Total Hip Replacement Improves Aerobic Capacity in Osteoarthritis Patients: A Prospective Experimental Study

Unyo C1*, Caceres E2, Salazar J3, Gich I4 and Coll R5

1Department of Physical Medicine and Rehabilitation, Egarsat-SUMA, Terrassa, Barcelona, Spain
2Department of Orthopaedic Surgery, Hospital Vall d’Hebron, Barcelona, Spain
3Department of Orthopaedic Surgery, Hospital Germans Trias i Pujol, Badalona, Barcelona, Spain
4Department of Epidemiology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
5Department of Physical Medicine and Rehabilitation, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain

*Corresponding author: Unyo C, Department of Physical Medicine and Rehabilitation, Egarsat-SUMA, c/Garcia Humet, 40, 08221-Terrassa, Spain, Tel: 96650660345; E-mail: cunyo@egarsat.es

Received date: 20 October 2014; Accepted date: 16 December 2014; Published date: 20 December 2014

Abstract

Background: Total hip arthroplasty (THA) is the treatment of choice in advanced hip osteoarthritis and has shown its effectiveness in reducing pain and improving the individual’s function and quality of life. The objectives of our study were to measure the effects of hip osteoarthritis and the post-arthroplasty changes on aerobic capacity and gait parameters.

Methods: This was a prospective, experimental study on 37 patients with hip osteoarthritis pending THA. We registered clinical parameters (range of motion, muscular balance, Visual Analogue Scale [VAS] and use of analgesics), functional scales (Lequesne, Johanson) and metabolic parameters (VO2, VCO2) in the preoperative period and at 6 months after the intervention. The development of the compromised hip was analysed with the Friedman test, the ergonomic parameters were analysed using ANOVA and the correlations between the clinical and ergonomic parameters were analysed with a multiple linear regression model.

Results: We analysed 40 THA in 25 men and 12 women, mean age of 63.9 ± 9.6 years. Hip flexion improved 20º, while the abduction and the rotations improved 10º. Muscular balance continued to show differences with the contralateral limb. The VAS value dropped 52%. In the postoperative follow-up, 71% of the patients did not require analgesics. The scores for the functional scales improved between 30% and 45%. Only 3 individuals exceeded the anaerobic threshold in the preoperative control, while 21 did so after the THA. Likewise, the maximum oxygen consumption rose 18% and the energy expenditure of walking decreased by 29%.

Conclusions: All of the clinical and ergonomic parameters showed statistically-significant improvements. The parameters that correlated best with decreased energy expenditure for walking were the reduction in pain and in use of analgesics and the increase in capacity and in walking speed.

Keywords: Total hip arthroplasty; Gait; Biomechanics; Kinetics; Recovery of function

Abbreviations

THA: Total Hip Arthroplasty; LL: Lower Limb; BMI: Body Mass Index; VAS: Visual Analogue Scale; ROM: Range of Motion; MB: Muscular Balance; SatO2: Oxygen Saturation; SD: Standard Deviation; ANOVA: Analysis of the Variance; DVT: Deep Vein Thrombosis; FL: Fasciae Latae; HR: Heart Rate; HRmax: Maximum HR; VO2: Oxygen Uptake; VCO2: Carbon dioxide output; RER: Respiratory Exchange Ratio; VO2/Kg/m: Gait Efficiency; RERmax: Maximum RER; METS: Metabolic Equivalents; VE/VO2: Ventilatory Equivalent; WOMAC: Western Ontario McMaster Universities Osteoarthritis Index; SF 36: Short Form 36 Health Survey

Background

The efficacy of the Total Hip Arthroplasty (THA) in reducing the pain attributable to severe degenerative pathology of that joint is well established. In fact, together with knee replacement, it is considered the orthopaedic surgery operation with the best cost-effectiveness ratio [1]. Many studies have also emphasised the improvement in functional capacity, measured with different functional assessment scales, after THA [2-5]. Other studies have shown that all the parameters of a generic quality of life test such as the Nottingham Health Profile improved after THA and that this improvement was maintained, without signs of loosening, for at least 5 years [3].

The human gait is a complex process, the result of the integration of a chain of physiological events involving the bone and joint, cardiorespiratory, circulatory, muscular and nerve systems. For this reason, pathology that affects any of these systems can bring movement alterations that can increase the energy expenditure of walking. Healthy individuals spontaneously adopt the walking speed that they
consider most comfortable, which is also the most economical. People that have unilateral alterations of the locomotor apparatus usually select for themselves a gait speed slower than that of healthy individuals of the same age; this represents an attempt to achieve greater stability and less pain, even though it involves greater energy consumption. On the other hand, the altered gait patterns and the pain lead to asymmetry and increased external mechanical work carried out by the unaffected limb. Consequently, THA efficacy can also be assessed quantitatively by the tendency towards normalisation of the energy expenditure of walking [4].

The main objectives of this study were to quantify the energy expenditure of walking in patients with uni or bilateral hip osteoarthritis before and after undergoing THA and to analyse the changes in functional capacity and effort after this surgery. A secondary objective was to ascertain which changes in the clinical examination correlated with improvements in ergometric variables, especially in energy efficiency of walking.

**Methods**

This was a prospective experimental study, in which the same tests were performed pre- and postoperatively. An informed consent approved by the Hospital Universitari Germans Trias i Pujol’s ethics committee was obtained in all of the cases.

**Subjects**

In this study, there were 37 individuals with severe primary or secondary hip osteoarthritis, uni- or bilateral, those were on the waiting list for THA implant. The cause limiting the ability to walk was hip involvement in all cases. We excluded patients receiving a substitute THA and those with severe osteoarthritis in other Lower Limb (LL) joints, or with cardiovascular, metabolic or neoplastic pathology that compromised the individual’s stress tolerance. It was necessary for the individuals to reside near the hospital in order to carry out the postoperative rehabilitation programme, with the individual being controlled by the same physician.

Of the subjects, 25 (67.56%) were males and 12 (32.43%), females. Mean age was 63.9 years standard deviation (SD) 9.6. Mean weight was 75.74 Kg. The body mass index (BMI) was normal in 5 patients, 17 were overweight, 13 had type I obesity and 2 had type II obesity.

The diagnosis that brought about the THA was unilateral primary hip osteoarthritis in 22 cases (59.45%) and bilateral in 7 cases (18.91%); unilateral avascular necrosis of the femoral head in 4 cases (10.81%) and bilateral in 2 cases (5.40%); 1 hip osteoarthritis secondary to a congenital hip luxation (2.70%); and 1 hip dysplasia (2.70%).

There was comorbidity in 32 patients (86.48%): 8 (21.62%) hypertension, 6 (16.21%) dyslipidemia, 6 (16.21%) circulatory disorders, 5 (13.51%) diabetes mellitus, 4 (10.81%) digestive disorders, 3 (8.10%) cancer in remission and 2 (5.40%) coronary disease.

**Experimental protocol**

All of the THA were cemented and were implanted by the same surgical team, using a lateral approach. The patients underwent the centre’s standard protocol for antibiotic and antithrombotic prophylaxis. On the 3rd postoperative day, the patients began rehabilitation, following a specific programme of physiotherapy, occupational therapy and hydrotherapy.

**Outcome measures**

The preoperative study was performed during the 3 months prior to surgery. We registered the pain (both resting and after walking) using a Visual Analogue Scale (VAS) [5] as well as the use of analgesics, and performed a clinical examination that included Range of Motion (ROM), Muscular Balance (MB), leg and thigh perimeters and measurement of the LL lengths. The MB was measured with Daniel’s scale that could included major MB differences. That was: 0 as 0, 1 as 1, 2 as 2, 3 as 3, 4 as 4, 5 as 5, 4+ as 7 and 5 as 8. We also used Johanson’s self-administered questionnaire of the Hospital for Special Surgery [6], Lequesne and Samson’s index of severity for osteoarthritis of the hip [7] and a self-care test applied by the occupational therapist at the beginning and the end of treatment (Table 1). A simple hip X-ray in anteroposterior and axial projection and a maximum effort test on treadmill were carried out. For the exercise test, we used a gas analyser (Mijnhardt Oxygen-4) connected to a treadmill with side bars (Enraf Nonius 3446.535 NR), simultaneously obtaining the electrocardiogram and oxygen saturation (SatO2) (504-US pulse oxymeter, Criticare Systems Inc). First, 3 minutes resting seated were registered. The individual then got up and began to walk (at 2 Km/h in the preoperative period and 3.5 Km/h in the postoperative), with increments of 0.5 Km/h every 30 seconds up to the maximum speed tolerated by the individual; after THA, if the patient exceeded 5 Km/h, 1% of slope was added every 30 seconds. The test was interrupted if arrhythmia or signs of myocardial ischemia were detected; if not, it ended due to fatigue. For the recovery stage, the individual continued walking for 6 minutes at a constant speed (1 Km/h in the preoperative period and 2.5 Km/h in the postoperative).

At 2 months postoperative, another follow-up of the clinical parameters and telemetry to rule out LL dysmetria were performed.
There were 37 individuals in the study, 3 with bilateral THA; that is, a total of 40 prostheses were studied. In the patients who already had 1 THA, the contralateral THA was performed after 1 year in 2 individuals and at 7 months in the other. Two months after THA, 1 patient had a recurrence of hepatocarcinoma, so that individual did not finish the follow-up. Of the 40 prostheses implanted, 36 (90%) were low friction Charnley arthroplasties and 4 (10%) were Auto-fit. All of the THA were performed by 2 surgeons using the same surgical technique. In 16 cases (40%), trochanteric osteotomy was used. There were 21 (52.5%) left THA and 19 (47.5%) right.

Complications occurred in 17 postoperative recoveries (42.5%); 8 (20%) deep vein thrombosis (DVT), 3 (7.5%) of them with pulmonary embolism, 2 (5%) pneumonia cases, 2 (5%) self-limited fevers, 1 (2.5%) urine infection, 1 (2.5%) knee osteoarthritis, 1 (2.5%) paresis of the common peroneal nerve that cleared up spontaneously, 1 (2.5%) abductor sprain and 1 (2.5%) surgical trochanter fracture that was treated by synthesis.

In the postoperative period, dysmetria exceeding 5 mm was found in 26 cases (65%), compensatory lift being indicated in 16 (40%).

All of the patients were followed-up by the corresponding author, following an established therapeutic protocol. In Table 2, the VAS pain scores and the scores for the functional scales used in the preoperative and postoperative periods are shown. In all cases, P<0.000. In the preoperative period, 23 (58.97%) patients required analgesics every day, while 27 (71.05%) did not require them 6 months after the THA.

### Results

All the subjects could perform the gait test and none must have been stopped for coronary symptoms.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Car out</th>
<th>Activities</th>
<th>Pick up objects</th>
<th>TOTAL</th>
</tr>
</thead>
</table>

### Table 1: Self-care questionnaire. The self-care test applied by the occupational therapist at the beginning and the end of treatment. OT: occupational therapy. 0: independence; 1: modified independence; 2: partial assistance; 3: total assistance; NO: need orthosis.

At 6 months, the questionnaires and clinical exam were repeated and another maximum effort test was performed.

### Data analysis

For the statistical analysis, the mean, SD, median, maximum and minimum values were obtained for the quantitative variables. For the qualitative variables, the frequencies and percentages were calculated. We made an exception with MB, applying also quantitative test that could better reflect changes. The Wilcoxon test was used to compare the healthy LL with the compromised one, while the Friedman test was used to analyse the development of the clinical parameters of the compromised LL throughout the follow-up. An analysis of the variance (ANOVA) was applied to the ergometric parameters. Finally, a correlation study with a linear regression model was performed for the variables that showed significant changes. The level of significance accepted was 5%.


In respect to joint, hip ROM in the compromised LL suffered statistically-significant changes (P<0.05) in all the movements except extension. There were no significant changes in knee ROM. The thigh and leg perimeters tended to end up the same for both LLs, although the changes were not very clinically relevant. In the preoperative period, the compromised LL was shorter. Despite the fact that MB was an ordinal variable, it was treated as a quantitative variable, calculating the mean because we considered that it provided useful information.

The differences between both LLs were significantly reduced following THA; however, in spite of this, differences remained in all the process for the anterior straight, the gluteus maximus and medius and the Fasciae Latae (FL) muscles (Tables 3 and 4).

We were able to analyse the ergometric parameters of the 36 THA. In the resting phase, differences were observed only when the Heart Rate (HR) decreased in the postoperative period. The effort phase lasted a mean of 14.83 minutes in the preoperative period and 14.72...
minutes 6 months post-THA. The patients walked almost 500 metres more, more quickly and tolerating slope. Only 3 (8.3%) patients exceeded the anaerobic threshold in the preoperative period; in contrast, in the postoperative period, 21 (58.3%) managed to do so. Resting HR decreased while maximum HR (HRmax) on effort increased, indicating a greater capacity for effort. In general, all of the parameters related with oxygen uptake (V\textsubscript{O\textsubscript{2}}) and carbon dioxide output (V\textsubscript{CO\textsubscript{2}}) increased, as well as the respiratory exchange ratio (RER). Gait efficiency (the coefficient between V\textsubscript{O\textsubscript{2}}/Kg and metres [V\textsubscript{O\textsubscript{2}}/Kg/m]), also improved significantly, 29.35%. In the recovery stage, significant differences were found in the same variables as in the effort phase (Table 5).

<table>
<thead>
<tr>
<th>Range of Motion (degrees)</th>
<th>pre</th>
<th>1 m</th>
<th>2 m</th>
<th>6 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion</td>
<td>75.28</td>
<td>80.25</td>
<td>88.00</td>
<td>93.15*</td>
</tr>
<tr>
<td>Extension</td>
<td>-0.66</td>
<td>-1.62</td>
<td>0.45</td>
<td>1.21</td>
</tr>
<tr>
<td>Abduction</td>
<td>20.29</td>
<td>26.42</td>
<td>29.97</td>
<td>32.52*</td>
</tr>
<tr>
<td>Adduction</td>
<td>18.35</td>
<td>23.32</td>
<td>24.47</td>
<td>28.47*</td>
</tr>
<tr>
<td>External rotation</td>
<td>25.61</td>
<td>29.30</td>
<td>32.10</td>
<td>35.92*</td>
</tr>
<tr>
<td>Internal rotation</td>
<td>-0.10</td>
<td>6.22</td>
<td>8.07</td>
<td>10.21*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lower limb differences (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thigh circumference</td>
</tr>
<tr>
<td>Calf circumference</td>
</tr>
<tr>
<td>Leg-length discrepancy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Muscle test change (0-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoas</td>
</tr>
<tr>
<td>Rectus femoris</td>
</tr>
<tr>
<td>Quadriceps</td>
</tr>
<tr>
<td>Hamstring</td>
</tr>
<tr>
<td>Adductors</td>
</tr>
<tr>
<td>Gluteus maximus</td>
</tr>
<tr>
<td>Gluteus minimus</td>
</tr>
<tr>
<td>Fascia Latae</td>
</tr>
</tbody>
</table>

Table 3: Clinical parameters. Muscle test change: differences in muscle balance between both hips preoperative and in the several controls postoperatives. * p<0.001.
correlations. Age was correlated with difference in resting VAS in the effort test. Gender was correlated with analgesic use at 6 months postoperative. HR: Heart Rate. Peak VO\(_2\): maximal oxygen uptake. Peak VCO\(_2\): maximal carbon dioxide output. VO\(_2\)kg/m: oxygen cost. RER: respiratory exchange ratio. %: changes from the preoperative to the postoperative values in percentage. *p<0.001. **p<0.01.

Table 4: Muscular Balance pre and postoperative.

<table>
<thead>
<tr>
<th>Adductors</th>
<th>Mean ± Sd</th>
<th>Differences</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluteus Maximus</td>
<td>6.48 ± 0.75</td>
<td>6.92 ± 0.27</td>
<td>8.42</td>
</tr>
<tr>
<td>Gluteus Minimus</td>
<td>5.23 ± 1.28</td>
<td>6.35 ± 0.98</td>
<td>3.38</td>
</tr>
</tbody>
</table>

Table 5: Differences in the maximal values of the exercise test pre and postoperative. HR: Heart Rate. Peak VO\(_2\) max: maximal oxygen uptake. Peak VCO\(_2\): maximal carbon dioxide output. VO\(_2\)kg/m: oxygen cost. RER: respiratory exchange ratio. %: changes from the preoperative to the postoperative values in percentage. *p<0.001. **p<0.01.

Discussion

The objective of THA is to decrease pain and improve functionality of individuals affected by hip osteoarthritis, without causing significant complications. In our study, the biomechanical gait parameters improved significantly following THA, but they did not reach normal levels. Walking speed increased 31% and VO\(_2\), 18%. The clinical parameters that best correlated with the improvements in energy consumption were the pain decrease and walking speed increase.

Numerous studies have shown that the greatest changes are registered in the 6 postoperative months, even though follow-up of improvements continues for 1 year. Consequently, this shorter follow-up period has been considered sufficient for trying to demonstrate the hypotheses posed [4,8-11].

In our study, resting VAS score and that after the effort test, as well as analgesic use, clearly decreased in the postoperative control; 71% of the population stopped using analgesics after THA. In this sense, there are studies that consider pain to be a more reliable indicator of loosening than radiology, given that an X-ray indicating loosening without pain probably does not need revision surgery [12]. Relief of pain is the main objective to increase the quality of life of these patients [13-15].

Our patients’ hip ROM improvements were significant, although flexion, extension and internal rotation did not equal the values for the contralateral LL. However, the values reached were totally functional. Horstmann et al [16], found corrections of bending of 14º and increases of 20º in flexion, 14º in abduction, 10º in adduction, 3º in external rotation and 8º in internal rotation, results very similar to those obtained by our sample. As other authors have mentioned, it is not known if early recovery of hip flexion affects long-term functional results [17]. In our series, it should be emphasised that the patients recovered flexion of nearly 90º in the 2nd postoperative month. With respect to manual MB, differences persisted in the anterior straight muscle of the quadriceps, the gluteus maximus and medius and the FL.

Revising the literature, previous studies had already confirmed that the greatest changes are shown in the isometric force of the abductor muscles improved but did not reach normal at 6 months after surgery and even at 2 years after it [16,18-21]. The atrophy and force of the quadriceps muscle were not recovered either [22,23]. Another study showed that the isokinetic force of the muscular groups of the hip reached 80% of the contralateral LL at 1 year post-THA [24].
Table 6: Age, sex and pain correlations with the other variables. VAS1post: Visual Analogue Scale punctuation at rest 6 month postoperative. VAS2post: Visual Analogue Scale punctuation after exercise test 6 month postoperative. Dif VAS1: Differences in Visual Analogue Scale punctuation at rest pre and postoperative. Dif VAS2: Differences in Visual Analogue Scale punctuation after exercise test pre and postoperative. Analg post: Analgesic use 6 month postoperative. Dif analg: Differences in use of analgesics pre and postoperative. HR: Heart Rate. Peak VO\textsubscript{2}: Maximal Oxygen uptake. Peak VCO\textsubscript{2}: Carbon dioxide output. RER max: Maximal Respiratory Exchange ratio. VEEQ: Ventilatory Equivalent of Oxygen (VE/VO\textsubscript{2}).

The incidence of postoperative complications was 42.5%, although in 25% of the sample these were relevant and caused a longer hospital stay due to DVT or pneumonia. As seen upon revising the literature, the complication rates varied greatly among the studies, ranging from 25% to 77% [2,25,26]. Functional capacity improvements are described in multiple studies. Several authors observed excellent results following THA, with 98.7% of THA carriers became independent for activities of daily living; this figure was 64.4% before surgery [3,13,27-29]. In another multicentre study, 71% of the patients that underwent THA obtained good or excellent results [2]. In our patients, the scores on the functional scales applied improved between 29% and 45%, with this being a statistically-significant change and one that was very relevant clinically.

Applying biomechanical studies to pathology of the musculoskeletal system, specifically to THA, makes it possible to quantify improvements in gait pattern and to identify small functional limitations that would otherwise not be detectable [30-38]. In our series, the biomechanical parameters improved significantly without reaching normal, as has been described in other research [39-45]. Patients with LL osteoarthritis have more cardiovascular risk factors and less aerobic capacity [46-49]. The inactivity caused by restricting physical activity due to pain involves a loss of physical condition that is reflected in a higher HR at a specific walking speed than that of a healthy population of similar age [50-53]. Musculoskeletal system pathologies that change the gait pattern and cause pain can lead to an increase in the energy expenditure of walking, and this increase is proportional to the degree of disability [54]. Individuals with such pathologies normally walk more slowly than healthy people of their age (even though this implies greater energy consumption) in an
attempt to achieve greater stability and reduce their pain. Consequently, the effectiveness of this intervention can also be assessed quantitatively through the tendency of the energy expenditure for walking to return to normal [55,56]. The first authors to publish data on VO\textsubscript{2} in THA-operated patients were Murray [3] and Pugh [49] in 1973. The former studied VO\textsubscript{2} before and after THA in a patient, observing an increase in walking speed, stride length and VO\textsubscript{2} and concluding that the energy expenditure of walking per kilometre decreased and efficiency thus improved. Pugh, in a wider sample, obtained similar results, introducing the concept of stress reduction for the cardio-respiratory system. Mattson et al. [57] later found a negative correlation between an increase in walking speed and its energy expenditure. They studied metabolic consumption in patients with hip osteoarthritis and gonarthrosis; however, in contrast to our results, they found little correlation between O2 expenditure and the appearance of pain, perception of effort while walking, Harris scale score and maximum walking speed. In our sample, the great improvement in walking efficiency is attributable to a clear increase in the maximum walking speed tolerated, which is clearly correlated with the improvements in the functional scores and the metabolic parameters that indicate a greater aerobic capacity. In our patients, we registered increases that ranged from 18% in maximum VO\textsubscript{2} and 17% in maximum VO\textsubscript{2}/Kg; maximum VCO\textsubscript{2} rose 27% and there was an increase of 31% in maximum speed tolerated and of 33% in the distance walked during the effort test despite performing part of the test with slopes of up to 5%. The mean drop in energy expenditure for walking (VO\textsubscript{2}/Kg/m) was 30% at 6 months post-THA, with improvement in cardiovascular condition as the patients were able to carry out more physical activity. These improvements were more distinct in the younger subjects and in the men.

The men in our sample obtained a greater degree of improvement than the women. This result coincides with the greater quality of life measured with the Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) in males with severe hip osteoarthritis [58].

In this study, we found good correlation between the improvement in the score for pain, clinical tests and walking parameters. Previous authors also found correlations between walking characteristics and the Short Form 36 Health Survey (SF 36) parameters related to pain and physical function [58]. Other studies using the WOMAC did not find correlation with walking parameters [57]. According to Kyriazis and Rigas [44] time parameters such as walking speed are good indicators of functional recovery after THA. Other authors encountered correlation between the distance covered in the 6-minute test, maximum VO\textsubscript{2} and improvement in functional condition based on the WOMAC [59]. In our results, we observed that walking speed, metres walked and postoperative pain score or use of analgesics were the easiest parameters to measure and were the most useful in assessing THA efficiency with respect to the reduction of energy expenditure for walking. Given that not all centres have access to the ergometer-performed effort tests, we feel that it can be useful to know which clinical examination parameters suggest favourable development following THA. Changes in scales that are as simple to apply as the VAS or tests measuring walking speed (such as the 2- or 6-minute walking tests) can be good indicators of the metabolic changes in the individual’s cardio-respiratory system.

Conclusions

Severe hip osteoarthritis entails an increase in the energy expenditure of walking. The efficiency of THA can be assessed by the tendency of this increased energy expenditure for movement to return to normal. The VO\textsubscript{2} value is related to pain, perception of effort, functional scales and walking speed.

This study demonstrated that THA was useful, not only in lessening pain during the stance phase during the gait cycle, but also in improving walking efficiency. At 6 months post-THA, the energy expenditure for walking had fallen by 30%. It is possible that the improvement in aerobic capacity following THA might affect the life expectancy of individuals with severe hip osteoarthritis.

The clinical parameters that can be obtained with a simple physical exam and that correlate best with improvements in variables related to walking efficiency were decrease in pain and increase in walking speed and in distance walked.

Acknowledgements

Authors wish to thank Dr. Abderrahim Baja and Dr. Enrique Rocha for their help in the initial phase of the present study.

References