1.97±0.6	1.98±0.7	1.86±0.6	1.93±0.5	2.06±0.5	1.99±0.6	1.99±0.6	0.005+ 0.03†,◊
GGT (U/L)	24±24	29±25	21±12	32±25	24±15	30±15	14±3	25±10	22±12	37±35	NS
AST (U/L)	28±7	35±15	29±12	43±37	26±8	56±64	21±6	33±7	32±13	39±22	0.032‖
ALT (U/L)	26±11	34±20	24±10	39±33	23±8	46±45	19±4	31±12	25±12	39±33	NS

Abbreviations: Data are expressed as mean ± SD. *Difference between female Drinkers and female Control; +Difference between male Drinkers and male Control; ‗Difference between female Hazardous group and female Control group; ‖Difference between male Hazardous group and male Control group; ‡Difference between female Abuse group and female Control group; †Difference between male Abuse group and male Control group; □Difference between female Dependence group and female Control group; ◊Difference between male Dependence group and male Control group. BMI: Body Mass Index; AUDIT: Alcohol Use Disorder Identification Test; Hb: Hemoglobin; Ht: Hematocrit; MCV: Mean corpuscular volume; NLR: Neutrophil to lymphocyte ratio; GGT: gamma-glutamyl tranferase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Kg/m²: kilogram per square meter; g/dl: Grams per Deciliter; %: percentage; μ: microns; mm³: cubic millimeters; U/L: Units per liter; SD: standard deviation; NS: Not significant.

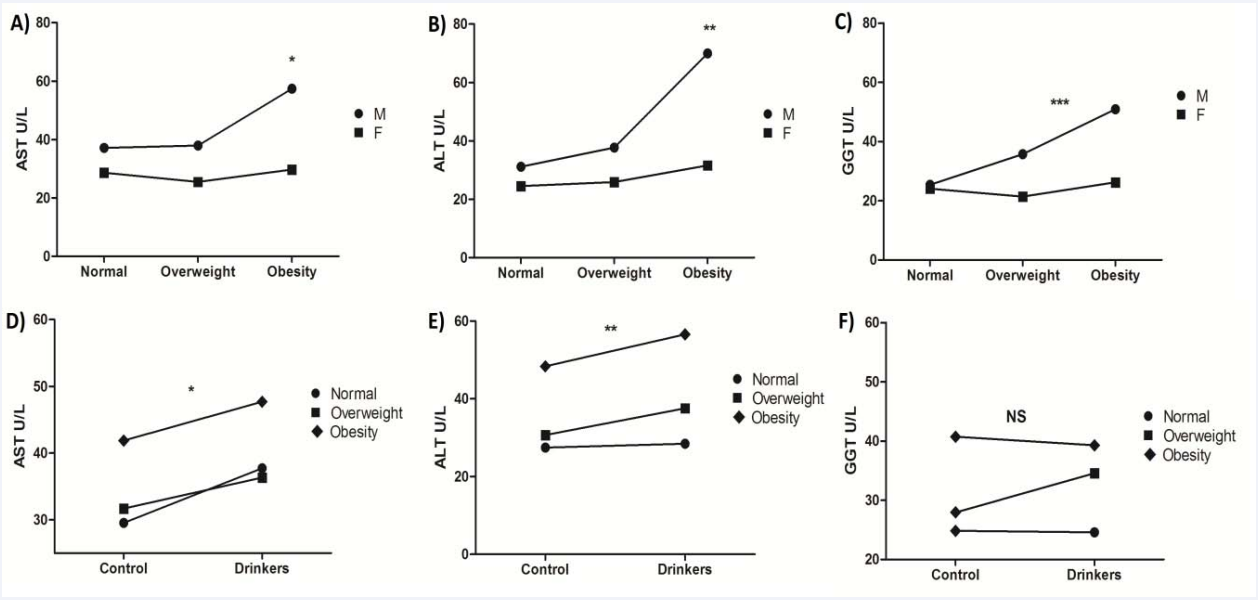

Figure 1 Interaction between AST, ALT and GGT levels according to the subjects' BMI and gender is shown in A) the levels of AST were higher in males with obesity than in males with normal BMI ($p<0.001^{*}$); B) ALT levels were higher in males with obesity than in males with normal weight ($p<0.001^{**}$); C) GGT levels were higher in males with obesity than in males with normal BMI ($p=0.016^{***}$).According to alcohol consumption and BMI; D) When analyzing alcohol consumption and body mass index, we found that the AST values were higher in the obese subjects than in those with overweight and normal weight in control group. Those values were also higher in the obese drinkers ($p=0.04^{*}$); E) ALT levels increased according to BMI in control subjects, while this effect increases even more in alcohol consumers ($p<0.001^{**}$); F) GGT levels were higher in controls and drinkers with obesity (NS). Data are expressed as mean values. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: gamma-glutamyl transferase; U/L Unit per liter; NS: Not Significant. Normal weight= 19.9-24.9 Kg/m^2, overweight is defined by 25.0-29.9 Kg/m^2, obesity is more than 30.0 Kg/m^2.

Table 3: Lymphocyte Profile according to alcohol consumption patterns and sex.

	Control n=107		Drinkers (All) n= 63		Hazardous n=13		Abuse n=15		Dependence n=35		*p*
	F	M	F	M	F	M	F	M	F	M	
T-cell (%)	66±8	63±9	64±7	62±9	62±13	62±6	61±6	63±12	66±5	61±8	NS
B-Cell (%)	16±7	14±6	17±5	13±4	22±6	14±4	19±8	14±4	15±3	12±4	NS
NK-Cell (%)	12±6	17±8	12±5	20±8	11±7	20±3	13±6	17±10	12±3	22±9	0.05[c]
NKT-Cell a (%)	3.3±2	2.7±2	4.4±3	4.4±3	1.8±0.6	3.5±	5.5±2	5.1±4	4.9±3	4.5±3	0.01[a, c] 0.05[‡, □]
T Lymphocytes (1000/mm^3)	1.4±0.7	1.1±0.6	1.3±0.6	1.1±0.5	1.1±0.7	1.3±0.7	1.9±0.3	1.1±0.2	1.2±0.6	1±0.5	NS
B Lymphocytes (1000/mm^3)	0.3±0.2	0.2±0.2	0.3±0.2	0.2±0.1	0.3±0.2	0.2±0.1	0.5±0.1	0.2±0.1	0.3±0.1	0.1±0.1	0.03[c]
NK cell (1000/mm^3)	0.2±0.1	0.3±0.2	0.2±0.1	0.3±0.2	0.1±0.1	0.4±0.1	0.4±0.2	0.3±0.2	0.2±0.1	0.3±0.2	NS
NKT cell (1000/mm^3)	0.06±0.06	0.04±0.05	0.08±0.07	0.08±0.06	0.03±0.03	0.07±0.04	0.1±0.07	0.09±0.07	0.08±0.06	0.07±0.07	0.02[a,b] 0.004[+] 0.04[‖]
Cytotoxic cells (1000/mm^3)	0.5±0.3	0.5±0.3	0.5±0.3	0.5±0.2	0.4±0.2	0.7±0.2	0.9±0.4	0.4±0.1	0.5±0.2	0.4±0.2	NS
Helper cells (1000/mm^3)	0.8±0.3	0.5±0.3	0.7±0.3	0.5±0.3	0.6±0.3	0.6±0.5	1±0.2	0.6±0.1	0.7±0.3	0.5±0.3	NS

Data are expressed as mean ± SD. [a] Difference between Drinkers and control group. [b] Difference between Abuse group and control group. [c] Difference between dependence subjects and control group. [*]Difference between female Drinkers and female Control; +Difference between male Drinkers and male Control; ▁Difference between female Hazardous group and female Control group; ‖Difference between male Hazardous group and male Control group; ‡Difference between female Abuse group and female Control group; †Difference between male Abuse group and male Control group; □Difference between female Dependence group and female Control group; ◊Difference between male Dependence group and male Control group.%: Percentage; mm^3: cubic millimeters; SD: standard deviation; NS: Not significant.

that AUDIT score related to alcohol consumption per occasion in females and males (r_s= 0.736, p<0.001), In males: AUDIT score with % and number of NKT cells (r_s= 0.382 and r_s= 0.247, p<0.02), number T lymphocytes related to number NK, number NKT, number B lymphocytes, number T CD8+ cells (r_s= 0.546, $r_{s=}$ 0.411, r_s= 0.584 , r_s= 0.584, respectively, p<0.001), also number NK cell with number NKT cells (r_s= 0.446, p<0.001) and number NKT cells related to T CD8+ cells (r_s= 0.408, p<0.001)

In females: AUDIT score with % CD 45+ (r_s= 0.315, p=0.003), number T lymphocytes related to number NK, number NKT, number B lymphocytes, number T CD4+ cells (r_s= 0.480, $r_{s=}$ 0.388, r_s= 0.486 , r_s= 0.850, respectively, p<0.001) r_s

Discussion

College students have a higher frequency of unhealthy drinking [19], known as binge drinking. Our study population showed this type of consumption, principally on weekends. Furthermore, the majority of the subjects in the HH drinking group were men. This result is similar to that reported by the 2011 National Addiction Survey. However, hazardous consumption has increased in young Mexican women [7].

Binge drinking is associated with an increased risk of developing acute and chronic liver diseases in young person's [2,5,20].

Although alcohol consumption in adolescents and youths is forbidden in Latin American countries, it is a common phenomenon [7,8,19]. However, the physiological consequences are still unknown for this age group. As a consequence, the incidence of chronic liver disease is likely to increase in young adults.

We have shown that BMI is an independent factor for increased levels of aminotransferases in obese drinkers. Hence, this is an additional factor involved in liver damage that has an impact on most of the populations worldwide in which the frequency of obesity has rapidly increased [2].

We found that BMI was similar between alcohol consumers and control groups. These results showed that university students have BMIs that range from normal to overweight (Table 2) and they demonstrated a positive relationship between BMI and alcohol consumption

In adolescents and young adults the increasing frequency of alcoholic liver disease is aggravated by the similar increase in the incidence of fatty liver caused by metabolic factors as a consequence of overweight and obesity in childhood [2,21,22].

The proportion of young person's that have died from alcohol-related liver disease has increased 8-fold in the last 10 years, as a result of alcohol abuse and nonalcoholic fatty liver disease (NAFLD) [2,23].

Craving for alcohol is common among alcohol-dependent persons. We evaluated the ability to decline drinks and found that it was moderate in both groups. A desire to drink and total craving were moderate in consumers. In the HH group, the score for the three items of craving was moderate and may be related to the onset of addiction [24]. We consider that evaluating craving in young people will improve diagnosis and prognosis and aid in finding prevention strategies for alcohol dependence.

Blood Cell Counts and Biochemical Tests

The findings showed differences in hematological parameters according to sex: erythrocytes, hemoglobin, hematocrit, and liver enzyme values were lower in women than in men and leukocyte and platelet levels were higher in women than in men (Table 2). Ours is the first report that indicates that there is a difference in the last two parameters between women and men.

Alcohol consumption in women increased red blood cell parameters such as hemoglobin, and hematocrit, also MCV was increased by alcohol consumption in female and male subjects. In men, white blood cells had a higher number of neutrophils and a lower number of lymphocytes, and the neutrophil to lymphocyte ratio (NLR) was higher in the HH group and in the abuse and dependence subgroups. AST levels were also elevated (Table 2). We believe our results may reflect the first biological alterations in alcohol consumers that have no clinical manifestations of disease. However, there is no evidence at present that hazardous and harmful alcohol consumption has an effect on neutrophils and lymphocytes. Only, in an animal study was it demonstrated that ethanol feeding increased the number of neutrophils [25]. In alcoholics, lymphopenia was shown to be a time-dependent and reversible event [26], and alcohol dependent adolescents had a higher risk of lymphopenia [10]. The NLR has only been identified as a systemic inflammatory marker in cancer patients and in those presenting with acute myocardial infarction [27,28], and can possibly be a tool in alcohol use disorders especially in males.

There were no significant differences between the HH subjects and the control group in relation to serum liver enzymes, but when we made a comparison by sex, male drinkers had slightly higher levels than their CT counterparts (p=0.06, Table 2). The men in the hazardous drinking group also had higher AST values than the control men (p=0.032), probably modified by alcohol consumption.

Many conventional assays have limited sensitivity and specificity when used alone, such as GGT, AST, and MCV [23,29–31]. However, these tests have not been used for HH drinking in young person's; our results showed that alcohol consumption slightly increased AST in men. An accurate and economic blood test for identifying persons at higher risk due to excessive alcohol consumption is needed.

Lymphocyte Profile

Alcoholism is known to alter the immune system [32]. The percentage of T lymphocytes in our control group was similar to the data from healthy young Mexican people [33]. Alcohol consumption did not modify the numbers of CD3+ T cells. Naude *et al.,* (2011) reported that adolescents with alcohol dependence had lower average absolute counts of CD3+ lymphocytes when compared with the control group. Experimental studies have shown that the number of T cells was not depleted, but their function was affected by ethanol [10,34].

Sacanella *et al.,* (1998) found a decrease in the number of B cells in alcoholic patients (13.1±4% vs 8.4±4%) [35]. A recent

study showed that ALD patients had a reduced number of lymphocyte subpopulations, including B cells [36]. Our results in alcohol dependent youths showed the same pattern in relation to B lymphocytes as previously reported in adult alcoholic patients.

Alcohol intake inhibits and deregulates the functions of T cells, NKT cells, dendritic cells, and Kupffer cells in the liver [37,38]. These cells orchestrate innate immune responses and initiate adaptive immune responses [39]. At present, the contribution of immune system activation is relevant to the development of ALD [9,37,38]. Alcoholic adults show different changes, in blood peripheral cytotoxic lymphocytes [9,40].

In regard to Natural Killer T cells, we found differences between alcohol consumers and the control group; there was a greater number of NKT cells in the HH drinkers (Table 3-A). There were also a higher percentage of NKT cells in the abuse subgroup than in the control group (Table 3-B). Current evidence of NKT changes due to alcohol consumption has only been reported in murine models [10,41–43].

NKT cells, alone, or in combination with NK cells, modulate the innate immune system through cytokine production and cytotoxic functions [41]. Other studies suggest that NKT cells promote liver fibrogenesis by producing pro-fibrotic cytokines. However, NKT cells may also attenuate liver fibrosis under certain conditions by killing hepatic stellate cells and by producing large amounts of anti-fibrotic cytokines, such as Interferon gamme [42]. Nevertheless, there are few studies on patients with alcoholic cirrhosis and the results of the number and function of NKT cells have not shown any significant changes [44].

This is the first study performed on youths with different alcohol consumption patterns. Hazardous and harmful alcohol consumption altered some biochemical parameters including the MCV in the HH group and red blood parameters in females, and the NLR in males, in the absence of clinical evidence of disease.

Drinkers showed higher absolute NKT cell counts, as did the alcohol abuse subjects. No studies have reported NKT modification in young drinkers. The alcohol dependent persons had a lower percentage of B cells. These outcomes are similar to published results on alcoholic liver disease [35,36]. Also, the absolute counts of these cells are probably one of the first changes that follow alcohol consumption. Therefore new research on young persons with alcohol use disorders is needed. Future studies will be aimed at analyzing the function and activation of different types of lymphocytic cells in the peripheral blood of youths.

CONCLUSION

The finding that alcohol use disorders induce biochemical and cellular alterations in young adults can be a useful tool to help design detection strategies and treatments for the prevention of excessive alcohol consumption and the consequent development of alcoholic liver disease and its burden in young subjects.

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