Effectiveness of Biofeedback Therapy on Quality of Life in Patients with Dyssynergic Defecation Disorder

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ABSTRACT

Background: *The first option to be considered in the treatment of functional defecation disorder is to correct the dyssynergia. However, limited studies exist to show the effectiveness of biofeedback.*

Objective: *We evaluated the effect of biofeedback on the severity of constipation, quality of life, and anorectal manometry in patients with dyssynergic defecation in which the biofeedback method was applied.*

Methods: *Effectiveness of biofeedback method on the quality of life of 24 dyssynergic defecation patients according to Rome III criteria after clinical and balloon expulsion tests (BETs) and colonic transit time was measured. Data were collected with patient identification form, Bristol Stool Chart, Constipation Quality of Life Scale forms, Visual Analogue Scale, diaphragmatic breathing exercises form, constipation diary, and constipation biofeedback monitoring form. Dyssnergic defecation cases received 6-week biofeedback training. For the same timeframe, the control group had a catheter into the rectum without any intervention.*

Results: *Constipation severity was reduced in both groups before biofeedback to post-biofeedback ($P < .05$). Anal canal pressure, BET, colonic transit time, and quality of life significantly improved in biofeedback patients compared with controls.*

Conclusions: *Biofeedback has a favorable effect on therapy and quality of life in dyssynergic defecation cases.*

Keywords: *biofeedback, defecation, dyssynergic defecation, nursing, quality of life*

INTRODUCTION

Constipation is a rather commonly seen disorder that involves subjective symptoms that are differently interpreted by those suffering from it. The disorder has been reported to affect 2-30% of the European population, with its differing prevalence depending on the definitions applied.[1] Annually, more than two and a half million people visit doctors to address constipation.[2] A study by Bor[3] in Turkey indicated that the rate of constipation among the Turkish population is 8.9%, with the rate in women being 12.1% but only 5.3% in men.

Defecation is a complex process, one that involves voluntary muscles and smooth muscles working in coordination. Diseases that occur as a result of the disruption of this process are called functional defecation disorders (FDDs). These are common functional bowel disorders seen in clinical practice, manifested as straining during defecation, lumpy or hard stools, and infrequent bowel movements in the absence of evident organic or structural diseases.[4] According to the pressure traces observed in anorectal manometry, 3 types of FDB have been identified: Type I, Type II, and Type III.[1] Anorectal biofeedback is the most successful treatment method for FDB, according to Rome III.[5] The main principle in the treatment is to eliminate the underlying pathophysiological causes. Therefore, the first option to be considered in the treatment of FDB is to correct the dyssynergia. Manometry biofeedback requires the insertion of a manometric probe, such as a pressure transducer, perfused catheter, or balloon, into the anal canal to measure anal canal pressure and contraction and relaxation of the pelvic floor. Contraction and relaxation of the anal sphincters and the pelvic floor are then displayed on a computer monitor with training techniques.[6] In health centers that offer biofeedback opportunities, it is recommended that the treatment be started with biofeedback.[7] Although behavioral therapies and the biofeedback method are advised for patients with dyssynergic defecation, standard treatment procedures have not been identified, and sham-controlled studies are lacking.[6] In addition, greater clarity on the effect of this treatment would help determine

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its impact on patients' quality of life. As there are many strategies that fall within the scope of nursing practice, nurses are in an important position to assist in assessing and managing constipation and biofeedback therapies.[8]

The aim of this prospective research is to determine the effect of the biofeedback method on patients with dyssynergic defecation disorder, in terms of constipation quality of life and anorectal manometry, by comparing these patients with a sham-controlled group.

METHODS

Design and Sample

This research was conducted in the outpatient clinic of the Department of Constipation, Incontinence and Biofeedback within the Gastroenterology Division of the School of Medicine at Ege University. Patients who were 18 years or older, at least primary school graduates, and not cognitively impaired and who had a diagnosis of Type I or Type III dyssynergic defecation disorder after clinical and anorectal evaluation were included in the research. Those who were unable to orally communicate, illiterate, or cognitively impaired; who had severe visual impairment, a diagnosis of irritable bowel syndrome, Type II dyssynergic defecation disorder, Hirschsprung's disease, a tumor history that could affect the research, Grade II, III, or IV hemorrhoids, pain and bleeding, anal fissures, or anal fistulas; who belonged to an abdominal operational group/colorectal operational group; who were pregnant; who had spinal cord injuries, muscular diseases, visceral myopathy, or neuropathy; who were on medication that caused constipation; who had uncontrolled diabetes and a similar treatment history in the past; and who declined to participate in the research were excluded from the study.

A total of 58 patients who presented with constipation complaints and were referred for biofeedback therapy by their physician formed the study population. The sample for the study included 24 of these patients, as 34 were excluded for the following reasons: 1 was cognitively impaired, 1 had IBS, 25 had solitary rectal ulcer syndrome, 2 had spinal cord injuries, 2 had an endocrinal disease, 1 was on medication that caused constipation, 1 had uncontrolled diabetes, 1 had muscular disease, and 1 had Type II dyssynergic defecation disorder.

Data Collection Instruments

The Rome III Functional Constipation Criteria and the Functional Defecation Diagnosis Criteria (FDDC) were used to diagnose constipation and FDD.[9] A patient description form developed by the researchers was used to collect the patients' personal information. The form consists of 2 parts: the first part includes questions on the patients' age, gender, and other sociodemographic items, and the second part includes questions related to constipation, such as whether or not the respondent experiences urgency before defecation, more-than-normal straining, or the feeling of incomplete emptying of stool. The second part was taken from the questionnaire developed by Bor et al. (2006). The Bristol Stool Chart (BSC)[10] and the Constipation Quality of Life Scale (CQLS) were used to measure the effect of constipation on patients' daily lives and to determine the patients' quality of life before and after biofeedback.[11] The Visual Analogue Scale (VAS) was used to determine the severity of constipation,[12] and the Abdominal or Diaphragmatic Breath Exercises form was presented to the patients to demonstrate how to perform breathing exercises. The patients were asked to fill out a Constipation Diary to assess constipation status before and after biofeedback.[13] Finally, the Patient Assessment of Constipation Quality of Life (PAC-QOL) questionnaire was used for assessing the patients' quality of life with constipation. This scale was adapted to Turks by Dedeli et al.[11] The highest score that can be obtained on the questionnaire is 140, while the lowest score is 28, with higher scores indicating lower quality of life.

Procedure

A gastroenterologist first assessed patients admitted to the outpatient clinic due to constipation complaints. All patients completed the patient description form, the BSC, CQLS forms, and the VAS via face-to-face interview. They were also trained on how to perform abdominal breathing exercises 3 times a day for a total of 20 minutes per day to increase the strength of their abdominal muscles during defecation. Patients were further asked to keep a constipation diary for 1 week. Anorectal manometry, colon transit time (CTT), and balloon expulsion tests (BETs) were performed as part of the functional assessment.

Anorectal Functional Tests

A 60-cm-long, 2-mm-diameter anorectal catheter polyethylene copolymer tube, with a film sensitive to pressure, 4 lumen, and a latex balloon for measuring rectal sensitivity, was used for the anorectal manometry test; anal canal resting pressure, anal canal squeezing pressure, anal canal pressure during attempts to defecate, intrarectal

pressure, first sensation, desire to defecate, urge to defecate, and maximum tolerated volume were measured.[14]

For BETs, a balloon that is 4-5 cm in length is filled with 50 mL of air and placed into the rectum, and the balloon is expected to be expelled within 1 minute.[15] The CTT test was performed with 1 abdominal film on the fifth day, 120 hours after the oral intake of 20 radiopaque markers.[16] After conducting all these tests and examinations, patients who met at least 2 FDDC with a Type I and Type III functional defecation diagnosis received biofeedback training according to the type of dyssynergic defecation.[5,9]

Monitoring Before Biofeedback

All of the patients were informed about normal bowel functions, dyssynergic defecation disorder, and pelvic floor functions before the study, and they received training on appropriate toilet sitting positions and on resting during defecation by not contracting the abdomen and not squeezing external sphincters. They were asked to sit for a maximum of 10-15 minutes on the toilet. Before performing the biofeedback, the patients were randomly placed into either the biofeedback group ($n = 12$) or the control group ($n = 12$). The first patient who met the inclusion criteria and was diagnosed with dyssynergic defecation was placed into the biofeedback group, while the next patient was put into the control group. A similar time frame was applied for the catheter for both the control (sham) group and the biofeedback group. However, the nurse did not give any verbal feedback or positive encouragement during the sham sessions.

The Biofeedback Program

Biofeedback training was performed by the same nurse researcher via computer software in 6 sessions, once a week, each session lasting for 30-45 minutes, and was administered with a tool used for anorectal manometry application and a standardized oral and visual feedback technique. Visual feedback was provided by observing changes in the pressure activities on the computer screen, while oral feedback was obtained by the researcher. Rectal sensitivity training was provided for the loss of rectal sensitivity using a balloon placed distally on the anorectal manometry catheter. At the end of the 6 weeks of biofeedback therapy, patients were given constipation diaries and the BSC, and they were followed up for 1 week. Patients were given check-ups at the end of the week. The anorectal manometry, CTT, and BET were repeated. The results were recorded by the researcher on the biofeedback form after the patients were administered the VAS and CQLS.

For the patients in the control group, an anorectal manometry probe was inserted. The procedure involved inserting a balloon into the rectum and then blowing it up with 50-60 mL of air to stimulate the stool. Patients were positioned on the commode in a manner to assume the position of physiological defecation. They were told that anal sphincter pressure movements were recorded, but they were given no oral or visual feedback. Nor were they provided any training on how to relax the anal sphincter muscles and on how to do any exercises that would increase their rectal sensitivity. However, a similar time frame was applied for the catheter for both the control (sham) group and the biofeedback group. The nurse did not provide any verbal feedback or positive encouragement in the sham sessions. After the sixth week, the patients in the control group were given biofeedback training by the researcher to not deprive them of the ethical right to receive treatment.

Data Analysis

Data were analyzed using the Statistical Program for Social Sciences 13.0 package program.[17] Crosstabs were drawn up for gender, and the χ^2 analysis was performed. For continuous variables, normality analysis was performed with Shapiro–Wilk test. The *t*-test was used for comparing 2 groups of variables with normal distribution, while the Mann–Whitney *U* test was used for comparing 2 groups with non-normal distribution characteristics. Before and after the biofeedback, the Wilcoxon signed rank test was used for comparing the 2 groups in terms of the variables with normal distribution, and the *t*-test was used for comparing the 2 groups in terms of variables with non-normal distribution.

Intention-to-treat (ITT) analysis, which is a treatment-oriented analysis, is the main method recommended to maintain the effect of randomization and to prevent reduction bias. ITT analysis is defined as the inclusion in the statistical analysis of each participant in the group to which they are assigned without taking into account the treatment they received, if any (i.e., regardless of separation, non-compliance with treatment or treatment/intervention withdrawal), after randomization. The main advantage of ITT is to maintain the balance of randomization, avoid side effects, and increase power. ITT analysis was used in this study and was performed for missing

data, with the results being considered significant at $P < .05$. Two out of the twelve patients in the biofeedback group failed to complete all the sessions. Of the remaining 10 patients, 7 showed improvement. Four of the 12 patients in the control (sham) group failed to complete all the sessions, possibly because they were not satisfied with the therapy.

RESULTS

It was observed that 75% of the biofeedback group and 66.7% of the control group were composed of women ($X^2 = 0.202$, $P > .05$). The mean age of the biofeedback group was 40 ± 17 years (18-68), while the mean age of the control group was 38 ± 9 years (22-54) ($P > .05$).

Findings on the Patients in the Biofeedback and Control Groups Before and After Biofeedback Therapy and Sham

The findings on anal canal pressure, BET, CTT, CQLS, and the severity of the patients' constipation in the biofeedback and control groups before and after biofeedback therapy and sham are presented in Table 1.

There was no statistically significant difference between the mean anal canal pressure values at the time of defecation attempt of the biofeedback (87.50 ± 35.00) and control (72.00 ± 29.00) groups before biofeedback therapy ($U = 57.50$, $P > .05$). After biofeedback therapy, however, there was a significant difference, in favor of the biofeedback group, between the mean anal canal pressure values during the defecation attempt of the biofeedback (49.00 ± 51.00) and control (97.50 ± 77.00) groups ($U = 23.00$, $P < .05$). While there was a statistically significant difference between the mean anal canal pressure values during the defecation attempt before and after biofeedback therapy in the biofeedback group ($Z = -2.552$, $P < .05$), there was no difference found in the control group ($Z = -1.820$, $P > .05$).

Before and after biofeedback therapy, there was no statistically significant difference between the mean scores determined for the biofeedback group (before 300.00±135.00 seconds; after 60.00±284.00 seconds) and control group (before 300.00 ± 135.00 seconds; after 300.0 ± 158.00) on the BET (before therapy $U = 67.50$, $P = .05$; post-therapy $U = 42.0$, $P = .059$). While there was a statistically significant difference between the mean scores before and after biofeedback therapy in the biofeedback group ($Z = -2.371$, $P = .018$), there was no difference found in the control group ($Z = -0.447$, $P = .655$).

Before biofeedback therapy, a statistically significant difference was observed between the mean scores on the CTT test of the biofeedback and control groups ($U = 38.00$, $P = .043$) but not after biofeedback therapy ($U = 63.00$, $P = .536$). While there was a statistically significant difference between the mean scores on the CTT test before and after biofeedback therapy in the biofeedback group ($Z = -2.366$, $P = .018$), there was no difference found in the control group ($Z = 0.00$, $P = 1.00$).

Before biofeedback therapy, there was no statistically significant difference between the mean scores on the total CQLS of the biofeedback (80.00 ± 14.63) and control groups (84.83 ± 20.45) ($t = -0.666$, $P = .513$). After biofeedback therapy, there was a significant difference between the biofeedback group (68.83 ± 17.62) and the control (87.67 ± 20.83) group, in favor of the biofeedback group ($t = -2.391$, $P = .026$). While there was a statistically significant difference between the mean scores on the CQLS before and after biofeedback therapy in the biofeedback group ($t = 2.234$, $P = .047$), there was no difference found in the control group ($t = -1.693$ $P = .119$).

There was no statistically significant difference between the mean scores on constipation severity for the biofeedback (8.3 ± 1.4) and control (7.5 ± 2.2) groups before biofeedback therapy ($t = 1.116$, $P = .277$). After biofeedback therapy, however, a significant difference was found between the biofeedback (4.6 ± 2.4) and control (6.9 ± 2.8) groups, in favor of the biofeedback group ($t = -2.176$, $P = .041$). There was a statistically significant difference between the mean scores on constipation before and after biofeedback therapy in the biofeedback group ($t = 5.461$, $P = .001$) but not in the control group ($t = 1.865$, $P = .089$).

The biofeedback treatment group showed significantly better results than those of the control group for the severity of constipation, anal canal pressure during attempted defecation, BET, CTT, and CQLS.

The findings related to the BSC for the biofeedback group before and after biofeedback therapy and for the control group before and after sham therapy are shown in Table 2. Most of the patients (66.7%) in both groups had Type 3 stool consistency before the therapy started. After the biofeedback therapy, the stool consistency of 4 patients from both the biofeedback and control groups improved. The stool consistency of the biofeedback group post therapy improved to Type 4 because of the benefit of receiving biofeedback therapy. However, the

Table 1. Test Results of Patients in the Biofeedback Group Before and After Biofeedback Therapy and in the Control Group Before and After Sham Therapy

	Biofeedback Group (n = 12)		Control Group (n = 12)	
	Before Biofeedback	After Biofeedback	Before Sham	After Sham
Anal canal pressure (mmHg)	Median ± IR			
	87.5 ± 35	49 ± 51	72 ± 29	97.5 ± 77
	Z = –2.552, P = .011		Z = –1.820, P = .069	
	Biofeedback Group—Control Group			
	Before Biofeedback Therapy		After Biofeedback Therapy	
	U = 57.50	P = .402	U = 23.00	P = .005*
Balloon expulsion test (sec)	Median ± IR			
	300 ± 135	60 ± 284	300 ± 135	300 ± 158
	Z = –2.371, P = .018		Z = –0.447, P = .655	
	Biofeedback Group—Control Group			
	Before Biofeedback Therapy		After Biofeedback Therapy	
	U = 67.50	P = .746	U = 42.0	P = .059
Colon transit test (CTT)	Median ± IR			
	6.50 ± 17.00	0.00 ± 7.00	0.00 ± 6.00	0.00 ± 5.00
	Z = –2.366	P = .018	Z = 0.00	P = 1.00
	Biofeedback Group—Control Group			
	Before Biofeedback Therapy		After Biofeedback Therapy	
	U = 38.00	P = .043	U = 63.00	P = .536
Constipation quality of life	X ± SD			
	80 ± 14.6	68.8 ± 17.6	84.8 ± 20.4	87.7 ± 20.8
	t = 2.234	P = .047*	t = –1.693	P = .119
	Biofeedback Group—Control Group			
	Before Biofeedback Therapy		After Biofeedback Therapy	
	t = –0.666	P = .513	t = –2.391	P = .026*
Severity of constipation	X ± SD			
	8.3 ± 1.4	4.6 ± 2.4	7.5 ± 2.2	6.9 ± 2.8
	t = 5.461	P = .001	t = 1.865	P = .089
	Biofeedback Group—Control Group			
	Before Biofeedback Therapy		After Biofeedback Therapy	
	t = 1.116	P = .277	t = –2.176	P = .041*

X ± S Mean plus/minus SD (standard deviation).
*P < .05.

stool consistency of the control group patients showed no significant difference before and after biofeedback therapy. These findings clearly show that in the case of stool consistency, the biofeedback group greatly benefited from biofeedback therapy.

Results from all the analyses showed that there was a significant improvement in the dyssynergic defecation disorder in 7 of 10 patients in the biofeedback group following biofeedback therapy, compared with the findings before biofeedback.

DISCUSSION

This study investigated the efficacy of the biofeedback method in the treatment of dyssynergic defecation

Table 2. Bristol Stool Scale Results of Patients in the Biofeedback Group Before and After Biofeedback Therapy and in the Control Group Before and After Sham Therapy

	Before Biofeedback		After Biofeedback		Before Sham		After Sham	
	n	%	*n*	%	*n*	%	*n*	%
Type 1	0	0	0	0	1	8.3	1	8.3
Type 2	3	25.0	0	0	0	0	1	8.3
Type 3	8	66.7	7	58.2	8	66.7	5	41.6
Type 4	1	8.3	5	41.6	1	8.3	1	8.3
Type 5	0	0	0	0	1	8.3	3	25.0
Type 6	0	0	0	0	1	8.3	1	8.3
Total	12	100.0	12	100.0	12	100.0	12	100.0

Bristol Stool Scale of types (Type 1, 2, 3, 4, 5).

disorder and in improving patients' quality of life by comparing a treatment group with a sham-control group, and it was determined that the biofeedback method decreased the severity of constipation, increased treatment satisfaction, provided the desired relaxation in the external anal sphincter, and positively affected patients' quality of life.

Biofeedback therapy is evaluated very positively in the literature. The reported effectiveness varies between 50% and 90%. Biofeedback therapy is a helpful and effective method for the treatment of functional constipation, and biofeedback exercises should be the first-choice method in the treatment of constipation caused by pelvic floor dyssynergia and the abnormal function of the anal sphincters, as confirmed by anorectal manometry.[18]

The study by Bassotti et al.[6] reported that more than 70% of the patients derived beneficial results from biofeedback training. Rao et al.[19] revealed that patients who received biofeedback had a higher level of satisfaction than that of those who received standard treatment. Different studies have pointed out that long-term patient satisfaction considerably increased after biofeedback according to the VAS analysis and that the recovery was made easier, with an increase in defecation frequency and a decrease in the use of laxatives.[20-23] The present study showed that there was a significant difference between the patients who received biofeedback and those who did not (i.e., a sham biofeedback was performed) for constipation severity according to the VAS analysis.

As part of biofeedback therapy, trained therapists, including physicians, nurses, and physical or occupational therapists, teach patients strengthening exercises or relaxation techniques that can be performed to reduce their symptoms.[24]

It has been suggested that the high success rate obtained in studies may have resulted from the fact that biofeedback treatment was performed by a single nurse therapist and that the psychological and motivational factors of the patients may have played a key role.[22] Myung[25] emphasized the importance of the use of special techniques in the biofeedback method and, to a lesser degree, the therapist's ability. In the current study, a total of 6 biofeedback sessions were given by the same nurse researcher, with 1 session per week and each session lasting 30-45 minutes.

Anorectal manometry has an important role in the determination of defecation pattern and type and in the evaluation of anorectal physiology. Yet, as it is not available in many centers, it is impossible to evaluate dyssynergic defecation in all cases.[26] Dyssynergic defecation disorder results from insufficient relaxation of the external anal sphincter during defecation.[27,28] In this study, it was found that 60% of the patients had rectoanal coordination disorder, 78% had insufficient relaxation, and 66% had deteriorated rectal sensitivity. As was the case in the present study, other studies too have reported a decrease in anal canal pressure during the attempt to defecate after patients received biofeedback.[6,20-23]

One of the diagnostic criteria used for identifying FDD is the BET. The BET is easy to perform, and patient compliance is also very high.[26] Minguez et al., in their study, reported that BET's specificity is 89%, its negative predictive value is 97%, its sensitivity is 88%, and its positive predictive value is 67%.[29] This test can help identify patients who have a dyssynergic defecation pattern

that requires them to be referred to appropriate specialists for biofeedback treatment. It has been reported that normal values for the BET in Turkey are shorter than 30 seconds for men aged below 40 and shorter than 1 minute for men aged above 40, whereas they are 1 minute for all age groups of women.[30] In a study by Rao et al.,[19] it was found that the BET results of patients who received biofeedback decreased significantly. In the present study, the BET results of the biofeedback group decreased to normal values, which was a positive indicator of the efficacy of the biofeedback method.

This study found that there was significant difference in the average pre- and post-biofeedback therapy scores on the CTT test for the biofeedback patient group (Table 1). No statistical significance, however, was found between the pre- and post-therapy scores in the control group. The average CTT values of the biofeedback group patients decreased from 6.5 ± 17.00 to 0. There were no markers after the biofeedback therapy to suggest the possibility that the method also improved dyssynergic defecation as well. In the study by Rao et al. (2007), the CTT test results dropped significantly in the biofeedback therapy patients compared with those of the non-therapy group.[19]

As the quality of life of patients with dyssynergia is poorer than that experienced by the normal population, it would bring relief to patients, physiologically, economically, and socially, to have a treatment process that was capable of improving their quality of life and that was low cost.[30]

Sahin et al. studied the quality of life of patients with dyssynergic defecation disorder and found that biofeedback was effective as a treatment method for constipation and improved the patients' quality of life.[31] Wald[32] reported that there was a great difference in the quality of life between individuals with and without constipation. Irvine[33] noted that constipation led to a statistically significant decrease in the quality of life related to health. It was also reported by Dennison[34] that constipation affected the quality of life related to health negatively in terms of the economic burden it placed on the health system, the health care providers, and the individuals themselves and that constipation was a difficult condition to address in terms of treatment and clinical studies. The study by Lee et al.[35] observed that there was improvement in the quality of life of patients whose long-term response to biofeedback treatment was positive. These findings were in agreement with those found in the present study.

In this study the quality of life of patients with dyssynergic defecation disorder was investigated. It was found that the biofeedback method decreased the severity of constipation, increased treatment satisfaction, had a positive effect in decreasing anal canal pressure during the attempt to defecate, provided the desired relaxation of the external anal sphincter as a result of the BET, increased CTT, and positively affected the quality of life. The primary limitation of the study was the small number of cases. However, it did not prevent these results from being statistically significant. Given that the research was carried out with a small sample group, the results of the research can be generalized only to this specific research group.

It is evident from the results of this study and others on dyssynergic defecation that behavioral medical techniques like biofeedback should not be considered simply as alternative medical therapies but rather as valuable routine management options that involve low costs and little to no side effects.

The results derived from the present study can provide a basis for the biofeedback treatment procedures used on patients with dyssynergic defecation disorder, considering the low cost of the biofeedback method and the improvement it has been demonstrated to give on the quality of life of patients. More patients should be referred to specialized centers that have facilities for further anorectal physiological assessments and biofeedback so that experienced staff, including nurses, can routinely use them in gastroenterology outpatient settings. Thus, both physicians and patients need to be trained and motivated to use biofeedback therapy more widely.

Ethics Committee Approval: Collection and analysis of these data have received approval from the Ethics Committee of the Ege University, Faculty of Medicine Research and Application Hospital and the Ege University, Faculty of Medicine Directorate of Research and Application Hospital, Turkey (08-7/7, title: "Effectiveness of Biofeedback Therapy on Quality of Life in Patients with Dyssynergic Defecation Disorder," approval date 6 August 2008), in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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