20.

RED BLOOD CELLS (RBC) DEFORMABILITY AND AGGREGABILITY: ALTERATIONS IN ALCOHOLISM

Vincenzo O. Palmieri, Giuseppe Cicco*, Francesco Minerva, Piero Portincasa, Ignazio Grattagliano, Vincenzo Memeo* and Giuseppe Palasciano[1]

1. INTRODUCTION

Alcohol is known to produce alterations in erythrocyte function leading to hemolysis association with alterations in shape and volume (macrocitosis, echinocitosis). [1,2]

The presence of macrocitosis is strictly associated with alcohol-correlated folate deficit even if modifications of the Mean Corpuscular Volume (MCV) do not correlate with the values of daily alcohol intake in alcoholic patients. [3,4,5]

Whether these effects are due to hemorheological alterations (deformability and aggregation) is not well understood.

Preliminary and isolated observations have reported some effect of *in vivo* alcohol on red blood cell (RBC) morphology and deformability evaluated by Rheodine SSD in healthy subjects consuming a large quantity of alcohol [6], but these changes have not been correlated with the selective action of alcohol per se or its metabolites (acetaldehyde, fatty acid ethyl esters) and could not be demonstrated in alcoholism (that according to WHO may be defined as a condition characterized by chronic consumption of alcohol associated with the presence of dependence and target organs damage).[7]

Recent studies have detected an *in vitro* action of ethanol, but not of acetaldehyde, on erythocytes resistance against hemolysis induced by sodium hypochlorite; the deleterious effect of ethanol consumption on erythrocyte *in vivo* may be, at least in part, the result of a direct effect of unmetabolized ethanol on erythrocyte components.[8]

No definitive data are available on *in vivo* RBC hemorheological properties in human alcoholics.

The Laser Assisted Optical Rotational Cell Analyzer (LORCA, Mechatronics, Hoon), first described by Hardeman et al.[9,10,11] is a valid technique for the measurement of various structural hemorheological parameters such as RBC deformability expressed by the elongation (EI: expressed as PA) and the RBC aggregation index (expressed as AI and T1/2).

This study therefore aimed to evaluate in patients with a history of alcoholism, RBC deformability and aggregation alterations using the LORCA, to correlate data on RBC

[1] Clinica Medica "A. Murri" University of Bari – *CEMOT Bari University of Bari

deformability with RBC morphological alterations and MCV values, to correlate data on RBC aggregability with factors able to modify this physical property, (such as the level of plasma fibrinogen), and evaluate the effects of alcohol withdrawal on these parameters.

2. MATERIALS AND METHODS

Nineteen consecutive alcoholic patients were enrolled in this study during their stay in the Clinica Medica "A. Murri" to evaluate alcohol-related damage and to plan their rehabilitation program.

The diagnosis of alcoholism was made according to the criteria suggested by the WHO[7] and confirmed by clinical questionnaires, biochemical markers (MCV, gamma GT, AST/ALT), and the presence of one or more target organs damage. In all patients liver disease was ascertained by histology. The mean daily alcohol intake at admission to the study was assessed according to a standardized questionnaire. Patients were queried about their alcohol intake during the previous 6-year period and the average daily consumption of beer, wine and spirits was quantified. Thereafter, the mean lifetime daily alcohol intake was calculated. General inclusion criteria were as follows: patient compliance, age $\geq$18$\leq$ 60yrs, history of problematic use of alcohol, daily alcohol consumption > 80g/day >4 days/week, absence of arterial hypertension or cardiac and hematological diseases.

The hemorheological study was conduced day 1, 7, 14 and 90 of alcohol withdrawal. Preliminary data are available on days 1 and 7 of abstinence.

Alcoholic subjects that did not comply with abstinence during the observation period were excluded from the evaluation. The maintenance of abstinence was ascertained by clinical and biochemical (MCV, gamma GT, AST/ALT) parameters.

18 subjects with a negative personal history of problematic alcohol consumption and with a mean daily alcohol intake <20 g/day served as controls. They underwent the same preliminary evaluation of hepatic and hematological functions as assessed in the alcoholic patients.

The hemorheological study was conducted with the LORCA equipment of the CEMOT in the University of Bari. Deformability and aggregation of RBC were expressed as previously reported[9,10]. According to the standard procedure, 25 μl blood samples from each subject were processed the same day of the observation and diluted in 5 ml of polyvinylpyrrolidone (PVP) before running the test on the LORCA equipment.

Statistical analysis: Student's t test was used for paired (between alcoholics) and unpaired data (comparison with controls), the Mann-Withney U test in case of failure of the normality test, chi-square with Fisher correction; according to previously published data on EI values obtained by the LORCA device, we considered the EI results at pressure values of 0.3, 3 and 30 AP making it appropriate to use a direct comparison of mean values.

Results are expressed as Means (M) $\pm$ Standard Deviation (SD).

3. RESULTS

Clinical and serological characteristics of the alcoholic patients and controls are reported in Table 1. Although no differences were found between the two groups regarding sex distribution and age, the alcoholics had much higher values of AST, ALT