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Original Article

Quality of life, clinical outcomes and cost utilization of endoscopic therapy in patients with Barrett's esophagus and early esophageal cancer—an 8-year Canadian experience

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Abstract

Background and Aims: Endoscopic treatment is a definitive and cost-effective management strategy for early neoplasia in Barrett's oesophagus (BE). However, little is known of its impact on quality of life (QoL). This study reports outcomes of endoscopic eradication treatment (EET), focusing on QoL and costs in a Canadian tertiary referral centre.

Methods: A retrospective cohort study using a prospectively maintained clinical database captured validated QoL metrics during and at the end of EET, risk factors for BE, treatment response, complications, costs, and follow-up response of all treated Barrett patients in Calgary and Southern Alberta, Canada.

Results: A total of 147 BE patients were treated from 2013 to 2021. All patients showed significant improvement in almost all QoL parameters except depression. There was significant improvement in 7 of the 8 QoL metrics in those who achieved complete eradication of intestinal metaplasia (CEIM). EET was successful in achieving complete eradication of dysplasia (CED) and CEIM in 93.4% and 74.3% of patients, respectively, with a median of 3 radio frequency ablation treatments. Longer circumferential segments of BE (Cx) predicted a lower likelihood of achieving CEIM. The average total cost to achieve CED and CEIM were $10 414.58 and $9347.93CAD, respectively (compared to oesophagectomy estimated at $58 332.30 CAD).

Conclusion: This Canadian cohort reports significant post-treatment improvement in QoL parameters in patients treated to CEIM or CED over an 8-year period. EET for BE eradication is cost-effective compared to oesophagectomy. There was a low rate of complications and recurrence post-CEIM.

Key words: complete eradication of dysplasia; complete elimination of metaplasia; endoscopic mucosal resection; endoscopic eradication therapy

Introduction

Barrett's oesophagus (BE) is a premalignant condition affecting 2%–6% of Canadians.[1] The diagnosis of BE is established when more than 1 cm segment of the distal oesophageal squamous epithelium transforms into metaplastic columnar intestinal epithelium.[2] BE can progress to a dysplastic state,[3] which increases the risk of oesophageal adenocarcinoma (EAC).[4] Progression from BE to EAC is influenced by cellular, environmental, inflammatory and genetic factors,[5] among others. Oesophagectomy was previously the definitive management, however, with advances in technology and techniques, endoscopic eradication treatments (EET) have become the first-line strategy for low-grade dysplasia (LGD), high-grade dysplasia (HGD) and T1a EAC.[6,7] EET for BE[8,9] involves 2 components: endoscopic resection (ER) [endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD)]; and ablation (radio frequency ablation (RFA), argon plasma coagulation (APC), or cryoablation). The diagnosis of BE is associated with negative impacts on quality of life (QoL).[10–12] However, it is not clear how EET and associated follow-up impacts QoL. We sought to understand the impact of EET for dysplastic BE on QoL among patients in Canada. We also assessed the cost of EET relative to oesophagectomy in the Canadian context, a comparison not previously reported in the literature.[2]

Patients and methods

Population

This study was approved by the University of Calgary Institutional Ethics Review Board. Patients with BE undergoing EET from July 2013 to March 2021 were identified within the 'Barrett's oesophageal and Early Oesophageal Cancer Endotherapy Program of Southern Alberta' prospective

database. This program centrally manages BE patients within Calgary, Southern Alberta, and interior British Columbia, for a catchment area of over 1.4 million people. All patients who underwent RFA, APC, EMR, and/or subsequent oesophagectomy in Calgary during this time were included in the study and represent a population-based cohort. The database includes BE risk factors, type of endoscopic treatment, type of device used (for RFA only), complications, emergency room visits following EET, and post-treatment follow-up. Chart reviews captured risk factors for BE, comorbidities, medications, post-treatment complications, emergency visits, and hospitalizations. Patients completed QoL questionnaires on the day of their procedure before sedation. All patients in the database consented to research; patients who withdrew their consent were removed from the database.

Endoscopic treatment protocol

Patients with histologically confirmed dysplastic BE, non-dysplastic BE, or EAC were treated according to ACG[2,6] and Alberta Health Services guidelines.[13] All patients had an initial diagnostic endoscopy (EGD) with a gastroenterologist or surgical endoscopist to document the BE length based on Prague criteria delineating the circumferential (Cx) and maximal extent (Mx) of Barrett's epithelium.[14] Biopsies of original segment and GEJ/gastric cardia were obtained in the initial diagnostic endoscopy per Seattle Protocol[2,15] (4 quadrant biopsies every 1 cm) and sent to an expert GI pathologist. In rural areas, pathology slides were re-read by Calgary GI pathologists to confirm dysplasia. All dysplastic BE and T1a EAC with nodular lesions underwent EMR or ESD followed by RFA to strive for complete eradication. Patients with non-dysplastic BE and high-risk features (diagnosis at age <40, family history of Barrett's related oesophageal cancer, and segment length >6 cm) were considered for EET after consultation with local BE experts (PB, MG). The program was briefly suspended in 2020 due to the COVID-19 pandemic, during which treatment times were delayed up to 6 months. In 2021, the original schedule was resumed. EET for NDBE was performed every 6–12 months due to lower risk and limited availability of anaesthesia/endoscopy time. Post-EMR flat BE without visible endoscopic abnormalities was treated with RFA until biopsy-confirmed complete elimination of intestinal metaplasia (CEIM).

Definitions for treatment outcomes and complications

Complete eradication of dysplasia (CED) was defined as a histologic assessment showing no evidence of dysplasia on 2 consecutive EGDs. CEIM was defined as EGD and histology showed no evidence of IM on 2 consecutive EGDs. After CEIM, patients underwent endoscopic surveillance to monitor for BE recurrence following ACG guidelines.[2] An oesophageal stricture in this study was defined as a noticeable narrowing in the oesophageal lumen that required dilation to alleviate patient-reported symptoms of dysphagia. All histological sampling to assess for CED and CEIM was carried out using Seattle protocol for Barrett's surveillance and management. This protocol requires biopsy samples from every 1–2 cm in 4 quadrants of the suspected Barrett's tissue. GEJ/Cardia was also sampled with 4–6 biopsy specimens.

Quality of life (QoL) and chart review

All patients recruited for this study completed a validated BE QoL analog scale[16] questionnaire pre- and post-EET (Supplementary Figure S1). This questionnaire was administered prior to their EET treatment at every visit. It is a combination of 2 yes/no questions and 8 visual analogue scale questions. A higher score reflects a higher health burden from BE and a lower QoL. Lower scores indicated improved QoL.

Evaluation of direct and indirect costs of CED and CEIM

Patients were stratified by stage of dysplasia at baseline, CED, and CEIM. The average number of RFA and EMR performed were calculated to obtain average rates of CED and CEIM, stratified by dysplasia status. Direct costs considered included device costs, physician fees (gastroenterology/anesthesia), and nursing time in a single-payer, public-funded health system. Physician costs, local costs for equipment, and non-physician staffing costs for endoscopic intervention were obtained from the Alberta Schedule of Medical Benefits.[17] Indirect costs[18] were approximated at 10 hours lost work for patients per day using the median wage of $26.72 CDN/hour in Alberta (8 hours for patients, 2 hours for caregivers for transportation). Costs are reported in 2022 Canadian dollars. Due to a limited number of treated patients, ESD patients were not included in the study population.

Statistics

Categorical variables are reported as counts and percentages and continuous variables as medians with 25th and 75th percentiles. For comparative analyses of parametric and non-parametric values, categorical values were carried out using chi-square test or Fisher's exact test for categorical values, respectively, and Students *t*-test or Mann–Whitney's *U* test for continuous variables, respectively. A stepwise logistic regression assessed predictors of CEIM and a linear regression assessed predictors of improvement in QoL. Patients with missing data for the corresponding variable were not included in the analysis. All statistical analyses were carried out using SPSS version 25.0 for Windows (Armonk, NY).

Results

A total of 147 patients undergoing EET were consented to participate in this study during 2013–2021 (Figure 1). Three patients withdrew their consent during the study period and were removed from analysis (originally 150 patients). The average age of patients in this cohort was 65.1 ± 12 years, and 87.1% of the population were male. Table 1 describes patient characteristics and comorbidities. Table 2 highlights risk factors stratified by dysplasia status.

Treatment outcomes

The treatment cohort was divided based on their diagnosis into dysplastic and non-dysplastic BE (Table 3). Treatments included a combination of EMR, RFA, and APC. There were no cryotherapy or ESD cases. Dysplastic patients were more likely to have dyslipidemia, hypertension, and smoking history (Table 2; $P < .05$). For RFA treatments, data was based on the type of catheter used (circumferential and focal). Three patients had an oesophagectomy due to

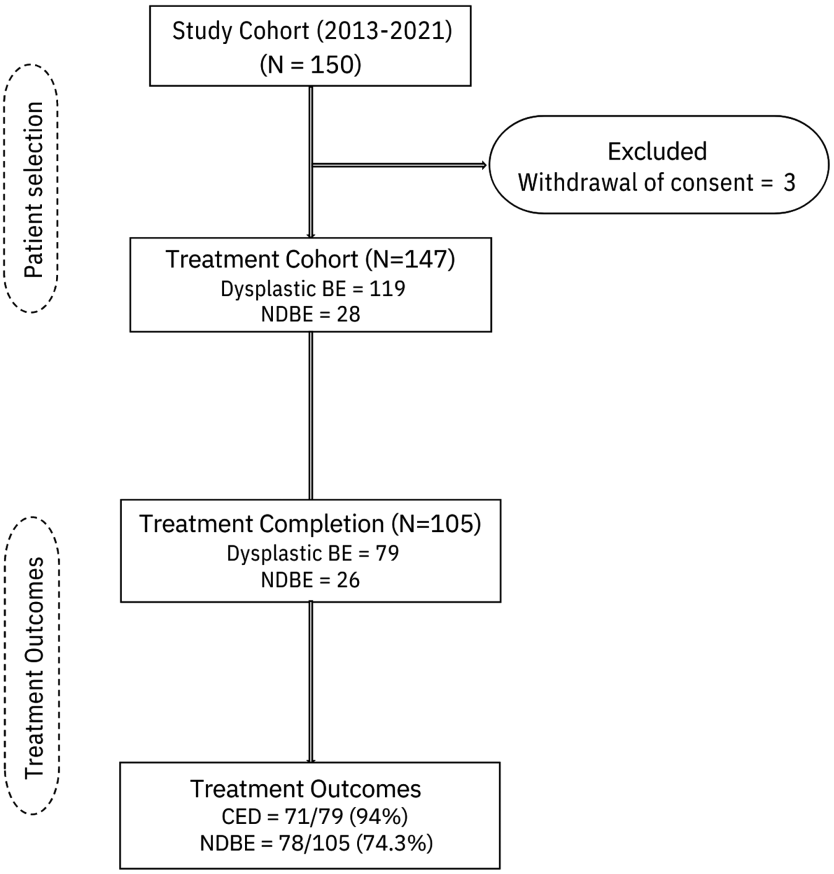

Figure 1. Patient selection, treatment, and outcomes flowchart.

lympho-vascular invasion, or advanced EAC. Three patients failed to follow-up after CED, and 3 died during the study from unrelated causes (non-oesophageal cancer and cardiac complications).

CED and CEIM rates were calculated for those who completed treatment (n = 105; Figure 1; Table 3). Circumferential (Cx) length of BE (OR = 0.48, 95% CI: 0.24–0.98), male sex (OR = 0.05 (95% CI: 0.01–0.45), and waist circumference (OR = 0.93, 95% CI: 0.88–0.99) were independent predictors of CEIM. The results from the stepwise logistic regression analysis are shown in Table 4 and Supplementary Table S4 shows all the inclusion and exclusion criteria for variables that are relevant to BE progression. Sixty patients (41%) did not achieve CEIM after an average treatment period of 31 ± 7 months. These patients continue to be followed and additional details about this population are in the appendix.

Quality of life

A pairwise comparison of patient-reported outcomes pre- and post-EET showed a significant reduction in all QoL measures, except for depression (Table 5). QoL outcomes were measured at each treatment visit including CED and CEIM. Of the 147 patients, 8 (5 %) did not have pre-treatment QoL measurement recorded and were removed from analysis. There was significant improvement in sleep and overall QoL between patients who achieved CEIM and those who did not (Supplementary Table 2). Multiple linear regression showed improvement in QoL with every unit increase in age and fewer number of EMR (Beta = 0.24, P = 0.02, 95% CI: 0.67–1.25).

Complications

Treatment for oesophageal strictures was completed in 21 of 147 patients (14.3 %). Logistic regression analysis showed a positive association with EMR and incidence of strictures, but this was not statistically significant (Beta = P = .08, 95% CI: 0.09–0.89) (Supplementary Table S3 has all variables and results). Eleven patients with CEIM and CED required oesophageal dilation (1 dilation per stricture). Four patients (5.1%), with an average BE length of C10M11, required 7 dilatations to treat strictures.

Post-eradication follow-up and recurrence rates

CEIM was achieved in 78 patients, and post-eradication surveillance EGDs were completed at our centre. The median post-eradication follow-up was 24 months (IQR 17–42). The median number of post-eradication EGDs were 2 for NDBE (IQR 2–3), 3 (IQR 2–4) for LGD, 4 (IQR 3–6) for HGD, and 4 (IQR 4–6) for IMCa patients. Seven patients (9%) were lost to follow-up after an average of 4 months. During the post-eradication follow-up, 2 patients (2.6%) had a recurrence of BE at 24 and 33 months. In the first case, the patient had T1a EAC at baseline and LGD recurrence at 24 months. In the second case, the patient initially had HGD, and recurrence of NDBE at 33 months. Both patients were re-treated with RFA and achieved CEIM within 6 months.

Table 1. Baseline patient characteristics.

Baseline characteristics (no. of missing values)		*N* (%)
Age (±SD)		65.1 (±12)
Sex		129 male (87.1%)
Waist-hip ratio (IQR)		1.0 (0.98–1.05)
Comorbidities	Dyslipidemia (12)	51 (34.4%)
	Hypertension (2)	67 (45.3%)
	Obstructive sleep apnoea (1)	19 (12.8%)
	Type 2 diabetes (0)	18 (12.1%)
	History of smoking in NDBE† (0)	9 (32.1%)
	History of smoking in dysplastic BE (1)	74 (61.6%)
	Proton pump inhibitor use (1)	138 (93.2%)
	Non-steroidal anti-inflammatory (0)	63 (42.6%)
	Statin (1)	47 (31.8%)
Diagnosis	Non-dysplastic BE (NDBE)	28 (18.9 %)
	Low-grade dysplasia (LGD)	38 (25.7 %)
	High-grade dysplasia (HGD)	53 (35.8%)
	Intramucosal carcinoma (IMCa)	28 (19.0%)
Barrett's measurements	Length of Cx‡ (IQR¶)	4 (1–8)
	Length of Mx§ (IQR¶)	6 (3–10)
Therapy	Number of RFA¥ (IQR¶)	3 (2–4)
	Number of EMR£ (IQR¶)	0 (0–1)

†Non-dysplastic Barrett's oesophagus, ‡circumferential BE length, §Maximal BE length, ¶Interquartile range, ¥Radiofrequency ablation, £Endoscopic mucosal resection.

Evaluation of direct and indirect costs of CED and CEIM

Table 6 provides a breakdown of the average costs for endoscopic procedures and equipment for Alberta in 2018/2019. The average oesophagectomy cost at our centre in 2018/2019 was $58 332.30 CAD. CED was obtained in 96 patients requiring an average of 3 RFA procedures and <1 EMR. The average cost per patient to obtain CED was $10 263.70 with direct costs being $9109.08. More advanced dysplasia had higher costs to obtain CED, and cost breakdown by dysplasia state is listed in Table 7. For patients who obtained CEIM (*n* =7 8), a median RFA of 3 (IQR2-4) treatments and 0 (IQR 0-1) EMRs were performed. The average cost to achieve CEIM per patient was $9374.93 (direct cost $8485.33) with the highest cost for obtaining CEIM seen in non-dysplastic BE (Table 8).

Discussion

This study reports QoL impact, treatment outcomes, and costs to achieve CED and CEIM for BE in an expert centre in Canada from 2013 to 2021. This study is the first to our knowledge to look at the impact of EET on QoL in BE patients over multiple points of contact.

QoL in BE patients is reduced by the burden of the disease, its treatment, and fear of progression to cancer.[19] These patients have a higher proportion of stress, anxiety, and depression.[20] We found improvement in QoL in all patients who received EET, regardless of achieving CED or CEIM. The negative effect of BE on sleep and daily QoL has also been observed in other studies.[20,21] The population was followed

Table 2. Distribution of risk factors amongst BE types.

Comorbidities (no. of missing values)	NDBE†	Dysplastic‡ BE	*P*-value
Dyslipidemia (12)	5 (17.9%)	46 (38.7%)	0.04
Hypertension (2)	7 (25.0%)	59 (49.6%)	0.02
Obstructive sleep apnoea (1)	3 (10.7%)	9 (7.6%)	0.6
Type 2 diabetes (0)	1 (3.6%)	17 (14.3%)	0.1
History of smoking (1)	9 (32.1%)	73 (61.3%)	0.01
Statin (1)	5 (17.9%)	42 (35.3%)	0.07
PPI§ (1)	27 (96.4%)	110 (92.4%)	0.5
Circumferential (Cx) length of BE	8 (6-10)	3 (1-6)	<0.01
Maximal (Mx) length of BE	10 (7.5-11)	5 (3-9)	<0.01
Number of RFA¶	4 (3-5)	3 (2-4)	0.03

†Non-dysplastic Barrett's oesophagus, ‡dysplastic BE inclusive of LGD, HGD and oesophageal adenocarcinoma, §Proton pump inhibitors, ¶radiofrequency ablation.

Table 3: Treatment outcomes based on BE types.

BE type	No. of patients	Avg BE length	Percentage of patients requiring EMR	Average number of months treated	Treatment outcomes at the end of the study period		
					No. of patients in the analysis	CED (no. of patients)	CEIM (no. of patients)
High-risk NDBE	28	C8M10	3.6%	30 ± 10 months	26	–	53.8% (14)
LGD	38	C4M5	13.2%	23 ± 12 months	25	92% (25)	68.5% (20)
HGD	53	C3M5	45.3%	23 ± 12 months	35	94% (35)	85.7% (30)
IMCA	28	C2M4	78.6%	24 ± 13 months	19	95% (18)	69.8% (14)
Total	147	–	–	–	105	71/79 = 93.6%	78/105 = 74.3%

Table 4. Logistic regression of predictors of response to endoscopic eradication therapy (EET).

Predictors	β	S. E	Wald	*P* value	Odds ratio (95% CI)
Circumferential BE (Cx)	–0.73	0.36	4.08	0.04	0.48 (0.24–0.98)
Sex (Male)	–2.99	1.10	7.21	0.01	0.05 (0.01–0.45)
Waist circumference	–0.07	0.28	6.33	0.01	0.93 (0.88–0.99)

Model statistics $X^2(25) = 49.26$, $P < .01$.

Table 5. Quality of life outcome analysis (139 records included in this analysis). Higher QoL scores/percentages reflected poor QoL. Score reduction reflected improvement in QoL. Bolded values are statistically signifcant (p<0.05).

QoL† measure	Pre-treatment	Post-treatment	*P* value
Oesophagectomy worry (yes/no)	27%	16%	**0.01**
Adenocarcinoma worry (yes/no)	67%	64%	0.782
Disease worry	2.75	0.89	**>0.001**
Depression	1.46	1.27	0.332
Daily QoL	1.77	1.23	**0.002**
Stress	1.96	1.41	**0.001**
Satisfaction	4.39	3.4	**0.005**
Sleep difficulty	1.81	1.12	**0.002**
Negative impact on life	1.75	1.01	**>0.001**
Fear of death	2.39	1.63	**>0.001**

†Quality of life.

Table 6. Cost of endoscopic procedures.

Item	Cost [CDN$]	Source
Physician cost for consult	189.99	SOMB
Physician cost EGD†	113.99	SOMB
Physician cost dilatation	59.99	SOMB
Physician cost RFA‡	113.99	SOMB
Physician cost for anesthesia	263.84	SOMB
RFA probe cost	1800	Local data
Banding device cost	327.50	Local data
Balloon dilator cost	140	Local data
Indirect costs (8 hours for patient, 2 hours for caregiver at $26.72/hour	267.20	–
Physician cost dilatation	89.22	AHW Guide

†Oesophagogastroduodenoscopy, ‡radio frequency ablation.
Abbreviation: AHW = Alberta Health Wellness Guide; SOMB = Schedule of Medical Benefits (Alberta).

for over 8 years, representing one of the largest and longest QoL studies published in BE.[22,23] This study used a BE-specific QoL scale to reflect patient symptoms and responses, providing more nuanced results. Our findings suggest that BE patients should be counselled to expect initial worsening of sleep after starting EET, with subsequent improvement as treatment efficacy is achieved. Our results suggest mental health is not worsened by treatment. The study did not collect QOL data for untreated BE population, and thus could not provide a comparison with the treated group.

EET was effective in achieving CED and CEIM. Our study maintained a stringent definition for CED/CEIM to reduce sampling error. Traditionally, publications with higher rates of eradication do not include the GEJ and were not stringent about 2 negative EGDs.[24,25] This study reports CEIM of 74.3%, which is in keeping with other studies reporting rates of 61.5% to 98%.[26–29] CEIM was higher (81%) in the dysplastic group compared to high-risk NDBE (57.8%). Studies have reported longer BE segments as predictive of poorer CEIM.[24,30–32] Our study had a median length of C8M9 for high-risk NDBE compared to C4M5 for dysplastic group. Offering high-risk NDBE patients EET is in line with 2016 ASGE recommendations,[2] and our CEIM rates were likely affected by their inclusion.

Sustained eradication of IM was seen in 97.4% of CEIM patients and the recurrence rate was <1% during an average 24-month follow-up. This is similar to other studies.[23,31,33–35] The most observed complication post-treatment was strictures (14.3%), which is similar to a large BE treatment outcomes study,[36] but higher compared to some publications.[29,37] Lower rates of complications published often restrict BE length and extent of EMRs performed.[35] Our study did not have such limitations and reflects real-world experience.

Table 7. Costs for patients obtaining complete elimination of dysplasia (CED). All values are in CAD (Canadian dollars).

	# EMR† pre	#RFA‡	#EMR† post	EMR† total	#Dilations	Total RFA‡ cost	Total EMR† cost	Cost complications	Grand total
CED§									
Average	0.58	3.58	0.33	3.90	0.30	$9321.58	$895.59	$197.41	$10 414.58
Total	48	297	27	324.00	25	$773 690.94	$74 334.00	$16 385.25	$864 410.19
Number	83								
LGD¶									
Average	0.24	3.62	0.10	0.34	0.24	$9431.97	$341.77	$158.20	$9931.94
Total	7	105	3	10.00	7	$273,27.10	$9911.20	$4587.87	$288 026.17
Number	29								
HGD¥									
Average	0.65	3.55	0.125	0.78	0.2	$9247.82	$768.12	$131.08	$10 147.02
Total	26	142	5	31.00	8	$369 912.84	$30 724.72	$5243.28	$405 880.84
Number	40								
IMCA£									
Average	1.07	3.57	1.36	2.43	0.71	$9303.64	$2407.01	$468.15	$12 178.80
Total	15	50	19	34.00	10	$130 251.00	$33 698.08	$6554.10	$170 503.18
Number	14								

†Endoscopic mucosal resection, ‡radiofrequency ablation, §complete eradication of dysplasia, ¶low grade dysplasia, ¥high grade dysplasia, £intramucosal cancer.

Table 8. Costs for patients obtaining complete elimination of intestinal metaplasia (CEIM). All values are in CAD (Canadian dollars).

	# EMR† pre	#RFA‡	#EMR† post	#Dilations	Total RFA‡ cost	Total EMR† cost	Cost of strictures	Grand total
CEIM§								
Average	0.54	3.32	0.15	0.09	$8650.00	$686.16	$66.49	$9374.93
Total	42	259	12	7	$674 700.18	$53 520.48	$4587.87	$737 396.40
Number	78							
IM¶								
Average	0.07	4.14	0.00	0.29	$10 792.23	$70.79	$187.26	$11 237.54
Total	1	58	0	4	$151 091.16	$991.12	$2621.64	$157 325.56
Number	14							
LGD¥								
Average	0.10	3.10	0.05	0.00	$8075.56	$148.67	$0.00	$8224.23
Total	2	62	1	0	$161 511.24	$2973.36	$0.00	$164 484.60
Number	20							
HGD£								
Average	0.63	3.10	0.03	0.00	$8075.56	$660.75	$0.00	$8736.31
Total	19	93	1	0	$242 266.86	$19 822.40	$0.00	$262 089.26
Number	30							
IMCA€								
Average	1.43	3.29	0.71	0.21	$8559.35	$2123.83	$140.45	$10 964.07
Total	20	46	10	3	$119 830.92	$29 733.60	$1966.23	$153 496.98
Number	14							

†Endoscopic mucosal resection, ‡radiofrequency ablation, §complete eradication of intestinal metaplasia, ¶intestinal metaplasia, ¥low grade dysplasia, £high grade dysplasia, €intramucosal cancer.

This study is one of the few Canadian reports on the efficacy and cost of EET (RFA and EMR) outcomes in BE. This cost comparison is relevant for BE management in community centre's, where endoscopic management is not as available as surgical interventions. Similar studies carried out in United States and Europe found EET to be cost-effective in comparison to quality-adjusted life years.[38–40] Our findings are in keeping with other cost analyses, but it is the first time a direct costing comparison has been undertaken between dysplastic and high-risk NDBE in the Canadian context. We postulate the length discrepancy between NDBE and dysplasia is an important contributor. During the 8 years, none of the high-risk NDBE patients developed EAC or dysplasia.

There are limitations to the study. The data for this study were collected prospectively, and there were some missing values for comorbidities and QoL outcomes. To assess

real-world outcomes, there were no pre-specified cut-offs for BE length or treatment duration. However, this led to higher variability in outcomes. Despite these limitations, we attempted to make up for this with rigorous patient follow-up and stringent definitions of CEIM/CED.

In conclusion, EET for high-risk NDBE, dysplastic, and early EAC in our centre is successful at eradicating dysplasia in 93.4% and IM in 74.3% over 8 years. Almost all QoL parameters significantly improved post-EET, with significant improvement in daily QoL and sleep for patients achieving CEIM or CED. Endoscopic treatment to achieve CRD and CEIM was more cost-effective than oesophagectomy.

Supplementary material

Supplementary data are available at *Journal of the Canadian Association of Gastroenterology* online.

Author contributions

Milli Gupta: conceived and supervised the study. Joel David, Mathew Woo, Thurarshen Jeyalingam, Paul J. Belletrutti, Milli Gupta: writing. Milli Gupta, Mathew Woo, Stephen Congly, Christopher N Andrews, Paul J Belletrutti, Thurarshen Jeyalingam, Joel David: data review and management. All authors reviewed the manuscript for intellectual content and credibility.

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Conflicts of interest

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Data availability

The data and analytical methods that support the findings of this article will be shared on reasonable request directly to the corresponding author.

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