INFLUENCE OF GENETIC AND ACQUIRED FACTORS THAT MODIFY SERUM BILIRUBIN LEVELS IN THE PORTUGUESE POPULATION

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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 where it is conjugated by the enzyme uridine-diphosphate-glucuronosyltransferase 1A1 (UGT1A1) and excreted[1]. 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) patients[2]. However, this polymorphism is not sufficient to explain the inter-individual variation and the presence of hiperbilirubinemia[3]. It is established that other non-genetic factors like gender[1], age[4], or smoking status[5], influence the inter-individual variation of bilirubin levels. There are few studies that compare the influence of genetic and non genetic variables in serum bilirubin concentration. Recently, it was described that increased red cell mass probably plays a role in the pathogenesis of GS[6]. Since hemoglobin degradation is the principal determinant in bilirubin production, it may be possible that phenotypical differences between subjects could be also explained by the influence of non-genetic factors.

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

2. MATERIAL AND METHODS

Subjects and assays

To perform this study we recruited 183 young adults (138 females and 45 males; aged 20,8 ± 2,1 years). Standardized interviewer-administered questionnaire was performed in all subjects, which included questions about smoking habits, oral contraceptive therapy, caloric intake, fasting time, and physical activity. Exclusion criteria included the presence of liver and/or hematological disorders. All volunteers gave their written informed consent.

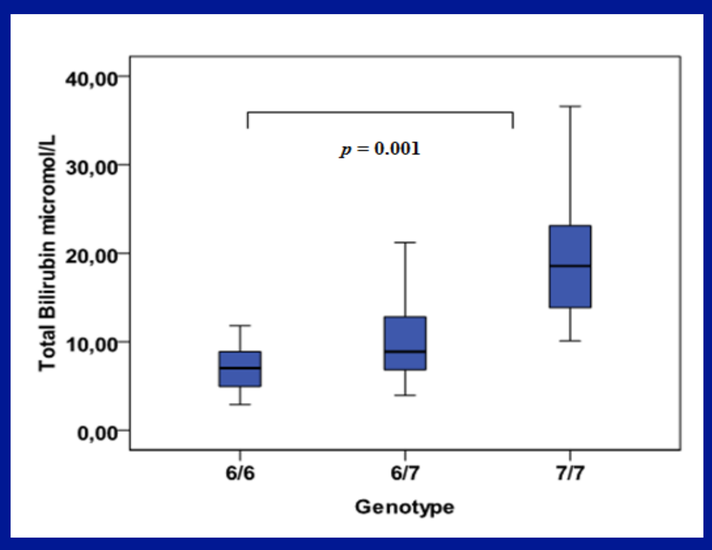
For each individual, after an overnight fasting, venous blood samples were collected in order to determine total and direct-reacting bilirubin and cell count. Genomic DNA was isolated for molecular study of *UGT1A1* gene promoter, followed PCR amplification as previously described[3].

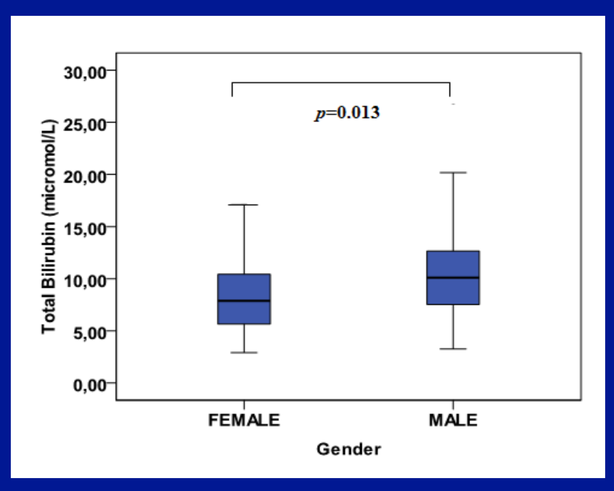
Data Analysis

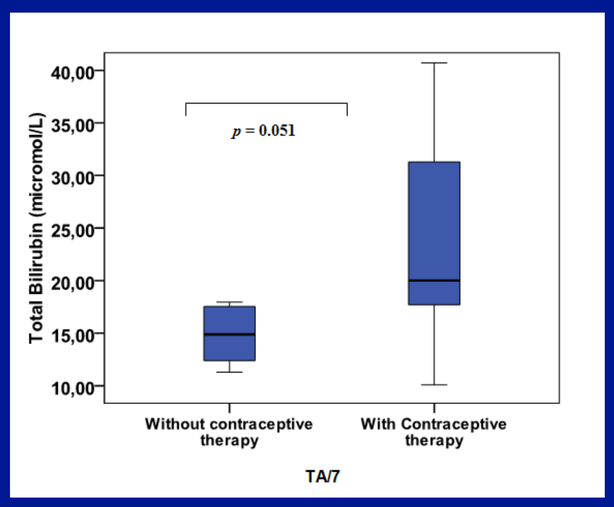
For statistical analysis, the Statistical Package for Social Sciences, version 17.0 was used. Kolmogorov Smirnov statistics were used to assess sample normality distribution. The variable *TA* repeat polymorphism was categorize in Normal (*6/6*), Heterozygous (*6/7*) and Homozygous (*7/7*). To compare groups we use Kruskal-Wallis test and the Mann-Whitney U test. We used the Spearman correlation coefficient to evaluate relationships between sets of data. Significance was accepted at $p<0.05$.

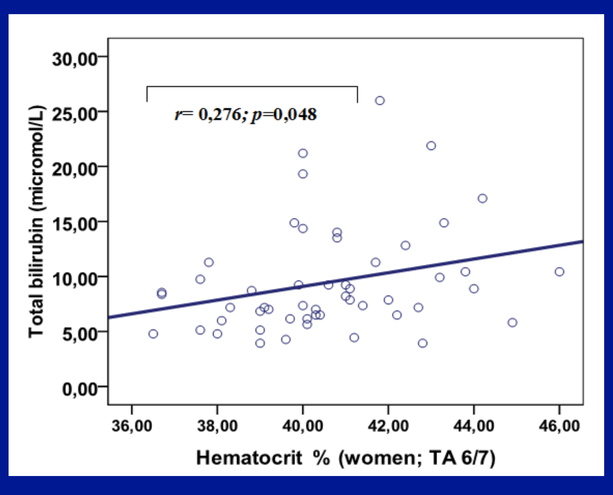
3. RESULTS and DISCUSSION

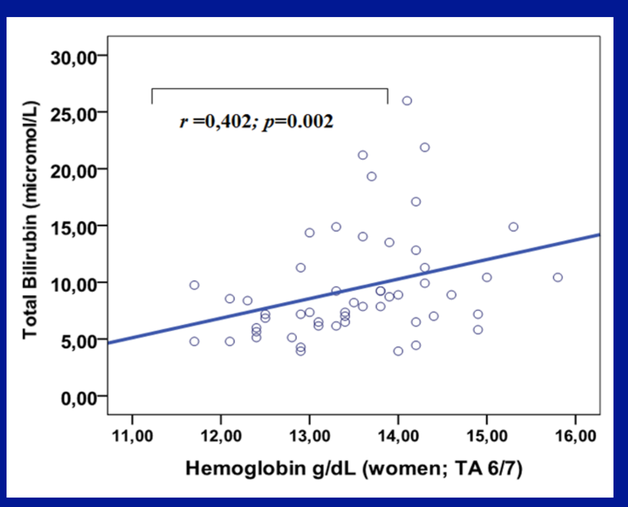
The first approach to data analysis was done using all subjects and we found statistical differences between serum bilirubin levels (SBL) and genotypes as well as gender. It is well-known that *TA* polymorphism is the *major* determinant in the hiperbilirubinemia observed in Gilbert syndrome patients. We also verified that *TA* polymorphism is determinant in SBL of the normal population and we can separate the groups by genotype. The second step to stratify the sample, eliminating the confounder effect of those two variables, and searching differences and associations on the different groups. We found differences only in homozygous women under contraceptive therapy, suggesting that this factor may condition a higher hiperbilirubinemic state behind the genotype. We also searched for a putative interference of different red blood mass in bilirubin levels since it is expectable that more hemoglobin concentration leads to more bilirubin production. Dependence on red cell mass explains why this condition is more frequent in males. Some studies refer that sub-clinical hemolysis and reduced life span of erythrocytes are important factors in hiperbilirubinemia, thus is not expected to have a positive correlation of red blood cells with SBL. Our results showed that the correlation between the red blood mass and bilirubin levels was not strong as described by Buyukasik and co-workers[6] which refer that this factor could explain an increment in SBL present in Gilbert syndrome patients.











Differences between total bilirubin concentration (µmol/L) according to: (A) Genotypes *6/6*, *6/7* and *7/7*; (B) Gender; (C) use of contraceptive therapy.

Correlations between total serum bilirubin levels (µmol/L) with: (D) hemoglobin and (E) hematocrit.

4. CONCLUSIONS

Our study showed:

- a clear association between the *TA* repeat polymorphism and higher bilirubin concentration (p = 0,001).
- statistical significant differences in serum bilirubin levels according to gender (p = 0,013) and to contraceptive therapy (p = 0,051).
- statistically significant positive correlations were found between bilirubin serum level and hematocrit (r = 0,276; p = 0,048) as well as with hemoglobin (r = 0,402; p = 0,002).
- no statically significant differences were found in smoking status, caloric intake, fasting time and physical activity.

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