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ORIGINAL ARTICLE

# Proposal for early detection of ethanol consumption in students of the *Universidad Autónoma del Estado de Morelos*

*Sara García-Jiménez[1]*, Miguel Erazo-Mijares[1], Cairo D. Toledano-Jaimes[2], Antonio Monroy-Noyola[2], Fernando Bilbao-Marcos[3], Miguel A. Sánchez-Alemán[4] and Myrna Déciga-Campos[5]*

*[1]Laboratory of Clinical Biochemistry, Faculty of Pharmacy; [2]CA Clinical Pharmacy and Molecular Diagnosis, Faculty of Pharmacy; [3]Faculty of Psychology. Universidad Autónoma del Estado de Morelos, Mexico City; [4]Center of Infectious Diseases, Instituto Nacional de Salud Pública, Cuernavaca, Mor.; [5]Postgraduate and Research Section, Higher School of Medicine, Instituto Politécnico Nacional, Mexico City. Mexico*

## Abstract

*The present study determined through analytic techniques the quantification of some biomarkers that have been useful to detect early ethanol consumption in a college population. A group of 117 students of recent entry to the Universidad Autónoma del Estado de Morelos was analyzed. The enzyme determination of aspartate aminotransferase, alanine aminotransferase, and gamma glutamyltransferase as metabolic markers of ethanol, as well as the carbohydrate-deficient transferrin (CDT) detected by high chromatographic liquid (up to 1.8% of CDT), allowed us to identify that 6% of the college population presented a potential risk of alcohol consumption. The use of the biochemical-analytical method overall with the psychological drug and a risk factor instrument established by the Universidad Autónoma del Estado de Morelos permit us to identify students whose substance abuse consumption puts their terminal efficiency at risk as well as their academic level. The timely detection on admission to college can monitor and support a student consumer's substance abuse.* (Gac Med Mex. 2016;152:132-7)

***Corresponding author:*** *Sara García-Jiménez, saragarcia@uaem.mx*

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## Introduction

In Mexico, school dropout and low academic performance in college students of the entire country are due, among several social factors, to the consumption of substances of abuse[1]. The consumption of alcoholic beverages is common practice among youths because they are easily accessible and the cost of beverages with very low analytical quality is low. Alcohol abusive consumption generates changes at the cerebral level, which produce tolerance and dependence; therefore, alcohol and tobacco consumers soon can easily move on to the consumption of other substances of abuse such as marihuana and cocaine[2,3]. According to the 2011 National Survey on Addictions (ENA 2011) of the Mexican Ministry of Health, alcohol consumption increased considerably from 2002 to 2011. In the 12 to 65-year-old population, a significant increase was observed in alcohol consumption: sporadic consumption (sometime in life) went from 64.9 to 71.3%, prevalence of consumption in the preceding 6 months, from 46.3 to 51.4% and prevalence in the preceding month, from 19.2 to 31.6%. A student admitted to the

**Correspondence:**
*Sara García-Jiménez
Laboratorio de Bioquímica Clínica, Facultad de Farmacia
Universidad Autónoma del Estado de Morelos
Av. Universidad, 1001
Col. Chamilpa
C.P. 62209, Cuernavaca, Mor., México
E-mail: saragarcia@uaem.mx

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university is on average 19 years old, and at this stage of life he/she has already been exposed to alcohol and tobacco consumption. According to the ENA 2011, this sector of the population considerably increased consumption. Sporadic consumption (sometime) in youths between 18 and 20 years went from 35.6 to 42.9%, both in males (11.5% to 17.4%) and females (2.7 to 11.6%). In 2013, a study conducted at the *Universidad Michoacana de San Nicolás de Hidalgo* was published, which determined an incidence of alcohol consumption of 76.9% in young college students and established that there were no differences between males and females (73.7 and 78.5%, respectively)[4]. In view of all this, it is necessary and indispensable for universities to have a program to timely detect the consumption of substances of abuse among young college students in order to eliminate the risk of school dropout, initiation of other substances of abuse and, in many occasions, cases of violence.

At the *Universidad Autónoma del Estado de Morelos,* a psychological assessment instrument named DROYFAR, which was intended to diagnose possible risks associated with the consumption of substances of abuse adolescents might be exposed to, was implemented and validated[5]. Previously, our working group correlated this instrument with the consumption of marihuana and cocaine by analytically analyzing the d9-THCA-A and benzoylecgonine metabolites, respectively. The instrument was determined to be adequate to detect young people at high risk for consumption of substances of abuse[6].

The purpose of this work was to determine the usefulness of some biomarkers of alcohol in order to, together with the DROYFAR instrument, be able to early detect alcohol consumption in students of the *Universidad Autónoma del Estado de Morelos.*

The biomarkers proposed were the gamma glutamyl transferase (GGT)[7], alanine aminotransferase (ALT)[8], aspartate aminotransferase (AST)[9] enzymes and carbohydrate-deficient transferrin (CDT)[10], in addition to the mean corpuscular volume (MCV)[11,12].

## Method

### *Application of the DROYFAR instrument*

According to the *Tierra y Libertad* official journal (2007)[13], the DROYFAR instrument is intended to identify factors influencing on students and putting their academic performance at risk, as well as their mental and physical health. This questionnaire evaluates 6 areas related to mental health, family relationships, level of education, aggressive behavior, academic performance and the information on effects and damages associated with substance use and abuse. The idea of applying this instrument enables university authorities, based on the results obtained in the population, to make decisions for prevention and/or opportune care of young students at risk for dropping out, with perceived low academic performance, poor communication with their families, frequent mood changes or overt aggression against the environment, among other problems. The instrument was applied to 117 students at admission to the *Universidad Autónoma del Estado de Morelos.*

### *Biological samples*

To select the students, a non-probabilistic, incidental-type sampling was carried out, i.e., students who were present at the moment the DROYFAR instrument was applied were included. The sample was comprised by 74 females and 43 males, students of different disciplines at the *Universidad Autónoma del Estado de Morelos.* The DROYFAR instrument was assessed by the Department of Psychology of the university under the direction of Dr. Fenando Bilbao. A blood sample was requested from each participant, which was obtained by venous puncture (5 ml), as well as a urine sample (10 ml). In order to determine the students' general health status, total blood was analyzed by means of blood count (leukocyte, hemoglobin and MCV quantification) and the urine samples, with test strips (determination of pH, density, nitrites, proteins, glucose, ketones and the corresponding microscopic assessment). Other part of the blood samples were centrifuged at 3,500 g for 5 min and the serum was stored at -20 °C for ethanol concentration and ALT, AST and GGT enzymes analysis.

### *ALT, AST and GGT enzymes and ethanol determination*

ALT, AST and GGT enzymes activity was determined with Roche-USA laboratories enzymatic kits in a Cobas c 111 automated equipment (Roche USA). 100 µl of serum were used with the corresponding substrate of each enzyme; measurement is based on NADH oxidation by dehydrogenase lactate and/or dehydrogenase malate, forming a compound able to absorb ultraviolet light (405 nm for GGT and 340 nm for ALT and AST).

### *Ethanol determination on blood samples*

Blood alcohol determination was performed using a colorimetric diagnostic enzyme assay kit Roche-COBAS USA. Ethanol present on the blood sample reacts with NAD+ and the alcohol dehydrogenase enzyme, which is proportional to the amount of alcohol on the sample.

### *CDT determination*

For the determination of glycosylated transferrin, an analytical method previously validated by Helander et al. (2003)[14] was implemented, using high performance liquid chromatography (HPLC). In a 200 µl sample, transferrin was saturated with iron with 40 µl of a 10 mmol/l ferric nitrilotriacetic acid (FeNTA) solution; each transferrin molecule binds at the most to two ions, which makes for incubation not to be required. The FeNTA solution was prepared with 275 mg of nitrilotriacetic acid monohydrate salt and 270 mg of $FeCL_3$ in 90 ml of water. On this sample, lipoproteins were precipitated by a dextran-$CaCl_2$ solution (20 g/l and 1 mol/l, respectively). The samples were placed on ice for 30-60 min and centrifuged at 3,500 g for 5 min. 100 µl of the supernatant were diluted in a 2:5 proportion with 10 mM Bis-Tris buffer solution (pH 6.2) to stabilize transferrin.

To quantify transferrin glycoforms, 100 µl of the sample were injected into a Waters HPLC using a ion-exchange column Waters Protein Pak Q 15 HR 15 µm (10 x 100 mm), with a UV detector at 470 nm and gradient elution of 4 buffer solutions with variable composition: solution A: Bis-Tris 10 nM pH 7; solution B: Bis-Tris 10 nM + 0.5 M NaCl pH 6.2; solution C: Bis-Tris 10 nM pH 6.2 and solution D: NaCl 2 M.

## Results

The present investigation included 74 females and 43 males who had been recently admitted to the *Universidad Autónoma del Estado de Morelos* and who were applied the DROYFAR instrument. An age range of 18-20 years was determined; 62% referred having consumed some initial substance of abuse (alcohol [44%], alcohol and tobacco [11%] and only tobacco [7%]). Most of the analyzed population were detected to still maintain familiar stability, since 86% of participants lived with relatives.

All surveyed subjects were taken a blood and urine sample, which underwent blood count and urinalysis testing, respectively. All analyzed samples, both from males and females, were found to be within normal reference values for the population of the city of Cuernavaca (Morelos); only 4% showed slight leucopenia and 5%, leukocytosis. Four percent of females and 14% of males had hypochromia.

On the other hand, the GGT, AST and ALT enzymes were quantified as possible alcohol markers and all samples, except for 7, were found within normal reference limits. Table 1 shows the number of samples found to be outside normal limits of the AST, ALT and GGT enzymes concentrations. AST concentration was high in all 7 samples, whereas those for ALT and GGT were elevated only in two samples. In order to rule out that the enzyme concentration increase was due to hepatitis B or C, rapid testing test strips were used and none of the subjects was determined to be positive to these tests.

MCV is regarded as a biomarker when alcohol consumption is high. In this work, no participant showed a significant increase of this parameter (94.6 fl) and for this reason it was not proposed as a biomarker in the present study.

The determination of serum ethanol was carried out using an enzymatic colorimetric method at 340 nm, validated with a 0.5% variation coefficient accuracy; this method enables to measure concentrations ranging from 10 to 500 mg/dl. All the results of the analyzed samples were below the detection limit (10 mg/dl).

To quantify transferrin glycoforms, 4 different solutions were assessed by HPLC. The chromatographic profile of the gradient obtained at a flow rate of 1 ml/min is shown in table 2.

To determine CDT, the HPLC ion exchange analytical method was implemented[14]. The different glycoforms were eluted for 20-30 min (Fig. 1). To determine reproducibility, the disialotransferrin area under the curve (AUC) was considered (AUC: 1565.4 ± 94.6; VC: 6.0%), with a precision of 6% being obtained. Linearity was determined with 4 different serum solutions at 50 (100 µl), at 100 (200 µl), at 150 (300 µl) and at 200 (400 µl), with a coefficient of 0.99 being obtained.

All analyzed samples showed CDT values lower than 2%; the seven samples with the highest levels displayed an average value of 1.8%. In order to establish that the quantification method for the different biomarkers was adequate, 8 positive samples of a group of subjects from a rehabilitation center of the state of Morelos, classified as high alcohol chronic consumers, were processed. The peak enzymatic level of this control population's enzymes ranged between the following

**Table 1. Number of students with increased enzymatic biomarkers**

| Enzyme | Reference values | Number of samples with increased values | |
|---|---|---|---|
| | U/l | Males | Females |
| AST | 31 in females and 36 in males | 4 (42, 44, 50, 52) | 3 (40, 42, 50) |
| ALT | 38 in females and 55 in males | 2 (64, 66) | 2 (48, 56) |
| GGT | 38 in females and 55 in males | 2 (79, 98) | 2 (42, 120) |

**Table 2. Profile of the gradient used for transferrin glycoforms separation by HPLC**

| Time (min) | Solution A (%)* | Solution B (%)† | Solution C (%)† | Solution D (%)§ |
|---|---|---|---|---|
| 0.00 | 100 | 0 | 0 | 0 |
| 1.00 | 100 | 0 | 0 | 0 |
| 1.01 | 0 | 0 | 100 | 0 |
| 30.00 | 0 | 30 | 70 | 0 |
| 30.01 | 0 | 0 | 0 | 100 |
| 35.00 | 0 | 0 | 0 | 100 |
| 35.50 | 100 | 0 | 0 | 0 |
| 37.00 | 100 | 0 | 0 | 0 |

*Solution A: 10 mmol/l Bis-Tris pH 7.
†Solution B: 10 mmol/l Bis-Tris + 0.5 mol/l NaCl pH 6.2.
†Solution C: 10 mmol/l Bis-Tris pH 6.2.
§Solution D: NaCl 2M.

values: ALT: 89-186 U/l; AST: 47-64 U/l and GGT: 69-315 U/l. CDT "positive" values (Y axis) with regard to the values obtained for the student samples (X axis) are depicted in figure 2. Ethanol chronic consumers samples appear in gray color and were higher than 2.2% with regard to those determined in the student sample.

According to the ALT, AST and GGT enzymatic determination, 7 students were detected at possible risk of alcoholism; these data are correlated with values closer to the CDT upper limit (1.8%). No statistically significant differences were found with regard to the lowest values detected in 4 CDT-positive samples (2-3%) by means of Student's t-test using a p-value $< 0.05$. Therefore, ALT, AST and GGT enzymatic analytical method and CDT chromatographic method can be considered to be adequate to detect alcohol consumption.

It is important pointing out that no CDT or enzymes (ALT, AST and GGT) levels as high as those in the positive control samples were detected, since the latter corresponded to alcohol chronic drinkers and the young college students were initial consumers.

## Discussion

The DROYFAR instrument was implemented in 2005 at the *Universidad Autónoma del Estado de Morelos* with the purpose to timely detect cases of students likely to be part of the school dropout or poor performance group due to the use of substances of abuse. Using this assessment instrument in the present study, 62% of the 117 evaluated students were detected to have been exposed to the consumption of substances of abuse; the subset of those who are part of a high-risk group likely to move on to other substances such as marihuana or cocaine is unknown.

With the purpose to really identify that high-risk students did not avoid detection with the applied DROYFAR instrument, we decided to analyze a series of ethanol biomarkers.

A biomarker is defined as a characteristic that is objectively measured and assessed as an indicator of a normal biological process, a pathogenic process or a pharmacological response. Biomarkers are classified as status markers, when they are absent

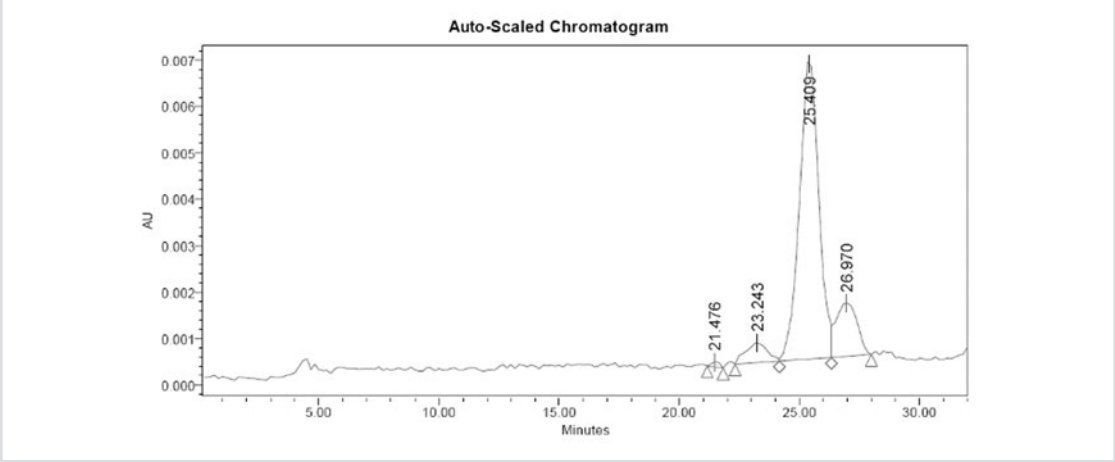


**Figure 1.** *CDT assessment chromatogram analysis: from left to right, the disialotransferrin (21'), trisialotransferrin (23'), tetrasialotransferrin (25') and pentasialotransferrin (27') transferrin glycoforms are observed.*

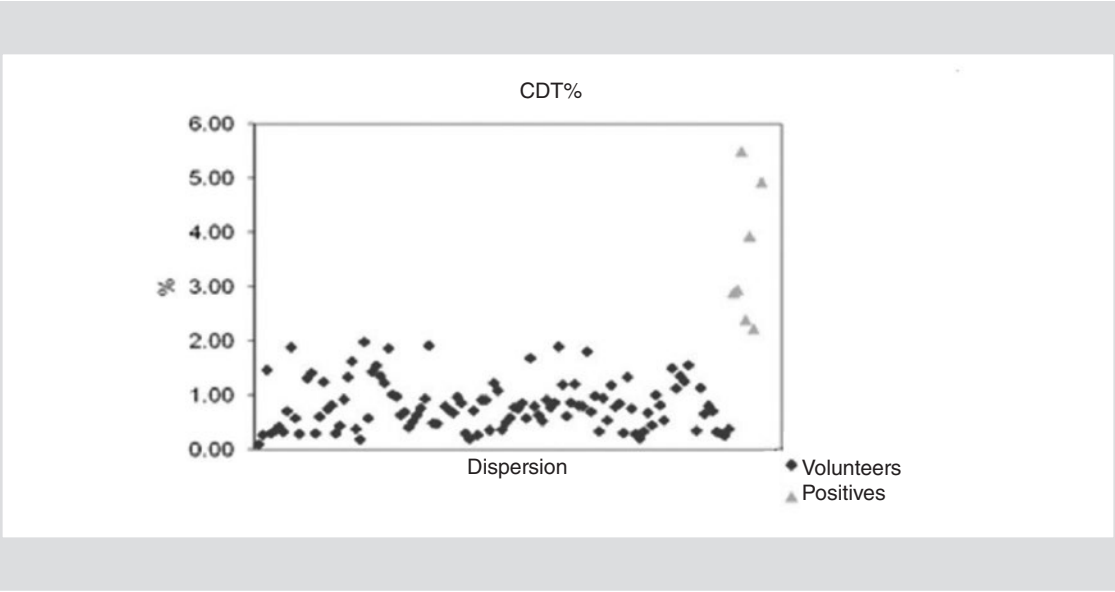


**Figure 2.** *CDT serum values dispersion; volunteer student values are shown in blue and, in red, those for the samples obtained from a rehabilitation center of Cuernavaca (Morelos).*

before and after the disease, and its presence can be useful for diagnosis and treatment, and trait makers, when they are indicators present throughout the individual's entire life and are able to coexist with disease[15].

According to the literature, the AST, ALT and GGT enzymes, as well as MCV and the CDT value, are considered as biomarkers to detect high ethanol consumption[8-10]. In the present study, MCV was excluded as a biomarker because no significant differences were found with regard to normal values. Ethanol concentrations determination was neither useful, since no concentrations higher than the reference value could be detemined[8-10]. Thus, only the AST, ALT and GGT enzymes were considered as biomarkers, in addition to the CDT value[8-10]. The increase in the AST, ALT and GGT enzymes values suggested that 7 individuals might be in the high-risk group, and this data was

correlated with the CDT values. Although the requirement of a CDT value of 2% to classify an individual in a high-risk group has been documented, in the present study, a CDT average value of 1.8% was established. It is important to consider that this data depends on the analyzed population; for example, in Korea, up to 2.4% has been recorded as reference for CDT[16]. In Mexico there is no CDT reference data available; in this first study, a value of 1.8% is proposed for a young population classified as being at high risk for ethanol consumption.

Next, some considerations that must be taken into account for future studies are explained. Although the biochemical method was adequate to detect high-risk consumers, it is important for a clinical diagnosis to be available, in addition to the DROYFAR instrument, since the levels of quantified enzymes can be modified by diseases such as hepatitis B and C, hepatic abscesses, biliary diseases or cirrhosis, as well as consumption of medications (at least one month before). In addition, the fact that markers depend on age, gender and study population has to be considered. Clearly, the study sample has to be broadened in order to establish a reference value for the AST, ALT and GGT enzymes and for CDT.

## Conclusions

The analytical method employed is adequate to detect values higher than 2% in positive individuals from a rehabilitation center; in the case of the student samples, the detected values were lower than 2%. However, the CDT value, together with the AST, ALT and GGT enzymes determination, is adequate to opportunely detect alcohol consumption in students admitted to the university. The analytical method, combined with the DROYFAR instrument, allows for a proposal to timely detect students with potential risk for dropping out due to the consumption of substances of abuse such as ethanol to be established.

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