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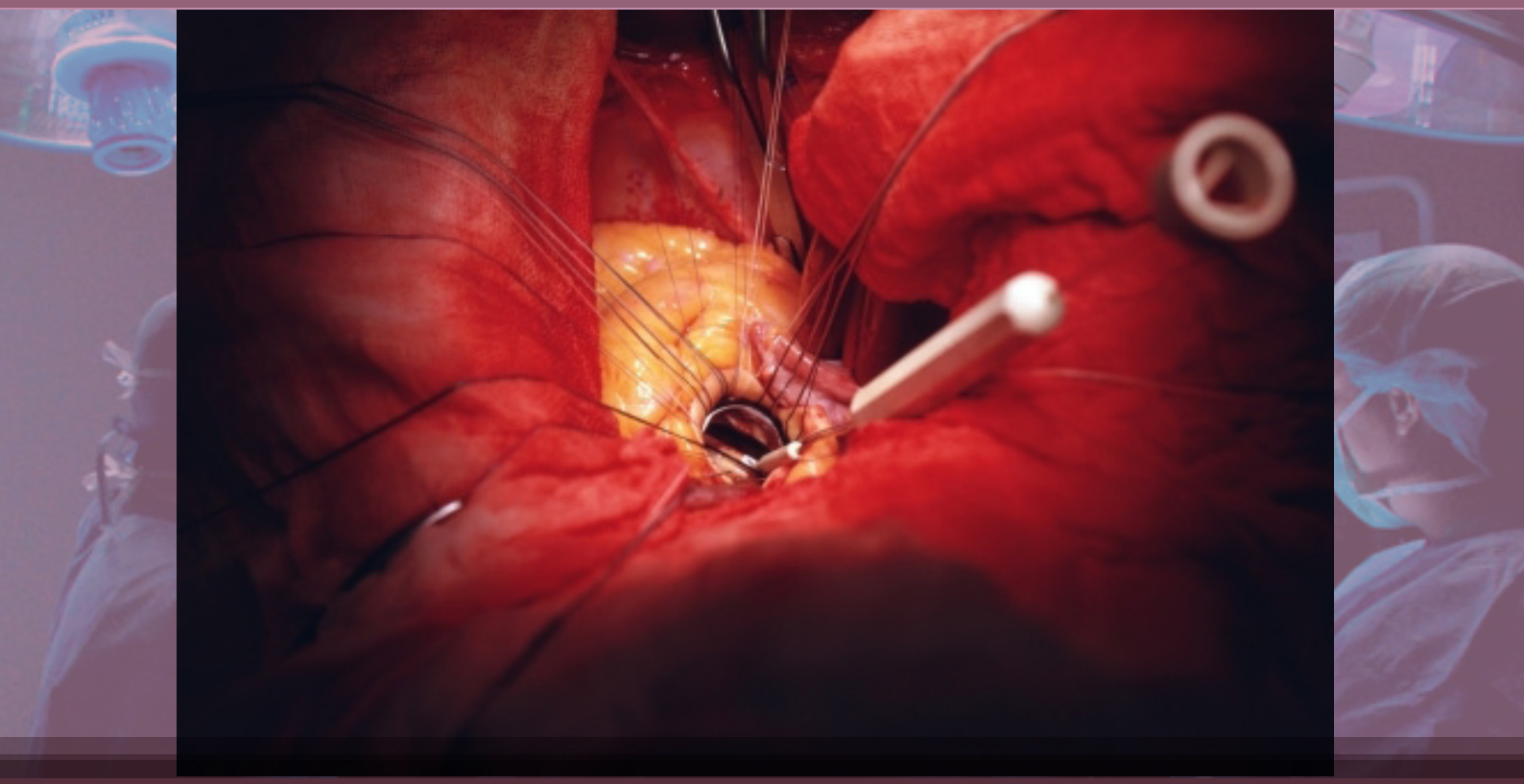

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TABLE OF CONTENTS

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Research

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The Effects of (-)-Epigallocatechin-3-Galate on Wound Closure and Infections in Mice

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ABSTRACT

In this study, we investigated the effects of (-)-epigallocatechin-3-gallate (EGCG) on wound healing both *in vitro* and *in vivo* with and without infection. EGCG has antimicrobial properties and could be useful as a topical agent to prevent and/or treat wound infections. Normal fibroblasts were isolated from the dermis of C57BL/6 mice and cultured with 0, 0.001 to 0.400 mg/ml of EGCG. *In vitro* assays demonstrated that migration, proliferation, and apoptosis were inhibited at EGCG concentrations of 0.100-0.400 mg/ml. Expression of α-smooth muscle actin (α-SMA) was also reduced. *In vivo* experiments measured closure/contraction of full-thickness dorsal wounds that were treated with 0, 0.3, 3.0, and 30.0 mg/ml of EGCG every 24 hours. Macrophages (F4/80), neutrophils (Ly-6G) and myofibroblasts (α-SMA) were assessed at 48 and 168 hours. By 168 hours there was a significant reduction in presence with the 30 mg/ml dose *vs.* 0.3 and 3.0 mg/ml ($p<0.009$, $p<0.006$, respectively). The percentage of wound closure at one week in EGCG treated wounds was 87.87% (0.03 mg), 85.23% (0.3 mg) and 40.06% (30 mg/ml) compared to controls. Reduced quantities of α-SMA myofibroblasts were observed in the 3 mg EGCG treatment group compared to controls at 168 hours. We previously demonstrated that EGCG has antimicrobial properties (MIC_{50} ~0.3 mg/ml). This data suggests that EGCG could potentially be applied to the wound surface as an antimicrobial without negatively influencing healing. To this end we applied EGCG (10 mg/ml) to a model of an infected traumatic wound. EGCG treatment significantly reduced bacterial load after one and two dose regimens.

KEYWORDS: EGCG; (-)-Epigallocatechin-3-Gallate; Wound healing.

INTRODUCTION

Epigallocatechin-3-gallate (EGCG) is the predominant polyphenol found in green tea. It is has been widely consumed for three millennia as a beverage and is known to have medicinal properties. Recent work with EGCG, and related catechins, has shown it has powerful anti-oxidant properties[1] and displays anti-tumor,[2] anti-inflammatory,[3,4] antikeloid scar formation,[5] alters apoptosis,[5-8] anti-metastatic,[9,10] and anti-atherogenic[11] activity both *in vitro* and *in vivo* settings. EGCG also has been demonstrated to inhibit growth of microbes such as *Acinetobacter baumannii*,[12] *Bordetella pertussis*,[13] *Helicobacter pylori*,[14,15] *Porphyromonas gingivalis*,[16,17] *Staphylococcus aureus* and *Eschericia coli*[18] and *Salmonella typhimurium*.[19] Moreover, EGCG has been shown to synergize with antibiotics in preventing growth of various microbes.[13,20-24] Because EGCG is generally considered safe for use in humans, it may be an attractive compound to augment or treat wounds topically, especially in light of its antimicrobial activity. While recent work with nude mice has demonstrated the effects of EGCG impregnated dressing on sterile wound,[25] it is not known if the concentrations needed to inhibit microbial growth are compatible with wound closure in immune competent mice.

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Recent work in our laboratory indicated that the EGCG concentration needed to kill Multi-Drug Resistant (MDR) *A. baumannii* would be difficult to achieve in the blood. Burns and cutaneous wounds, therefore, represent an ideal situation in which EGCG could be applied directly to the wound. The superficial location of wounds allow for topical applications of high concentrations of the antimicrobial polyphenol. Kim et al. showed in leptin receptor deficient mice used as a model for diabetic wound healing that an implanted collagen sponge infused with EGCG enhanced wound closure.[23] Additionally, EGCG at various concentrations promoted differentiation and proliferation of keratinocytes. These results suggest that EGCG may promote wound healing. However, in this same study the researchers found that high concentrations of EGCG (1000 ppm) appear to inhibit wound contraction and reduce granulation tissue formation. Klass et al. demonstrate that EGCG reduces the effects of Transforming Growth Factor beta (TGFβ1) on fibroblast contraction as well as differentiation of myofibroblasts.[26] Furthermore EGCG in a number of systems has been shown to inhibit the production of collagen[27] and fibroblast contraction.[28] In a full-thickness excisional rat model of wounds EGCG increased angiogenesis and Vascular Endothelial Growth Factor (VEGF) expression, decreased arginase activity, and more mature collagen deposition within the wound site.[29]

To determine the effects of EGCG on murine wound healing we investigated its effects following a full-thickness excisional wound made on the dorsal surface of C57BL6 mice. EGCG was applied at various concentrations and wound closure, apoptosis, alpha-smooth muscle actin (α-SMA) expression, migration and proliferation of cultured primary mouse fibroblasts, inflammation and granulation formation were monitored. Finally we wished to determine if the antibacterial properties of EGCG observed *in vitro* are effective in a surgical wound with infection model.

MATERIALS AND METHODS

General Approach

EGCG has been previously shown to alter many biological functions. In this study we examined the effects of this compound on healing (contraction) of sterile wounds *in vitro* and *in vivo* and on viability of grossly contaminated wounds. Some of the potential mechanisms for the results obtained were also examined. Studies performed *in vitro* included an examination of the effects of EGCG on the migration of skin fibroblasts into the damaged are of a monolayer, These *in vitro* studies were expanded to include an examination of the effects of EGCG on cell proliferation and cell death by apoptosis, In the next series of experiments two *in vivo* models were utilized to examine the effects of EGCG: a punch biopsy model to measure the effects on wound closure and an incision/infection model to measure the effects on wound infections. Immunohistochemical methods were applied to the sections from the wound to determine the effects of EGCG on potential scarring and leukocyte infiltration.

Reagents

EGCG was provided by S.H Hyon (Institute for Frontier Medical Sciences, Kyoto University. Japan), with purity in excess of 90%. EGCG was reconstituted at the required concentration in 1X PBS unless otherwise noted. Annexin V-APC was obtained from BD Biosciences (Franklin Lakes, NJ, USA). α-smooth muscle actin rabbit monoclonal (α-SMA, clone E184) was purchased from Abcam (Cambridge, MA, USA). Anti-Gr-1 and anti-F4/80 antibodies were purchased from eBioscience (San Diego, CA, USA). For immunohistochemical staining the Vectastain ABC kit was purchased from Vector Laboratories (Burlingame, CA, USA).

Cell culture

Primary cell cultures were initiated by removing a 3 by 3cm section of dorsal skin from 3 female C57BL/6 mice per experiment (Charles River Laboratories Wilmington, MA, USA, 8 week-old, 20-22 g). Before excision, hair was shaved and skin disinfected with povidone-iodine. Dermis was mechanically separated and placed in a 0.1% gelatin coated 6-well plate. After 15 minutes, cells were cultured in Dulbecco's Modified Eagle Medium containing 10% Fetal Bovine Serum (FBS), penicillin, and streptomycin at 37°C in 5% CO_2. Cells at passage 6 were seeded into culture dishes and tested with various concentrations of EGCG.

Cell apoptosis

Apoptotic cells were detected by flow cytometry using Annexin V-APC and propidium iodide.[30] Cells were seeded in 100-mm dishes, and 0, 1.0, 10, 100, 200, and 400 μg/ml of EGCG in culture medium were added for 48 hours. The cells were then trypsinized and 1×10^6 cells were washed in ice-cold azide free flow buffer (1X PBS, 1% BSA, 25 mM $CaCl_2$). Cells where then stained with Annexin V-APC and 1 μg/ml of propidium iodide in 1X binding buffer (0.01 M HEPES, pH 7.4; 0.14 M NaCl; 2.5 mM $CaCl_2$), on ice, and in the dark for 15 minutes. Cells were immediately analysed on an LSR II flow cytometer (BD Biosciences) equipped with 488 nm, 633 nm laser excitation and appropriate filters. 10,000 cells were collected per sample.

Flow cytometry

Flow cytometry was performed at the Shriners Hospital for Children in Cincinnati Flow Cytometry Core Facility. All cytometry, with the exception of DNA cell cycle determinations, was performed on an LSRII flow cytometer (BD Biosciences). The 488 and 633 nm lasers on LSR II were utilized and fluorescence was collected with appropriate filters. The data obtained from the LSR II were analyzed using Diva Software v5.0.2 (BD Bioscience). For DNA cell cycle

measurements, data were collected on a Coulter Epics XL system (Beckman-Coulter, Fullerton, CA, USA) with 488 nm excitation with System II software (v3) as described previously.[31] Listmode files were analyzed using ModFit v3.1 (Verity Software House, Inc., Topsham, ME, USA) to determine quantities of cells in G_0/G_1, S, and G_2/M phases of the cell cycle. At least 10,000 cells were measured per sample.

Immunohistochemistry

Immunohistochemical staining was used to detect alpha smooth muscle actin (α-SMA). Cells were grown to 80% confluence with each concentration of EGCG in 24 well culture dishes (six wells/sample), and fixed with 4% paraformaldehyde. Cells were stained with the Vectastain ABC kit according to manufacturer's directions. Briefly, after washes with PBS, cells were incubated in 0.5% H_2O_2 in MeOH for 30 minutes and then washed with 1X PBS. Samples were stained with primary antibodies to rabbit monoclonal anti-α-SMA for 1 hour. Anti-mouse biotinylated secondary antibodies were added followed by 1 hour incubation before the strepavidin-HRP was applied for 30 minutes at room temperature. All samples were visualized with 3,3'-diaminobenzidine colorimetric substrate (DAB). Wound tissues, 8 samples per treatment, were fixed with 10% paraformaldehyde in PBS, embedded in paraffin, and cut into three 5-6 µm slices. Sections where then washed with xylene to remove paraffin. The sections were stained with anti-mouse monoclonal Gr-1 anti-mouse monoclonal F4/80and anti-rabbit alpha smooth muscle actin.

Migration assay

Cells were seeded into 24-well plates at a density of 1×10^4 cells per well dish, allowed to attach overnight, and then treated with culture medium containing various concentrations of EGCG for 48 hours using 6 samples per concentration. Cell monolayers were then wounded by making a scratch with a sterile 200 µl pipet tip perpendicular to the bottom of the plate. After fixation with 4% paraformaldehyde, cells were stained with hematoxylin & eosin and migration distance measured by light microscopy.

Proliferation assay

For cell-cycle analysis, flow cytometry was performed as previously described by Osterburg et al.[SS] Briefly, cells were seeded into duplicate 100-mm dishes at a density of 1×10^6 cells per dish, allowed to attach overnight, and then treated with 0, 1.0, 10, and 100 µg/ml EGCG in culture medium for 48 hours. Cells were harvested using trypsin and fixed with 90% methanol overnight at -20°C. After washing 2X with flow buffer (1X PBS, 1% BSA, 25 mM $CaCl_2$, and 0.1% sodium azide), cells were incubated in propidium iodide staining solution (50 µg PI/mL, 0.1% Triton X-100, 25µg RNase A, 1X PBS) for 30 minutes and analyzed.

Wound healing model

Specific pathogen-free C57BL/6 mice (Charles River, 8-12 week-old, female, 20-22 grams) were purchased from Charles River Laboratories (Wilmington, MA, USA). All animal procedures were approved by the University of Cincinnati's Institutional Animal Care and Use Committees (IACUC). Eight mice were used for each experiment and the experiments were repeated for consistency. Following hair removal from the dorsal surface, the skin was disinfected and two full-thickness skin wounds were created on each side of the midline using 4-mm biopsy punch. Then 100 µl of EGCG in PBS (0,0.3, 3.0, and 30 mg/ml) was applied to the wound surface of the right side, and 100 µl of PBS as a control was added to the left side. Each wound was individually covered with a rectangle Theragauze (Soluble Systems, VA, USA) which was sutured into place. Sterile gauze was placed over the Theragauze and both were covered with Opsite (Smith & Nephew Medical Limited, Hull, United Kingdom). Finally, the dressings and wound site were covered with Coban (3M, US). Concentrations of EGCG or PBS were loaded into the TheraGauze dressing every 24-hours by injecting through the Opsite using 1 mm syringe. For planimetric and immunohistological wound assessments, tissue biopsies were taken from the wound area and the surrounding normal tissue at 48 hours and 1 week after wounding.

Traumatic Wound with Infection Model

A full thickness excision (2x2 cm) was made mid-dorsal of 26-32 gram CF1 mice, 8/group, maintaining the integrity of the panniculus carnosus under isofluorane sedation. The mice were then infected with a clinical strain of *Pseudomonas aeruginosa* (SBIN) by pipetting the organisms directly into the wound (~10^3 organisms/wound). Four groups were utilized, two EGCG (single and multiple doses), Ciprofloxacin, and PBS. The wounds were then covered with TheraGauze® which was preloaded with 200 microliters of EGCG (10 mg/ml), PBS, or Ciprofloxacin (20 µg/ml). TheraGauze® which was loaded using a micropipette allows for the continue release of a constant quantity of liquid over a set time period. Wound margins were overlaid with OpSite and wrapped with Coban. In two dose groups, 200 microliters was injected through OpSite 24 hours later.

Statistics

Experiments were performed in triplicate, and results are expressed as means ± SE. Data were analyzed using Student's t-test, ANOVA or Kruskal-Wallis One-way Analysis of Variance. *p*-values <0.05 were considered to be significant. Kaplan-Meier, with multiple test corrections was utilized in wound with infection model.

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RESULTS

Effects of EGCG on scratch assay with cultured fibroblasts

In vitro wounding assays (scratch assays) were performed to determine if EGCG altered cellular migration into the scratch. Confluent cultures where perturbed with a pipet tip and migration into the wound measured over a three-day period. The data in figure 1A demonstrate a dose-dependent effect EGCG (200µg/ml and 400µg/ml) with fewer cells appearing in the scratched area with these concentrations versus the lower concentrations of EGCG and the control. Figure 1B quantifies the size of the initial scratch over time. The data indicate that over time the higher doses of EGCG (400, 200, and 100 µg/ml) significantly (P<0.05) reduced the extent of cell migration into the scratch.

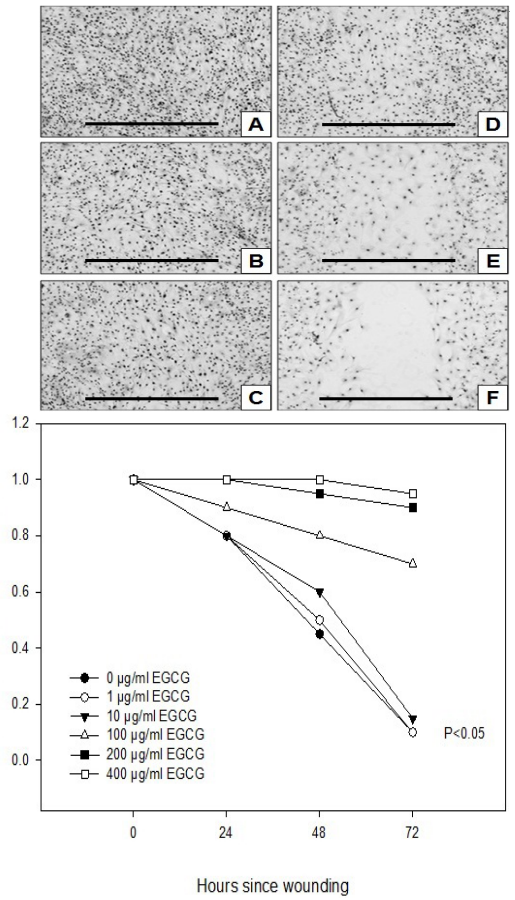

Figure 1: Scratch wounds of primary mouse fibroblasts treated with EGCG. A) Hematoxylin & eosin counter stained fibroblasts. Black bar represents 1 mm. Figure is representative of multiple experiments. B) Scratch width *vs.* the initial size. Widths where measured every 24 hours. Error bars omitted for clarity. P<0.05 of 100-400 ug/ml EGCG *vs.* controls. N=4.

Inhibition of migration could be due to increased apoptosis of cells in culture. Therefore we examined the effects of EGCG on apoptosis in treated fibroblasts. Apoptosis was as -sayed by staining with Annexin V-APC and propidium iodide followed by analysis using flow cytometry. As demonstrated in Figure 2, at the 100 µg/ml dose of EGCG (p<0.003 *vs.* lower doses) there were increasing quantities of cells in early and late apoptosis/necrosis. The 0, 1 and 10 µg/ml doses of EGCG had near baseline percentages of both early and late apoptosis/necrosis. At the very highest dose of EGCG (400 µg/ml) there was a large increase (75.7% +/- 1.68 SE) in predominantly late apoptosis/necrosis. The reduction in early apoptosis at this quantity of EGCG is due to increased progression to late apoptosis/necrosis in this sample.

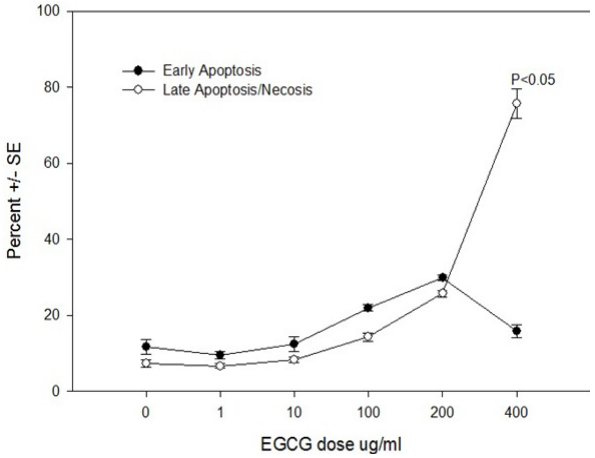

Figure 2: Apoptosis of primary mouse fibroblasts treated with EGCG. Early apoptosis represents cells that are annexin V positive, but negative for propidium iodide. Late apoptosis/necrosis represents cells that are propidium iodide positive and either annexin V positive or negative. N=3 +/- SE. P<0.05.

Decreased migration into the scratch wound could also be indicative of a decrease in cellular proliferation of the primary fibroblasts. In order to determine the effects of EGCG on cell cycle, cells were treated with various doses of EGCG, stained with propidium iodide, and analyzed by flow cytometry. Figure 3 indicates that cell cycle progression is altered at 100 ug/ml of EGCG. As can be seen in the figure the quantity of cells in G_0/G_1 are significantly increased (P<0.05) *vs.* the controls, and S and G_2/M phases were significantly decreased (P<0.05) *vs.* the controls. Lower doses of EGCG did not appear to have any effect on cell cycle kinetics.

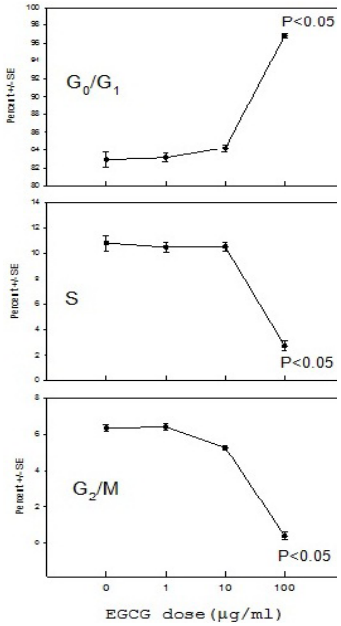

Figure 3: Effects of EGCG on cell cycle of primary mouse fibroblasts. Propidium iodide stained cells were analyzed by flow cytometry and the quantity of cells in G_0/G_1, S and G_2/M determined by analysis of the appropriate listmode files with ModFit. N=3+/-SE.

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Wound healing and Immunochemistry

Previously published studies suggest that EGCG inhibits the α-SMA production. We stained primary fibroblasts cultures with an anti-α-SMA and visualized them by immunohistochemistry. The data in Figure 4 indicate that as the dose of EGCG increases the quantity of α-SMA decreases. At the two highest doses of EGCG (200, and 400 µg/ml) α-SMA appears to be almost entirely absent. There are fewer cells at these doses but this does not account for the reduction in the quantity of α-SMA staining.

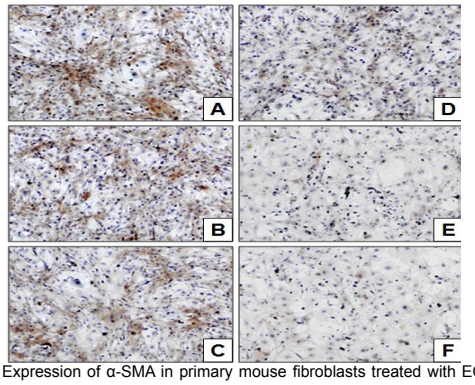

Figure 4: Expression of α-SMA in primary mouse fibroblasts treated with EGCG. Primary mouse fibroblasts where treated and then immunohistochemically stained for α-SMA. Figure is representative of multiple experiments.

Next, a series of animal experiments were performed in which two full thickness excisional wounds were made with 4 mm punch biopsy probes on the backs of C57BL6 mice to determine if EGCG inhibited wound contraction and/or closure. The pictures in Figure 5A depicts the wounds after 7 days. The highest dose of EGCG (30 mg/ml) shows a clearly greater size of wound. The data in Figure 5B quantifies closure rates at day 2 and 7 post-wounding. A significant delay in closure/contraction at the 30 mg/ml is visible at day 7. The high dose of EGCG significantly inhibited closure/contraction 40.06% +/- 8.63SE *vs.* 88.23%+/- 5.30SE (p<0.009) for 30 mg/ml *vs.* 3.0 mg/ml, respectively. Likewise, there was an inhibition of closure/contraction for the 0.3 mg/ml EGCG (87.87% +/-3.46) dose *vs.* the 30 mg/ml dose (p<0.006).

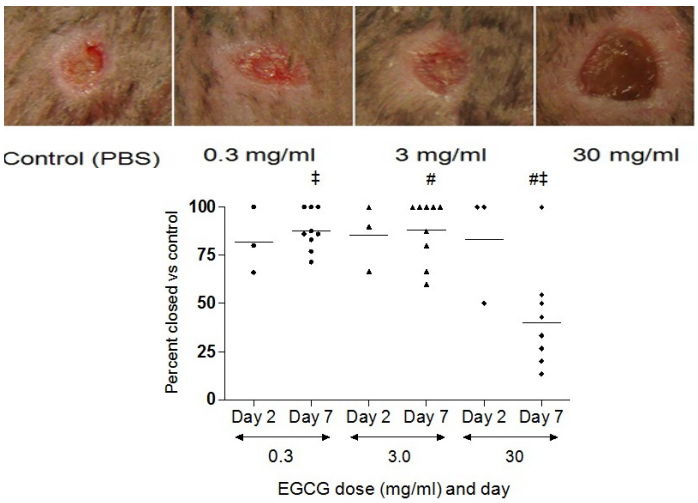

Figure 5: Wound contraction/closure in C57BL6 mice exposed to EGCG. Wounds were treated as listed every 24 hours. Wound size was measured at days 2 and 7. A) Images taken at day 7 that represent the extent of closure/contraction. B) Plot of the wound size at days 2 and 7. Wounds were normalized by dividing the size of the EGCG treated wound with the PBS treated control wound. On day two n=3 and on day 7 n=9. # P<0.009 for 30 mg/ml *vs.* 0.3 mg/ml at day 7, ‡ P<0.006 for 3.0 mg/ml *vs.* 0.3 mg/ml at day 7.

We sought to determine if the decreased closure/contraction of the 30.0 mg/ml dosed wounds was due to decreased numbers of α-SMA expressing myofibroblasts. Figure 6 depicts the immunohistochemical staining of tissue sections from EGCG treated wounds 7 week post-wounding. There is a clear reduction in the α-SMA positive cells. The lower doses of EGCG do not appear to alter α-SMA staining *vs.* controls. We then sought to determine if there where changes in F4/80 macrophage and Ly-6G neutrophil populations in EGCG treated wounds. The data in Figure 7 indicate there are no differences in the extent and location of staining at day 2 or 7 for either F4/80 or Ly-6G.

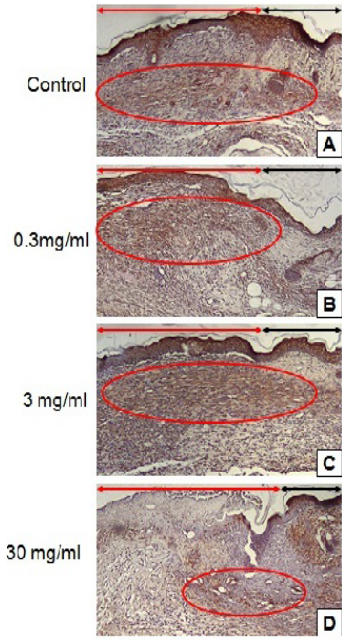

Figure 6: Immunohistochemical staining for α-SMA of wound beds from mice. Brightfield images where taken at 7 days and 40x magnification. Circled areas represent locations of α-SMA staining. Figure is representative of multiple experiments.

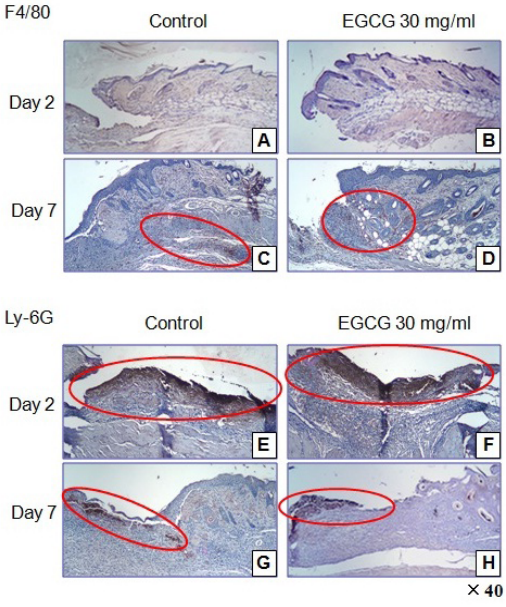

Figure 7: Immunohistochemical staining for F4/80 macrophages and Ly-6G neutrophils in EGCG treated wounds. Wounds were probed for F4/80 (macrophage) and Ly-6G (neutrophil) epitopes. Tissue sections where stained at days 2 and 7. Circles represent areas of either macrophage or neutrophils. Figure is representative of multiple experiments.

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Traumatic Wound with Infection model

Our combined *in vitro* results (antibacterial and wound healing neutral) culminated with an *in vivo* application of EGCG to an infected murine wound. The results were far superior to vehicle control, approaching results obtained with Ciprofloxacin. While data in these studies were not put through the same battery of analysis as in wound closure model, we saw no sign that wound closure was inhibited. Tracing data indicated no closure at 7 days whether treated with Ciprofloxacin, EGCG, or PBS.

DISCUSSION

The rise in antibiotic resistant microorganisms has renewed the interest in agents that can supplement current antibiotics. Recent work demonstrates that polyphenol EGCG has significant antimicrobial activity against a number of microorganisms.[13,32-34] Furthermore, microorganisms with resistance to systemic antibiotics have increased resistance to many topical antimicrobial treatments currently in use.[35] There is a need to evaluate the efficacy of topical agents that can be used to supplement or enhance current systemic antimicrobial therapies. In the clinical arena, especially following severe trauma, both wound healing and infections are major concerns. Our laboratory has reported that EGCG exhibits broad anti-microbial activity especially against multi-drug resistant organisms such as *A. baumannii.*[13] We found that the minimum inhibitory concentration of EGCG against *A. baumannii* is ~300 µg/ml, and additionally, against clinical isolates of *Pseudomonas aeruginosa* and MRSA MICs, ~600 µg/ml and ~60 µg/ml, respectively.[36] To use EGCG as a topical anti-microbial agent on wounds or burns, it should have limited deleterious effects on wound healing. In this study, we examined the effects of EGCG on migration, apoptosis, proliferation and alpha-smooth muscle actin (α-SMA) expression in cultured primary mouse fibroblasts *in vitro*. Murine wound closure/contraction was also investigated following a full-thickness excisional wound made on the dorsal surface of C57BL6 mice. EGCG was applied *in vivo* at various concentrations with wound closure, α-SMA, inflammation and granulation tissue formation monitored. It was important to determine not only the effects of EGCG on wound healing but also its effects at concentrations that are both anti-microbial and within the concentrations that may be used clinically.

EGCG significantly inhibited migration and proliferation of fibroblasts using an *in vitro* scratch assay. This is in agreement with data published by Park et al.[32] who reported that EGCG inhibited proliferation and migration of keloid and to lesser extent normal fibroblasts *in vitro*. We also demonstrated that at higher doses of EGCG (100 µg/ml and above) treated fibroblasts demonstrate a statistically significant ($P<0.05$) increase in the quantity of cells in G_0/G_1 compared to controls indicating that a smaller fraction of cells were dividing. Absence of cell proliferation is one explanation for the lack of closure of the scratch wound. Additionally, EGCG may have induced programmed cell death in cultured primary fibroblasts. Our studies indicated that apoptosis was significantly increased at high dosages of EGCG. These data indicate that both alterations in cell cycle progression and increased apoptosis are responsible for the reduction in fibroblast proliferation following treatment with EGCG. Increased EGCG-dependent apoptosis of transformed cells is well known. Additionally, EGCG has been reported to increase apoptosis in normal non-transformed cells.[34,36]

Myofibroblasts produce contractile forces in the wound and promote wound closure. We examined the effects of EGCG on the expression of α-SMA in the wound margins. EGCG could reduce recruitment of fibroblasts/myofibroblasts from circulating bone marrow derived cells. This opens the possibility that EGCG is anti-scarring. However, Barisic-Dujmovic et al. demonstrated that myofibroblasts within the wound are derived from resident fibroblasts.[35] EGCG has been previously demonstrated to inhibit the differentiation of fibroblasts into myofibroblasts.[26] *In vitro*, there was a significant reduction in α-SMA staining at the higher doses of EGCG. In culture, EGCG has been shown to inhibit transforming growth factor beta-receptor signaling as well as inhibiting AP-1 and NF-κB in fibroblasts.[26] In the animal wound, this would be consistent with our observations of reduced α-SMA staining in sections from the wounds treated with 30 mg/ml EGCG.

The *in vivo* wound model used in these studies clearly indicated that only at very high concentrations EGCG delayed wound contracture/closure. This is in contrast to several studies that reported that EGCG enhanced wound closure .[37,32] Kim et al. implanted collagen sponges impregnated with EGCG, and that at low doses (10 ppm), healing was observed in diabetic wounds.[33] Park et al. examine keloid formation *in vivo* with and without EGCG.[32] These are very different wound healing models and while these data suggest an effect of EGCG at low doses, the studies involve the response of a diabetic pathology and not the direct effect of EGCG on normal wound healing. Our group examined the inflammatory infiltrate in EGCG and non-treated tissues and found no difference, indicating the observed differences were not due to alterations in inflammation.

The data presented here demonstrate that concentrations of EGCG used for *in vitro* and *in vivo* assays are not directly comparable and thus vary in the amounts necessary to produce biological function. For example, the *in vitro* data indicated that there are changes in migration, proliferation and apoptosis at EGCG concentrations of 0.1-0.4 mg/ml while the *in vivo* data indicated that a significantly higher dose of EGCG was needed to alter wound closure (Figure 5A/B). Our laboratory demonstrated that EGCG was bactericidal at concentrations of 0.300 mg/ml. This dose would clearly fall into the concentration that inhibited apoptosis, migration and proliferation *in vivo*. However, the 0.300 mg/ml concentration had little impact on the degree of wound closure/contraction

in vivo. Caution should be exercised in evaluating *in vitro* evidence to interpret the effects of EGCG *in vivo*. We clearly show that the dose of EGCG that inhibits negatively cell cycle, apoptosis, and scratch wound closure is much lower than the dose required *in vivo*. The discrepancy between *in vitro* and *in vivo* data suggests that cells are directly exposed to EGCG in culture systems in contrast to *in vivo exposures* in which tissues are more likely exposed to metabolized products of EGCG rather than intact EGCG.[35] Additionally, the wound is a complex environment and the dose of EGCG that cells see may highly depended on the microenvironment of the wound. As a topical agent, EGCG warrants further investigation into its properties in the context of healing and infection control.

In conclusion, the detrimental effects of EGCG on wound closure appear minor, with reduced closure only at high doses. However the antimicrobial effects of EGCG are impressive and the well-known safety of external and internal use of EGCG suggests that further investigations into its use to supplement and treat topical lesions are warranted.

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REFERENCES

1. Kanadzu M, Lu Y, Morimoto K. Dual function of (--)-epigallocatechin gallate (EGCG) in healthy human lymphocytes. *Cancer letters*. 2006; 241(2): 250-255. doi: http://dx.doi.org/10.1016/j.canlet.2005.10.021

2. Tran PL, Kim SA, Choi HS, Yoon JH, Ahn SG. Epigallocatechin-3-gallate suppresses the expression of HSP70 and HSP90 and exhibits anti-tumor activity in vitro and in vivo. *BMC cancer*. 2010; 10: 276. doi: 10.1186/1471-2407-10-276

3. Yang F, de Villiers WJ, McClain CJ, Varilek GW. Green tea polyphenols block endotoxin-induced tumor necrosis factor-production and lethality in a murine model. *The Journal of nutrition*. 1998; 128(12): 2334-2409

4. Syed F, Bagabir RA, Paus R, Bayat A. Epigallocatechin-3-gallate ameliorates seawater aspiration-induced acute lung injury via regulating inflammatory cytokines and inhibiting JAK/STAT1 pathway in rats. *Mediators Inflamm*. 2014; Epub 2014 Feb 20. doi: 10.1155/2014/612593

5. Liu W, Dong M, Bo L, et al. Ex vivo evaluation of antifibrotic compounds in skin scarring: EGCG and silencing of PAI-1 inde pendently inhibit growth and induce keloid shrinkage. *Lab Invest*. 2013; 93(8): 946-960. doi: 10.1038/labinvest.2013.82

6. Chung LY, Cheung TC, Kong SK, et al. Induction of apoptosis by green tea catechins in human prostate cancer DU145 cells. *Life sciences*. 2001; 68(10): 1207-1214. doi: 10.1016/S0024-3205(00)01020-1

7. Giakoustidis DE, Giakoustidis AE, Iliadis S, et al. Attenuation of liver ischemia/reperfusion induced apoptosis by epigallocatechin-3-gallate via down-regulation of NF-kappaB and c-Jun expression. *J Surg Res*. 2010; 159(2): 720-728. doi: 10.1016/j.jss.2008.08.038

8. Hayakawa S, Saeki K, Sazuka M, et al. Apoptosis induction by epigallocatechin gallate involves its binding to Fas. *Biochemical and biophysical research communications* 2001; 285(5): 1102-1106. doi: 10.1006/bbrc.2001.5293

9. Park HJ, Shin DH, Chung WJ, et al. Epigallocatechin gallate reduces hypoxia-induced apoptosis in human hepatoma cells. *Life sciences*. 2006; 78(24): 2826-2832. doi: 10.1016/j.lfs.2005.11.001

10. Dell'Aica I, Dona M, Sartor L, Pezzato E, Garbisa S. (-) Epigallocatechin-3-gallate directly inhibits MT1-MMP activity, leading to accumulation of nonactivated MMP-2 at the cell surface. *Laboratory investigation; a journal of technical methods and pathology.* 2002; 82(12): 1685-1693.

11. Garbisa S, Sartor L, Biggin S, Salvato B, Benelli R, Albini A. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. *Cancer*. 2001; 91(4): 822-832.

12. Kuriyama S, Shimazu T, Ohmori K, et al. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *JAMA*. 2006; 296(10): 1255-1265. doi: 10.1001/jama.296.10.1255

13. Osterburg A, Gardner J, Hyon SH, Neely A, Babcock G. Highly antibiotic-resistant Acinetobacter baumannii clinical isolates are killed by the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG). *Clin Microbiol Infect.* 2009; 15(4): 341-346.

14. Horiuchi Y, Toda M, Okubo S, Hara Y, Shimamura T. [Protective activity of tea and catechins against Bordetella pertussis]. *Kansenshogaku zasshi.* 1992; 66(5): 599-605.

15. Yanagawa Y, Yamamoto Y, Hara Y, Shimamura T. A combination effect of epigallocatechin gallate, a major

compound of green tea catechins, with antibiotics on Helicobacter pylori growth in vitro. *Current microbiology.* 2003; 47(3): 244-249. doi: 10.1007/s00284-002-3956-6

16. Sakanaka S, Aizawa M, Kim M, Yamamoto T. Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, Porphyromonas gingivalis. *Bioscience, biotechnology, and biochemistry*.1996; 60(5): 745-749. doi: http://dx.doi.org/10.1271/bbb.60.745

17. Sakanaka S, Okada Y. Inhibitory effects of green tea polyphenols on the production of a virulence factor of the periodontal-disease-causing anaerobic bacterium Porphyromonas gingivalis. *J Agric Food Chem.* 2004; 52(6): 1688-1692. doi: 10.1021/jf0302815

18. Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. *Biochimica et biophysica acta*. 1993; 1147(1): 132-136.

19. Si W, Gong J, Tsao R, Kalab M, Yang R, Yin Y. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. *Journal of chromatography*. 2006; 1125(2): 204-210. doi: 10.1016/j.chroma.2006.05.061

20. Hu ZQ, Zhao WH, Asano N, Yoda Y, Hara Y, Shimamura T. Epigallocatechin gallate synergistically enhances the activity of carbapenems against methicillin-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy*. 2002; 46(2): 558-560. doi: 10.1128/AAC.46.2.558-560.2002

21. Lee YS, Han CH, Kang SH, et al. Synergistic effect between catechin and ciprofloxacin on chronic bacterial prostatitis rat model. *Int J Urol.* 2005; 12(4): 383-389. doi: 10.1111/j.1442-2042-.2005.01052.x

22. Yam TS, Hamilton-Miller JM, Shah S. The effect of a component of tea (Camellia sinensis) on methicillin resistance, PBP2' synthesis, and beta-lactamase production in Staphylococcus aureus. *The Journal of antimicrobial chemotherapy*. 1998; 42(2): 211-216. doi: 10.1093/jac/42.2.211

23. Kim HL, Lee JH, Kwon BJ, et al. Promotion of full-thickness wound healing using epigallocatechin-3-O-gallate/poly (lactic-co-glycolic acid) membrane as temporary wound dressing. *Artif Organs*. 2014; 38(5): 411-417. doi: 10.1111/aor.12190

24. Zhao WH, Asano N, Hu ZQ, Shimamura T. Restoration of antibacterial activity of beta-lactams by epigallocatechin gallate against beta-lactamase-producing species depending on location of beta-lactamase. *The Journal of pharmacy and pharmacology.* 2003; 55(6): 735-734.

25. Kin HL, Lee JH, Kwon BJ, et al. Promotion of full-thickness wound healing using epigallocatechin-3-O gallate/poly (lactic-co-glycolic acid) membrane as temorary wound dressing. *Artifical Organs*. 2014; 38(5): 411-417. doi: 10.1111/aor.12190

26. Klass BR, Branford OA, Grobbelaar AO, Rolfe KJ. The effect of epigallocatechin-3-gallate, a constituent of green tea, on transforming growth factor-beta1-stimulated wound contraction. *Wound Repair Regen*. 2010; 18(1): 80-88. doi: 10.1111/j.1524-475X.2009.00552.x

27. Zhang Q, Kelly AP, Wang L, et al. Green tea extract and (-)-epigallocatechin-3-gallate inhibit mast cell-stimulated type I collagen expression in keloid fibroblasts via blocking PI-3K/AkT signaling pathways. *The Journal of investigative dermatology.* 2006; 126(12): 2607-2613. doi: 10.1038/sj.jid.5700472

28. Suzuki Y, Hattori S, Isemura M. Epigallocatechin-3-O-gallate inhibits fibroblast contraction of floating collagen gel: interaction between epigallocatechin-3-O-gallate and platelet derived growth factor. *Bioscience, biotechnology, and biochemistry.* 2004; 68(8): 1817-1820. doi: 10.1271/bbb.68.1817

29. Kapoor M, Howard R, Hall I, Appleton I. Effects of epicatechin gallate on wound healing and scar formation in a full thickness incisional wound healing model in rats. *Am J Pathol.* 2004; 165(1): 299-307. doi: http://dx.doi.org/10.1016/S0002-9440(10)63297-X

30. Rodeberg DA, Meyer JG, Babcock GF. Heat shock response: presence and effects in burn patient neutrophils. *Journal of leukocyte biology.* 1999; 66(5): 773-780.

31. Knauff JA, Ouyang B, Knudsen ES, Fukasawa K, Babcock G, Fagin JA. Oncogenic RAS induces accelerated transition through G2/M and promotes defecs in the G2 DNA damage and mitotic spindle checkpoins. *The Journal of biological chemistry.* 2006; 281(7): 3800-3809.

32. Park G, Yoon BS, Moon JH, et al. Green tea polyphenol epigallocatechin-3-gallate suppresses collagen production and proliferation in keloid fibroblasts via inhibition of the STAT3-signaling pathway. *The Journal of investigative dermatology.* 2008; 128(10): 2429-2441. doi: 10.1038/jid.2008.103

33. Kim H, Kawazoe T, Han DW, et al. Enhanced wound healing by an epigallocatechin gallate-incorporated collagen sponge in diabetic mice. *Wound Repair Regen.* 2008; 16(5): 714-720. doi: 10.1111/j.1524-475X.2008.00422.x

34. Hofmann CS, Sonenshein GE. Green tea polyphenol epigallocatechin-3 gallate induces apoptosis of proliferating

vascular smooth muscle cells via activation of p53. *Faseb J*. 2003; 17(6): 702-704. doi: 10.1096/fj.02-0665fje

35. Barisic-Dujmovic T, Boban I, Clark SH. Fibroblasts/myofibroblasts that participate in cutaneous wound healing are not derived from circulating progenitor cells. *Journal of cellular physiology.* 2010; 222(3): 703-712. doi: 10.1002/jcp.21997

36. Kawai K, Tsuno NH, Kitayama J, et al. Epigallocatechin gallate induces apoptosis of monocytes. *The Journal of allergy and clinical immunology.* 2005; 115(1): 186-191. doi: http://dx.doi.org/10.1016/j.jaci.2004.10.005

37. Kroon PA, Clifford MN, Crozier A, et al. How should we assess the effects of exposure to dietary polyphenols in vitro? *The American journal of clinical nutrition.* 2004; 80(1): 15-21.

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Research

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Diagnostic Yield of Flexible Sigmoidoscopy in Symptomatic Population: An Insight to Rapid Access Sigmoidoscopy Clinic

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ABSTRACT

Aim: The study aim was to investigate the correlations between suspicious lower GI symptoms and endoscopic findings in symptomatic population using flexible sigmoidoscopy.

Methods: Retrospective review of all rapid access sigmoidoscopy clinic referrals was performed. Clinical, sigmoidoscopy findings and outcome were reviewed. Patients were further monitored for over five years for new cancers.

Results: A total of 445 patients met the inclusion criteria. Most patients (87.2%) had a flexible sigmoidoscopy in their first visit; 41.2% had barium enema to investigate proximal bowel. Polyp detection rate was 12.6% with an average distance of 23.1±18.9 cm from the anal verge. Passing mucus (p=0.05) and incontinence (p=0.035) were the only predictive symptoms for polyps. Cancer detection rate was 7%. Almost 93% of cancers were detected with the flexible sigmoidoscope alone with an average distance of 23.1±16.6 cm with majority being advanced as 17.9% had Duke's A. Weight loss (p=0.005), tenesmus (p=0.006) and passing mucus per rectum (p=0.008) were three predictive symptoms on univariate and multivariate analysis. One patient developed a primary cancer 5 years from his index sigmoidoscopy. Substantial savings were achieved using this model of investigation.

Conclusion: Flexible sigmoidoscopy is easy, safe, sensitive and cost effective investigation for patients with suspicious lower colonic symptoms.

KEYWORDS: Symptomatic; Sigmoidoscopy; Cancer.

INTRODUCTION

Worldwide the incidence of Colorectal Cancers (CRC) is approximately one million per year with an annual mortality approaching 5,00,000.[1] Despite medical advances and the high quality primary health care in Europe, the annual incidence is around 4,12,000 with an annual mortality reaching 50 percent.[2] The Republic of Ireland has a similar trend with an annual incident rate of close to 1,900 with an annual mortality of approximately 930 patients. In the report published by GLOBOCAN in 2002, Ireland had the highest mortality rates in Western European and the fourth worldwide.[3] Furthermore, the World Health Organisation has estimated that the number of newly diagnosed colorectal cancers will increase by 79% in males and 56% in females by 2020.[4] In response to these figures, the launch of a national colorectal screening program is anticipated to start this year targeting patients between the age of 60-69 in its first phase until it finally involves a screening population between 55-74 years old.[4]

Despite the high incidence of colorectal cancers and the intensive studies of the disease,

the early symptoms remain very vague resulting in 19-44% of patients presenting with advanced disease and requiring emergency surgery.[5] Part of the delay in the diagnosis can be linked to the reluctance of patients and primary care physicians to refer for endoscopy in secondary care centres.[6] The problem is further complicated by the universal delay to the index outpatient review and colonoscopy.[7]

The early stages of cancer are asymptomatic but clinically detectable. Cancer screening programmes target this stage as the cancer is still at an early stage and is believed to be curable. The advanced stages are symptomatic and will logically have a poorer outcome.[8] Although many studies recommend colonoscopy as a preferred investigation, most colorectal studies have been performed in asymptomatic cohorts with limited data in symptomatic population.[9]

Flexible sigmoidoscopy has been shown to reduce cancer related mortality.[10] Once-off flexible Sigmoidoscopy screening test for bowel cancer can reduce mortality from the disease by 43% (31% on a population basis) and reduce the incidence of bowel cancer by 33%.[11] Furthermore, sigmoidoscopy is more convenient for patients to evaluate their bowel compared to colonoscopy as it does not require an intense bowel preparation and it is a less stressful procedure.[12]

What is important is that despite all available diagnostic modalities, the vast majority of patients present only after developing worrying symptoms.[13] Therefore, the main objective of this study was to provide an evaluation of symptomatic patients presenting with suspicious symptoms of colorectal cancer to our rapid access colorectal clinic where they had flexible sigmoidoscopy in their index visit. Diagnostic yield, polyps and cancer detection rates, histological grade, anatomical location, presenting symptoms and economic viability were analysed. A secondary outcome was to look at new cancers diagnosed within the first five years following negative sigmoidoscopy.

METHODS

The Rapid access colorectal clinic in Cork University Hospital was established in June 2001. The main objective of this clinic was to identify patients with symptoms suggestive of colorectal cancer and to review them within two weeks from referral. General Practitioners (GPs) in the hospital capture area were invited to refer all patients using a standardised referral form. The referral symptoms included in the form were rectal bleeding, passing mucus per rectum, Faecal Occult Blood (FOB) positive stool samples, and change in bowel habits, episodes of faecal incontinence, weight loss, anorexia, tenesmus and family history. GPs had to specify the duration of the symptom or symptoms and if there was a family history; age, sex, relationship to patient and the type of cancer should be included. All physical findings including per rectal examination had to be clearly highlighted.

Rapid access patients were seen as part of the general outpatient clinic. Upon arrival to the clinic, patients were reviewed with a full history and clinical examination. Half an hour before the flexible sigmoidoscopy, a phosphate enema was given by the endoscopy nurse. All sigmoidoscopies were performed by a consultant or a specialist registrar with a full sigmoidoscopy report written immediately after the procedure and before reviewing the next patient. No sedation was administered and banding of haemorrhoids was performed in the same visit if indicated. Patients with high risk polyps (multiple or high grade) or with symptoms suggestive of neoplasia were scheduled for a completion colonoscopy. Bowel imaging was completed in appropriate patients using barium enema, CT colonography or colonoscopy.

All patients referred to the rapid access colorectal clinic between 2001 and 2006 were included in the study. Patients younger than 40 years old have been excluded from analysis as it has been shown that their cancer diagnostic yield is limited.[12] Other exclusion criteria were history of inflammatory bowel disease, incomplete data, refusal of sigmoidoscopy and incomplete sigmoidoscopy reports. All new cancers diagnosed within five years from the initial sigmoidoscopy were recorded.

Statistical analysis

The statistical analysis was performed using SPSS 20 (SPSS Inc. Chicago, IL, USA). Descriptive statistics such as rates and percentages were used for categorical data while mean ± Standard Deviations (SD) were used for continuous data. The categorical variables were tested using X^2 test, Fisher's exact test or Wilcoxon rank–sum test. Continuous variables were tested using a Student's t-test. Standard logistic regression analysis was used to calculate the relative risk as Odds Ratios (OR) with 95% Confidence Intervals (CI). A p-value of less than or equal to 0.05 was considered significant.

RESULTS

After exclusion, a total of 445 patients were included in the study and deemed suitable for analysis. None of the excluded patients was diagnosed with cancer throughout the study period.

The average age of referred patients was 58.7±13.05 years (52 years mean). Under half of the referred patients (49.4%, n=220) were males and 50.6% (n=225) were females. Most patients (87.2%, n= 388) had their flexible sigmoidoscopy in their index visit. The average waiting time from referral to clinical review was 19.5 (± SD 16.7) days. The average distance of assessed colon from the anal verge was 52.4±16.8 cm (55.95 cm in males and 48.95 cm in females). Some pa-

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-tients (41.1%) then required a completion barium enema (n=183) and 4.5 % of patients had other tests to investigate their bowel (n=20) (Table 1).

Patients characteristics	N = 445
Age Mean	58.7(± SD 13.1)
Median	52
Male	220(49.4%)
Female	225(50.6%)
Sigmoidoscopy at first visit	388(87.2%)
Clinic waiting time(days)	19.5(± SD 16.7)
Sigmoidoscopy distance in cm	54.4(± SD 16.8)
Completion barium enema	183(41.1%)
Other investigations	20(4.5%)
Indication for referral	
Bleeding PR	233(52.4%)
Change in bowel habits	239(53.7%)
Weight loss	33(7.4%)
Family history	58(13 %)
Abdominal pain	66(14.8%)
Tenesmus	70(15.7%)
Mucus	25(5.6%)
Incontinence	24(5.4%)
Other complaints	8(1.8%)
Diagnosis	
Diverticular disease	66(14.8%)
Haemorrhoids	65(14.6%)
Colitis	29(6.5%)
Irritable bowel syndrome	12(2.7%)
Inflammatory bowel disease	9(2%)
Anal fissure	10(2.2%)
Celiac disease	4(0.9%)
Polyps	56(12.6%)
Cancers	31(7%)
NAD	141(31.7%)
Others	22(4.9%)

Table 1: Patients characteristics, presenting symptoms and diagnosis

The commonest presenting symptom of the referred patients was change in bowel habit accounting for 53.7% (n=239) followed by 52.4% of patients presenting with bleeding per rectum (n=233). The commonest diagnosis was diverticular disease (14.8 %, n=66) followed by haemorrhoids (14.6 %, n=65). Almost one third of patients (31.7%) did not show any pathology in their investigations (n=141). The polyp detection rate was 12.6% (n=56), and the cancer detection rate was 7 % (n=31). (Table 1).

The commonest presenting symptom of the referred patients was change in bowel habit accounting for 53.7% (n=239) followed by 52.4% of patients presenting with bleeding per rectum (n=233). The commonest diagnosis was diverticular disease (14.8 %, n=66) followed by haemorrhoids (14.6 %, n= 65). Almost one third of patients (31.7%) did not show any pathology in their investigations (n=141). The polyp detection rate was 12.6% (n=56), and the cancer detection rate was 7 % (n= 31) (Table 1).

The mean age of the 56 patients diagnosed with colonic polyps was 60.8 years ± 12.3 (61 years median). The mean age in male patients in this group was 56.3 years while females were slightly older (62.1 years). The average detection distance for polyps was 23.1 cm (± SD 18.9). Half of the polyps were in the sigmoid colon (50%, n= 28) followed by the rectum (37.5%, n=21). The commonest detected polyp was an adenomatous polyp (30.4%, n= 17) followed by hyperplastic polyps (28.6%, n=16). The characteristics of detected polyps are shown in Table 2.

Polyp characteristics	N = 56, 12.6%
Patients age Mean	60.8 ± SD 12.3
Median	61
Average polyp distance	23.1 cm ± SD
Anatomical location	
Rectum	21(37.5%)
Rectosigmoid junction	6(10.7%)
Sigmoid colon	28(50%)
Descending colon	1(1.8%)
Polyp histology	
Adenoma	17(30.4%)
Villous	11(19.6%)
Hyperplastic	16(28.6%)
Dysplastic	1(1.8%)
Inflammatory	4(7.1%)
Mucosa	7(12.5%)

Table 2: Characteristic of colorectal polyps

The commonest complaint was change in bowel habits (60.7%, n=34) followed by 51.8% reporting bleeding per rectum (n=29). Passing mucus per rectum (12.5%, n=7, p= 0.05) and episodes of faecal incontinence (12.5%, n=7, p=0.035) were the only significant symptoms seen in the univariate analysis. However, faecal incontinence was the only significant symptom identified by multivariate analysis (p=0.043, OR 3.143, CI 1.04-9.53) (see Table 3)

Thirty-one cancers (7%) were diagnosed in the referred population. Three patients were diagnosed with non-colonic cancers which were pancreatic, anal and small bowel lymphoma on subsequent investigations. The total number of the detected colonic cancers was 28 patients (6.3%) with mean age of 66.4±13.3 years (65.9 years old in males and 67.4 years old in females) and median of 65 years. The average tumour distance was 21.95±16.6 cm from the anal verge. The majority of cancer patients presented with advanced disease. The rec-

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Colonic polyps		Polyps N = 56, 12.6%	No polyps N = 389	Univariate analysis *p* value	Multivariate analysis *p* value, OR & CI
Age		60.8 ± SD 12.3	58.72 ± SD 12.6	0.340	-
Gender					
	Male	35 (62.5%)	185 (47.6%)	0.069	
	Female	21 (37.5%)	204 (52.4%)	-	-
Presenting symptom					
	Bleeding PR	29 (51.8%)	204 (52.4%)	0.89	-
	Change in bowel habits	34 (60.7%)	205 (52.7%)	0.176	-
	Weight loss	1 (1.8%)	32 (8.2%)	0.051	-
	Family history	10 (17.9%)	48 (12.3%)	0.377	-
	Abdominal pain	10 (17.9%)	56 (14.4%)	0.999	-
	Tenesmus	8 (14.3%)	62 (15.9%)	0.814	-
	Mucus	7 (12.5%)	18 (6.2%)	0.05	0.058,OR 2.89 (CI 0.96 -8.68)
	Incontinence	7 (12.5%)	17 (4.4 %)	0.035	0.043,OR 3.143 (CI 1.04-9.53)

Table 3: Univariate and multivariate analysis of polyps symptoms

tum was the commonest site for cancer (53.6%, n=15) followed by rectosigmoid junction (28.6%, n=8) (see Table 4). Within this group, the commonest symptoms were bleeding per rectum which was seen in 57.1% (n=16) and change in bowel habits in 57.1% (n=16). Weight loss, tenesmus and passing mucus per rectum were the three significant symptoms seen in univariate and multivariate analysis of this group (see Table 5).

Cancer characteristics		N = 28, 6.3%
Patients age (Mean)		66.4±13.3
	Median	65
Average cancer distance		21.95 ± SD 16.64
Anatomical location		
	Rectum	15 (53.6%)
	Rectosigmoid junction	8 (28.6%)
	Sigmoid colon	3 (10.7%)
	Hepatic flexure	1 (3.6%)
	Cecum	1 (3.6%)
Cancer type		
	Duke A	5 (17.86%)
	Duke B	8 (28.57%)
	Duke C	15 (53.57%)

Table 4: Characteristic of colorectal cancers

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Colonic cancers		Cancer N = 28, 6.8%	No Cancer N = 417	Univariate analysis *p* values	Multivariate analysis *p* values, OR & CI
Age		66.4 ± SD 13.29	57.76 ± SD 12.77	0.004	-
Gender					
	Male	19 (67.9%)	201 (48.2%)	0.062	
	Female	9 (32.1%)	214 (51.8%)	-	-
Presenting symptom					
	Bleeding PR	16 (57.14%)	217 (52.03%)	0.971	-
	Change in bowel habits	16 (57.14%)	223 (53.5%)	0.390	-
	Weight loss	7 (25%)	26 (6.2%)	0.005	0.01,OR 4.5, CI 1.4-14.1
	Family history	5 (17.86%)	53 (12.7%)	0.458	-
	Abdominal pain	3 (10.7%)	63 (15.1%)	0.745	-
	Tenesmus	9 (31.1%)	61 (14.6%)	0.006	0.014, OR 3.3, CI 1.27-8.64
	Mucus	5 (17.86%)	20 (4.8%)	0.008	0.031, OR 3.96,CI 1.13-13.9
	Incontinence	1 (3.6%)	23 (5.5%)	0.093	-

Table 5: Univariate and multivariate analysis of colorectal cancers symptoms

One patient was diagnosed with right sided Duke's A colonic cancer five years after his initial visit. His initial diagnosis was adenomatous polyp in the sigmoid colon and the cancer was detected during his follow up visit.

The estimated cost difference between flexible sigmoidoscopy and a day case colonoscopy is around 220 Euros. If all patients in this study (n=388) had undergone a colonoscopy, an additional cost of 80,309 Euros would have been incurred without any difference in the detection of major disease compared to the combination of flexible sigmoidoscopy and selective barium enema. Even with the addition of all patients who required completion colonoscopy for polyps or cancer or other means of bowel evaluation, our savings were around 27,500 Euros.

DISCUSSION

Flexible sigmoidoscopes reach 70% of bowel cancers and their combination with barium enema increases their sensitivity to 94% and specificity to 99% for the detection of bowel neoplasia[14] Moreover, the ability of a physician to perform this test in most health care settings makes it more accessible. This combined with the relative ease of performing sigmoidoscopies could enable primary care physicians and para-medical staff to perform the procedure after relatively short training.[15]

The early detection and removal of adenomas has a positive impact on the subsequent development of cancers.[12] The national polyp study demonstrated a 76-90% reduction in the development of colorectal cancer after successful endoscopic removal of polyps even after a prolonged surveillance period.[16] Although screening an already symptomatic population with more advanced disease will not result in any survival benefit;[8] the elective treatment of colorectal cancer can significantly improve patients prognosis compared to outcomes after emergency surgery for an obstructing or perforated tumor.[17]

In our study, colonic polyps were detected on 12.6% of patients with the median age at the time of detection being 61 years. As seen in many studies, there is a preponderance of the disease in the male population.[18] We identified that passing mucus per rectum and episodic faecal incontinence are unique presenting symptoms for distal colonic polyp which has not been described in previous reports. However, the relatively small number of polyps detected and the overall small patient pool may have resulted in a Type I error or false positives in other words Therefore large studies looking specifically at symptoms related to distal polyps will be required to further validate these findings.

Although there is a myriad of symptoms a colorectal cancer patients can present with, few of these are unique. It is very important to understand that these symptoms are more likely to occur in clusters rather than in isolation. Most of isolated symptoms lack sensitivity and specificity and in clusters they increase the likelihood of cancer.[19] Most patients referred to secondary centres with colorectal cancers have more than one symptom (85%).[13] Furthermore, these symptoms are subjected to selection bias which affects their predictive value in both the primary and secondary care settings.[20]

Rectal bleeding is one of the most widely known and feared symptom. It is important to evaluate both the nature and duration of bleeding before considering flexible sigmoidoscopy or any other investigation. The estimated predictive value of bleeding per rectum for colorectal cancer in the general population is around 1 in 1000.[21] Furthermore, 6% of the population above the

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age of 40 years had recent rectal bleeding.[22] In most cases, bleeding per rectum is a symptom of benign pathology rather than from a cancerous origin. This was reflected in our study as 233 patients (52.4%) were referred with bleeding per rectum and only 16 (6.9%) were diagnosed with cancer.

When looking at change in bowel habits, constipation is considered as a low risk symptom for colorectal cancer.[23] Many studies reported that increased bowel frequency and loose motion is associated with 60-91% in distal and 40-61% in proximal cancers.[24] However, it was reported that the predictive value of diarrhoea is 0.63% in patients below 70 years and 1.7% in those over 70.[25] Similar to rectal bleeding, out of 239 patients (53.7%) referred with change in bowel habits, only 16 (6.7%) were diagnosed with cancer.

Unintentional weight loss is one of the most alarming symptoms of colorectal cancers despite being shared with benign conditions. The incidence of weight loss in general population is about 2.3-3.3 %.[26] In our study, weight loss was a significant predictor for colorectal cancers. Out of 33 patients referred with unintentional weight loss, 7 (21.2%) had colorectal cancer ($p<0.05$). A higher incidence rate was seen in a study by Majumdar et. al in which 39% of a cohort of 194 cancer patients presented with weight loss. The study also showed that weight loss was less common in distal cancers without reaching statistical significance.[8] This high figure drops to 1.2% in non-selected primary care patient cohort.[19,25] Likewise, colonoscopy findings in patients with abdominal pain compared to asymptomatic patients showed no difference in the detection of major colorectal diseases.[27]

The combinations of two or more symptoms can increase the predictive ability to detect colorectal cancer.[19] The association of rectal bleeding and change of bowel habit is described in many studies.[25] Likewise, the combination of weight loss and rectal bleeding or change in bowel habits increases the positive predictive value.[19] Despite the low predictive value of abdominal pain, its combination with rectal bleeding can raise its predictive value significantly.[25] In our study, 79% of patients had more than one symptom in their presentation.

The colorectal cancer detection rate of 6.3% in this study is comparable to the detection range of 4 -16 % described in previous reports.[5,12,28] Moreover, the 57 % incidence of rectal bleeding and change in bowel habits in bowel cancer was very similar to 58 and 51% in a symptomatic colorectal study.[13] In our study, 92.8% of all cancers were within the reach of the sigmoidoscope and 7.2% were on the right side which is in keeping with previous reports.[29] Unexplained weight loss, peri-anal pain and passing mucus per rectum were the most significant independent predictors of colorectal carcinoma in our symptomatic population.

Currently, our rapid access clinic has further transformed and it is almost entirely managed by our advanced nurse practitioner who is supervised by consultant colorectal surgeons. This has further reduced the operator cost from an average of 50 Euros per hour to 20 Euros per hour. Furthermore, twice the number of sigmoidoscopies can be performed compared to colonoscopies in each endoscopy session. This further reduced the cost, the endoscopic waiting time and the burden on the day-case services. Within 2011, our service was able to deliver 818 flexible sigmoidoscopies through the rapid access clinic.

One potential weakness of this study is the variability of symptoms among different referring GPs. It is quite possible that they might be more thorough in their referrals if there was a suspicion of malignancy. As such, the predictive value of the symptoms could be overestimated. We were also hampered by our inability to accurately collect information from the referral forms about dietary habits and medications that may have had relevance to the patient presentation.

In conclusion, in the presence of the alarming symptoms identified in this study or combination of two or more, patient's referral should be flagged as urgent. Most importantly, flexible sigmoidoscopy is relatively easy, safe, sensitive and cost effective investigation for patients with lower colonic symptoms especially in such a difficult economic climate.

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REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin*. 2009; 59(4): 225-249. doi: 10.3322/caac.20006

2. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol*. 2007; 18(3): 581-592. doi: 10.1093/annonc/mdl498

3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005; 55(2): 74-108.

4. National cancer screenig service. Recommendations for a colorectal cancer screening programme in Ireland. Letter of submission and executive summary. Website: http://www.cancerscreening.ie/publications/Colorectal-Recommendations.pdf 2009; *HSE press.* 2-7.

5. Vellacott K, Roe A, Mortensen N. An evaluation of a direct access flexible fibreoptic sigmoidoscopy service. *Ann R Coll Surg of Engl*. 1987; 69(4): 149-152.

6. Lamah M, Ahmad SM, Charalampopoulos A, Ho J, Leicester RJ. Three-year evaluation of a rapid-access coloproctology clinic. *Dig Surg*. 2000; 17(2): 150-153. doi: 10.1159/000018819

7. Bruster S, Jarman B, Bosanquet N, Weston D, Erens R, Delbanco TL. National survey of hospital patients. *BMJ*. 1994; 309(6968): 1542-1546. doi: 10.1136/bmj.309.6968.1542

8. Majumdar SR, Fletcher RH, Evans AT. How does colorectal cancer present? Symptoms, duration, and clues to location. *Am J Gastroenterol*. 1999; 94(10): 3039-3045. doi: 10.1111/j.1572-0241-.1999.01454.x

9. Lieberman DA, Holub JL, Moravec MD, Eisen GM, Peters D, Morris CD. Prevalence of colon polyps detected by colonoscopy screening in asymptomatic black and white patients. *JAMA*. 2008; 300(12): 1417-1422. doi: 10.1001/jama.300.12.1417

10. Selby JV, Friedman GD, Quesenberry CP Jr, Weiss NS. A case–control study of screening sigmoidoscopy and mortality from colorectal cancer. *N Engl J Med*. 1992; 326: 653-657. doi: 10.1056/NEJM199203053261001

11. Atkin WS, Edwards R, Kralj-Hans I, et al. Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: a multicentre randomised controlled trial. *The Lancet*. 2010; 375: 1624-1633. doi: 10.1016/S0140-6736(10)60551-X

12. Vipond M, Moshakis V. Four-year evaluation of a direct-access fibreoptic sigmoidoscopy service. *Ann R Coll Surg Engl*. 1996; 78(1): 23-26.

13. Flashman K, O'Leary DP, Senapati A, Thompson MR. The Department of Health's "two week standard" for bowel cancer: is it working? *Gut*. 2004; 53(3): 387-391. doi: 10.1136/gut.2003.020503

14. Jensen J, Kewenter J, Asztély M, Lycke G, Wojciechowski J. Double contrast barium enema and flexible rectosigmoidoscopy: a reliable diagnostic combination for detection of colorectal neoplasm. *Br J Surg*. 1990; 77(3): 270-272.

15. Duthie GS, Drew PJ, Hughes MA, et al. A UK training programme for nurse practitioner flexible sigmoidoscopy and a prospective evaluation of the practice of the first UK trained nurse flexible sigmoidoscopist. *Gut*. 1998; 43: 711-714. doi: 10.1136/gut.43.5.711

16. Winawer SJ, Zauber AG, Ho MN, et al. Prevention of colorectal cancer by colonoscopic polypectomy. *N Engl J Med*. 1993; 329(27): 1977-1981. doi: 10.1056/NEJM199312303292701

17. Umpleby H, Williamson RC. Survival in acute obstructing colorectal carcinoma. *Dis Colon Rectum*. 1984; 27: 299-304. doi: 10.1007/BF02555634

18. Binda V, Pereira-Lima J, Nunes CA, Falkemberg LT, Azambuja DB, Cruz JV. Is there a role for sigmoidoscopy in symptom atic patients? Analysis of a study correlating distal and proximal colonic neoplasias detected by colonoscopy in a symptomatic population. *Arq Gastroenterol*. 2007; 44(1): 2-7. doi: 10.1590/S0004-28032007000100002

19. Selvachandran S, Hodder RJ, Ballal MS, Jones P, Cade D. Prediction of colorectal cancer by a patient consultation questionnaire and scoring system: a prospective study. *Lancet*. 2002; 360(9329): 278-283. doi: 10.1016/S0140-6736(02)09549-1

20. Knottnerus J, Knipschild P, Sturmans F. Symptoms and selection bias: the influence of selection towards specialist care on the relationship between symptoms and diagnoses. *Theoretical Med and Bioeth*. 1989; 10(1): 67-81.

21. Fijten GH, Blijham GH, Knottnerus JA. Occurrence and clinical significance of overt blood loss per rectum in the general population and in medical practice. *Br J Gen Pract*. 1994; 44(384): 320-325.

22. Goulston K, Chapuis P, Bokey L. Significance of bowel symptoms. *Med J Aust*. 1987; 146(12): 631-633.

23. Thompson M. ACPGBI Referral guidelines for colorectal cancer. *Colorectal Dis*. 2002; 4: 287-297. doi: 10.1046/j.1463-1318.2002.00348.x

24. Neugut A, Garbowski G, Waye J, et al. Diagnostic yield of colorectal neoplasia with colonoscopy for abdominal pain, change in bowel habits, and rectal bleeding. *Am J Gastroenterol*. 1993; 88: 1179-1185.

25. Hamilton W, Round A, Sharp D, Peters TJ. Clinical features of colorectal cancer before diagnosis: a population-based case–control study. *Br J Cancer*. 2005; 93(4): 399-405. doi: 10.1038/sj.bjc.6602714

26. Summerton N, Mann S, Sutton J, et al. Developing clinically relevant and reproducible symptom-defined populations for cancer diagnostic research in general practice using a community survey. *Fam Pract*. 2003; 20(3): 340-346. doi: 10.1093/fampra/cmg317

27. Lieberman DA, de Garmo PL, Fleischer DE, Eisen GM, Chan BK, Helfand M. Colonic neoplasia in patients with nonspecific GI symptoms. *Gastrointest Endosc*. 2000; 51(6): 647-651.

28. Imperiale TF, Wagner DR, Lin CY, Larkin GN, Rogge JD, Ransohoff DF. Risk of advanced proximal neoplasms in asymptomatic adults according to the distal colorectal findings. *N Engl J Med*. 2000; 343: 169-174.

29. Kronborg O, Fenger C, Olsen J, Jørgensen OD, Søndergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet*. 1996; 348(9040): 1467-1471.

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Cost Effectiveness analysis of Surgical Treatment of Stress Urinary Incontinence Using Single-Incision Mini-Slings *vs.* Tension-Free Vaginal Obturator in Spain

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ABSTRACT

Objectives: Stress Urinary Incontinence (SUI) is highly prevalent, noticeably deteriorating quality of life. The current surgical treatment is performed through minimally invasive techniques that are quite rapidly evolving. The objective of this study is to analyze the comparative efficiency, from the perspective of the health system, of surgical treatments for patients with SUI through the use of the single-incision mini-sling (SIMS), MiniArc™ (American Medical Systems, Inc), in relation to the transobturator sling, TVT-O.
Methods: Cost-effectiveness analysis based on the results of interventions performed with TVT-O (2005-2008) and MiniArc™ (2008-2011) in women with a diagnosis of SUI. The clinical result was an objective cure at 12 months (pad-test <1 g/h). The costs were the direct healthcare costs (diagnostic and surgical procedures, medical devices, medications, hospital stay times and staff). Later, different sensitivity analyses were conducted.
Results: The two groups were homogenous regarding the principal base characteristics. No statistically significant difference in effectiveness between MiniArc™ (93.2%) and TVT-O (86.5%) was observed. The total annual cost per patient was less with MiniArc™ (2,059€, 95% CI: 1,914; 2,285; 2,543$, 95% CI: 2,364; 2,822) than with TVT-O (2,821€, 95% CI: 2,661; 2,997; 3,484$, 95% CI: 3,287; 3,701), causing an estimated savings of 762€, 95% CI: 516; 987 (-941$, 95% CI: -1,219; -637). The sensitivity analysis showed that the probability of association MiniArc™ with the lowest cost was close to 100% in almost all the cases.
Conclusions: The use of MiniArc™ is associated with a 762€, 941$ per patient reduction in the average annual cost, compared to TVT-O, while maintaining a similar effectiveness.

KEYWORDS: Cost-effectiveness; Single-incision mini-sling; Transobturator.

ABBREVIATIONS: SIMS: Single-incision mini-sling; TVT-O: Tension-free Vaginal Tape Obturator; SUI: Stress Urinary Incontinence; ICIQ-SF: International Consultation on Incontinence Questionnaire-Short Form; ICER: Incremental Cost Effectiveness Ratio; 95% CI: 95% Confidence Interval; SD: Standard Deviation.

INTRODUCTION

Stress Urinary Incontinence (SUI) is the involuntary loss of urine triggered by physical activity such as coughing, laughing, etc., which coincides with an increase in abdominal pressure. It is most commonly caused when the urethra is hyper mobile because of problems with the muscles of the pelvis and hyperactivity of the detrusor. This pathology affects a significant proportion of women, especially beginning at 30 years of age, notably deteriorating

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quality of life.[1] The objective of its treatment is improving the patients' quality of life. Once pharmacological treatment or muscular rehabilitation of the pelvic floor is not enough, surgery is used.[2] In 1996, Ulmstein et al[3] used a tension-free suburethral vaginal sling (Tension-free Vaginal Tape, TVT), performing an intraoperative review cystoscopy to test bladder integrity. Later, to reduce complications associated with retropubic placement of the TVT, Delorme et al[4] approached the technique more simply as it did not require the review cytoscopy, by implanting a transobturator sling (Tension-free Vaginal Tape, TVT-O). Finally, a third generation of slings appears, with a single incision as well as reduced size, called mini-slings,[5,6] among which are TVT-Secur™ and MiniArc™, which are affixed using an obturator needle.

The Spanish National Health System presents a universal coverage, and it is funded from taxes and predominantly operates within the public sector. Provision is free of charge-with the exception of the drugs prescribed. Nowadays, knowledge of the efficiency of new technology has become indispensable, for the sake of a rational allocation of health resources. For this reason, the objective of the present study is to estimate the incremental cost effectiveness ratio of surgical treatment of patients with SUI using the MiniArc™ compared to TVT-O, from the perspective of the health system.

MATERIAL AND METHODS

Data obtained from medical records of patients who had undergone interventions consecutively in one hospital for SUI between 2005 and 2011 were analyzed retrospectively. The follow-up period for data collection was 12 months. All patients signed the corresponding informed consent form; additionally, prior approval from the hospital's corresponding Research Ethics Committee was obtained. The basal severity was measured with Sandvick's test[7] (consists of two questions, regarding frequency and amount of leakage), and the International Consultation on Incontinence Questionnaire-Short Form, ICIQ-SF[8] (that provides a measure to assess the impact of symptoms of incontinence on quality of life and outcome treatment by using 4 items, being severe incontinence if there is an score bigger than 12). An economic evaluation of the surgical options, MiniArc™ and TVT-O, was performed to estimate the incremental costs and benefits of surgical treatments for the patients who presented with stress urinary incontinence. The analysis was carried out in a 1 year time horizon and from the perspective of the Spanish National Health System. The type of analysis was cost effectiveness; a cost minimization analysis would be carried out if there was not a significant statistical difference of clinical result. The result of the analysis is expressed as the Incremental Cost Effectiveness Ratio (ICER), calculated using:

$$ICER \frac{= \left[Cost_{MiniArc} - Cost_{TVT-O} \right]}{= \left[Effectiveness_{MiniArc} - Effectiveness_{TVT-O} \right]}$$

In the case of performing a cost minimization analysis, the final result corresponds to the difference between the costs of each option.

A mathematical model was designed to estimate the overall cost per patient during 12 months of follow-up. The model was based on the flow of follow-up processes in care of patients in the hospital (Figure 1)

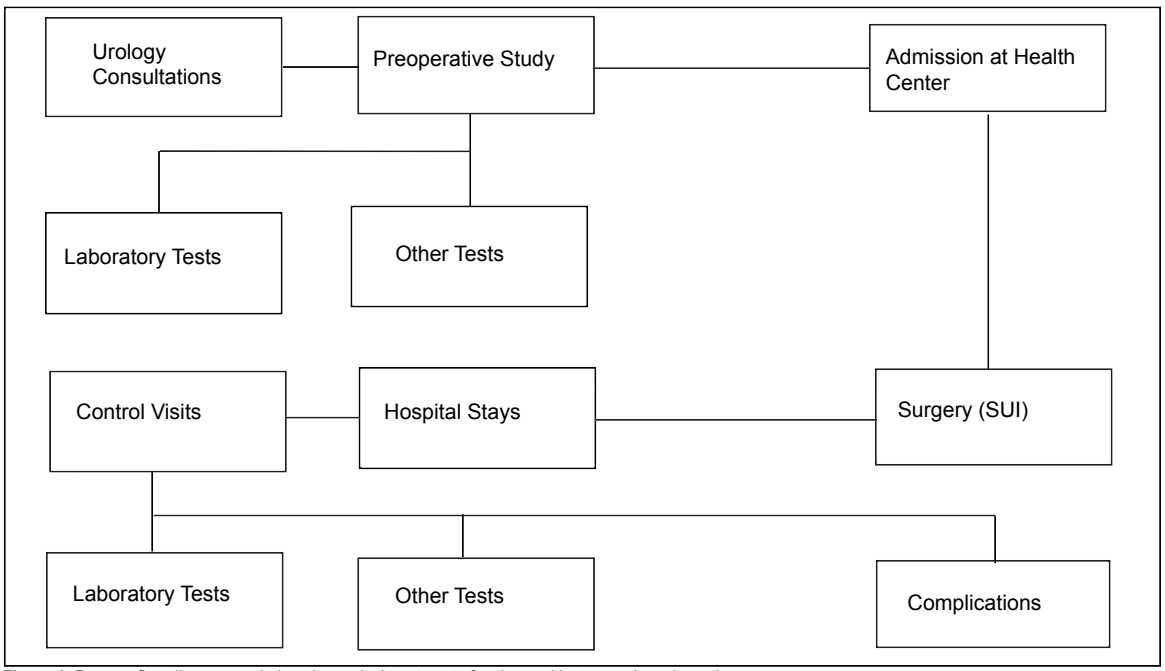

Figure 1: Process flow diagram carried out in surgical treatment of patients with stress urinary incontinence

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Given the health system's perspective, only direct healthcare costs were included.[9] The cost analysis was carried out using the *bottom-up* method. Briefly, this method identifies, quantifies and evaluates the individual costs of each phase of the process:

a) Preoperative, which includes everything from the visit when the decision to surgical intervention is made (consultations for clinical history and overall evaluation, diagnostic and laboratory tests and urodynamic evaluation);

b) Interventional, including everything from the moment of the intervention until patient's discharge from the hospital (time of the surgical intervention and the health professionals and hospital stay);

c) Post-operative, where the events that have occurred since hospital discharge after surgery up to 12 months follow-up (consultations, diagnostic and laboratory tests, and management of complications) are analyzed.

The unit prices of consultations, diagnostic testing and laboratory tests are taken from the hospital's analytical accounting; the prices of medications used were extracted from the BOT Plus database from the General Council of Spanish Pharmacists;[10] operating room times and the times of the health professionals were taken from the hospital registries of each patient who underwent surgery. All costs are expressed in 2013 euros (and US dollars), updating them to that year if necessary, according to the general CPI of the National Statistics Institute of Spain.

The total cost (T_c) is calculated using the corresponding cost of each phase (i):

$$T_c = \sum_{1}^{n} q_{(i)} * c_{(i)}$$

q being the frequency of use of each resource and c the unit price.

Once the total cost was estimated, the bootstrap method[11,12] was used to analyze the uncertainty associated with the obtained result of each surgical option. Briefly, bootstrap is a nonparametric method, which, through a resampling process with replacement, generates a large quantity of samples with the same size as the original, allowing one to estimate a probabilistic distribution to calculate the statistic of interest, in our case, the dispersion of the average cost using its confidence interval at 95% (95% CI).

Finally, the average cost of the difference between the use of TVT-O and MiniArc™ was calculated, then proceeding to estimate the probability that MiniArc™ was less costly than TVT-O, analyzing for each iteration obtained in the bootstrap:

$$[Prob\{(Cost_{MiniArc} - Cost_{TVT-O}) < 0\}$$

Clinical effectiveness was expressed using the objective cure rate at 12 months follow-up, defined as the absence of urine loss, manifested with a negative pad test result (urine loss of <1g in 1 hour).[13] Additionally, the secondary complications of each surgical option were analyzed: intraoperative complications such as bladder perforation, early complications (in the first month after surgery) such as hematoma, groin pain or urethral obstruction, and late complications (after the first month) such as vaginal erosion, difficulty urinating, new urgency or urinary tract infection (UTI) (repeated).

When comparing the two groups, continuous variables were analyzed using the Student's t test, and categorical ones using Chi-square test; difference between proportions was estimated using Wald method. The statistical package used was SPSS 17.

Different sensitivity analyses were performed, evaluating the robustness of the result through variation of the value of the most relevant variables. As such, the hospital stay of patients who received the TVT-O was reduced to bring it in line with the stay of MiniArc™ patients (0 days), the cost of the hospital stay was varied within a wide margin (±25%), attending to the variability existing between different centers; additionally, taking the variability of the price of the devices into account, the corresponding price of MiniArc™ varied some ±25%.

RESULTS

Patient Characteristics

The information corresponding to 81 patients was collected, 37 of whom had undergone surgery through the available technique at the time of the TVT-O (2005-2008) and the remaining 44 of whom with MiniArc™, beginning when this device was available (2008-2011). The patients' clinical parameters that were most relevant to the economic study are included in Table 1.

	MiniArc™ (SD)	TVT-O (SD)	*p*
Number of patients	44	37	
Age (yr)	58.9 (11.0)	58.3 (11.3)	0.816
Body Mass Index (kg/m²)	27.9 (4.7)	31.1 (5.7)	0.009
Parity (n)	2.5 (1.2)	2.3 (1.3)	0.547
Sandvik's test	4.32 (1.22)	4.38 (1.32)	0.833
ICIQ-SF Questionnaire	14.50 (1.77)	14.95 (1.39)	0.208

Table 1: Baseline characteristics of patients who underwent the surgical interventions

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Clinical Results

The principal clinical result, objective cure of the patient at 12 months, was slightly greater with MiniArc™ (41/44; 93.2%; SD: 3.8) than with TVT-O (32/37; 86.5%; SD: 5.6), although a significant statistical difference was not present (6.7%, 95% CI: -6.6; 20.0; χ^2: 0.40; p: 0.527). For that reason, the adopted economic analysis was cost minimization, exclusively evaluating the costs associated with each surgical option, since there was a statistical equivalence of clinical results.

No intraoperative complications were observed in either of the analyzed surgical options. Only 3 cases of early complications were observed (1 with MiniArc™ and 2 with TVT-O) without showing a statistically significant difference between the two techniques p: 0.553). With regard to late complications, statistically different differences in vaginal erosion (1 case with TVT-O) difficulty urinating (2 with MiniArc™ and 3 with TVT-O; p: 0.506), new urgency (6 with MiniArc™ and 10 with TVT-O; p: 0.131) were not observed either. The only difference was with repeated UTIs (1 with MiniArc™ and 13 with TVT-O; $p<0.05$)

Costs

The relevant resources used in patients with one or the other therapeutic option, as well as the average number of units used are expressed in Table 2.

	MiniArc ™	TVT-O	*p*
Preoperative phase			
Gynecological visit	1.00	1.14	0.129
Ultrasound scan	1.09	1.00	0.061
Urodynamic test	1.05	0.97	0.095
Urine sediment	0.98	0.58	0.013(*)
Rehabilitation	4.91	2.54	0.097
Intervention phase			
Theater (min)	39.66	54.24	<0.001(*)
Gynecologist (min)	24.68	42.65	0.001(*)
Anaesthetist (min)	57.57	50.68	<0.001(*)
Nurse (min)	46.61	54.38	<0.001(*)
Hospital stay (days)	0	3.14	<0.001(*)
Post-operative phase			
Gynecologist (visit)	4.09	3.84	0.046(*)
Ultrasound scan	2.18	2.41	0.345
Urodinamic test	1.02	1.08	0.535
Urine sediment	1.98	0.00	<0.001(*)
Rehabilitation	0	0.65	<0.001(*)

Table 2: Healthcare resources used for patient management with each therapeutic option. (*): statistically significant difference

The main difference is found in the intervention phase, showing a significant reduction in resources used during that phase with MiniArc™.

To estimate the overall cost of patient care, the unit prices described in Table 3 were used, which multiplying the frequency of use of each to estimate the cost of each phase.

Health Resource	Unit Price (€; ($))
Gynecologist visit	35.00€ ; (43.23$)
Ultrasound scan	43.00€ ; (53.11$)
Urodynamic test	201.72€ ; (249.14$)
Urine sediment	7.00€ ; (8.65$)
Rehabilitation Session	14.07€ ; (17.38$)
Theater (min)	2.50€ ; (3.09$)
Gynecologist (min)	0.81€ ; (1.00$)
Anaesthetist (min)	0.90€ ; (1.11$)
Nurse (min)	0.46€ ; (0.57$)
Nurse's Assistant (min)	0.29€ ; (0.36$)
MiniArc™	680.40€ ; (840.34$)
TVT-O	646.76€ ; (798.79$)
AMS Room	76.17€ ; (94.07$)
Hospital stay (days)	288.90€ ; (356.81$)

TVT-O: Tension free Vaginal Tape Obturator; MAS: Major ambulatory surgery

Table 3: Unit prices of the principal resources used (€; ($), 2013).

The result of the analysis after performing the bootstrap indicated that the average cost associated with MiniArc™ was lower (2,059€; 2,543$), showing a statistically significant difference with respect to TVT-O (2,821€; 3,485$), with an average savings of 762 euros (941$) (t: -5.6636; p: 1.99) per patient in the first year of follow-up (Table 4). Additionally, a 100% probability of producing this cost savings was estimated.

Therapeutic Alternative	Cost (€;$) (95% CI)	Difference (95% CI)
MiniArc™	2,059€ (1,914; 2,285); 2,543$ (2,364; 2,822	-762€ (-987; -516); -941$ (-1,219; -637)
TVT-O	2,821€ (2,661; 2,997); 3,484$ (3,287; 3,701)	

Table 4: Results of costs obtained with MiniArc™ and TVT-O in the base case

The results of the costs, disaggregated according to the previously defined phases, show that the intervention phase constitutes the cost determinant in the group of patients who had MiniArc™ placement (Table 5).

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Phase	Alternative	Cost (95% CI)	Difference (95% CI)
Preoperative	MiniArc™	369 (339; 399)	51 (11; 90)
	TVT-O	317 (293; 344)	
Intervention	MiniArc™	950 (941; 961)	-984 (-988; -817)
	TVT-O	1,832 (1,770; 1,934)	
Post-Operative	MiniArc™	740 (591; 915)	64 (-135; 286)
	TVT-O	676 (583; 820)	

Table 5: Average cost disaggregated by time components of the process

That is essentially due to the fact that the surgery is performed as an outpatient procedure, which is less normal in the case of TVT-O (mean stay: 3.14 days, range: 2-9) because of pain.

Sensitivity Analysis

In the univariate sensitivity analysis, a reduction of costs associated with the use of MiniArc™ was maintained when the hospital stay was reduced by 25% (555€, 95% CI: 252; 779; 685$, 95% CI: 311; 962) or was increased by the same proportion (966€, 95% CI: 692; 1,242; 1,193$, 95% CI: 855; 1,534). Additionally, given that the length of the hospital stay forms a relevant variable in the cost, a sensitivity analysis was carried out, modifying the time in an interval of 2 to 0 days, estimating that the probability of cost savings with MiniArc™ was 100%, 90% and 9% for an average stay of patients treated with TVT-O of 2, 1, and 0 days, respectively (Figure 2). On the other hand, if the cost of MiniArc™ were reduced by 25% the reduction of the overall cost would increase to 919€ (95% CI: 651; 1,163),1,134$ (95% CI: 804; 1,436) ; in the case of a 25% increase in the price of the device, there would still be a cost savings, estimated at 575€ (95% CI:313; 832), 710$ (95% CI%: 387; 1,028). (Figure 2)

DISCUSSION

Based on the results found, surgical treatment with-MiniArc™ for patients diagnosed with stress urinary incontinence is associated with a comparable clinical effectiveness to that obtained with Tension-free Vaginal Tape Obturator (TVT-O),without any significant difference in the occurrence of complications observed, except for repeated UTIs, which were fewer in patients who had undergone surgery with MiniArc™. However, the use of MiniArc™ produces a significant reduction of 762€ (95% CI: 516; 987), 941$ (95% CI: 637; 1,219), in the total cost.

The equivalent effectiveness between the two surgical options has already been shown previously. An exploratory, randomized phase 2 study[14] analyzed the objective response, measured using the pad test at 12 months, and shows a slight difference in favor of MiniArc™. Later, a randomized controlled trial,[15] which has evaluated the objective cure rate of MiniArc™ compared to the transobturator standard midurethral sling,

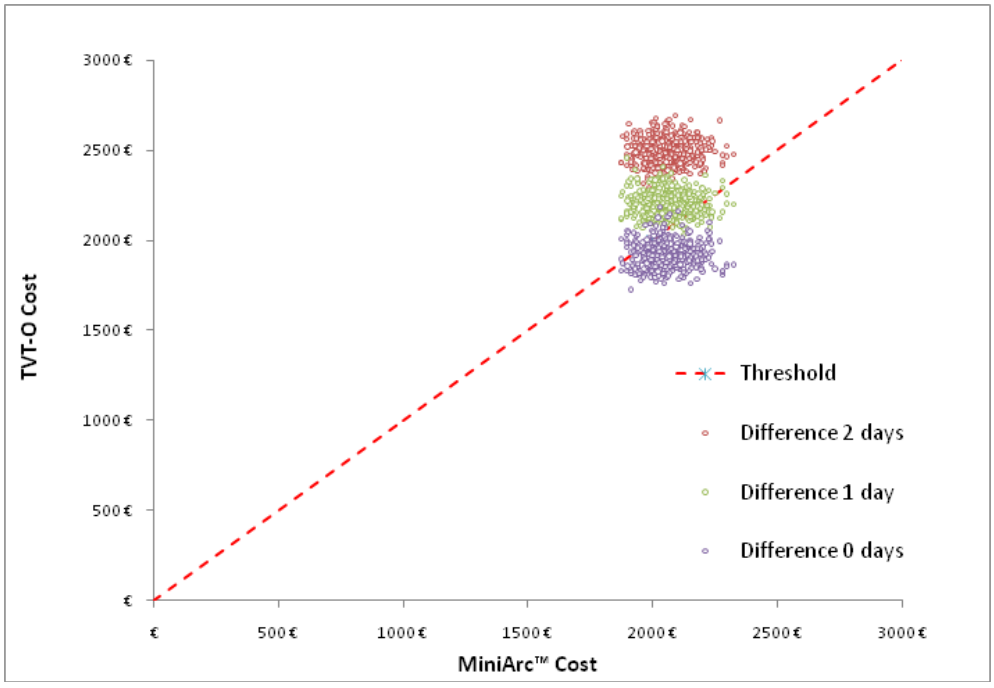

Figure 2: Graphic representation of the bootstrap (after 1000 iterations) to estimate the probability that the treatment with MiniArc™ would be less costly with respect to the different stays with TVT-O. The points above the threshold indicate a lower MiniArc™ cost; those below indicate a greater cost.

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Monarc™, concludes that MiniArc™ presents an objective cure rate that is not inferior to the other option (89% v. 91%, respectively; p: 0.65) nor subjective cure (83% v. 86%, respectively; p: 0.46) while post-operative pain is greater ($p<0.01$). Additionally, systematic reviews have been performed that confirm this fact: Mostafa et al[16] have compared the effectiveness of the single-incision minislings *vs*. TVT-O and similar slings, concluding that there is no significant difference in the objective cure rate (RR: 0.98; 95% CI: 0.94; 1.01 at 18.6 months) although MiniArc™ shows a shorter recovery time to return to daily activities (5.08 v. 7.20 days). The demonstration of equivalence in the effectiveness results is essential for the choice of the type of economic analysis.[17] This fact led to the decision to perform a cost minimization analysis, for which the variable in the result is the difference between the total average cost of each evaluated option, having estimated a reduction of costs associated with the use of MiniArc™.

The cost determinants, which explain the result, consist of the shorter intervention time and the performance of the surgery with MiniArc™ as an outpatient procedure. The hospital stay is a relevant factor in the cost difference, which is why that variable was analyzed in depth. On one hand, the duration obtained in our study is in accordance with other practices in our country, as is shown in the recent study from Castroviejo-Royo et al,[18] who indicated a stay of 3.83±2.46 days in patients treated using TVT-TOT. Girvent et al[19] analyzed interventions with TVT-O, obtaining an average stay of 2.7 days, equal to what was reported by others, such as Úbeda et al[20] or Álvarez Cañadas et al.[21] A slightly shorter stay was described by Navazo et al[22] (1.5 days, range: 1-5) or Zullo et al[23] (1, 1±0.3 days). However, the performance of the technique under anesthesia-sedation as an outpatient procedure has been described,[24] for which there was no stay. Thus a great variability can be observed in our country regarding hospitalization time after surgical intervention. For these reasons, the sensitivity of the obtained result to the length of the hospital stay was analyzed, estimating the average cost associated with TVT-O according to different lengths of hospital stays (between 3 and 0 days), showing a higher probability of a lower cost with MiniArc™ in almost all analyzed cases.

We have not found an economic study that compares the cost of MiniArc™ with that of TVT-O. Nevertheless, Montesino et al[25] have recently evaluated the efficiency of surgical treatment – with mini-slings or TVT-O, compared to no surgery in a sample of women with stress urinary incontinence, mixed urinary incontinence, and incontinence associated with prolapse. However, as comparison of the evaluated procedures is not the objective of their study, they did not disaggregate the result by type of procedure or incontinence. They concluded, however, that surgery with minis lings is an efficient option for the National Health System. In another recent study, Boyers et al[26] had estimated the relative efficiency of TVT-O and another single-incision minis ling, Ajust™, showing a higher cost of TVT-O in comparison; the said increase in cost is still greater when the investigators carried out the study from a social perspective, based on a faster recovery with the single-incision minisling, a fact that has also been indicated for MiniArc™ in a recent systemic review.[16]

The study carried out presents some limitations. The sample size is not enough to give the study a large statistical power. For that reason, although the average value of effectiveness was higher with MiniArc™, it could not be demonstrated that there was a significant difference. Nevertheless, a nonparametric bootstrap has been applied to the costs, allowing us to more precisely estimate the measure of interindividual dispersion. A second limitation is the retrospective nature of the study, since the TVT-O group corresponds to a historical control, which could reduce homogeneity between the patients according to some variation in clinical practice. However, this fact was minimized, since the same group of professionals treated all the patients in both groups, which minimizes variability in clinical practice; residents were not involved. Finally, randomized assignment of the surgical options was not done; it was made sequentially in two time periods, before and after the appearance of MiniArc™, which could have an influence in that the samples were not perfectly homologous; however, the samples faithfully reflect common practice, which increases its external validity.

For future investigations, it is suggested that prospective studies are designed with a large enough sample size and that the studied options are randomly assigned to patients. Also, the follow-up times should be longer to analyze the relative efficiency of the two surgical options in the medium and long term.

In conclusion, the results obtained indicate that the use of MiniArc™ in surgical intervention for patients with stress urinary incontinence shows a comparable effectiveness as well as a 762€, 941$ reduction of the annual cost per patient, in comparison with TVT-O. The results suggest that MiniArc™ isa dominant alternative in comparison with TVT-O due to its lower cost and comparable effectiveness.

FINANCIAL DISCLAIMER / CONFLICT OF INTEREST

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OBSERVATIONS

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REFERENCES

1. Modroño M, Sánchez M, Gayoso P, Valero M, Blanco M, Cuña F. [Study on the prevalence of stress urinary incontinence in women from 18-65 years of age and its influence on quality of life]. *Aten Primaria*. 2004; 34(3): 134-139.

2 Lucas M, Bosch R, Burkhard F, et al. EAU Guidelines on surgical treatment on urinary incontinence. *Eur Urol*. 2012; 62: 1118-1129. doi: 10.1016/j.eururo.2012.09.023

3. Ulmsten U, Henriksson L, Johnson P, Varhos G. An ambulatory surgical procedure under local anesthesia for treatment of female urinary incontinence. *Int Urogynecol J Pelvic Floor Dysfunct*. 1996; 7: 81-86. doi: 10.1007/BF01902378

4. Delorme E. Transobturator urethral suspension: mini-invasive procedure in the treatment of stress urinary incontinence in women. *Prog Urol*. 2001; 11(6):1306-1313.

5. Solà Dalenz V, Ricci Arriola P, Pardo Schanz J. [Stress urinary incontinence surgical correction with third generation sub-mid-urethra sling: TVT-Secur]. *Actas Urol Esp*. 2008; 32(5): 522-526. doi: 10.1016/S0210-4806(08)73877-4

6. Debodinance P, Delporte P. MiniArc: preliminary prospective study on 72 cases. *J Gynecol Obstet Biol Reprod (Paris)*. 2009; 38(2): 144-148. doi: 10.1016/j.jgyn.2008.11.006

7. Sandvik H, Seim A, Vanvik A, Hunskaar S. A severity index for epidemiological surveys of female urinary incontinence: comparison with 48-hour pad-weighing tests. Neurourol Urodyn . 2000; 19:137-145. doi: 10.1002/(SICI)1520-6777-(2000)19:2<137::AID-NAU4>3.0.CO;2-G

8. Avery K, Donovan J, Peters T, Shaw C, Gotoh M, Abrams P. ICIQ: a brief and robust measure for evaluating the symptoms and impact of urinary incontinence. Neurourol Urodyn. 2004; 23(4): 322-330. doi: 10.1002/nau.20041

9. The University of York. Centre for Health Economics. Mogyorosy Z, Smith P. The main methodological issues in costing health care services. A literature review. CHE Research Paper Website: http://www.york.ac.uk/che/pdf/rp7.pdf; Accessed April 16, 2005.

10. Organización Farmacéutica Colegial [General Council of Spanish Pharmacists]. BOT Plus 2.0. Website: http://www.portalfarma.com; Accessed June, 14, 2013.

11. Efron B. Bootstrap methods: Another look at the jackknife. *Ann Statist*. 1979; (1): 1-26.

12. O'Brien B, Briggs A. Analysis of uncertainty in health care cost-effectiveness studies: an introduction to statistical issues and methods. *Stat Methods Med Res*. 2002; 11(6): 455-468. doi: 10.1191/0962280202sm304ra

13. European Association of Urology. Lucas M, Bedretdinova D, Bosch J, et al. Guidelines on Urinary Incontinence. Update March 2013. Website: http://www.uroweb.org/gls/pdf/19_Urinary_Incontinence_LR.pdf; Accessed May 7, 2013.

14. Oliveira R, Botelho F, Silva P, et al. Exploratory study assessing efficacy and complications of TVT-O, TVT-Secur, and Mini-Arc: Results at 12-month follow-up. *Eur Urol*. 2011; 59(6): 940-944. doi: 10.1016/j.eururo.2011.01.018

15. Schellart R, Rengerink K, Van der A, et al. A randomized comparison of a single-incision midurethral sling and a transobturator midurethral sling in women with stress urinary incontinence: Results of 12-mo follow-up. *Eur. Urology*. doi: 10.1016/j.eururo.2014.07.027

16. Mostafa A, Lim C, Hopper L, Madhuvrata P, Abdel-Fattah M. Single-incision mini-slings versus standard midurethral slings in surgical management of female stress urinary incontinence: an update systemic review and meta-analysis of effectiveness and complications. *Eur Urology*. 2014; 65(2): 402-427. doi: 10.1016/j.eururo.2013.08.032

17. Sanz Granda A. [Is it worth the cost?] Majadahonda, Madrid. Spain: Ed. Ergon, 2007.

18. Castroviejo-Royo F, Martínez-Sagarra J, Marina-García C, Conde-Redondo C, Rodríguez-Toves L, González Tejero C. [Treatment of female stress urinaryincontinenceusingsuburethralslings: a retrospective, comparative, observationalstudy of 2 surgicaltechniques]. *Actas Urol Esp*. 2013; 37: 149-153.

19. Girvent M, Monclús M, Lineros E, Pereda A, Ojeda F. [A comparison of techniques for correcting stress urinary incontinence with tension-free tapes: transobturator and needle-free]. Gynecology and Obstetrics E-Archives. 2008; 13. E-magazine. Website: http://e-archivos.org/e-AGO200802/comptotnedleless.

pdf; Accessed June 21, 2013.

20. Úbeda D, Gómez N, Pages C, Macho Y, Cañete M. [Treatment of stress urinary incontinence with TVT-O suburethral tape: Results and complications]. National Pelvic Floor Congress VII. Bilbao, April 3-5, 2014. Website: http://www.sego.es/Content/microsites/congresos2014/suelo_pelvico/pdf/posters/131.pdf; Accessed September 7, 2014.

21. Álvarez Cañadas V, Cano S, Girvent M, Pereda A, Porta O, Ojeda F. [Comparison between TVT and TOT techniques: effectiveness and cure percentage]. Gynecology and Obstetrics e-Archives. 2006; 8. E-magazine. Website: http://e-archivos.org/e-AGO200601/COMPTVT-TOT.pdf; Accessed September 18, 2014.

22. Navazo R, Moreno J, Hidalgo C, et al. [Contasure needleless: single-incision TOT for treating stress urinary incontinence]. *Arch Esp Urol*. 2009; 62(9): 719-723.

23. Zullo M, Plotti F, Calcagno M, et al. One-year follow-up of tensión-free vaginal tape (TVT) and trans-obturator suburethral tape from inside to outside (TVT-O) for surgical treatment of female stress urinary incontinence: A prospective randomized trial. *Eur Urology*. 2007; 51: 1376-1384. doi: 10.1016/j.eururo.2006.10.066

24. Navalón V, Navalón P, Pallás Y, Ordoño F, Monllor E. [Outpatient Surgical treatment of female stress urinary incontinence under local anesthesia-sedation with contasure needleless incision]. *Actas Urol Esp*. 2014; 38(1): 49-54. doi: 10.1016/j.acuro.2013.01.011

25. Montesino M, Jimenez J, Cabases J, Sanchez E, Hualde A, García D. Cost-effectiveness analysis on the surgical treatment of female urinary incontinence using slings and meshes. *Eur J Obstet Gynecolo Reprod Biol*. 2013; 171(1):180-186. doi: 10.1016/j.ejogrb.2013.08.035

26. Boyers D, Kilonzo M, Mostafa A, Abdel-Fattah M. Comparison of an adjustable anchored single-incision mini-sling, Ajust, with a standard mid-urethral sling, TVT.O: a health economic evaluation. *BJU Int*. 2013; 112(8): 1169-1177. doi: 10.1111/bju.12388

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Research

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Epidemiologic Analysis of the Surgical Specimens of Breast Carcinosarcomas Examined at a Reference Laboratory in a Nigerian Community

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ABSTRACT

The idea that carcinoma and sarcoma can exist together dated to as early as 1804. Pictorial illustrations of this phenomenon appeared by 1882. Nowadays, with regard to the breast, it is reportedly rare, there being but the documentation of one or two cases. Therefore, i.e. a series of 12 cases is presented from one ethnic group in Nigeria. The ages ranged from 22 to 52 years (average, 35 years). Two cases were diagnosed clinically as benign, 2 others as Cystosarcoma Phyllodes and the rest as carcinoma. A 2 cm wedge was submitted whereas the rest ranged up to 20 cm in size. The appearances were described often as huge, fleshy and fungating. The right: left ratio was 1.4:1. Most patients were attended to at the cosmopolitan Enugu Teaching Hospital, the rest being from the hinterland.

KEYWORDS: Breast; Carcinosarcoma; Reference Laboratory; Nigeria.

INTRODUCTION

The concept of the co-existence of not only carcinoma but also sarcoma was known for many years. Thus, going by my historical collections, Abernethy,[1] of the fascia eponym, wrote concerning "carcinomatous sarcoma." Indeed, he noted that "the disease extends through the medium of the absorbing vessels." By 1883, David Finlay[2] of the Middlesex Hospital of London presented to the Pathological Society of that City the illustrations of the different carcinomatous and sarcomatous components found in the one patient's fibroid uterus (Figures 1 and 2).

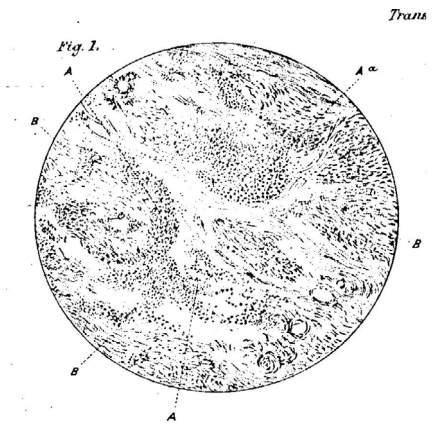

Figure 1: Drawings from the cited reference: a section of the growth under a low power. A refers to areas of rounded-cell growth. B refers to areas of spindle-cell growth.

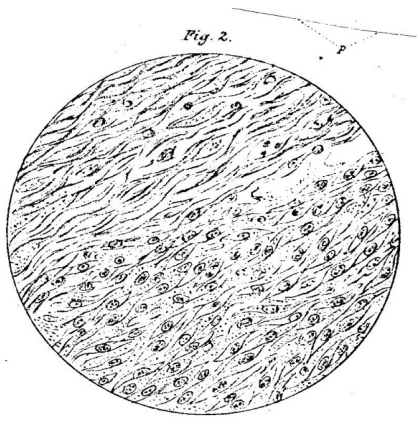

Figure 2: Represents a portion where an area of spindle-shaped cells closely adjoins a portion of the normal structure of the fibromyoma.

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Nowadays, with particular reference to the breast, contributors from countries as far apart as Jamaica,[3] USA[4] and Islamabad[5] emphasized the rarity of mammary carcinosarcoma as contained in the literature. Therefore, the Nigerian picture is subjoined in terms of the epidemiologic picture based on an ethnic group called the Igbos or Ibos.[6] The total was possible because of the establishment of a histopathology data pool as was proposed by a Birmingham, UK group.[7]

INVESTIGATION

The establishment in 1970 of a Reference Pathology Laboratory serving the large ethnic group called the Igbo's who lives mainly in the Eastern Region of Nigeria served until other laboratories came on stream by 2000. During this period, the author was the sole pathologist. This facilitated the analysis of surgical specimens. The only proviso was that clinical details must be supplied in the Standard Request Form. Incidentally, as many as 420 cases of teenage fibro adenomas were encountered during this period.[8] Concerning the special tumor called carcinosarcoma, it was not until 1986 that the first case was recognized personally. It is of interest to reproduce my very report as thus: (Table 1)

The microscopic appearances are odd. There are two components, each showing high mitotic activity. On the one hand, there are deeply basophilic epithelial elements which form recognizable acini in places and are epidermoid in one focus. On the other hand, there is a granulation-like component in which hyperchromatic bizarre cells are scattered.

DISCUSSION

Now, these cases pertain to biopsy materials, whose parameters do not include surgical treatments and outcomes. Therefore, these aspects are outside the scope of this paper. Accordingly, epidemiologic considerations alone are deemed to be apposite.

Firstly, there are the above histological descriptions which are typical. It is to be noted that this particular example displays malignancy of two different breast tissues. Such tissues are regarded as being homologous. In fact, the encyclopedic Ackerman's Surgical Pathology[9] distinguished between the homologous type, which displays the malignant change of the component tissues, as opposed to the heterologous group which may show "skeletal muscle, cartilage, bone or fat."

Secondly, apart from the three cited examples concerning the breast from Jamaica,[3] USA[4] and Islamabad,[5] there are interesting records from the UK,[10] Taiwan,[11] and Morocco,[12] in respect of another female organ, i.e., the ovary.

Thirdly, there is the debate in the UK as to "whether a satisfactory histopathology service can ever be delivered to a hospital remote from the providing pathologists and their laboratory."[13] Certainly, in this series, most requests were made in Enugu where the Pathology Laboratory is situated. However, requests made by surgeons practicing in Missionary Hospitals and Private Hospitals situated in the hinterland of this developing community were attended to. In point of fact, the statistically the usefulness of such a designated laboratory has been

Series	Patient	Lab No	Age	Description	Size (cm)	Side	Appearance	Town
1	A I	011196	36	Carcinoma	2	R	Ulcer	Afikpo
2	A N	52/91	33	Fibroadenoma	4	L	Lump	Enugu
3	I C	584/88	22	Fibrocystic	4	R	Huge	Umuahia
4	I F	040555	37	Carcinoma	12	L	Lump	Enugu
5	N C	06122	49	Carcinoma	8	L	Lump	Owerri
6	N V	416/87	24	Carcinoma	16	R	Lump	Enugu
7	O C	249/87	41	Carcinoma	9	R	Fungating	Enugu
8	O N	1607/87	37	Carcinoma	18	L	Fungating	Enugu
9	O E	9205137	52	Carcinoma	7	L	Lump	Enugu
10	U E	001092	28	Cystosarcoma	20	R	Fishy	Ehime
11	O P	9301116	25	Cystosarcoma	11	R	Mass	Enugu
12	O C	2224/86	42	Carcinoma	4	R	Lump	Enugu

Table 1: Epidemiologic data: Shows the findings concerning this unique tumour from 1986 to 2006.

demonstrated by courtesy of a British journal[14] and a Nigerian journal.[15] Moreover, the present study provides concrete evidence that surgeons trained in a cosmopolitan center can opt to render skilled professional services in remote hospitals.

Finally, an interesting question arises as follows; "Of what use is breast cancer classification?" From the Netherlands, Weigelt`s group[16] sought the refinement of cancer classification along molecular lines. As they summarized, "Taken together, our results represent a step forward towards a taxonomy that not only best reflects the biology of breast cancers, but also paves the way for a refinement in the prognostication of breast cancer patients and the identification of novel tailored therapies." From UK and Australia, Reis-Filho and Lakhan,[17] who classified "Carcinosarcoma" as "Metaplastic Carcinoma," were able to conclude with an eye on the future prospects thus:

Understanding the molecular basis of histological features is a crucial step for unravelling the complexity and heterogeneity of breast cancer and for effectively tailoring the therapies of breast cancer patients. Let us hope that these efforts pave the way for better classification systems and hence more tailored management of patients with cancer.

REFERENCES

1. Abernethy J. Surgical observations containing a classification of tumours. *London: Longman and Reeves.* 1804; 72.

2. Finlay DW. Fibro-myoma of the uterus, becoming sarcomatous, with secondary growths in pleura, heart and kidney. *Trans Pathol Soc London.* 1883; 34: 177-180.

3. Harris M, Persaud V. Carcinosarcoma of the breast. *J Pathol.* 1974; 112: 99-105.

4. Esses KM, Hagmaier RM, Blanchard SA, et al. Carcinosarcoma of the breast: two case reports and review of the literature. *Cases Journal.* 2009; 2: 15. doi: 10.1186/1757-1626-2-15

5. Abbasi MA, Mahmood H, Faheem M, et al. Carcinosarcoma of the breast. *J Coll Phy Surg Pakistan.* 2012; 22: 333-334.

6. Basden GT. Niger Ibos. *London: Frank Cass & Co Ltd.* 1966; 417.

7. Macartney JC, Rollason TP, Codling BW. Use of a histopathology data pool for epidemiological analysis. *J Clin Pathol.* 1980; 33: 351-359.

8. Onuigbo WIB. Breast fibroadenoma in teenage females. *Turkish Journal of Pediatrics*. 2003; 45: 326-328.

9. Rosai J. Ackermam's Surgical Pathology. (2(8) Ed). *St Louis: Mosby.* 1996; 1423.

10. Chang J, Sharpe JC, A'Hern RP, Fisher C, et al. Carcinosarcoma of the ovary: Incidence, prognosis, treatment and survival of patients. *Ann Oncol.* 1995; 6: 755-758.

11. Chang JS, Chua C-C, Lee C-C. Borderline mucinous cystic ovarian tumor with mural nodules (carcinosarcoma). *Ann Saudi Med.* 2012; 32: 415-420.

12. Belbaraka R, Taleb A, Errihani H. A rare tumour of the ovary: Carcinosarcoma. *J Med Cases.* 2010; 1(2): 55-57.

13. Lilleyman J. From the President. *Bull Roy Coll Pathol.* 2002; 117: 3.

14. Onuigbo WIB, Mbanaso AU. Urban histopathology service for a remote Nigerian hospital. *Bull Roy CollPathol.* 2005; 132: 32-34.

15. Onuigbo WIB, Anyaeze CM. Biopsy service for surgical practice in mission hospital. *Owerri Med J.* 2009; 3: 7-10.

16. Weigelt B, Horlings HM, Kreike B, et al. Refinement of breast cancer classification by molecular characterization of histological special types. *J Pathol.* 2008; 216: 141-150. doi: 10.1002/path.2407

17. Reis-Filbo JS, Lakhani SR. Breast cancer special types, why bother? *J Pathol.* 2008; 216: 394-398. doi: 10.1002/path.2419

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Case Report

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A Rare Presentation of a Patient with Limb-Shaking TIA due to Severe Carotid Artery Stenosis

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ABSTRACT

Background: A limb-shaking Transient Ischemic Attack (TIA) is a rare neurological symptom of hemodynamic origin caused by severe carotid artery disease. Physicians should be aware of its presence because it has a non-typical presentation and is cured by endarterectomy or stenting of the stenosed carotid artery. The aim of the present study was to describe a rare case of limb-shaking TIA.
Description of case: A 76 year-old man with a 80% right internal carotid artery stenosis presented with numerous episodes of involuntary jerky movements of his left arm and leg, associated with episodes of brief weakness of the left leg, caused by preoperative optimization of his hypertension. No stroke ensued despite the daily appearance of symptoms for several months. Limb-shaking TIA was diagnosed and all symptoms disappeared immediately after an uncomplicated carotid endarterectomy was performed.
Conclusion: The clinical presentation of this limb-shaking TIA case, the first to our knowledge to appear during treatment of hypertension, supports further the hemodynamic theory of limb-shaking TIAs.

KEYWORDS: Brain TIA; Arterial disease, carotid; Carotid endarterectomy.

INTRODUCTION

Limb-Shaking Transient Ischemic Attack (LS-TIA) is a rare form of a TIA characterized by involuntary limb movements or "shaking", caused by severe stenosis or occlusion of the carotid artery ipsilateral to the appropriate brain hemisphere. LS-TIAs were first described by C. Miller Fisher of Massachusetts General Hospital some 50 years ago.[1] Contrary to the vast majority of TIAs, hemodynamic mechanisms are thought to be involved in the pathogenesis of LS-TIAs.[2-4]

Because LS-TIA may mimic focal motor seizures, clinicians should be aware of its existence and make the correct diagnosis.[2] Timely carotid endarterectomy can not only cure LS-TIA, but also prevent carotid occlusion and/or stroke. Herein we present a rare case of LS-TIA caused by preoperative management of hypertension leading to deterioration of brain hypoperfusion.

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DESCRIPTION OF CASE

A 76 year-old male patient with a 80% asymptomatic right carotid stenosis and a negative stress echo was on the waiting list for endarterectomy pending control of his otherwise poorly controlled long-standing hypertension (systolic blood pressure values around 170 mmHg). The initial diagnosis was made by Duplex ultrasound and confirmed by Computed Tomography (CT) angiography. The latter showed a mixed plaque (type II) at the right carotid bulb obstructing the lumen by 80%, according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) methodology (Figure 1a). The contralateral carotid had a 30% stenosis, while the aortic arch and supra-aortic arteries were all patent. There was no evidence of anatomic variations of the circle of Willis (Figure 1b). Both vertebral arteries were patent with antegrade flow on Duplex. The patient was referred back to his primary care physician for optimization of his blood pressure before carotic endarterectomy was performed, while he was instructed to immediately report symptoms of amaurosis fugax, TIA or stroke. Although such symptoms did not occur, he started getting involuntary jerky movements five months after he was originally seen. He failed to report these symptoms for five months up to the point he was called in order to re-examine his situation and schedule an endarterectomy. These movements affected his left arm and leg, occurred several times every day each one lasting for a few seconds, and were associated with episodes of weakness of his left leg, short in duration; a positioning effect was not reported. Based on the clinical characteristics of his symptoms and the history of severe carotid stenosis, the diagnosis of LS-TIA was made.

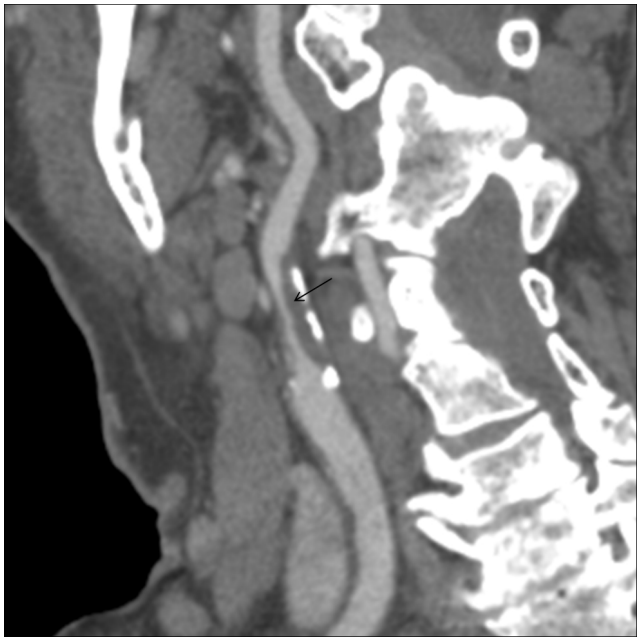

Figure 1a: Sagittal MPR image of the right carotid, shows mixed atherosclerotic plaque (type II) at the level of carotid bulb (black arrow).

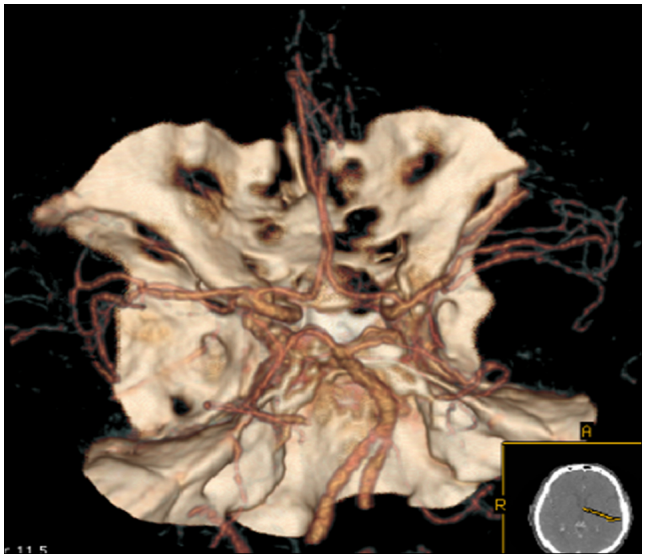

Figure 1b: VRT (Volume Rendering Technique) reconstruction of the Circle of Willis (CoW), shows no anatomic variations of CoW.

Past medical history included hypertension, diabetes mellitus, hyperlipidemia and excision of bladder papillomas. Medications taken were clopidogrel 75 mg OD, losartan/hydrochlorothiazide 50 mg/12.5 mg BID, lasipidin 4 mg BID, atenolol/chlorothalidone 50 mg/12.5 mg OD, glimepiride 4 mg OD, metformin 850 mg BID, simvastatin 40 mg OD and alfuzosin 10 mg OD.

Upon admission to the hospital he was normotensive (with the exception of one abnormal blood pressure reading), a right carotid bruit was noted and he had a normal neurological examination. CT scanning excluded brain infarction or a tumor and a repeat duplex scanning excluded interval carotid occlusion not amenable to endarterectomy. He underwent carotid endarterectomy under general anesthesia with cerebral oximetry monitoring; hypoperfusion during carotid clamping was not detected. Immediately after the operation all neurological symptoms resolved completely. Postoperative course was uneventful and he was discharged home on the second postoperative day on an enhanced antihypertensive regimen (losartan tb 50 mg OD in the afternoon); three months later he had a twenty minute Electroencephalogram (EEG) that was normal with no signs of any epileptiform activity. Our patient remains asymptomatic 16 months postoperatively, with no evidence of restenosis on carotid Duplex and his hypertension being fully controlled.

DISCUSSION

This is the first case of a LS-TIA caused by management of hypertension in a patient with a tight carotid stenosis, leading to hypoperfusion of a critical watershed brain territory, and to the best of our knowledge the first LS-TIA to be managed with endarterectomy in Greece.

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Our patient reported short in duration episodes of left leg weakness, in addition to the involuntary limb movements. Frequently other typical TIA manifestations co-exist in patients with LS-TIA,[5] these can be missed if careful history is not taken. LS-TIA may mimic a focal motor seizure, and thus should be meticulously differentiated. Neurological deficits in TIAs are maximal at onset whereas symptoms in a focal seizure tend to evolve over seconds. The lack of a Jacksonian march,[2] together with the sparing of the facial muscles characterizes the LS-TIA. The presence of other vascular paroxysmal dyskinesias, such as ataxia, myoclonic jerks, dystonic limb posturing and parkinsonism, may aid the clinical diagnosis of LS-TIA, which is further supported by a normal EEG, which rules out epileptic seizures.

It is interesting that despite the repetitive long-standing TIAs, our patient did not develop stroke, perhaps because of the transient hemodynamic nature of LS-TIA,[2] whereas the classical TIAs are caused by embolism from an unstable carotid lesion, not present in this patient. The hemodynamic mechanism of LS-TIAs is further supported not only by the observation that in our patient all symptoms started after the attempts to manage his poorly-controlled hypertension, but also from other reports where symptoms were elicited by orthostatic hypotension,[6,7] postprandial hypotension,[8] hypertension control in association with complete carotid occlusion successfully managed with antihypertensive dose reduction,[9] external compression,[10] or balloon occlusion,[11] of the carotid artery. During evolvement of symptoms in a patient in one study, a dramatic decrease of flow velocities in the left middle cerebral artery was observed on transcranial Doppler, a finding that further support the hemodynamic theory.[12]

Carotid endarterectomy led to immediate disappearance of limb shaking of our patient, similarly to a previous report on six patients.[5] No complications occurred, including the hyperperfusion syndrome and hemorrhage into the revascularized brain territory, which might be seen more often in patients with LS-TIAs because of loss of cerebrovascular vasomotor reactivity due to the high grade carotid stenosis. Although suboptimal hypertension control may prevent LS-TIA symptoms, on the other hand it may increase the frequency of the hyperperfusion syndrome of the brain.[13] Carotid revascularization (endarterectomy or stenting) is considered the treatment of choice for patients with LS-TIA due to severe stenosis because of better patient prognosis compared to medical treatment.[14] Indeed our patient remains asymptomatic more than a year after his carotid endarterectomy was performed. On the other hand LS-TIA in patients with complete carotid artery occlusion can respond to blood pressure optimization.[2,15]

In conclusion, a rare case of LS-TIA is described. Clinicians should be aware of its existence to make the correct diagnosis, while its clinical presentation-association with attempts to manage hypertension-supports further the hemodynamic nature of this entity.

CONFLICTS OF INTEREST: None

REFERENCES

1. Fisher CM. Concerning recurrent transient cerebral ischemic attacks. *Can Med Assoc J*. 1962; 86: 1091-1099.

2. Ali S, Khan MA, Khealani B. Limb-shaking Transient Ischemic Attacks: case report and review of literature. *BMC Neurol*. 2006; 6: 5. doi: 10.1186/1471-2377-6-5

3. Tatemichi TK, Young WL, Prohovnik I, Gitelman DR, Correll JW, Mohr JP. Perfusion insufficiency in limb-shaking transient ischemic attacks. *Stroke*. 1990; 21(2): 341-347. doi: 10.1161/01.STR.21.2.341

4. Persoon S, Kappelle LJ, Klijn CJ. Limb-shaking transient ischaemic attacks in patients with internal carotid artery occlusion: a case-control study. *Brain*. 2010; 133(Pt 3): 915-922. doi: 10.1093/brain/awq009

5. Baquis GD, Pessin MS, Scott RM. Limb shaking--a carotid TIA. *Stroke*. 1985; 16: 444-448. doi: 10.1161/01.STR.16.3.444

6. Zaidat OO, Werz MA, Landis DM, Selman W. Orthostatic limb shaking from carotid hypoperfusion. *Neurology*. 1999; 53(3): 650-651. doi: 10.1212/WNL.53.3.650

7. Adatia SP, Chauhan *VS*, Hastak SM. First do no harm - a case of limb shaking TIA. *Neurol India*. 2009; 57(6): 807-808. doi: 10.4103/0028-3886.59486

8. Cheshire WP, Jr., Meschia JF. Postprandial limb-shaking: an unusual presentation of transient cerebral ischemia. *Clin Auton Res*. 2006; 16(3): 243-246. doi: 10.1007/s10286-006-0344-5

9. Leira EC, Ajax T, Adams HP, Jr. Limb-shaking carotid transient ischemic attacks successfully treated with modification of the antihypertensive regimen. *Arch Neurol*. 1997; 54(7): 904-905. doi: 10.1001/archneur.1997.00550190090019

10. Braakman HM, Knippenberg SA, de Bondt BJ, Lodder J. An unusual cause of transient neurologic deficits: compression of the carotid artery by a thyroid cystic nodule. *J Stroke Cerebrovasc Dis*. 2010; 19(1): 73-74. doi: 10.1016/j.jstrokecerebrovasdis.2009.02.007

11. Swinnen B, Schreurs A, Heye S, Lemmens R. Limb-shaking TIA during balloon test occlusion of the internal carotid artery. *Acta Neurol Belg*. 2014. doi: 10.1007/s13760-014-0366-z

12. Nedelmann M, Kolbe M, Angermueller D, Franzen W, Gizewski ER. Cerebral hemodynamic failure presenting as limb-

shaking transient ischemic attacks. *Case Rep Neurol.* 2011; 3: 97-102. doi: 10.1159/000327683

13. Switzer JA, Nichols FT. Are limb-shaking transient ischemic attacks a risk factor for postendarterectomy hemorrhage? Case report and literature review. *J Neuroimaging*. 2008; 18(1): 96-100. doi: 10.1111/j.1552-6569.2007.00172.x

14. Ma QF, Huang Q, Zhang Q, Fan CQ, Guo XH, Wu J. Association between clinical features and prognosis of patients with limb-shaking transient ischemic attack. *Chin Med J (Engl)*. 2013; 126(22): 4354-4357.

15. Das A, Baheti NN. Limb-shaking transient ischemic attack. *J Neurosci Rural Pract*. 2013; 4(1): 55-56. doi: 10.4103/0976-3147.105615