

CONTRIBUTION OF RED CELL MASS AND *UGT1A1* ALLELES IN SERUM BILIRUBIN LEVELS OF THE PORTUGUESE POPULATION

Carina Rodrigues^{1,2}, Elísio Costa^{3,5}, Rosário Santos⁴, Alice Santos-Silva^{1,5}
& Elsa Bronze-da-Rocha^{1,5}

1 - Faculdade de Farmácia da Universidade do Porto; 2 - Escola Superior de Saúde do Instituto Politécnico de Bragança;
3 - Instituto de Ciências da Saúde da Universidade Católica, Portugal; 4 - Instituto de Genética Médica Dr. Jacinto Magalhães INSARJ,
Porto; 5 - Instituto de Biologia Molecular e Celular da Universidade do Porto, Portugal.



1. INTRODUCTION

The reticuloendothelial system breaks down old red blood cells and bilirubin is one of the waste products that must be made water-soluble to be excreted. The unconjugated, bilirubin is carried by albumin to the liver, is in a lipid-soluble form, where it is conjugated by the enzyme uridine-diphosphate-glucuronosyltransferase 1A1 (*UGT1A1*) and excreted¹. A polymorphisms in *UGT1A1* gene, consisting in a *TA* duplication [(*TA*)₇ allele] in the repetitive *TATA*-box sequence of the gene promoter encoding this enzyme, result in a decreased capacity to glucuronidate bilirubin, a characteristic observed in Gilbert syndrome (GS)². However, this polymorphism is not sufficient explain the inter-individual variation and the presence of hiperbilirubinemia³. Recently, it was described that increased red cell mass probably plays a role in the pathogenesis of GS⁴. Since hemoglobin degradation is the principal determinant in bilirubin production, it may be possible that phenotypical differences between subjects related to red blood mass could explain some of the inter-individual variation.

The aim of this work is to investigate the putative role of increased red cell mass and the (*TA*)₇ allele in bilirubin serum levels, in the Portuguese population.

2. MATERIAL AND METHODS

Subjects and assays

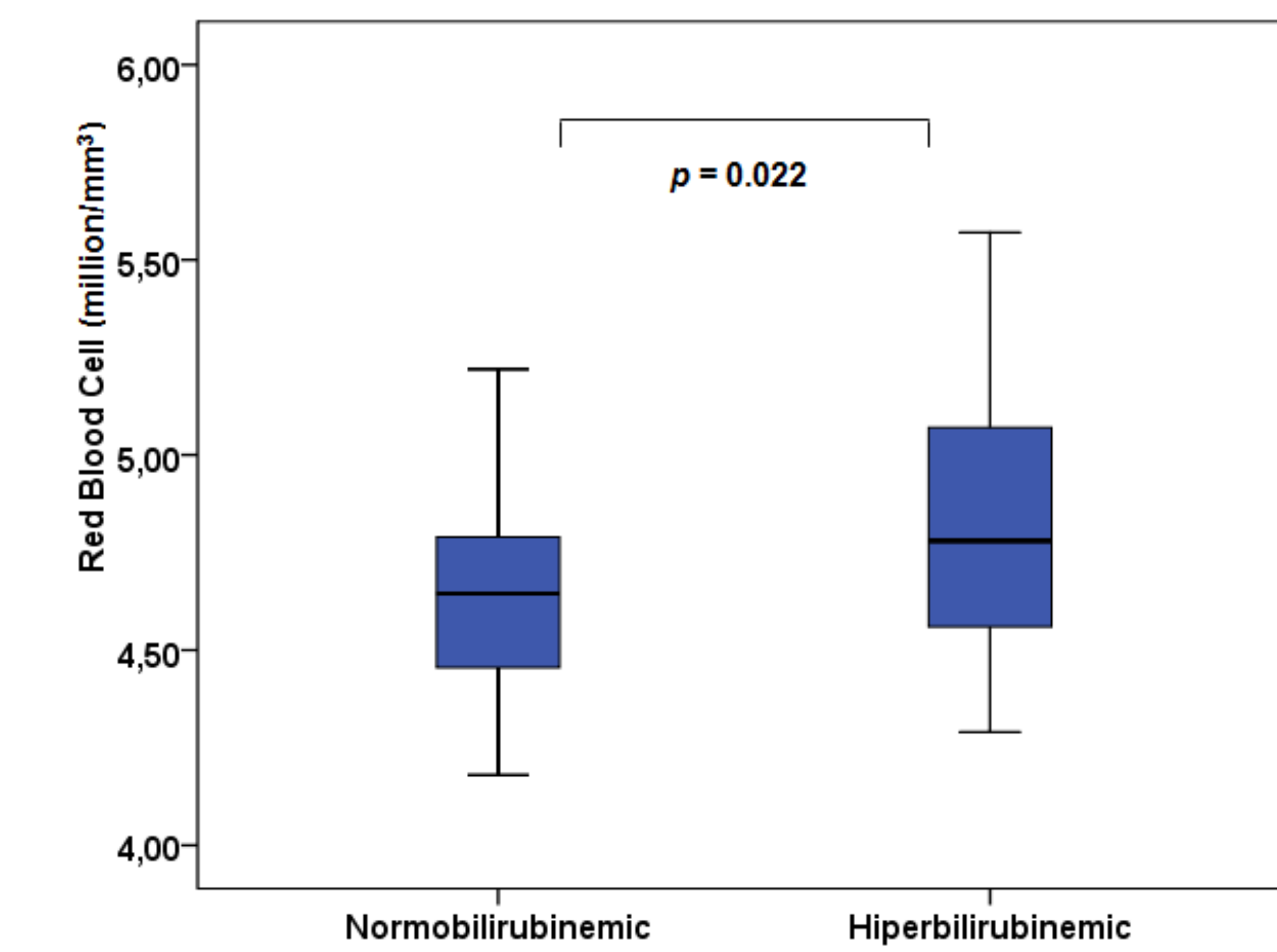
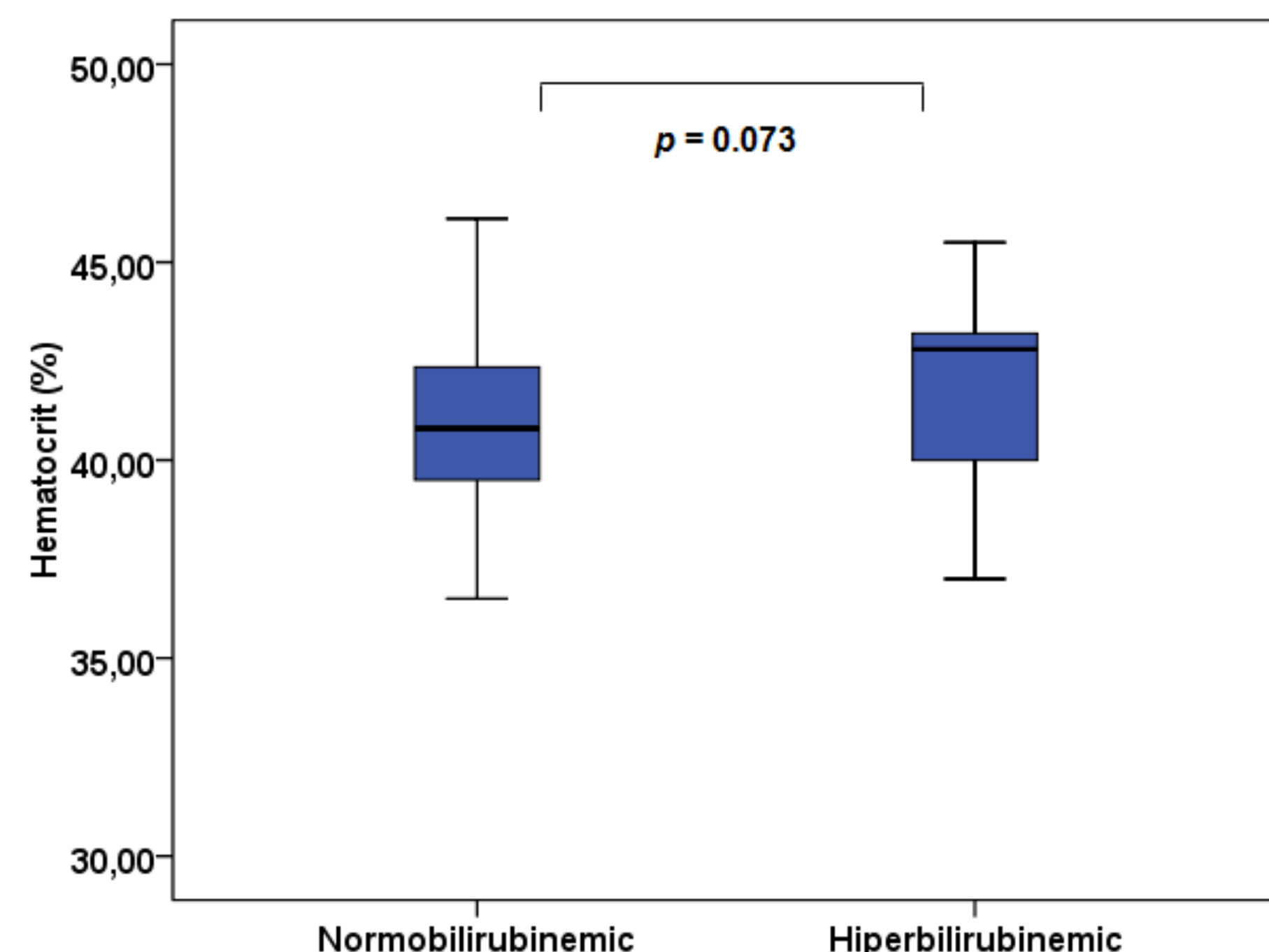
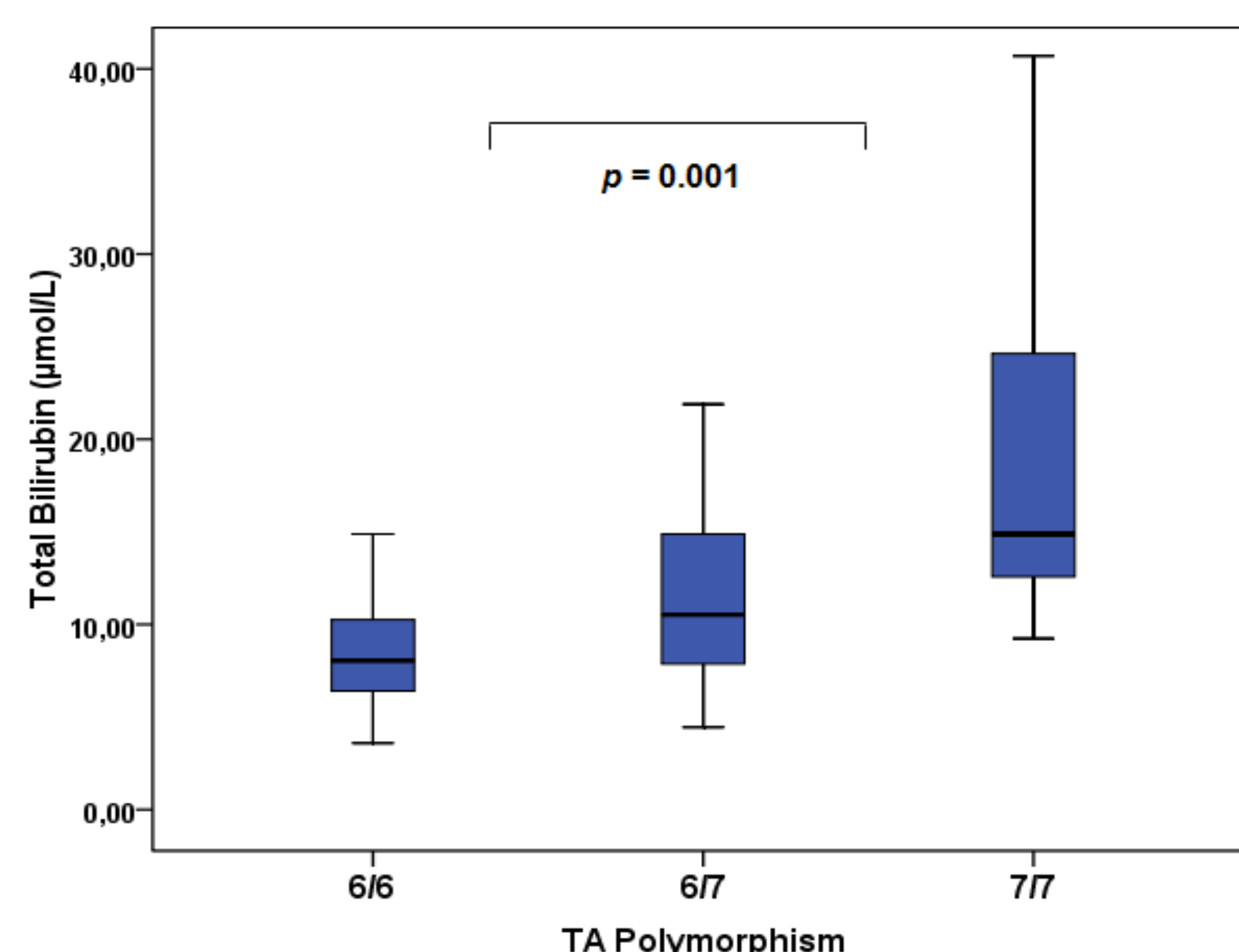
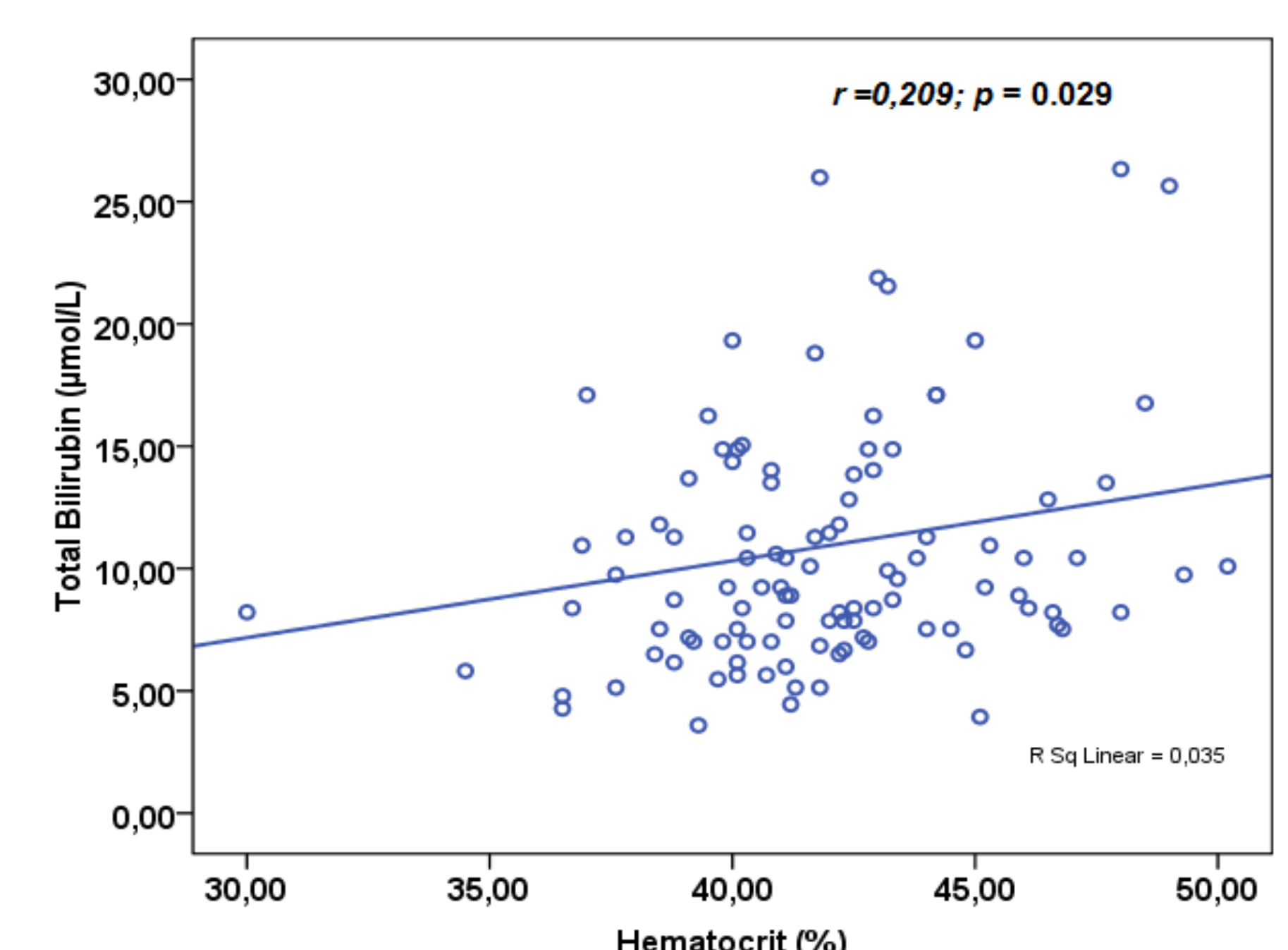
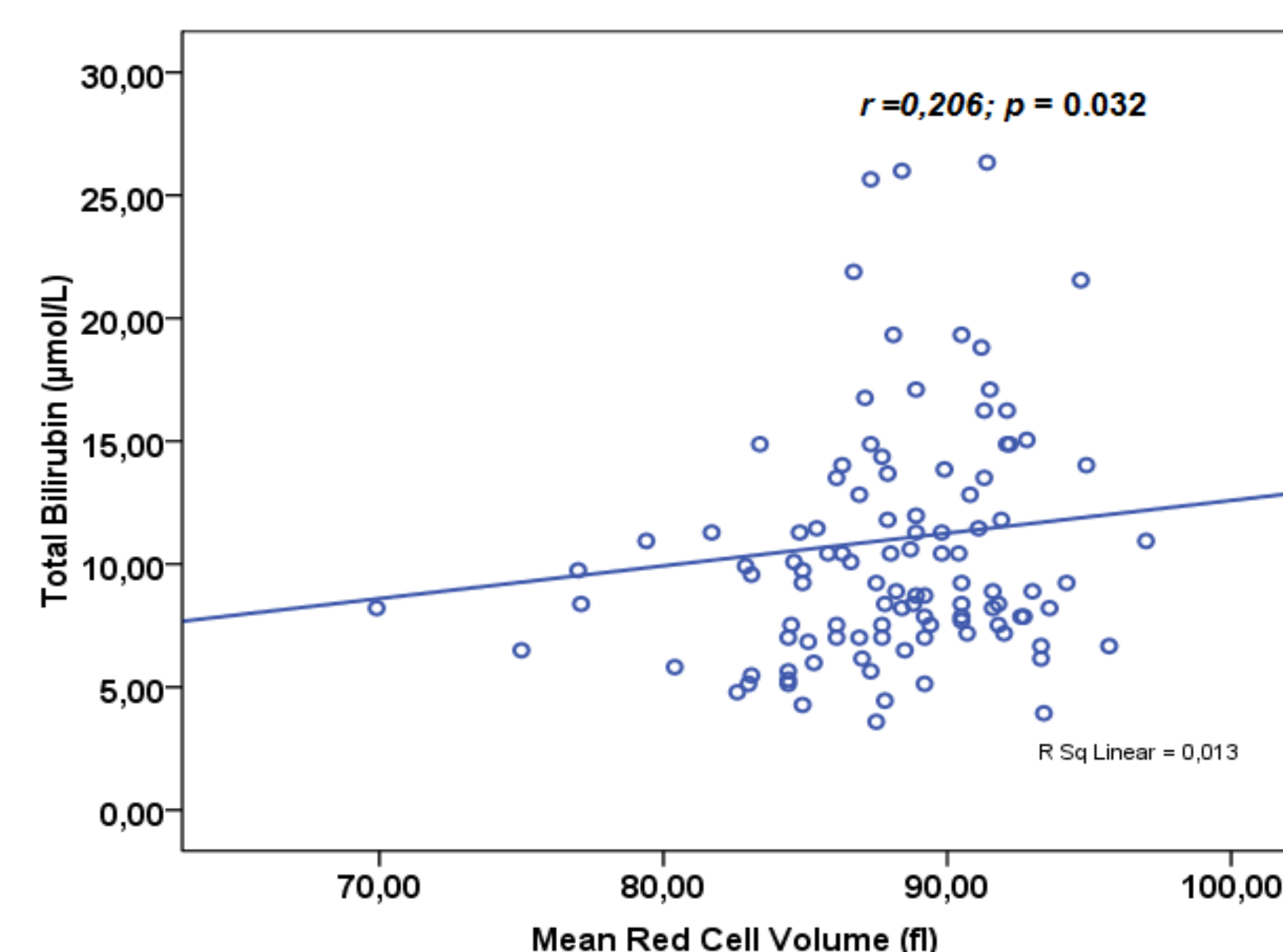
This study was performed in 109 volunteer healthy young adults (20.3±1.9 years) without liver and/or hematological disorders. Blood samples were collected and processed in order to determine bilirubin serum levels, complete blood cells count, and DNA extraction. The *TATA*-box region was analyzed by PCR amplification followed by subsequent analysis by automated capillary electrophoresis as previously described⁵.

Data Analysis

For statistical analysis, we used the Statistical Package for Social Sciences (SPSS; version 16.0 for Windows, Inc. Chicago, IL, USA). Kolmogorov Smirnov statistics were used to evaluate sample normality distribution. Spearman's rank correlation coefficient was used to evaluate relationships between sets of data. Total bilirubin values were categorized in normal and hiperbilirubinemic (>17,1µmol/L). The variable *TA* Repeat Polymorphism was categorize in normal (6/6), Heterozygous (6/7) and Homozygous (7/7). Comparison between groups were performed using the Kruskal-Wallis test and the Mann-Whitney U test. Significance was accepted at $p < 0.05$.

3. RESULTS

Fig. 1. Correlations between serum bilirubin levels and: Mean Red Cell Volume (A) and Hematocrit (B). Differences between normobilirubinemic and hiperbilirubinemic subjects: in *TA* Repeat Polymorphism (C), Hematocrit (D) and Red Blood Cell Count (E)



4. DISCUSSION

Results obtained in this work showed that:

- among our population, 7 subjects were homozygous for the (*TA*)₇ allele, 54 were heterozygous (*TA*)₆ / (*TA*)₇ and 48 were homozygous for the normal allele;
- comparing bilirubin serum levels (SBL) according to the *UGT1A1* genotype, we found statistically differences in SBL between genotypes ($p < 0.001$);
- a positive significant correlation was found between SBL and hematocrit ($r=0,209$; $p=0,029$) and between SBL and mean cell volume ($r=0,206$; $p=0,032$);
- statistically differences in Red Blood Cell Count were found between hiperbilirubinemic and normobilirubinemic subjects ($p=0,022$) and a trend to higher hematocrit was found in hiperbilirubinemic subjects ($p=0,073$).

Taken in account that some studies refer sub-clinical hemolysis and reduced life span of erythrocytes⁶ as important factors in hiperbilirubinemia it is remarkable having red blood cell mass correlated positively with serum bilirubin levels. Further studies including a larger group of controls and GS patients [homozygous for the (*TA*)₇ allele] are required to analyze the same parameters separated by gender since males have higher red cell blood mass and higher bilirubin turnover⁷. Dependence on red cell mass explains why this condition is more frequent in males⁴.

This data suggests that in our population the presence of abnormal number of *TA* repeats in the *UGT1A1* gene is associated with increased bilirubin levels and that increased red blood mass could contribute for this phenotype.