Abstract. – Background: Bariatric surgery is the most effective long-term treatment for morbid obesity, reducing obesity-associated co-morbidities. We decide to investigate the role of the polymorphism (G1359A) of the cannabinoid (CB)1 receptor gene on clinical outcomes 1 year after biliopancreatic diversion in morbidly obese patients.

Design: A sample of 66 morbidly obese patients (BMI >40 kg/m²) were operated. Weight, fat mass, blood pressure, basal glucose, triacylglycerols, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol were measured at basal visit and at each visit. The frequency of metabolic comorbidities was recorded at each visit.

Results: Thirty-nine patients (59.1%) had genotype G1359G (wild type group) and 27 (40.9%) patients had genotype G1359A (mutant type group). In wild and mutant type groups, body mass index, weight, waist circumference, systolic blood pressure, glucose, total cholesterol, low-density lipoprotein cholesterol and triacylglycerols concentrations decreased, without statistical between genotype groups. Initial weight percent loss at 1 year of follow up was similar in both genotypes (33.1% vs 33.6%; ns).

Conclusion: The present study demonstrates that polymorphism G1359A in the CB1 receptor did not have a significant effect on biochemical and anthropometric improvements after biliopancreatic diversion surgery.

Key Words: Bariatric surgery, Cannabinoid receptor gene, Polymorphism, Morbidly obese, G1359A

Introduction

Obesity and type 2 diabetes mellitus are spreading rapidly worldwide. Weight reduction is known to be an effective treatment for overweight-obese patients with risk factors of metabolic syndrome. Bariatric surgery is the most effective long-term treatment for morbid obesity, reducing obesity-associated co-morbidities.

Biliopancreatic diversion (BPD) of Scopinaro et al is a mixed operation that has shown good results regarding weight loss. Nevertheless, long-term follow up is known to be heterogeneous in some surgery studies. Perhaps the genetic background of these patients could influence in follow up and outcomes.

Herbal Cannabis sativa (marijuana) has been known to have many psychoactive effects in humans including increases in body weight. The endogenous cannabinoid system mediates and it’s positioned both functionally and anatomically to be an important modulator of normal human brain behavior. This system consists of endogenous ligands 2-arachidonoylglycerol (2-AG) and anandamide (ADA) and two types of G-protein-coupled cannabinoid receptors: cannabinoid type-1 receptor (CB1), located in several brain areas and in a variety of peripheral tissues including adipose tissue, and cannabinoid type-2 receptor (CB2), present in the immune system. A silent intragenic biallelic polymorphism (1359 G/A) of the CB1 gene resulting in the substitution of the G to A at nucleotide position 1359 (Thr), was reported as a common polymorphism in the German population, reaching frequencies of 24-32% for the allele (A).

Considering the evidence that endogenous cannabinoid system plays a role in metabolic aspects of body weight and feeding behavior, we decide to investigate the role of missense polymorphism (G1359A) of CB1 receptor gene on outcomes 1 year after biliopancreatic diversion in morbidly obese patients.
Subjects and Methods

Subjects

A sample of 66 morbidly obese patients (BMI >40) was operated on from December 2004 to December 2008 (Table I). We analyzed a consecutive series of patients who underwent open BPD by the Scopinaro et al technique.3

The BPD consisted of an average of 200-cm alimentary limb and 80-cm common limb. Gastric volume was measured after stapling with sterile water. Intestinal limbs were measured during the surgery with a sterile tape measure. Follow-up visits were carried out at intervals (3, 9, and 12 months). The following variables were specifically recorded: age, weight, BMI, waist circumference, and associated morbidity.

Evaluation and Follow-up Time

The frequency of patients with diabetes mellitus, hypertension and hyperlipidemia was recorded at each visit (3, 9, and 12 mo after surgery).

Hypertension and hyperlipidemia were diagnosed in patients taking hypotensive and hypolipemic drugs, respectively. Hypertension or hyperlipidemia according to National Cholesterol Education Program standards also was diagnosed.9 Diabetes mellitus was diagnosed in patients taking hypoglycemic drugs or insulin or according to the American Diabetes Association standard of diagnosis.10

Weight, BMI, fat mass, blood pressure, basal glucose, triacylglycerols, total cholesterol, LDL cholesterol, HDL cholesterol were measured at basal visit (before surgery) and at each visit (3, 9, and 12 mo).

Methods Use for Each Determination

Blood pressure was measured twice after a 10 minutes rest with a sphygmomanometer OM-RON Mx3 (Omron Matsusaka Co. Ld, Tokio Japan), and averaged.

Body weight was measured to an accuracy of 0.1 Kg and body mass index computed as body weight/(height^2).

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Serum total cholesterol, HDL-cholesterol and triacylglycerol concentrations were determined by enzymatic colorimetric assay (Roche Diagnostics, Mannheim, Germany).

Genotyping of CB1 Gene Polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International, LA, CA, USA). The polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 µL of each oligonucleotide primer (primer forward: 5'-TTC ACA GGG CCG CAG AAA G-3' and reverse 5'-GAG GCA TCA GGC TCA CAG AG-3'), and 0.25 uL of each probes (wild probe: 5'-Fam-ATC AAC AGC AGC TCA GTC AAG ATT GCC-BHQ-1-3') and (mutant probe: 5'-Texas red- ATC AAC ACA GTC AAG ATT GCC -BHQ-1-3') in a 25 µL final volume (Termociclador iCycler IQ (Bio-Rad), Hercules, CA, USA). DNA was denatured at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95°C for 15 s, and annealing at 59.3°C for 45 s). The PCR were run in a 25 µL final volume containing 12.5 uL of IQTM Supermix (Bio-Rad, Hercules, CA, USA) with hot start Taq DNA polymerase. Hardy Weinberger equilibrium was assessed.

Statistical Analysis

The results were expressed as means ± SD. The normal distribution of variables was analyzed with Kolmogorov-Smirnov test. Non-parametric variables were analyzed with the Mann-Whitney test and Wilcoxon test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher’s test. Sample size estimation was performed based on the effects on weight loss using polymorphism frequency (30%) in morbid obese subjects. A p-value under 0.05 was considered statistically significant. The statistical analysis was performed for the combined G1359A and A1359A as a group and wild type G1359G as second group, with a dominant model. A p-value <0.05 was considered statistically significant.

Results

Thirty-nine patients (59.1%) had genotype G1359G (wild type group) and 27 (40.9%) patients had genotype G1359A (mutant type group). In the wild type group, mean age was 41.6±14.6 years and there were 9 men and 30 women. In the mutant type group, mean age was 43.3±10.4 years and there were 6 men and 21 women, without statistical differences.

The preoperative characteristics of the patients are shown in Table I.
Table II presents anthropometric parameters and blood pressure levels. In the wild type group, BMI, weight, waist circumference and systolic blood pressure decreased. In the mutant type group, the same parameters improved, without statistical differences between groups. No differences were detected between mutant and wild genotypes in all anthropometric parameters. Initial weight percent loss at 1 year of follow up was similar in both groups (33.1% vs 33.6%; ns).

Table III shows the biochemical parameters. In the wild type group, glucose, total cholesterol, LDL cholesterol and triacylglycerol concentrations decreased. In the mutant type group, the same parameters improved. No differences were detected between mutant and wild genotypes in all these biochemical parameters.

A decrease in the percentage of cardiovascular risk factors was detected in both groups. After one year, hypertension frequency decreased in wild-type patients from 35.8% to 7.7% and from 37.1% to 7.4%, in mutant-type patients. Diabetes mellitus disappeared in all patients (wild and mutant genotypes). Oral drugs or subcutaneous insulin were discontinued in all patients after surgery. After one year, dyslipemia frequency decreased in wild-type patients from 10.3% to 2.6% and from 11.1% to 3.7%, in mutant-type patients.

Discussion

The finding of this study is the lack of association of the G1359A and A1359A CB1 genotypes with clinical and biochemical outcomes after bariatric surgery. Weight, waist circumference, systolic blood pressure, glycemia, plasma lipid levels improved during follow up after BPD in carriers of the homozygous wild-type or heterozygous variant genotype.

The literature supports the notion that endocannabinoid system is positioned for regulation of endocannabinoid levels that could influence craving and reward behaviors through the relevant neuronal circuitry and metabolic parameters. Also, the CB1 receptor is expressed in some peripheral human tissue studied in relation to the pathogenesis of obesity and obesity-associated metabolic disorders. A marked down-regulation of the fatty acid amide hydrolase (FAAH) gene expression was found in the adi-

Table I. Preoperative characteristics of the patients.

| Morbid obese | 51 |
| obese | 15 |
| Gender (men/women) | 15/51 |
| Age (years) | 42.9 ± 13.3 |
| BMI (kg/m²) | 48.8 ± 7.6 |
| Hypertension (%) | 36.6% |
| Diabetes mellitus (%) | 7.6% |
| Dyslipemia (%) | 10.6% |

Table II. Anthropometric and blood pressure course.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Basal time</th>
<th>3 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
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<tbody>
<tr>
<td>Wild group (G1359G)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>48.3 ± 6.5</td>
<td>40.7 ± 6.8*</td>
<td>36.2 ± 6.2*</td>
<td>33.2 ± 7.7*</td>
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<tr>
<td>Weight (kg)</td>
<td>126.1 ± 20.1</td>
<td>104.1 ± 18.3*</td>
<td>95.3 ± 15*</td>
<td>87.5 ± 18*</td>
</tr>
<tr>
<td>IEWL (%)</td>
<td>–</td>
<td>15.8</td>
<td>25.3</td>
<td>33.1</td>
</tr>
<tr>
<td>SBP mmHg</td>
<td>151± 35</td>
<td>136.1 ± 18*</td>
<td>131 ± 16*</td>
<td>129.1 ± 20*</td>
</tr>
<tr>
<td>DBP mmHg</td>
<td>90 ± 18</td>
<td>78.8 ± 17</td>
<td>81.3 ± 11</td>
<td>81.2 ± 7.6</td>
</tr>
<tr>
<td>WC</td>
<td>127 ± 18</td>
<td>114.4 ± 15*</td>
<td>100.8 ± 16*</td>
<td>101.6 ± 20*</td>
</tr>
<tr>
<td>Mutant group (G1359A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>48.9 ± 7.6</td>
<td>41.1 ± 8.4*</td>
<td>37.4 ± 5.1*</td>
<td>33.7 ± 5.7*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>126.3 ± 20</td>
<td>107.9 ± 18*</td>
<td>93.5 ± 13*</td>
<td>84.3 ± 13*</td>
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<tr>
<td>IEWL (%)</td>
<td>–</td>
<td>14.5</td>
<td>26.2</td>
<td>33.6</td>
</tr>
<tr>
<td>SBP mmHg</td>
<td>135 ± 16</td>
<td>132.5 ± 20*</td>
<td>135.1 ± 14</td>
<td>131.4 ± 9.2*</td>
</tr>
<tr>
<td>DBP mmHg</td>
<td>85.1 ± 6.9</td>
<td>82.2 ± 9</td>
<td>83.1 ± 6.3</td>
<td>86.2 ± 10.2</td>
</tr>
<tr>
<td>WC</td>
<td>126 ± 17</td>
<td>112.8 ± 14*</td>
<td>101.6 ± 12*</td>
<td>102.9 ± 16*</td>
</tr>
</tbody>
</table>

IEWL%: initial excess weight percent loss. WC: Waist circumference. SBP (systolic blood pressure). DBP (diastolic blood pressure). * p<0.05 with basal value in each group.
pose tissue, suggesting that adipose tissue may be an important contributor to endocannabinoid metabolism.

In our study the prevalence of GA genotype was 59.1%, higher than previous studies: 43.5%\(^{13}\), 19.6%\(^{14}\) and 33.1%\(^{15}\). Perhaps, our sample with a high average BMI than previous studies could explain this difference. A second finding is the lack of association between BMI and genotypes of this polymorphism. This fact is in contrast with the association detected by Gazzerro et al\(^{13}\) with SNP G1359A of CB1 receptor, and other polymorphisms of this receptor such as A3813A and A4895A SNPs\(^{16}\) and (G1422A) SNP of CB1 receptor\(^{17}\). The inconsistencies between association studies may reflect the complex interactions between multiple population-specific genetic and environmental factors.

The effects of different polymorphisms after bariatric surgery are an interesting area of investigation. Some Authors have demonstrated, that melanocortin-4 receptor gene variant determine the outcome of bariatric treatment of severe obesity\(^{18,19}\). In other study, Sesti et al\(^{20}\) have demonstrated that after laparoscopic adjustable gastric banding, carriers of G-174G IL-6 genotype had lost more weight than G-174C or C-174C and carriers of A866A uncoupling protein 2 genotypes have lost more weight as compared with G866G.

Only one interventional study with this polymorphism has been published. Aberle et al\(^{14}\) have shown that carriers of at least one A allele in CB1 lost more weight and reduced LDL cholesterol than wild type patients. Perhaps, these discrepancies with our results could be explained by different inclusion criteria of subjects in this previous work. First, the average BMI was different in our bariatric surgery study than previous hypocaloric diet study. Second, weight loss and the maintenance are different. Therefore, the question arises of whether weight loss achieved by a BPD in our research overrides the subtle polymorphism-dependent effects seen in Aberle’s study\(^{14}\). However, this topic area is an interesting area of investigation with unclear therapeutical implications\(^{21,22}\).

In conclusion, the present study demonstrates that polymorphism G1359A in the CB1 receptor did not have a significant effect on biochemical and anthropometric improvements after bariatric surgery.

### References

