Original article

Frequency of cardiovascular risk factors before and 6 and 12 months after bariatric surgery

Maria Alayde Mendonça da Silva, Ivan Romero Rivera, Emília Maria Wanderley de Gusmão Barbosa, Maria Angélica Correia Crispim, Guilherme Costa Farias, Alberto Jorge Albuquerque Fontan, Rodrigo Azavedo Bezerra, Larissa Gabriella de Souza Sá

Hospital Universitário Professor Alberto Antunes, Universidade Federal de Alagoas, Maceió, AL, Brazil

ARTICLE INFO

Article history:
Received 30 October 2012
Accepted 21 February 2013

Keywords:
Obesity
Hypertension
Dyslipidemia
Diabetes mellitus type 2
Risk factors
Bariatric surgery

ABSTRACT

Objective: To compare the frequency of cardiovascular risk factors (CVRFs) in obese patients of the Brazilian Unified Health System (Sistema Único de Saúde – SUS) with indication of bariatric surgery during the preoperative period and after the sixth month and the first year of the procedure.

Methods: An observational, longitudinal, prospective, and analytical study was performed, with consecutive selection of obese patients with indication for surgery referred to preoperative cardiac evaluation. The protocol consisted of: medical history, physical examination, electrocardiogram, echocardiogram, and biochemical analysis. This study analyzed the following variables: weight, body mass index (BMI), waist circumference (WC), systemic arterial hypertension (SAH), diabetes mellitus type 2 (DM), dyslipidemia (high LDL cholesterol; low HDL cholesterol; hypertriglyceridemia), and metabolic syndrome (MS). The chi-squared test and the Tukey-Kramer method were used for statistical analysis.

Results: The sample was composed of 96 obese people, among which 86 were women, aged between 18 and 58 years old (median 35 years old). At the end of six months, significant reductions of 88%, 95%, 71%, 89%, and 80% in the frequency of SAH, high LDL cholesterol, hypertriglyceridemia, DM, and MS could already be observed. A significant and small reduction in the frequency of low HDL cholesterol (24%) and abnormal WC (31%) was observed only at the end of 12 months. After six months and one year, weight and BMI experienced reductions of 33.4 kg and 44.3 kg, and 13.1 kg/m² and 17.2 kg/m², respectively.

Conclusion: The positive impact on weight loss and the reduction in BMI, WC, and in the frequency of CVRFs are already extremely significant after six months and remain so one year after bariatric surgery.

© 2012 Elsevier Editora Ltda. All rights reserved.
Frequência de fatores de risco cardiovascular antes e 6 e 12 meses após gastroplastia

RESUMO

Objetivo: Comparar a frequência dos fatores de risco cardiovascular (FRCV) em obesos com indicação de gastroplastia no pré-operatório e após o sexto mês e o primeiro ano do procedimento, em usuários do Sistema Único de Saúde.

Métodos: Foi realizado estudo observacional, longitudinal, prospectivo e analítico, com seleção consecutiva de obesos com indicação cirúrgica, encaminhados para avaliação cardiológica pré-operatória. O protocolo foi constituído de: história clínica, exame físico, eletrocardiograma, ecocardiograma e dosagens bioquímicas. No presente estudo, foram analisadas as seguintes variáveis: peso, índice de massa corporal (IMC), circunferência abdominal (CA), hipertensão arterial Sistêmica (HAS), diabetes mellitus-tipo 2 (DM), dislipidemia (colesterol LDL elevado; colesterol HDL baixo; hipertrigliceridemia) e síndrome metabólica (SM). Para análise estatística foram utilizados os métodos do Qui-quadrado e Tukey-Kramer.

Resultados: A amostra foi constituída de 96 obesos. Desses, 86 eram mulheres com idades entre 18 e 58 anos (mediana de 35 anos). Ao final de seis meses, foi observada redução significante de 88%, 95%, 71%, 89% e 80% na frequência de HAS, colesterol LDL elevado, hipertrigliceridemia, DM e SM. Apenas ao final de 12 meses houve significante e modesta redução na frequência de colesterol HDL baixo (24%) e CA anormal (31%). Em seis meses e um ano, o peso e o IMC sofreram reduções respectivas de 33,4 e 44,3 kg e de 13,1 e 17,2 kg/m².

Conclusão: O impacto positivo na perda de peso, na redução do IMC, da CA e da frequência dos FRCM mostrou-se extremamente significante após seis meses, e se manteve após um ano da gastroplastia.

© 2012 Elsevier Editora Ltda. Todos os direitos reservados.

PALAVRAS-CHAVE: Obesidade, Hipertensão, Dislipidemia, Diabetes mellitus tipo 2, Fatores de risco, Gastroplastia

Introduction

Obesity is currently a public health problem due to its increasing prevalence, the high mortality rate determined by it, and because it increases the frequency of other diseases, which contributes to further increase the morbi-mortality in this group of individuals. These diseases include the cardiovascular risk factors (CVRFs) represented by systemic arterial hypertension (SAH), diabetes mellitus type 2 (DM), and dyslipidemia.

Among the anthropometric methods used to assess excessive body fat, the body mass index (BMI), which divides weight by height squared, has been the most frequently used in adults. Obesity is defined as BMI equal or higher than 30 kg/m².

In a complementary manner, waist circumference (WC) serves as an anthropometric measurement to indicate intra-abdominal fat, and was incorporated as one of the criteria used in the diagnosis of metabolic syndrome (MS), characterized by a group of CVRFs and insulin resistance, which increases cardiovascular mortality in patients with this disease. MS is observed in over 70% of obese patients seeking bariatric surgery.

In Brazil, 13.9% of the population is obese, while 46.6% is overweight (defined as BMI between 25 kg/m² and 29.9 kg/m²). In 2006, these prevalences were 11.4% and 42.7%, respectively.

Despite the positive impact of clinical treatment on obesity, there is an indication for bariatric surgery when the BMI is higher than 40 kg/m² or higher than 35 kg/m² if associated with chronic diseases aggravated by obesity. Bariatric surgery thus became an important therapeutic strategy for obesity; nowadays, gastric bypass and adjustable gastric band are the most widely used methods.

The Brazilian Ministry of Health approved in 2000 the indications for bariatric surgery in the Brazilian Unified Health System (Sistema Único de Saúde – SUS). It is estimated that 344,221 bariatric surgeries were performed in 2008, 25,000 of which in Brazil. Currently, in Brazil, approximately 65,000 bariatric surgeries are performed on an annual basis.

A meta-analysis of 134 studies involving approximately 22,000 obese patients demonstrated that bariatric surgery determines an average reduction of 61% of the excess body weight, 39.7 kg of body weight, 13.2 kg/m² in the BMI, and an important improvement or resolution of comorbidities. The greatest loss of excess weight and beneficial effect on comorbidities occur approximately one year after the surgery, remaining stable in most patients; much of this effect, however, can already be observed in the first six months.

The objective of this study was to compare the frequency of CVRFs in obese patients with indication of bariatric surgery (gastric bypass, Fobi-Capella) during the preoperative period and after the sixth month and the first year of the procedure, in patients of the SUS.
Methods

An observational, longitudinal, prospective, and analytical study was performed with a sample of patients with diagnosis of obesity and indication of bariatric surgery (gastropasty) included in the Bariatric Surgery Program of the Hospital Universitário Professor Alberto Antunes (HUPAA) of the Universidade Federal de Alagoas (UFAL).

Patients were consecutively referred for preoperative cardiac evaluation and submitted to the same protocol for evaluation, which included: medical history (including the use of medicines on a regular basis), physical examination (including measurement of blood pressure, weight, height, WC), 12-lead electrocardiogram, echocardiogram, and biochemical analyses (fasting glucose, total cholesterol, LDL cholesterol, triglyceride levels). Other heart tests were requested pursuant to the individual clinical indication (24-hour Holter monitoring, myocardial scintigraphy, coronary angiography, among others).

For the diagnosis of SAH, dyslipidemias, DM, and MS, the parameters included in the respective guidelines from the Brazilian Society of Cardiology, currently described in the I Brazilian guideline for diagnosis and treatment of metabolic syndrome, were used.

For the diagnosis of obesity based on the BMI, the criteria of the World Health Organization, also adopted in Brazil, were used: low weight (BMI < 18.5 kg/m²), normal weight (BMI between 18.5 and 24.9 kg/m²), overweight (BMI between 25 and 29.9 kg/m²), obesity grade I (BMI between 30.0 and 34.9 kg/m²), obesity grade II (BMI between 35.0 and 39.9 kg/m²), and obesity grade III (BMI > 40 kg/m²). Severely obese patients were also classified as super obesity (BMI between 50 and 59.9 kg/m²) and super-super obesity (BMI > 60 kg/m²). WC, measured at halfway between the costal margin and the iliac crest, was considered normal when below 88 cm in women and below 102 cm in men.

The following variables were analyzed for this study: levels of obesity based on the BMI, abdominal obesity based on the WC, SAH, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, DM, and MS.

The Chi-squared test was used to compare proportions of the categorical variables, and the Tukey-Kramer method was used for multiple comparisons of the average continuous variables, before and after the bariatric surgery (after six months and after one year). The significance level was set at 5% (p < 0.05).

The research project was approved by the Research Ethics Committee of the UFAL (process No. 011407/2010-93 of August 30, 2010).

Results

During the period from January 2005 to June 2010, 155 obese patients with indication of bariatric surgery pursuant to the criteria established by the Brazilian Health Ministry were evaluated from the cardiovascular standpoint. Of these patients, 101 were submitted to bariatric surgery pursuant to the Fobi-Capella technique (one death occurred in the first days of the postoperative period; no complications were observed in the other patients), and 96 concluded at least one year of postoperative clinical follow-up, constituting the sample of this study. From the 54 other patients, 32 abandoned the program and 22 were waiting for surgery.

In this study, the sample was constituted of 10 men (10%) and 86 women (90%) aged between 18 and 58 years old, median of 35 years, and average of 37.2 ± 4.8 years.

The average and standard deviation values of the continuous variables in the preoperative evaluation were as follows: a) weight: 124.1 ± 23.3 kg; b) BMI (kg/m²): 48.1 ± 7.0; c) WC: 129.7 ± 14.0 cm; d) fasting glucose: 96.5 ± 26.6 mg/dL; e) total cholesterol: 200.0 ± 32.7 mg/dL; f) LDL cholesterol: 125.4 ± 29.0 mg/dL; g) HDL cholesterol: 41.5 ± 8.6 mg/dL; and h) triglycerides: 159.2 ± 80.0 mg/dL.

Table 1 shows the distribution of the sample based on the BMI values before the bariatric surgery and 6 and 12 months after the intervention. In the table, it is notable that, one year after the bariatric surgery, there was a reduction of 99% in the frequency of grade III obese patients (only one individual remained super obese), emergence of overweight patients (which became 38.5% of the sample), and only four individuals (4.2%) with normal BMI. It is also noted that, at the end of one year, 56.3% of the sample showed grade I or II obesity.

Table 2 shows that the impact of the bariatric surgery on the control of blood pressure, LDL cholesterol, DM, and hypertriglyceridemia, and, consequently, MS affected a lower number of patients after six months of surgery, without a significant decrease in this proportion of individuals in the following six months. Reduction in WC and increase in HDL cholesterol significantly encompassed more patients at a later stage, after only one year from the procedure. The metabolic results observed above were obtained even with only a small number of patients presenting normal HDL cholesterol concentration (24%), WC (31%), and BMI (4%) within six months or one year after the bariatric surgery.

Table 3 shows the average of the different values of the abovementioned continuous variables, from the numerical standpoint, between (a) the preoperative period and the 6th postoperative month, (b) the preoperative period and the 12th postoperative month, and (c) the preoperative period and the 24th postoperative month.

Table 1 – Distribution of patients as to the degree of obesity determined by the body mass index (BMI) before bariatric surgery, six and twelve months later.

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>Before (%)</th>
<th>After six months (%)</th>
<th>After 12 months (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 25 (Regular)</td>
<td>0</td>
<td>0</td>
<td>4 (4.2)</td>
</tr>
<tr>
<td>25.0-29.9 (Overweight)</td>
<td>0</td>
<td>16 (16.7)</td>
<td>37 (38.5)</td>
</tr>
<tr>
<td>30.0-34.9 (Grade I)</td>
<td>0</td>
<td>36 (37.5)</td>
<td>41 (42.7)</td>
</tr>
<tr>
<td>35.0-39.9 (Grade II)</td>
<td>4 (4.2)</td>
<td>33 (34.4)</td>
<td>13 (13.6)</td>
</tr>
<tr>
<td>40.0-49.9 (Grade III)</td>
<td>67 (69.8)</td>
<td>8 (8.3)</td>
<td>0</td>
</tr>
<tr>
<td>50.0-59.9 (Grade III)</td>
<td>19 (19.8)</td>
<td>2 (2.1%)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>≥ 60 (Grade III)</td>
<td>6 (6.2)</td>
<td>1 (1.0)</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>96 (100)</td>
<td>96 (100)</td>
<td>96 (100)</td>
</tr>
</tbody>
</table>
the 12th postoperative month, and (c) the sixth and the 12th postoperative month, as well as the significance level of these differences.

In the same table, it is possible to note that there was a significant reduction in body weight, WC, and BMI in the first phase, between the preoperative period and the sixth month \(p < 0.001\), which also significantly persisted between the sixth and 12th month \(p < 0.001\).

Regarding fasting glucose, total cholesterol, LDL cholesterol, and triglyceride levels, there was already a significant reduction in their concentrations at the end of the sixth postoperative month \(p < 0.001\), with no statistically significant reduction from that moment until the end of the first year after surgery \(p > 0.05\).

**Discussion**

It is estimated that there are currently one billion overweight adults and 500 million obese people in the world.\(^{18}\) In obese patients, the clinical treatment (behavioral changes and pharmacological treatment) results in reduction of only 5% to 10% in body weight (rarely maintained), while the bariatric surgery (usually indicated for obese patients with BMI > 40 kg/m\(^2\)) determines a reduction of approximately 30% in body weight, with such results maintained in the long term.\(^{19}\)

Due to this important reduction in body weight and in visceral fat, bariatric surgery determines, starting in the short term, a positive impact on the control/resolution of the comorbidities associated with obesity, including DM, dyslipidemia, SAH, liver disease, apnea/hypopnea sleep syndrome, and cardiovascular disorder.\(^{20}\) This beneficial effect is maintained in the long term, as observed in patients

---

**Table 2** Distribution of the frequency of cardiovascular risk factors before and after bariatric surgery \((n = 96\) patients).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preoperative period (a)</th>
<th>Sixth postoperative month (b)</th>
<th>12th postoperative month (c)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAH</td>
<td>51 (53%)</td>
<td>6 (6%)</td>
<td>8 (8%)</td>
<td>*axb and *axc &lt; 0.0001 bxc = 0.78</td>
</tr>
<tr>
<td>High LDL</td>
<td>20 (21%)</td>
<td>1 (1%)</td>
<td>3 (3%)</td>
<td>*axb and *axc &lt; 0.0001 bxc = 0.62 axb = 0.48</td>
</tr>
<tr>
<td>Low HDL</td>
<td>78 (81%)</td>
<td>73 (76%)</td>
<td>59 (61%)</td>
<td>*ax = 0.004; *bxc = 0.04 *axb and *axc &lt; 0.0001 bxc = 0.0025</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>42 (44%)</td>
<td>12 (12.5%)</td>
<td>1 (1%)</td>
<td>*axb and *axc &lt; 0.0001 bxc = 1.0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>27 (28%)</td>
<td>3 (3%)</td>
<td>2 (2%)</td>
<td>*axb and *axc &lt; 0.0001 bxc = 0.807</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>66 (69%)</td>
<td>13 (14%)</td>
<td>5 (5%)</td>
<td>*axb and *axc &lt; 0.0001 bxc = 0.014 axc and *bxc &lt; 0.0001</td>
</tr>
<tr>
<td>Waist circumference: &gt; 102 cm in men; &gt; 88 cm in women.</td>
<td>96 (100%)</td>
<td>89 (93%)</td>
<td>66 (69%)</td>
<td>*ax = 0.014 axc and *bxc &lt; 0.0001</td>
</tr>
<tr>
<td>BMI &gt; 25 kg/m(^2)</td>
<td>96 (100%)</td>
<td>96 (100%)</td>
<td>92 (96%)</td>
<td>axc and bxc = 0.12</td>
</tr>
</tbody>
</table>

BMI, body mass index; HDL, HDL cholesterol; LDL, LDL cholesterol; SAH, systemic arterial hypertension.

Chi-squared test.

\(^{*}p < 0.05.\)

**Table 3** Distribution of the average difference between the variables compared at two different moments. (A) between the preoperative period and the sixth postoperative month; (B) between the preoperative period and the first postoperative year and (C) between the sixth postoperative month and the first postoperative year.

<table>
<thead>
<tr>
<th>Variable</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>33.4*</td>
<td>44.3*</td>
<td>10.9*</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>13.1*</td>
<td>17.2*</td>
<td>4.1*</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>23.3*</td>
<td>33.9*</td>
<td>10.6*</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>14.9*</td>
<td>17.8*</td>
<td>2.9</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>31.1*</td>
<td>37.1*</td>
<td>6.0</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>18.9*</td>
<td>25.9*</td>
<td>7.0</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>–1.6</td>
<td>–5.4*</td>
<td>–3.8*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>62.3*</td>
<td>77.5*</td>
<td>15.1</td>
</tr>
</tbody>
</table>

BMI, body mass index; HDL, HDL cholesterol; LDL, LDL cholesterol.

\(^{*}p < 0.001.\)
monitored for ten years after the procedure, and results in the reduction of cases and in cardiovascular mortality, thus making bariatric surgery the treatment of choice for severely obese patients in which the drug-behavioral therapy has failed to control weight and comorbidities.

A meta-analysis of 89 studies performed in other countries regarding the impact of the bariatric surgery on the treatment of obesity showed that the average age of patients is 38 years, and that more than one third of these patients are women. Another meta-analysis involving 134 studies and an aggregate of 22,094 patients showed that 73% are women, with an average age of 39 years. In a sample of 35 obese patients submitted to bariatric surgery in a Brazilian university hospital, Monteiro Junior et al. identified 88.5% as women, with an average age of 38 years. As in this study, the abovementioned data show that the population undergoing surgical treatment for obesity is predominantly female and in the fourth decade of life, probably when the complications resulting from excess body weight appear from the clinical point of view. Additionally, obese women appear to be more motivated than obese men to lose weight, as a result of social pressures with respect to aesthetics.

Most authors report the impact of bariatric surgery on the reduction of body weight and control of cardiovascular risk factors one and three years after surgery, because of its greater clinical relevance. Within this period, a reduction of 20 kg to 30 kg in the body weight is expected, approximately 35% of the BMI, and resolution of SAH, DM, and dyslipidemia in 62%, 76%, and 70% of the individuals, respectively, with a lower impact on the increase in HDL cholesterol and reduction in WC. In the present study, it was observed that, at the end of the sixth month after bariatric surgery, these expected results were already observed, and were maintained until the end of the first year.

Regarding the anthropometric data, the results presented here showed an average reduction of 33.4 kg in body weight, of 13.1 kg/m² in the BMI (a reduction of 27%), and of 23.3 cm in the WC six months after bariatric surgery. Santos, when evaluating 15 obese patients six months after bariatric surgery, observed an average reduction of 41 kg in the body weight and 15.4 kg/m² in the BMI (a reduction of 28%). Both works identified an acceleration of the positive results of bariatric surgery regarding body weight and BMI, referred to in other groups as relevant only at the end of the first year.

When analyzing the frequency of several cardiovascular risk factors, in the present a drastic reduction in the prevalence of SAH (53% X 7% X 8%), high LDL cholesterol (21% X 11% X 3%), DM (28% X 5% X 2%), and MS (69% X 14% X 5%) was already observed in the sixth month, with no significant difference in these frequencies between the sixth month and the first year after the procedure. This reduction, ranging from 71% to 95%, exceeds that of meta-analyses involving over 30,000 patients undergoing bariatric surgery (between 60% and 70%), which evidences the importance of the earlier assessment of these patients for nutritional adjustments necessary due to a rapid weight loss and, consequently, a rapid loss of essential nutrients.

HDL cholesterol concentrations showed a late increase, so that a significant reduction in the frequency of individuals with improvement in this variable (81% X 76% X 61%) was observed only at the end of one year; a similar trend to that observed with the reduction in the WC, which showed a significant normalization in 31% of the sample only at the end of one year (100% X 93% X 69%). Monteiro et al. observed a reduction of 45.8% in the WC of obese patients three years after the bariatric surgery, so the observed reduction of 31% in the present study appears to follow the expected natural course after surgical intervention. A slow increase in HDL cholesterol concentrations has been observed after reduction in body weight in all individuals submitted to bariatric surgery, with higher impact on those who had bypass surgery.

There was a significant reduction in the triglyceride concentrations during the first year after surgery, with a significant reduction in the prevalence of hypertriglyceridemia both at the end of the sixth month and at the end of the first year of follow-up (44% X 12,5% X 1%). It is known that the triglyceride concentrations are consistently reduced immediately after surgery and maintained in the long term, even if the patient regains weight. More significant reductions are observed after gastric bypass surgery, when compared to the restrictive surgery.

The improvement of the lipid profile in obese patients determined by the bariatric surgery, as observed in this investigation, appears to be related to weight loss and the consequent improvement of the hepatic insulin sensitivity; however, the exact mechanism of these benefits is not fully clear.

The findings above unquestionably show the efficacy of the bariatric surgery in the treatment of obesity in selected patients, and it is worth mentioning that the beneficial effect observed in the metabolic and cardiovascular profile of the study population occurred after six months or one year of follow-up, even with only 4% of the sample reaching the normalization of BMI at that moment. Together, the results showed the importance of maintaining the nutritional and behavioral actions that will contribute to obtain an ideal weight and to prevent any weight gain that could lead these patients back to the risk situations they faced before the bariatric surgery.

Additionally, given that patients undergoing bariatric surgery evolve with higher risk of developing nutritional deficiencies caused by their consequent limitation in the intake and absorption of different nutrients, this study also evidenced the need for a nutritional assessment immediately after the bariatric surgery, in order to continuously guide this supplementation. Thus, the essential measures for ensuring the replacement of the necessary nutrients can be quickly identified.

In 1998, 40,000 bariatric surgeries were performed across the world, 146,301 in 2003, and 344,221 in 2008. Thus, between 1998 and 2003 there was an increment of 266% in the number of surgeries performed; between 2003 and 2008, the increment was of 125%; and considering the interval between 1998 and 2008, the increment was of 761%. The safety of this procedure, evidenced by the low rates (4.3%) of early and late complications (venous thromboembolism, surgical reinterventions, and long hospital stays) and a mortality rate of 0.3% in the first month of the postoperative period in
samples with thousands of patients, together with the good results, support its insertion as an important strategy in the treatment of severe obesity. In the present study, a mortality rate of 0.9% (1/101 patients) was observed in the first month of the postoperative period, caused by fatal pulmonary thromboembolism; no other complications were identified.

Based on the results presented here, it can be concluded that the positive impact of the bariatric surgery on weight loss, reduction in BMI, WC, and in the frequency of cardiovascular risk factors is already extremely significant after six months and is maintained one year after the procedure.

**Conflicts of interest**

The authors declare no conflicts of interest.

**Acknowledgements**

The author would like to thank the CNPq/UFAL Institutional Program of Research Initiation Scholarships for granting scholarships to the students who participated in this work.

**REFERENCES**