Rivista Italiana di Colon-Proctologia Founded in 1982

PELVIPERINEOLOGY

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EDITORIAL

Dear Reader;

We present you with the first issue of 2024. We started to accept articles not only in the field of Pelviperineology but also in other disciplines as well. The main reason for this is to increase the readability of our journal; it's about not getting stuck in just one area. Naturally, we do not forget that our main field is Pelviperineology.

On the other hand, we have made our PubMed application and are in the evaluation process. I believe that we will give you good news about this as soon as possible.

See you in the next issue.

Stay healthy,

Prof. Dr. Ahmet Akın SİVASLIOĞLU

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Separate anatomical pathways for urge and pain mandate definition change for interstitial cystitis/bladder pain syndrome

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Keywords: Interstitial cystitis IC; bladder pain syndrome; chronic pelvic pain; speculum; xylocaine injection

Interstitial cystitis (IC)/bladder pain syndrome (BPS) "persistent or recurrent chronic pelvic pain (CPP), pressure or discomfort perceived to be related to the urinary bladder accompanied by at least one other urinary symptom such as an urgent need to void or urinary frequency.

Posterior fornix syndrome (PFS): "Predictably co-occurring group of pelvic symptoms, CPP, urge, frequency, nocturia, emptying difficulties/urinary retention, caused by uterosacral ligament (USL) laxity, and cured/improved by USL repair."

The background for this contribution was a debate within ESSIC whether or not to categorize Hunner lesion (HL) IC as a distinct disease entity from BPS.[1,2]

IC/BPS as defined by the ICS, has two main symptoms, pain and one bladder dysfunction. The PFS, first described in 1993 comprised (variously) co-occurring CPP, urge, frequency, nocturia, abnormal bladder emptying.[3,4] Though PFS symptoms could be simultaneously cured or improved by lax USL repair, the pain and bladder symptoms had very different anatomical pathways, Figure 1.

The catalyst for this editorial was a case report, serendipitous histologically-validated cure of "HL" treated by PFS protocols,[5] which included the speculum test, Figure 1, and USL repair. The question raised was, "were PFS and IC/BPS one and the same condition?"[5]

This question was tested for truth or falsity by examining data from 3 studies in 902 women assessed by PFS protocols, (including the speculum test), and treated by a posterior USL sling for uterine/apical prolapse.[6-8] The 902 women had 438 CPP symptoms and 1.351 bladder symptoms (urge, frequency, nocturia, abnormal bladder emptying) which accorded with ICS definitions for IC/BPS.[3] No evidence of HL was found in any of the 902 women when examined cystoscopically.

The CPP part of PFS was investigated by a laparoscopically controlled trial in women with severe pain who had native USL ligament repair.[9] Laparoscopy revealed no obvious pathology. At 12-month review, 70% were cured of CPP in multiple co-occurring sites. It was hypothesized that CPP originated from gravity stimulation of T12-Ll visceral plexuses (VP) because of inability of weak USL to support them.

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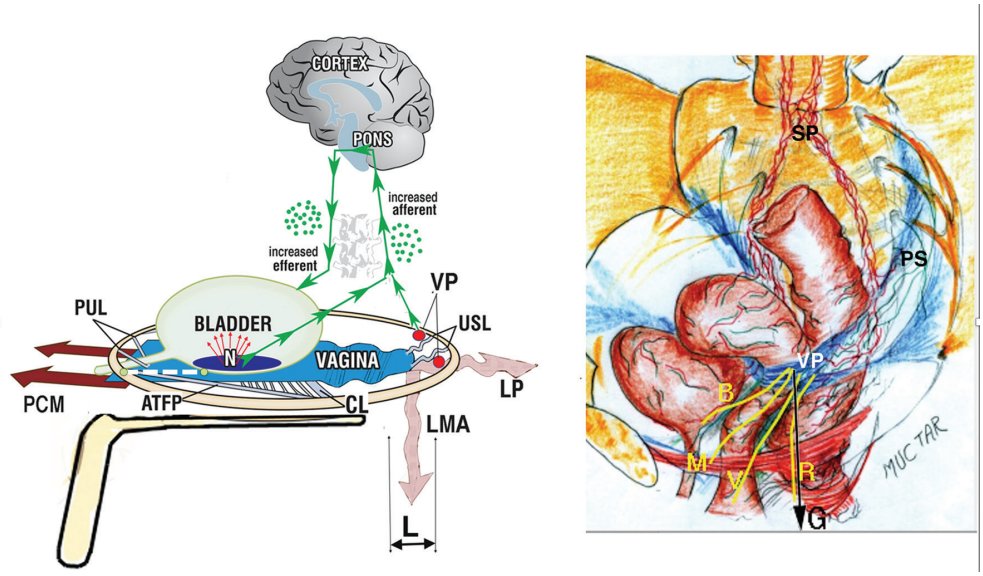

Figure 1. The speculum test relieves pain and urge

Left image: The speculum relieves urge by supporting "N" and pain by supporting VPs. Loose USLs ("L") cannot directly support pelvic visceral nerve plexuses "VP" and they weaken the LP/LMA muscles which contract against them to stretch the vagina to support the stretch receptors "N". Wavy lines in LP and LMA indicate weakened muscle forces, as LP/LMA require firm USLs to contract against for optimal force. LP=levator plate; LMA=conjoint longitudinal muscle of the anus; PUL=pubourethral ligaments; CL=cardinal ligaments.

Right image: 3D view of pelvic organs. VP comprises sympathetic plexus "SP", and parasympathetic plexus "PS". The yellow lines from VP represent visceral nerves to and from the end organs, M (muscles), V (vagina/vulva), B (bladder), R (rectum). G=force of gravity acting on VPs. Right figure by permission, Muctar S. and Karger

The VP causation of CPP was tested by local anesthetic (LA) injection into each USL, at the posterior fornix in 10 women with vulvodynia; 8/10 reported complete disappearance of introital sensitivity and 2/10 in one side only.[10]

The xylocaine injection test was applied to 3 women with IC/BPS.[11] Abdominal, urethral, introital and cervical tenderness and pain, objectively confirmed pre-test, disappeared, or substantially improved, within 5 minutes.[11]

With reference to Figure 1, although pre-operative speculum tests relieved both pain and urge,[6-8] the LA test.[10,11] demonstrated that the various manifestations of CPP originating from the VP were quite independent of urge symptoms originating from unsupported urothelial stretch receptors. This suggests pain and urge are separate entities, both caused by lax USLs, but from separate anatomical pathways. The 438 PFS data wholly incorporate non-Hunner IC/BPS symptoms as defined,[3] with no evidence of HL.[6-8] On this basis alone, neither BPS nor PFS belong in any HL definition. Definition change is urgently needed to separate HL from BPS. Incorporating a simple speculum test in assessment of IC/BPS patients, may indicate if the pain and urge are potentially curable by USL support, surgically, or in time, non-surgically.

ETHICS

Contributions

Surgical and Medical Practices: B.L., K.G.; Concept: B.L., K.G.; Design: B.L., K.G.; Data Collection or Processing: B.L., K.G.; Analysis or Interpretation: B.L., K.G.; Literature Search: B.L., K.G.; Writing: B.L., K.G.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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Molecular and *in silico* evidence: *DR4* gene rs20576 and rs6557634 variants are effective in the development of uterine leiomyoma

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ABSTRACT

Objectives: Leiomyomas are the most common benign tumours in women of reproductive age. In this study, we aimed to investigate the role of death receptor 4 (*DR4*) gene polymorphisms in the pathogenesis of leiomyoma.

Materials and Methods: In our study, 76 patients were diagnosed with leiomyoma and 81 patients without leiomyoma as healthy controls. The polymerase chain reaction-restriction fragment length polymorphism method was used to identify DR4 polymorphisms. We also determined the protein function and stability of rs20576 and rs6557634 variants by *in silico* approaches.

Results: There was a difference in the distribution of the genotypes of *DR4* gene rs20576 and rs6557634 polymorphisms between leiomyoma patients and health control groups (p=0.014 and p=0.039, respectively). In addition, the distribution of C alelle frequency of rs20576 and G allelle frequency of rs6557634 were significantly higher in leiomyoma patients (p=0.018 and p=0.029, respectively) and the A allele of both rs20576 and rs6557634 has had a protective effect against leiomyoma. According to *in silico* analysis results, rs20576 and rs6557634 have deleterious effects on protein function and structure.

Conclusion: The present results showed the association of gene variants of DR4 with leiomyomas. In addition, *DR4* gene rs20576 and rs6557634 polymorphisms effectively decrease protein stability and function. Further studies may be done on various polymorphisms belonging to DR4 receptors.

Keywords: Apoptosis; death receptor 4; gene polymophism; leiomyoma

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INTRODUCTION

Leiomyomas are monoclonal benign tumours originating from the smooth muscle cells of the uterus. Although most of the leiomyomas are asymptomatic in women of reproductive age, their incidence reaches 70% by the age of 50.[1] It is the most common cause of hysterectomy. Race, age, family history, parity, hypertension (Ht) and diabetes mellitus (DM) are associated with the risk of developing leiomyoma.[1-4] Genetic factors are associated with leiomyoma development as well. Clonal chromosomal rearrangements, including translocations, duplications and deletions in chromosomes 6, 7, 12, and 14 have been shown to be present in 40-50% of leiomyomas. Mediator complex subunit 12, fumarate hydratase, *HMGA1* and *HMGA2* of gene mutations are involved in the pathogenesis of leiomyoma.[5]

Apoptosis is programmed cell death that occurs in normal and pathological tissues. There is increasing evidence that genetic, environmental and hormonal factors impair apoptotic pathways in leiomyoma development.[6] It has been reported that the regulation of many genes related to apoptosis is disrupted in the development of leiomyoma. Disruption of apoptosis and proliferation in cells is thought to be an important step in the pathogenesis of leiomyomas.[7] There are many studies on apoptotic pathways and tumour formation in the literature.[8-10] The death receptor family is a member of the tumor necrosis receptor superfamily. It has 8 known members. DR4 is known as TRAILR1 or APO-2.[7] It has also been shown in the literature that *DR4* receptor gene polymorphisms, which play a role in the extrinsic pathway of apoptosis, are associated with tumour formation.

Loss of functionality of the apoptosis process at any stage may result in tumour formation. In our literature review, we found that the rs20576 and rs6557634 variants of the *DR4* receptor gene, which plays a role in the extrinsic pathway of apoptosis, have been studied in other diseases. Still, no study has yet been conducted to examine their relationship with leiomyomas. With this study, we aim to contribute to the literature to elucidate leiomyoma's etiopathogenesis by examining the effects of *DR4* gene rs20576 and rs6557634 variants on leiomyoma formation.

MATERIALS AND METHODS

Study Cohort

Our study was conducted within the ethical principles regarding medical research on humans included in the Declaration of Helsinki. The study protocol was approved by Muğla Sıtkı Koçman University Faculty of Medicine Medical Ethics Committee (decision dated: 22.12.2021, numbered: 27/VIII) and all participants provided written informed consent. One hundred-sixty two women over the age of 18, who were not pregnant and had no known malignant disease, and who applied to Muğla Training and Research Hospital Obstetrics and Gynaecology Outpatient Clinic were included in the study. After transabdominal and transvaginal ultrasonographic evaluations of the patients, necessary information was given to a total of 162 women 81 women who were diagnosed with leiomyoma in the case group and 81 healthy women in the control group, and informed consent was obtained from the individuals. Of the patients in the leiomyoma group, 5 were excluded from the study due to the diagnosis of cancer, including 1 leiomyosarcoma, 2 adenocarcinoma *in situ*, 1 smooth muscle cell tumour of undetermined malignant potential, and 1 breast cancer. The study was continued with a total of 157 participants, including the case group consisting of 76 female patients with leiomyoma and the control group consisting of 81 healthy women without leiomyoma. 2 mL were separated from the blood taken routinely and taken into tubes containing 2% ethylenedimethyltetraacetic acid and included in the study.

Genotyping Determination

We isolated DNA from peripheral blood leukocytes with a Hibrigen Blood DNA Isolation Kit (MG-KDNA-02-250; Hibrigen Biotechnology R&D Industry and Trade Inc., Gebze, Kocaeli, Türkiye). By using polymerase chain reaction (PCR)- restriction fragment length polymorphism (RFLP) method, the rs20576 and rs6557634 SNPs in the *DR4* gene were identified. PCR was performed with a 25 µL volume of 100 ng DNA, 20 pmol of each primer, 1.5 mM $MgCl_2$, Thermo Scientific PCR MasterMix. Amplification was performed on an automated Thermal Cycler (Thermo, ABI). Fragment separation at 120 V for 40-50 minutes on a 3.5% agarose gel containing 0.5 mg/mL ethidium bromide was used to determine the RFLP products. For each gel lane, a 100-bp DNA ladder (Fermentas Vilnius, Lithuania) was used as a size reference. The gel was viewed using a gel electrophoresis visualizing system (Cleaver Scientific Ltd., Clear View UV Trans illuminator, Rugby, UK) under UV light. PCR and RFLP conditions are shown in Table 1.

Statistical Analysis

The sample size of the study was calculated as 153 people.[11] Descriptive statistics; numbers for categorical variables, mean and standard deviation values for continuous variables and percentages is used. It is planned to use Kolmogorov-Smirnov, Shapiro-Wilk tests for normality in univariate analyzes, Student's t-test in cases where two means with the parametric condition are compared and for non-parametric cases, it is planned to

use the Mann-Whitney U test. Pearson chi-square value for the parametric condition and Fisher's Exact test result for the non-paremetric condition were given for the categorical variables. In the analytical analyzes, $p<0.05$ was accepted as the significance limit. SPSS 23.0 package program was used for statistical analysis.

Prediction of Deleterious Missense SNPs

PolyPhen-2 (Polymorphism Phenotyping v2) (http://genetics.bwh.harvard.edu/pph2/) tool was used to show the deleterious effect of the missense variants (rs20576 and rs6557634) on the protein. The PolyPhen-2 predicts the possible impact of amino acid changes on the stability and functionality of human proteins using structural and comparative evolutionary considerations.[12]

Protein Stability Change Prediction

The change in protein stability of rs20576 and rs6557634 variants was analysed with the MUpro (http://mupro.proteomics.ics.uci.edu/) and I-Mutant tools (https://folding.biofold.org/i-mutant/i-mutant2.0.html). MUpro tool uses support vector machine and neural networks machine learning methods. If the confidence score was <0, the mutation has decreased protein stability. But if the confidence score was >0, the mutation has increased protein stability. I-Mutant2.0 is a tool that automatically forecasts changes in protein stability brought on by single-point mutations using support vector machines.[13,14]

Prediction of Gene-gene Interactions

DR4 gene of its association with other genes in order to predict was used, GeneMANIA (https://genemania.org/) (accessed on 27 August 2023). The prediction of gene-gene interaction predictor GeneMANIA is based on the basis of co-localization, pathways, protein domain similarity, co-expression and genetic and protein interaction.[15]

RESULTS

Associations of DR4 Variants with Uterine Leiomyomas

We included 157 participants in our study, including 76 case groups and 81 control groups. While 56.7% of the participants applied to our outpatient clinic due to any gynaecological symptoms, 43.3% applied for routine annual control. We obtained anamnesis information from the participants. Accordingly, we detected Ht in 26.1% and DM in 17.2% of them. The socio-demographic and disease characteristics of the research group are shown in Table 2.

We compared socio-demographic variables such as body mass index (BMI), age and parity of the participants between the case and control groups (Table 3). Accordingly, the mean age of the case group was 44.81±6.97 years and the mean age of the control group was 43.23±9.78 years. There was no statistically significant difference between the case and control groups in terms of age ($p=0.248$). The mean BMI of the case group was 26.27±3.18 and the mean BMI of the control group was 25.61±3.36. We compared the mean BMI of the case and control groups and we did not find a statistically significant difference ($p=0.550$). The mean parity number in the case group was 1.89±1.05 and the mean parity number in the control group was 1.05±0.87. We compared the mean parity numbers between the case and control groups and we did not find a statistically significant difference ($p=0.210$).

We examined the status of Ht disease in 157 people who participated in the study. While 22 people have Ht disease in the case group, in the control group 19 people. Accordingly, the incidence of Ht in patients with leiomyoma is 28.9%, while the incidence of Ht in the control group is 23.5%. We did not find a statistically significant difference between the case group

Table 1. PCR and RFLP conditions used for the polymorphisms of *DR4* gene

(a) PCR conditions used for the polymorphisms of *DR4* gene

Gene	Polymorphism	Primers	Temperature of annealing	Product size
DR4	rs20576	P1 P2	61 °C	201 bp
	rs6557634	P3 P4	59 °C	230 bp

(b) Restriction enzymes, digestion conditions and restriction fragment sizes

Gene	Polymorphism	Restriction enzyme	Digestion conditions	Restriction fragment sizes
DR4	rs20576	*Taq*I	37 °C, 3 h	C allele: 201 bp A allele: 110 bp, 91 bp
	rs6557634	*Bse*GI	37 °C, 3 h	G allele: 230 bp A allele: 160 bp, 70 bp

P1: 5'-ATCCCACCTGGCCAGCTTTCCA–3' P2: 5'-AGACAGGAGTCTCGGGCTGCT–3'; P3: 5' ATCCTCTGGGAACTCTGTGG–3' P4: 5'-TACCACTCCCACCTTCACTGC–3'; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism

Table 2. Distribution of the research group according to socio-demographic and disease characteristics			
Variables		**Number (n)**	**Percentage (%)**
Research group (n=157)	**Case**	76	48.4
	Control	81	51.6
Symptom (n=157)	+	89	56.7
	-	68	43.3
Symptom type (n=89)	**Bleeding**	44	49.4
	Pain	19	21.3
	Other	26	29.2
Hypertension (n=157)	+	41	26.1
	-	116	73.9
Diabetes mellitus (n=157)	+	27	17.2
	-	130	82.8

Table 3. Evaluation of the principled consistency in the selection of the control group versus the case group in determining the risk posed by the factor in the study with some socio-demographic variables			
Variables	**Research group (n=157)**		***p****
	Case (n=76)	**Control (n=81)**	
	Mean ± SD	**Mean ± SD**	
Age	44.81±6.97	43.23±9.78	0.248
BMI	26.27±3.18	25.61±3.36	0.550
Parity	1.89±1.05	1.05±0.87	0.210
SD: standard deviation; BMI: body mass index			

and the control group in terms of the incidence of Ht (p>0.05) (Table 4). We examined the DM disease status of 157 people who participated in the study. While 17 people have DM disease in the case group, in the control group 10 people. Accordingly, while the incidence of DM was 22.4% in the case group, it was 12.3% in the control group, and we did not find a statistically significant difference between the case group and the control group in terms of DM frequency (p>0.05) (Table 4).

Genotype distributions of *DR4* gene rs20576 polymorphism were examined, we detected 48.7% CC, 50% CA and 1% AA genotype in the case group and 29.6% CC, 63% CA and 7.4% AA genotype in the control group. Considering the genotype frequencies, the rs20576 polymorphism was significant between the two groups (p=0.014) (Table 5). Genotype distributions of *DR4* gene rs6557634 polymorphism are examined, and we detected 60.5% GG, 23.7% GA and 15.8% AA genotype in the case group and 40.7% GG, 39.5% GA and 19.8% AA genotype in the control group. Considering the genotype frequencies, the rs6557634 polymorphism was significant between the two groups (p=0.039) (Table 5). rs20576 for the CC genotype and rs6557634 for the GG genotype was the risk genotype for uterine leiomyomas. Allele frequencies differed significantly between the two groups. In the patient group of rs20576, the C allele was higher than the control group, while the G allele of rs6557634 was higher than the control group (p=0.018 and p=0.029, respectively) (Table 5). However, we found that the increased frequency of A allele was protective in terms of the risk of myoma formation.

In order to find the genotype that made the difference, we evaluated the genotypes in the case and control groups in the four-eyed table with pairwise comparisons (Table 6). Accordingly, when the difference between the mean percentages of CC and CA genotypes of rs20576 polymorphism in the case and control groups is considered; we found the mean percentage of CC genotype in the case group (49.3%) to be statistically significantly higher than in the control group (32%) (p=0.031). The risk of leiomyoma in those with the CC genotype was 2.069 times higher than the control group. Considering the difference between the mean percentages of CC and AA genotypes of rs20576 polymorphism in the leiomyoma and control groups; we found the mean percentage of CC genotype (97.4%) in the case group to be statistically significantly higher than in the control group (80%) (p=0.038). The risk of leiomyoma in those with the CC genotype was 9.250 times higher than in the control group. Considering the difference between the average percentages of

Table 4. Evaluation of the relationship of the research group between the presence of Ht and DM and the presence of leiomyoma

Variables						*p**
		Case		Control		
		Number	Percentage (%)	Number	Percentage (%)	
Ht (n=157)	+	22	28.9	19	23.5	0.434
	-	54	71.1	62	76.5	
DM (n=157)	+	17	22.4	10	12.3	0.096
	-	59	77.6	71	87.7	

* Pearson chi-square; DM: diabetes mellitus

Table 5. Distributions of DR4 genotypes and allele frequencies in between case and control groups

	Case n (%)	Control n (%)	χ^2 *p*-value	OR (95% CI)
Genotype rs20576				
CC	37 (48.7)	24 (29.6)	**0.014**	
CA	38 (50)	51 (63)		
AA	1 (1.3)	6 (7.4)		
Allele rs20576				Reference
A	40 (26.3)	63 (38.9)	**0.018**	0.561 [0.347-0.962]
C	112 (73.7)	99 (61.1)		1.304 [1.056-1.609]
Genotype rs6557634				
GG	46 (60.5)	33 (40.7)	**0.039**	
GA	18 (23.7)	32 (39.5)		
AA	12 (15.8)	16 (19.8)		
Allele rs6557634				Reference
A	42 (27.6)	64 (39.3)	**0.029**	0.591 [0.367-0.949]
G	110 (72.4)	99 (60.1)		1.275 [1.033-1.573]

OR: odds ratio; CI: confidence interval

CA and AA genotypes of rs20576 polymorphism in the case and control groups; the mean percentage of CA genotype in the case group (97.4%) was not statistically significant compared to the control group (89.5%) (p=0.235).

To find the genotype that made the difference, we evaluated the rs6557634 genotypes in the case and control groups in the four-eyed table with pairwise comparisons (Table 6). Accordingly, when the difference between the mean percentages of GG and GA genotypes of rs6557634 polymorphism in the case and control groups is examined; we found the mean percentage of GG genotype (71.9%) in the case group to be statistically significantly higher than in the control group (50.8%) (p=0.014). The risk of leiomyoma in those with GG genotype was 2.478 times higher than the control group. Considering the difference between the mean percentages of GG and AA genotypes of rs6557634 polymorphism in the case and control groups; we did not find the mean percentage of GG genotype (79.3%) in the case group to be statistically significant compared to the control group (67.4%) (p=0.161). Considering the difference between the mean percentages of GA and AA genotypes of rs6557634 polymorphism in the case and control groups; we did not find the mean percentage of GA genotype (60%) in the case group to be statistically significant compared to the control group (66.7%) (p=0.550).

Prediction of Deleterious Missense SNPs

The deleterious effects of rs6557634 (H141R) and rs20576 (E228A) were demonstrated *in silico*. As a result, deleterious effects were detected in the Polyphen-2 tool. The expected confidence score is for rs6557634 and rs20576 variant, respectively, 0.824 and 0.712.

Protein Stability Change Prediction

The protein stability was decreased in both the MUpro tool and the I-Mutant 2.0 tool (Table 7). rs6557634 and rs20576 can have a serious effect on the phenotype.

Table 6. Pairwise comparison of the rs20576 and rs6557634 genotypes to determine the group that makes the difference in the relationship between the genotype presence of leiomyoma and the evaluation of the risk of leiomyoma presence

Variables		Genotype				p*	OR [95% CI]
		Case	Control				
		Number	Percentage (%)	Number	Percentage (%)		
rs20576	CC	37	49.3	24	32.0	0.031*	2.069 [1.066-4.017]
	CA	38	50.7	51	68.0		
rs20576	CC	37	97.4	24	80.0	0.038**	9.250 [1.047-81.701]
	AA	1	2.6	6	20.0		
rs20576	CA	38	97.4	51	89.5	0.235**	4.471 [0.516-38.698]
	AA	1	2.6	6	10.5		
rs6557634	GG	46	71.9	33	50.8	0.014*	2.478 [1.194-5.144]
	GA	18	28.1	32	49.2		
rs6557634	GG	46	79.3	33	67.3	0.161*	1.859 [0.777-4.445]
	AA	12	20.7	16	32.7		
rs6557634	GA	18	60.0	32	66.7	0.550*	0.750 [0.291-1.930]
	AA	12	40.0	16	33.3		

*: Pearson chi-square; **: Fisher's Exact test; OR: odds ratio; CI: confidence interval

Table 7. Effect of variants in protein stability

SNP ID	Aminoacide change	I-Mutant 2.0	RI (kcal/mol)	MUpro	ddG
rs6557634	H141R	Decrease	5	Decrease	-0.67550955
rs20576	E228A	Decrease	9	Decrease	-1.4814493

ddG: delta-delta G; RI: reliability index

Prediction of Gene-gene Interactions

Our findings revealed that DR4 (TNFRSF10A) is physical interaction with 20 genes (*DAP3, CASP8, ARAP1, TNSF10, FADD, TNFRSF10C, MOAP1, TNFRSF10B, TNFRSF10D, TRADD, RASSF1, CFLAR, RIPK1, CASP10, TRAP1, MAO3K1, LRRC47, CPVL, UBL4A* and *NDUFA5*) co-expressed with 19 genes (*DAP3, CASP8, ARAP1, TNSF10, FADD, TNFRSF10C, MOAP1, TNFRSF10B, TNFRSF10D, TRADD, RASSF1, CFLAR, RIPK1, CASP10, TRAP1, MAO3K1, LRRC47, UBL4A* and *NDUFA5*), shared a domain with 11 genes (*CASP8, ARAP1, FADD, TNFRSF10C, TNFRSF10B, TNFRSF10D, TRADD, RASSF1, CFLAR, RIPK1* and *CASP10*) (Figure 1). A total of 408 connections were determined.

DISCUSSION

Leiomyomas are a common pathology in women of reproductive age and its etiopathogenesis has not been fully elucidated yet. We believe that any pathology that may affect apoptosis mechanisms may trigger monoclonal proliferation in myometrial cells and therefore may play a role in leiomyoma etiopathogenesis. Although single nucleotide polymorphisms do not directly cause disease, they can affect the formation of the disease and the responses to the treatments to be applied. We see that the DR4 receptor, one of the TRAIL receptors in the apoptosis extrinsic pathway, is frequently studied in the literature in terms of its association with diseases. In our study, we found that CC and GG genotype variants, respectively, of homozygous genotypes of *DR4* gene rs20576 and rs6557634 polymorphisms, were statistically significantly higher in patients with leiomyomas. Among the allele frequencies of *DR4* gene rs20576 and rs6557634 polymorphisms, we detected statistically significantly higher frequencies of C and G alleles in myoma patients, respectively, and we also found that the increase in A allele frequencies in both rs20756 and rs6557634 polymorphisms was statistically significantly protective in terms of the risk of leiomyoma formation. In addition, we determined that the *DR4* gene

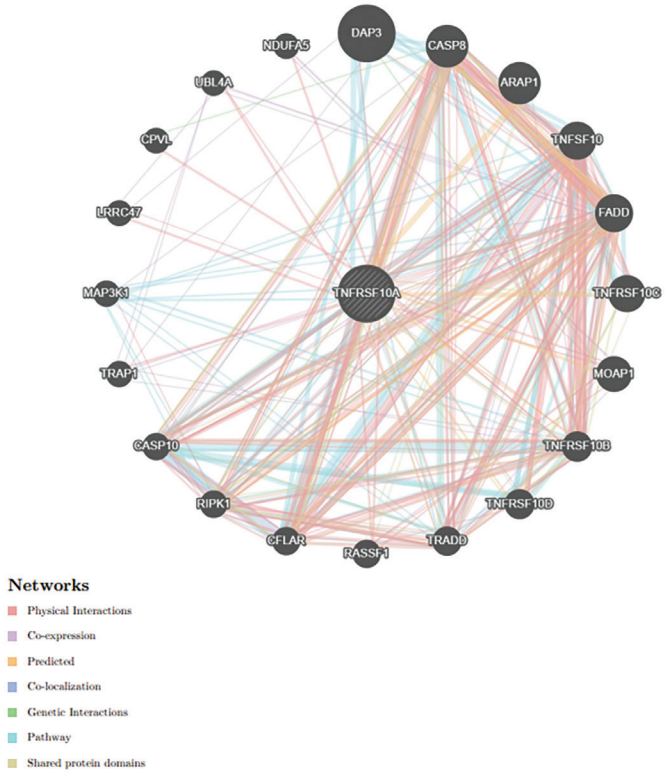

Figure 1. Gene-gene interaction network of DR4

rs20756 and rs6557634 variants significantly reduced protein stability and function.

Previous studies investigating the association of *DR4* gene variants with different diseases support our molecular results. Edgünlü et al.[11] found that the *DR4* gene rs6557634 polymorphism has a protective affect on Alzheimer's disease. In a study that investigated the association of *DR4* gene variants with prostate cancer risk in a North Indian population, it was found that rs6557634 polymorphism AA genotype and A allele, rs4871857 polymorphism GG genotype and G allele increased the risk.[16] Rai et al.[17] investigated the *DR4* gene polymorphisms rs20576 and rs6557634 in their study, in which they investigate the relationship of complex interactions of ADRB3 variation with other candidate gene variants with gallbladder cancer. In their study, they found that heterozygous genotypes of *rs20576* and *rs6557634* gene polymorphisms increased the risk of gallbladder cancer.[17] Körner et al.[18], in their study on the relationship between *DR4* gene rs20575 and rs20576 polymorphisms in hepatitis C virus (HCV) infected patients, and the risk of hepatocellular carcinoma (HCC) stated that genotype distribution were not different between healthy controls and HCV-positive patients without HCC. However, they found the frequencies of rs20575 and rs20576 to be statistically significantly high in patients with HCC. They associated the risk of HCC with the allele of rs20575 and the homozygous AA genotype of rs20576 and determined the coexistance of the two as indenpendent risk factors in the development of HCC. In adition, they found the HCV viral load to be statistically significantly higher in patients carrying the allele of rs20575 and the homozygous AA genotype of rs20576 simultaneously.[18] Kim et al.[19] in their study investigating the relationship between polymorphisms of TRAIL, TRAIL receptors and osteoprotegerin genes, and endometriosis, stated that *DR4* gene rs20575 and rs2230229 polymorphisms were not observed, and that there was no statistically significant difference in the genotype distributions of TRAIL, DR4, DR5 and OPG and the allele frequency distributions of single or combined polymorphisms between the groups with endometriosis disease and those without endometriosis disease.

CONCLUSION

In our study, we investigated the role of allele and genotype frequencies of *DR4* gene rs20576 and rs6557634 variants in leiomyoma etiopathogenesis and we believe that these variants may have a role in the development of leiomyomas. In addition, our *in silico* analyses showed that rs20576 and rs6557634 variants may play a role in the pathogenesis of various diseases, especially leiomyomas. Our study is the first to investigate the relationship between *DR4* gene rs20576 and rs6557634 variants and leiomyoma etiopathogenesis. The relationship of DR4 with leiomyomas may be manifested more precisely if polymorphisms of the *DR4* gene, gen expression, epigenetic mechanisms, and TRAIL protein levels in serum are analysed and evaluated together in a larger group of patients.

ETHICS

Ethics Committee Approval: The study protocol was approved by Muğla Sıtkı Koçman University Faculty of Medicine Medical Ethics Committee (decision dated: 22.12.2021, numbered: 27/VIII).

Informed Consent: All participants provided written informed consent.

Contributions

Concept: T.H., E.A., T.E.; Design: T.H., E.A., B.S., T.E.; Data Collection or Processing: T.H., Ç.Ö., B.D., T.E.; Analysis or Interpretation: T.H., E.A., Ç.Ö., B.D., B.S., T.E.; Literature Search: T.H., E.A., Ç.Ö.; Writing: T.H., E.A., B.D., B.S.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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Restorative effects of myricetin and hyaluronic acid on vaginal epithelial atrophy in ovariectomized rats

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ABSTRACT

Objectives: Postmenopausal women often suffer from genitourinary syndrome (GUS), which is vulva-vaginal atrophy. The aim of this study is to evaluate the effect of hyaluronic acid and myricetin on GUS caused by ovariectomy.

Materials and Methods: Twenty-eight Wistar Albino female adult rats were randomly divided into 4 equal groups. Vaginal smear and pH were collected from all groups before the procedures. To the vaginal walls, for Sham group (Group 1, n=7): The abdomen was opened and closed, then 0.9% saline/0.5 cc/subcutaneously, for ovariectomy group (Group 2, n=7): Two weeks after ovariectomy, 0.9% saline/0.5 cc/subcutaneously, for ovariectomy + myricetin group (Group 3, n=7): Two weeks after ovariectomy myricetin 5 mg/0.5 cc/subcutaneously, for ovariectomy + hyaluronic acid group (Group 4, n=7): 2 weeks after ovariectomy hyaluronic acid 5 mg/0.5 cc/subcutaneously was applied. After 2 weeks of the injection, vaginal smear and pH were checked again in all rats. Vaginal smears were stained with May-Grunwald Giemsa and Pap. Meisel's vaginal maturation index was calculated. Vaginectomy was performed. hematoxylin eosin and caspase-3 immunostaining was performed and scored. Biochemically tissue SOD, AOPP, and TSH were measured.

Results: Vaginal Maturation Index and pH were significantly increased in group 3 and 4 compared to group 2. Vaginal epithelial thickness was increased in Group 3 and 4 compared to group 2. The vaginal epithelial thickness of group 3 increased more than group 4. No significant change in biochemical parameters was observed between groups.

Conclusion: We believe that myricetin will be a promising option for non-hormonal treatment methods in women with GUS symptoms.

Keywords: Hyaluronic acid; menopause; myricetin; ovariectomy; vaginal maturation index

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INTRODUCTION

Genitourinary syndrome (GUS), replacing the old terminology of vulva-vaginal atrophy, is defined as the sum of symptoms and signs associated with a decrease in estrogen and other sex steroids, including changes in the genitourinary organs. About half of postmenopausal women are affected by GUS.[1] Common symptoms according to the degree of atrophy include vaginal dryness (75% in postmenopausal women), painful sexual intercourse (dyspareunia) (38%), and vaginal itching-discharge (15%).[2] Differential diagnoses of GUS may include infections (bacterial vaginosis, trichomoniasis, candidiasis), contact irritants, foreign bodies, and sexual trauma.[3] Treatment methods used in GUS are hormone replacement therapy, ospemiphene which is one of the selective estrogen receptor modulators, laser applications and topical estrogen creams. Intravaginal oxytocin gel and dehydroepiandrosterone applications are being researched.[4,5] Due to possible side-effect profile of hormonal methods, interest in non-hormonal methods has increased recently.[6] Among the non-hormonal methods in the treatment of GUS, vitamins, phytoestrogens and hyaluronic acid (HA) are promising.[7]

HA is involved in important biological functions such as regulation of cell adhesion and motility, cell differentiation, proliferation and tissue repair in most connective tissues.[8] The cross-linked form (filler) is used for hydrodynamic volume change of the extracellular matrix to reduce the clinical effects of aging and volumize the skin.[9] The free, non-cross-linked form absorbs water and stimulates the dermal tissue biologically and this form is used in the treatment of atrophy due to its fibroblast cell activation property.[10]

Myricetin is a flavonoid derived from plant source.[11] Myricetin has been shown to have a therapeutic effect on many diseases, including different types of tumors, inflammatory diseases, atherosclerosis, thrombosis, cerebral ischemia, diabetes, Alzheimer's disease, and pathogenic microbial infections.[12] Myricetin has been shown to have anti-inflammatory, analgesic, antitumor, and antibacterial effects.[13-17]

In our study, it is predicted that myricetin can be used as a non-hormonal treatment option such as HA in women with GUS complaints due to its anti-inflammatory properties.

MATERIALS AND METHODS

A total of 28 adult, virgin, 10-12 weeks old female Wistar albino rats were allocated in this study. The animals (200-250 g) were under conditions of 22±1 °C, 12-hours of day/night cycles and 50-60% humidity. The Muğla Sıtkı Koçman University Ethics Committee for Animal Research approved the animal protocol for this study (approval no: 06/22). Animal research investigations were guided in line with the Committee for Human Care. The rats were given water and standard rat feed. After being randomly divided into 4 groups, daily vaginal smears were taken from all groups. The rats having at least two documented 4-5 day menstrual cycles were included to the study. Vaginal pH and vaginal smears were checked for all experimental animals before the operation.

Surgical Procedure

Anesthesia was induced by intraperitoneal 10 mg/kg (Rompun, Bayer, Leverkusen, Germany) xylazine hydrochloride and 50 mg/kg (Ketalar, Eczacıbaşı, İstanbul, Türkiye) ketamine administration. After shaving and cleaning the surgical sites with antiseptic solution (Baticon, Drogsan, Türkiye), 2.5 cm midline vertical incisions were made in the periumbilical region. A surgical menopause model was created by performing ovariectomy. During the ovariectomy, the ovary was taken out of the abdomen by using the uterus catch hook. By pulling the tubas which are located at the end of the uterine horns are pulled. Then both ovarian arteries were ligated respectively. Then, the ovaries were excised by cutting. After the removal of the ovaries the abdomen was closed.

Groups

Ovariectomy was not performed on the rats in the Sham group (Group 1). The abdomen of the rats in the sham group was opened and closed, smears were taken and cytology was examined 4 weeks later. During this time, 0.5 cc 0.9% saline (subcutaneous) was applied to the vaginal walls. In groups 2, 3 and 4 ovariectomies were performed. 4 weeks after the ovariectomy procedures, vaginal cytology was also evaluated by taking vaginal pH and smear in all groups that underwent ovariectomy and the rats that developed atrophy according to cytology results were included in the study. 0.9% saline (0.5 cc/subcutaneous) was injected to the vaginal walls of the rats in ovariectomy + saline group (Group 2). Myricetin (25 mg/kg/subcutaneous) was injected to the vaginal walls of the rats in the ovariectomy + myricetin group (Group 3). HA (25 mg/kg/subcutaneous) was injected to the vaginal walls of the rats in the ovariectomy + HA group (Group 4). Vaginal pH and vaginal smears were checked again in all rats 4 weeks after the vaginal injections of 0.9% saline, myricetin and HA. Vaginectomy was performed in all rats which were then sacrified by injecting high-dose anesthetics.

Vaginal Smear and Vaginal Maturation Index

Two smear samples were taken from each rat. One of the samples was fixed by the air drying method and the other was fixed by

the alcohol fixation method. The air dried samples were stained with May-Grunwald Giemsa stain. The smears which were fixed with alcohol were stained using the Pap staining method.

For vaginal maturation index; Parabasal (P), Intermediate (I) and Superficial (S) cell counts are performed. It is multiplied by 0, 0.5 and 1.0 respectively. The sum of the three values gives the Meisel's vaginal maturation index (VMI). These procedures were performed by the gynecology and obstetrics physician.

Histopathological Analysis

Vaginal tissues were placed in 10% neutral formalin for fixation and prepared for routine paraffin embedding. Paraffin blocks were cut at 5 µm thick, mounted on slides, stained with hematoxylin-eosin (H-E). Vaginal changes including structure of lamina propria and blood vessels in the lamina propria were evaluated and vaginal epithelial thickness was measured in 5 different areas for each vaginal tissues under a light microscope.[18] All sections were examined with a Nikon Eclipse 80i light microscope and Nikon image analysis system (Digital Sight-L2, Ver=450.1032.3220.100531).

Immunohistological Analysis

For analysis by immunohistochemistry, the sections were placed on slides covered with polylysine. After rehydration, samples were heated in citrate buffer (pH 7.6) and microwave oven for 20 minutes. After waiting for 20 minutes at room temperature, the sections were rinsed with phosphate-buffered saline (PBS), then placed in 0.3% H_2O_2 for 7 minutes and rinsed with PBS. Sections were incubated with a primary rabbit-polyclonal anti-Caspase-3 (ab13847; Abcam) for 2 hours. They were washed in PBS. The biotinylated goat was incubated with an anti-polyvalent for 10 minutes. They were incubated with streptavidin peroxidase for 10 minutes at room temperature. They were performed using chromogen + substrate for 15 minutes, and slides were counterstained with Mayer hematoxylin for 1 minutes, rinsed in tap water, and then dehydrated. Anti-caspase-3 antibody was used following the manufacturer's instructions. Brownish granules in the cytoplasm were recognized as positive staining for Caspase-3. All of the sections were examined with a Nikon Eclipse 80i light microscope and a Nikon Image Analysis system (Digital Sight-L2, Ver=450.1032.3220.100531).

IHC evaluation: The semiquantitative H-Score was calculated by counting the positively stained cells in 5 randomly selected areas for each group [H-Score: ∑Pi (i+1) (Pi: % number of positively stained cells; i: staining intensity].[19]

Biochemical Analysis

Determination of SOD activity

For super oxide dismutase (SOD) activity, 150 µL of supernatant was taken, 150 µL of equal volume of chloroform/ethanol (3:5, V/V) was added to it and vortexed for 10 seconds.[20] The extraction mixture was centrifuged at 12000 xRCF (xg) (10519 RPM) for 30 min at +4 °C. After phase separation, enzyme activity and protein determination were made in the clear upper part. Reagent mixture was prepared by mixing 0.45 mg/mL Stock Xanthine, 0.6 mM EDTA, 150 µM NBT, 400 mM Na_2CO_3 and 1 mg/mL BSA. Then, samples were prepared with the reagent mixture and incubated for 20 min at room temperature (25 °C) in the dark. 0.8 mM $CuCl_2$ was added at the end of the incubation. The results were immediately read at a wavelength of 560 nm in a Multiskan Go microplate reader of Thermo Fischer company. Results were calculated according to the SOD activity inhibition rate. Enzyme activity was calculated considering that 50% inhibition provides 1U activity. SOD enzyme activity was divided by protein amounts and the results were given as U/mg protein.

Determination of advanced oxidation protein products (AOPP)

The spectrophotometric method described by Witko-Sarsat et al.[21] was used to measure AOPP levels.[19] The supernatant obtained after tissue homogenization was diluted with potassium phosphate pH=7.4 buffer at the appropriate rate. Diluted samples were vortexed by adding 1.16 M KI and acetic acid, and spectrophotometric measurements were made against blank at 340 nm absorbance. 0-100 µM Chloramine T standards were run as a sample. The results were calculated from the standard curve and given as µmol/g protein.

Determination of total sulfhydryl (TSH)

The spectrophotometric method described by Taylan and Resmi[22] was used to determine the total sulphhydryl content. The supernatant obtained after tissue homogenization was dissolved with an equal volume of 1:1 (v/v) 6% SDS. Afterwards, 4 mg/mL DTNB was added to the samples and incubated for 15 minutes at room temperature in the dark. At the end of the incubation, spectrophotometric measurements were made against the blank at a wavelength of 412 nm in the Multiskan Go cuvette reader of Thermo Fischer company. Standards of 0-500 µM reduced glutathione (GSH) were run as samples. The results were calculated from the standard curve and given as µmol/mg protein.

Determination of protein

The amount of protein in the samples was measured according to the Lowry method (ref). 2 mL of the reagent mixture (0.1 N NaOH in 0.5 mL 2% Na_2CO_3, 0.5 mL 2% Na-K tartrate, 16.5 mL 1% $CuSO_4$) was added to the appropriately diluted supernatant and vortexed, and incubated for 15 min at room temperature in the dark. At the end of the incubation, 200 µL of Folin ciocalteu's phenol was added and mixed and incubated in the dark for 30 min at room temperature. Samples were read in a microplate reader (Multiskan Go of Thermo Fischer) at a wavelength of 750 nm. The results were calculated on the standard curve prepared with bovine serum albumin and given as mg/mL.[23]

Statistical Analysis

One-Way ANOVA test was used to analyze quantitative variables. *P*-value <0.05 was considered significant. Multivariate test was used to compare pre- and post-treatment variables and because it contained repetitive variables. *P*-value <0.05 was considered significant. All data were analyzed by SPSS Statistics for Windows, version 22.0 software (Chicago, IL, USA).

RESULTS

Histopathological Findings

Vaginal tissues belonging to the Sham group were in normal histological structure (Figure 1A, B). Vaginal epithelial thickness was 119.71±5.28 µm in the sham group. In the histopathological examination of the ovariectomy group, it was observed that the vaginal epithelium was 1-2 rows. (Figure 1C, D). Vaginal epithelial thickness was 17.45±1.42 µm in the ovariectomy group. It was statistically significantly decreased in the ovariectomy group compared to the sham group (p=0.000). In the ovariectomy + myricetin group, the vaginal epithelium had a 2-3 layered appearance (Figure 1E, F). Vaginal epithelial thickness was 23.57±1.60 µm in the ovariectomy + myricetin group. When the ovariectomy group and the ovariectomy + myricetin group were compared, a statistically significant increase in epithelial thickness was observed in the myricetin treatment group (p=0.002). In the ovariectomy + HA group, the vaginal epithelium was 2-3 layered, similar to the ovariectomy + myricetin group (Figure 1G, H). Vaginal epithelial thickness was 21.33±1.02 µm in the ovariectomy + HA group. Compared with the ovariectomy group, the increase in epithelial thickness in the ovariectomy + HA group was statistically significant (p=0.012). There was no statistically significant difference between the ovariectomy + myricetin and ovariectomy + HA groups (p>0.05). Vaginal epithelial thickness was shown in Table 1.

Immunohistochemical Findings

Mild staining was detected with anti-Caspase-3 antibody in the sham group (Figure 2A). In the ovariectomy group, the intensity of the staining increased (Figure 2B). When the sham and ovariectomy groups were compared, a statistically significant increase was observed in the staining intensity with anti-Caspase-3 antibody (p=0.002). Staining intensity was decreased in the ovariectomy + myricetin group compared to the ovariectomy group (Figure 2C). When the ovariectomy and ovariectomy + myricetin groups were compared, a statistically significant decrease was observed (p=0.002). Staining intensity

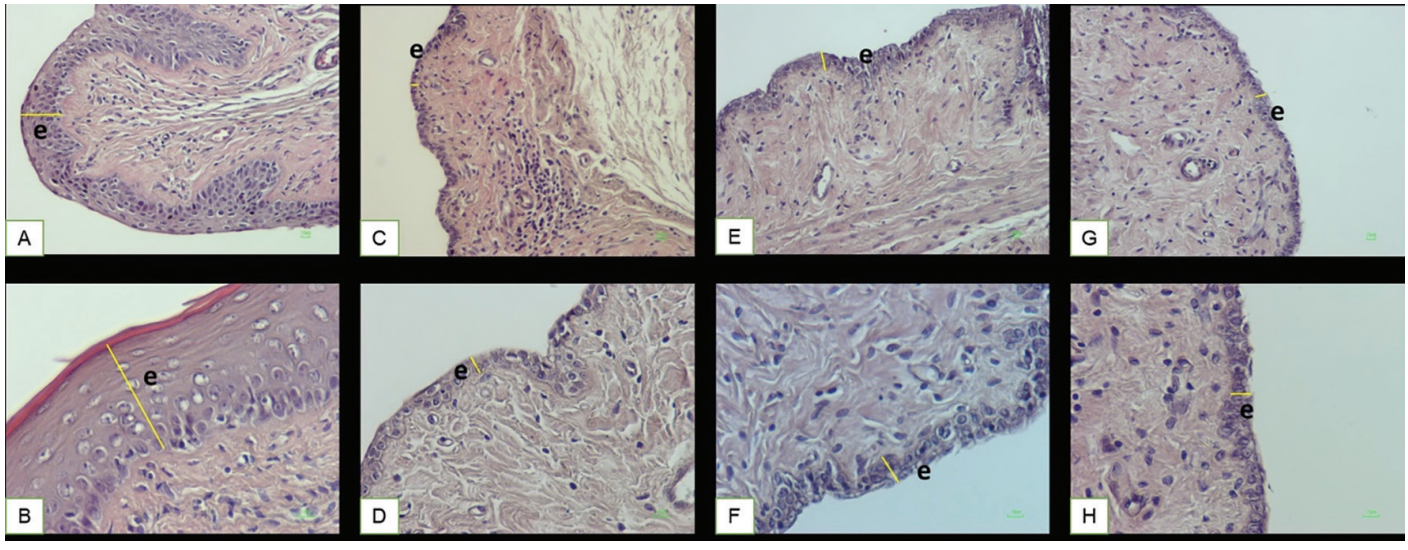

Figure 1. Vaginal epithelium (e) consist of 6-8 layered squamous cells. A. Group 1: Sham, H-E; X20. B. Group 1: Sham, H-E; X40. C. Group 2: Ovariectomy, H-E; X20. D. Group 2: Ovariectomy, H-E; X40. E. Group 3: Ovariectomy + Myricetin, H-E; X20. F. Group 3: Ovariectomy + Myricetin, H-E; X40. G. Group 4: Ovariectomy + Hyaluronic acid, H-E; X20. H. Group 4: Ovariectomy + Hyaluronic acid, H-E; X40

was decreased in the ovariectomy + HA group compared to the ovariectomy group (Figure 2D). When the ovariectomy and ovariectomy + hyaluronic groups were compared, a statistically significant decrease was observed (p=0.041). When the ovariectomy + myricetin groups were compared with the ovariectomy + hyaluronic groups, no statistically significant change was observed in the staining intensity with anti-Caspase-3 antibody (p>0.05). H-score values were shown in Table 2.

Vaginal Maturation Index

Vaginal maturation index value, when the samples taken were compared, the differences in the data were found to be statistically significant (p=0.000). Vaginal maturation index values were shown in Table 3.

Biochemical Findings

When the SOD value was compared among all four groups, 41.3±11.07 U/mg protein in the sham group, 42.51±10.64 U/mg protein in the ovariectomy group, 45.41±26.94 U/mg protein in the ovariectomy + myricetin group, while it was found to be 34.73±3.09 U/mg protein in the ovariectomy + HA group. No statistically significant difference was found between the groups (p=0.663). When the AOPP value was compared among all four groups, 22.22±7.18 µmol/g protein in the sham group, 20.34±11.22 µmol/g protein in the ovariectomy group, in the ovariectomy + myricetin group 21.87±14.78 µmol/g protein, while it was 17.42±2.67 µmol/g protein in the ovariectomy + HA group. No statistically significant difference was found between the data of the four experimental groups (p=0.793). TSH value was 119.76±46.92 µmol/mg in the sham group, 101.98±31.93 µmol/mg in the ovariectomy group, 98.40±35.01 µmol/mg in the ovariectomy + myricetin group and it was calculated as 122.47±46.01 µmol/mg in the ovariectomy + HA group. Biochemical values were shown in Table 4. No statistically significant difference was observed between the groups by means of TSH levels (p=0.650). When the experimental groups were compared, a statistically significant difference was found after injection compared to after ovariectomy in vaginal pH value (p=0.022). Vaginal pH values were shown in Table 5.

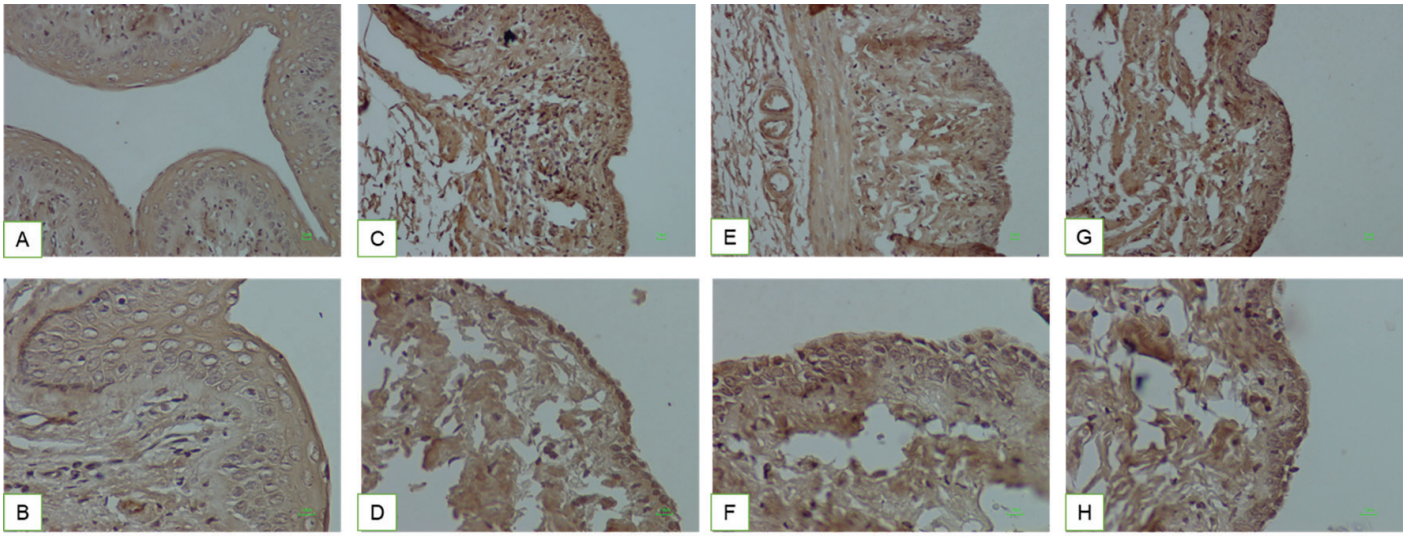

Figure 2. A. Group 1: Sham, anti-Caspase-3; X20. B. Group 1: Sham, anti-Caspase-3; X40. C. Group 2: Ovariectomy, anti-Caspase-3; X20. D. Group 2: Ovariectomy, anti-Caspase-3; X40. E. Group 3: Ovariectomy + Myricetin, anti-Caspase-3; X20. F. Group 3: Ovariectomy + Myricetin, anti-Caspase-3; X40. G. Group 4: Ovariectomy + Hyaluronic acid, anti-Caspase-3; X20. H. Group 4: Ovariectomy + Hyaluronic acid, anti-Caspase-3; X40

Table 1. Vaginal epithelial thickness (µm)

Groups	Vaginal epithelial thickness (µm)
Group 1: Sham	119.71±5.28
Group 2: Ovariectomy	17.45±1.42[a]
Group 3: Ovariectomy + myricetin	23.57±1.60[a,b]
Group 4: Ovariectomy + hyaluronic acid	21.33±1.02[a,c]

Data are expressed arithmetic mean ± SEM (n=7).
[a]=p=0.000 Group 1 vs. Groups 2, 3, 4; [b]=p=0.002 Group 3 vs. Group 2; [c]=p=0.012 Group 4 vs. Group 2

DISCUSSION

According to current literature, our study is the first to examine and compare the effects of both myricetin and HA in experimental rat menopause models. Our study also reports the improval of vaginal Ph, vaginal maturation index, vaginal epithelial thickness and vaginal epithelial nucleus morphological changes after myricetin application in rats which were confirmed to have vaginal atrophy after menopause model construction. Wang et al.[24] reported that HA injection reduced oxidative stress markers, nevertheless it did not make a difference in SOD levels in osteoarthritis synovial fluid. In our study, we also observed that both myricetin and HA did not create a significant difference in SOD levels in menopausal rat vaginas. This finding might be due to short interval between injections and vaginectomies. Almeida et al.[25], demonstrated that AOPP levels induced by myocardial infarction do not change with estradiol injection in female rats that underwent ovariectomy. In our study, we observed that myricetin and HA did not make a significant difference in AOPP levels in rat vaginas meanwhile the histopathological and immunohistochemial findigs are promising. Those irrevelant findings might also be due to short interval between injections and vaginectomies. Korkmaz et al.[26] reported that oxytocin which is being used for its anti-inflammatory effects, did not affect thiol levels in animals under chronic stress, but decreased serum levels in rats under acute stress. In despite of these results when TSH levels were analyzed, no statistically significant difference was observed in our study. This finding might be due to gradual settlement of atrophy after menopause model application in rats.

Nappi et al.[27] reported that an improvement in vaginal pH was observed after HA-based pessary treatment on vulvovaginal atrophy in postmenopausal women. In our study, our findings

Table 2. H-score values

Groups	H-score
Group 1: Sham	198.33±15.05
Group 2: Ovariectomy	328.67±15.25[a]
Group 3: Ovariectomy + myricetin	277.5±25.64[a,b]
Group 4: Ovariectomy + hyaluronic acid	293.33±33.4[2a,c]

Data are expressed arithmetic mean ± SEM (n=7).
[a]=*p*=0.002 Group 1 vs. Groups 2, 3, 4; [b]=*p*=0.002 Group 3 vs. Group 2;
[c]=*p*=0.041 Group 4 vs. Group 2

Table 3. Vaginal maturation index values

Groups	Before overectomy	After overectomy	After injection
Group 1: Sham	73.5±11.53	80.3±7.83	75.00±13.30
Group 2: Ovariectomy	81.78±7.59	2.25±0.64[a]	3.50±1.77[a]
Group 3: Ovariectomy + myricetin	78.21±7.59	3.71±3.08[a]	57.14±17.52[a]
Group 4: Ovariectomy + hyaluronic acid	78.57±9.33	5.64±2.79[a]	42.85±11.49[a]

Data are expressed arithmetic mean ± SEM (n=7).
[a]=*p*=0.000 Group 1 vs. Groups 2, 3, 4

Table 4. Biochemistry values

Groups	SOD (U/mg)	TSH (µmol/mg)	AOPP (µmol/g)
Group 1: Sham	41.33±11.07	119.76±46.92	22.22±7.18
Group 2: Ovariectomy	42.5±10.6	101.98±31.9	20.3±11.2
Group 3: Ovariectomy + myricetin	45.41±26.94	98.4±35.01	21.87±14.78
Group 4: Ovariectomy + hyaluronic acid	34.73±3.09	122.47±46.01	17.42±2.67

Data are expressed arithmetic mean ± SEM (n=7).
SOD=super oxide dismutase; TSH=total sulfhydryl; AOPP=advanced oxidation protein products

Table 5. Vaginal pH values

Groups	Before overectomy	After overectomy	After injection
Group 1: Sham	5.02±0.66	5.35±0.59	5.22±0.44
Group 2: Ovariectomy	4.84±0.72	5.4±0.70	5.90±0.57[a]
Group 3: Ovariectomy + myricetin	4.60±0.56	5.50±0.60	5.90±0.57[a]
Group 4: Ovariectomy + hyaluronic acid	4.7±0.39	5.32±0.33	4.87±0.51[a]

Data are expressed arithmetic mean ± SEM (n=7).
[a]=*p*=0.022 Group 1 vs. Groups 2, 3, 4

are relevant and we found that HA caused a statistically significant improvement in vaginal pH.[27] Hersant et al.[28] reported that in postmenopausal women with a history of breast cancer, HA and platelet-enriched concentrate treatment caused an improvement in vaginal pH. In our study, findings are relevant with the literature and it was also reported that the effect of myricetin on pH was more effective than the HA application.

Doğanay et al.[29] reported that estrogen and vitamin E treatment increased vaginal maturation index on postmenopausal women in cervicovaginal smear preparations. In our study, an increase in the maturation index was observed in the cervicovaginal smear preparations of both HA-treated rats and myricetin-treated rats.[29] Tersigni et al.[30] reported that vaginal epithelial thickness measurements improved after the Isoflavones (Perilei Pausa®) application.

In the findings of this study, which was conducted with the treatment strengthening hypothesis by combining HA, the vaginal epithelial thickness measurements obtained after treatment were positive and were similar to the effects on epithelial thickness in the HA group in our study.[30]

Lima et al.[31] demonstrated that isoflavone gels could be effective on atrophic changes of the vagina by an increase in the thickness of the vaginal epithelium. Myricetin is also in the isoflavone class, and our findings on the thickness of the vaginal epithelium were similar with the findings of this study. The changes in the vaginal epithelium might be due to anti-oxidative and anti-apoptotic properties of isoflavones.[31] Liu et al.[32] reported that HA gel could be effective an increase on vaginal epithelial thickness in ovariectomized rats. In this study, Western blot analysis performed in rats treated with HA gel. In our study, we observed that myricetin increased epithelial thickness with anti-inflammatory and anti-apoptotic effects, and its effect on vaginal HA concentration is still a matter of curiosity. Through its anti-oxidative and anti-apoptotic effects, menopausal aging effects like changes in vaginal pH, vaginal maturation index, vaginal epithelial thickness, vaginal epithelial nucleus morphological changes were improved by myricetin.

CONCLUSION

In our study, by examining the effects of myricetin and HA, we detected that myricetin is as effective as HA in the treatment of GUS, and that it is an effective substance that can be used in cases where HA and estrogen are contraindicated.

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ETHICS

Ethics Committee Approval: The experiments were conducted at the Department of Experimental Animals Application and Research Center. The Muğla Sıtkı Koçman University Ethics Committee for Animal Research approved the animal protocol for this study (approval no: 06/22).

Informed Consent: Animal research investigations were guided in line with the Committee for Human Care.

Contributions

Surgical and Medical Practices: S.K.M., M.N.A.; Concept: S.K.M., B.K.; Design: S.K.M., B.K.; Data Collection or Processing: S.K.M., D.Ç., M.N.A.; Analysis or Interpretation: S.K.M., B.K., H.E., D.Ç., Ü.Ö.T.; Literature Search: S.K.M.; Writing: S.K.M., B.K., A.A.S.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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Evaluation of ovarian reserve parameters in patients who underwent detorsion because of ovarian torsion

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ABSTRACT

Objectives: Ovarian torsion is a gynecologic emergency that can cause loss of ovarian function. The aim of the study was to evaluate the ovarian reserve of the patients who were operated for detorsion because of ovarian torsion.

Materials and Methods: The medical records of the patients who underwent detorsion for ovarian torsion in our clinic between January 2013 and June 2017 were investigated. Ovarian reserve parameters including ovarian volume, antral follicle count and ovarian artery resistance index (RI) were evaluated by ultrasonography in post-operational period. Patients that had oophorectomy due to torsion or who had another ovarian surgery before or after torsion and pregnant women were not included in the study. The ovarian volume, antral follicle count and ovarian artery RI in the operated side were compared with the contralateral ovary.

Results: Thirty-four women meeting the inclusion criteria were examined by ultrasonography in the early follicular phase of their cycles. Antral follicle count, ovarian volume and ovarian artery RI were decreased significantly ($p<0.001$, $p<0.001$ and $p<0.001$, respectively) in the operated ovary compared to the contralateral ovary. Sixteen patients who were treated by detorsion, underwent cystectomy at the same time. No significant difference was observed in ovarian reserve of the patients who had cystectomy together with detorsion compared to those who were operated only with detorsion.

Conclusion: Antral follicle count, ovarian volume and ovarian artery RI were significantly decreased after detorsion operation in the operated side compared to the contralateral ovary.

Keywords: Ovarian torsion; detorsion; ovarian reserve; antral follicle count; ovarian volume

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INTRODUCTION

Ovarian torsion is a gynaecologic emergency as a result of rotation of adnexal structures or the ovary on its ligamentous support.[1] Its prevalence is 2.7% and is usually seen in reproductive-age women.[2]

Ovarian torsion may cause necrosis, peritonitis, and ovarian function loss. Therefore, a timely diagnosis is important to maintain ovarian function and future fertility.[3] However, because of non-specific signs and symptoms, diagnosis and surgical intervention may be delayed. Colour Doppler ultrasonography might be useful that an abnormal flow or the absence of ovarian blood flow is detected when correlated with clinical findings.[4]

In ovarian torsion, damage to ovarian tissue occurs because of both ischemia and reperfusion after detorsion. In ovarian detorsion, reactive oxygen species accumulate in cells and lead to a decrease in the hormonal function of the ovary and ovarian reserve.[5]

In the current study, we aimed to evaluate ovarian reserve by counting antral follicle count (AFC), estimating ovarian volume, and assessing the ovarian artery resistance index (RI) in the operated ovary compared to the contralateral ovary.

MATERIALS AND METHODS

The medical records of the patients who underwent detorsion for ovarian torsion in our clinic between January 2013 and June 2017 were investigated. Patients treated with detorsion between the age 20-35 years were included in the study. The exclusion criteria were as follows: Patients treated with oophorectomy, those with a history of ovarian surgery before or after ovarian torsion, and those who were pregnant. The study was approved by the Local Ethics Committee of University of Health Sciences Türkiye, Taksim Training and Research Hospital (date: 07.02.2018, no: 101). All participants provided written informed consent, according to principles outlined by the Declaration of Helsinki (2013).

There were 48 patients operated on the relevant dates in our clinic because of ovarian torsion. A total of 34 patients who met the inclusion criteria were evaluated by ultrasonography (DC-8 EXP Ultrasound System; Mindray Medical, Colombia). All transvaginal ultrasounds (TVUSGs) were performed by the same specialist (NK) during the follicular period of the menstrual cycle (3^{rd}-5^{th} days of the cycle). The specialist who performed TVUSG was not informed about on which side of the ovary the surgery was performed. The following parameters were evaluated using TVUSG:

1. The AFC of the detorsioned ovary and the ovary on the other side-follicles between 2 and 10 mm in the ovary were evaluated as antral follicles.

2. The volumes of the detorsioned ovary and the ovary on the other side (length X, width X, and depth X 0.52) were calculated using the method defined by Gohari.[6]

3. The RIs of the detorsioned ovary and the ovary on the other side were calculated. Ovarian arteries were seen in the infundibulopelvic ligament in the inferolateral ovary.[7] The RI was measured three times and averaged after waveforms were seen. It was calculated by dividing the peak systolic flow and end-diastolic mean blood flow by the peak systolic blood flow.

AFC, ovarian volume, and RI were compared between the detorsion ovary and contralateral ovary. Comparison of ovaries operated on with detorsion and cystectomy with detorsion. Additionally, the same parameters were compared between the ovaries operated on with detorsion and the ovaries performed on with detorsion cystectomy. Demographic characteristics of the patients, sides operated, whether or not concomitant cystectomy was applied, and operation method were evaluated.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) for Windows 20.0 program was used for statistical analysis of the findings obtained from the study. The distribution of continuous variables was evaluated by the Kolmogorov-Smirnov test. The variables with a normal distribution and belonging to two groups were compared with paired-samples t-test. The Mann-Whitney U test was used to compare variables without a normal distribution. Continuous variables were given as mean ± standard deviation; $p<0.05$ was considered statistically significant.

RESULTS

Our study was performed on 34 patients who underwent detorsion because of torsion. The mean patient age was 27.9±7.3 years. The mean parity number was found to be 1.3±1.2. The period from the operation to the evaluation of TVUSG was 15.5±12.8 months. As presented in Table 1, 16 of the 34 patients were operated with concomitant cystectomy. Torsion was observed in the right side in 58.8% of the patients and in the left side in 41.2%. None of the patients required a reoperation.

The mean AFC was 3.4±2.0 in the detorsioned ovary and 7.1±2.8 in the ovary on the other side. The mean AFC was significantly lower in the detorsioned ovary compared to the ovary on the other side ($p<0.001$). Ovarian volume was found to be 5.5±3.1 cm^3 in the detorsioned ovary and 11.2±4.8 cm^3 in the ovary on the other side. The volume of the detorsioned ovary was significantly

lower (p<0.001; Table 2). The ovarian artery RI was 1.06±0.2 in the detorsioned ovary and 0.80±0.1 in the ovary on the other side. The ovarian artery RI in the detorsioned ovary was significantly higher than that in the ovary on the other side (p>0.001).

Sixteen patients underwent concomitant cystectomy with detorsion. Of 16 of these cystectomy pathology results, 7 were reported as dermoid, 5 as serous cystadenoma and 4 as corpus luteum cyst. Patients who underwent concomitant cystectomy with detorsion did not show any difference in terms of AFC, ovarian volume, and ovarian artery RI compared to those who underwent only detorsion (p=0.360, p=0.574, p=0.673, respectively). No difference was found between the patients who underwent only detorsion and who underwent concomitant cystectomy with respect to the AFC and volume difference of the contralateral ovary (Table 3).

Table 1. Demographic data of the patients

	n=34
Age (years)	27.9±7.3
Gravida (n)	1.4±1.2
Parity (n)	1.3±1.2
Torsion side Right ovary Left ovary	 20 (58.8%) 14 (41.2%)
Operation Laparotomy Laparoscopy	 25 (73.5%) 9 (26.5%)
Operation Detorsion+cystectomy Detorsion	 16 (47%) 18 (53%)
Time after surgery (months)	15.5±12.8

Data are expressed as mean (± standard deviation) or number (%)

DISCUSSION

In ovarian torsion, damage to the ovarian tissue occurs because of torsion and reperfusion after detorsion. Because ischaemia, congestion, haemorrhage, and necrosis would occur in the tissue because of torsion, this also reflects on ovarian functions and affects them negatively. Nowadays, the treatment approach in ovarian torsion is detorsion even if it purports as ovary necrosis, and thus, it is aimed to protect ovarian functions and reserve.[8] Ovarian reserve tests are known to represent the ovarian follicle pool that remains in the ovary.[9] The commonly used ovarian reserve tests are antimullerian hormone, AFC, and basal follicle stimulating hormone.[10,11] In this study, we aimed to evaluate the ovarian volume, AFC, and ovarian artery RI of the detorsioned ovary compared to the ovary on the other side. We found that the AFC and ovarian volume were significantly decreased in the detorsioned ovary compared to the ovary on the other side and observed that the ovarian artery RI was significantly increased in the detorsioned ovary compared to the ovary on the other side. We found that the application of cystectomy or only detorsion had no effect on these parameters.

In their study that evaluated the ovarian parenchyma of the patients on whom oophorectomy was performed because of torsion, Galinier et al.[12] have shown that in most of the cases, although ovaries seemed necrotic macroscopically, they were viable microscopically. They have concluded that applying a conservative approach by the detorsion of ovaries with a blue-black appearance is safe and effective. However, in terms of ovarian reserve, the presence of a viable tissue in the detorsioned ovary may not be a sign of a healthy cohort pool.[13] As shown in many studies, not only ischaemia but reperfusion may also adversely affect the follicle cohort.[14] The basic mechanism

Table 2. Ovarian reserve parameters of torsioned ovary compared to contralateral ovary

	Torsioned ovary	Contralateral ovary	p-value
Antral follicle count (n)	3.4±2.0	7.1±2.8	**<0.001**
Ovarian volume (cm^3)	5.5±3.1	11.2±4.8	**<0.001**
Ovarian artery RI	1.06±0.2	0.80±0.10	**<0.001**

RI: Resistance index, data are expressed as mean (± standard deviation), p<0.05 was considered statistically significant

Table 3. Comparison of ovaries operated with detorsion and cystectomy versus with detorsion

	Detorsion+cystectomy (n=16)	Detorsion (n=18)	p-value
Age	28.1±8.26	28.1±6.03	0.996
Antral follicle count (n)	2.5 (2)	4.0 (2)	0.360
Ovarian volume (cm^3)	5.0 (5.6)	5.4 (3.7)	0.574
Ovarian artery RI	1.10 (0.3)	1.10 (0.3)	0.673

RI: Resistance index, data are expressed as median (interquartile), p<0.05 was considered statistically significant

here is the free radicals and reactive oxygen species formed by providing excessive oxygen with reperfusion to the tissue that is ischaemic tiessue.[15] In addition, some authors claim that damage to ovarian tissue increases after detorsion.[16]

In a study that included the highest number of patients on follicular development after detorsion (102 patients), more than 90% of ovaries were found to have a normal follicular development.[17] This study demonstrates follicle development, but no evaluation has been made for ovarian reserve.

Although there is no ideal test showing ovarian reserve, it is typically evaluated by hormonal tests and ultrasonographically.[18] According to the design of the current study, evaluation of AFC was the most appropriate approach. Whether or not there is a change in ovarian reserve compared to the AFC in normal ovaries of the patients was presented. In our study, we found that AFC was significantly decreased in the torsioned ovary compared to the ovary on the other side. In contrast to the study where Bozdag et al.[17] have evaluated AFC after 18 detorsions, no difference was found between the mean AFC of the torsioned and normal ovaries. However, in this study, AFC was greater than 12 in eight patients, suggesting that a significant part of this patient group could be having polycystic ovary syndrome and that the change in AFC would not be obvious because of the high follicle cohort in these patients after detorsion.[18] Similarly, in a study where Yasa et al.[5] evaluated AFC after 11 detorsions, it was found that the mean AFC that they observed in the first month post-operatively decreased compared to the normal ovary, although they did not reach statistical significance. Another factor may be the time passed from the beginning of the symptoms of the patients to the surgery because the change in ovarian reserve is associated with the duration of torsion. Yasa et al.[5] found the mean time to surgery as 13.1±9.5 hours. The fact that these cases were diagnosed early and were rapidly taken into operation may be another reason for the absence of a change in ovarian reserve. We cannot comment on whether or not there was a delay in the diagnosis and operation decision in the patient group because we have not seen patients who underwent surgery before the operation and did not question these times, which is one of the limitations of our study.

According to a recent study, ovarian reserve can recover with time even though a significant decrease might initially be noticed after ovarian surgery.[19] Therefore, we evaluated ovarian reserve at least 6 months after the operation.

Cagnacci et al.[20] evaluated patients undergoing laparoscopic cystectomy by ovarian volume, AFC, and ovarian artery RI. They concluded that cystectomy was associated with decreased ovarian reserve, regardless of the histological type and size of the removed cyst.20 Cystectomy was performed in addition to detorsion in 14 of the 34 patients, but when we compared the detorsion-only group with the detorsion-cystectomy group, we found no difference between the two groups in terms of AFC, ovarian volume, and ovarian artery RI. Therefore, we can say that the decrease in AFC and ovarian volume is only due to damage secondary to torsion.

Ovarian velocimetry is not frequently used in the evaluation of post-operative damage. The RI is an indicator of vascular resistance to blood flow and is associated with flow in the microvascular bed.[21] Studies of endometrioma and non-endometrioma cystectomy have also shown increased RI in the operated ovary.[22] In our study, similar to these two studies, the RI of the detorsioned ovary was significantly higher.[21] The differences in the RI between the torsioned ovary and the ovary on the other side may be due to volume differences between the two.

Study Limitations

The most important limitation of our study is that these patients were not evaluated pre-operatively. Moreover, there was no record of the time between the beginning of the symptoms of the patients to the operation. Another limitation of our study is that the time elapsed after surgery and the time the patients were evaluated differed due to the design of our study. However, the strength of our study is the lack of previous studies on this topic and the comparison of both detorsion ovaries, detorsion and cystectomized patients concerning surgical technique. Another strength is that a single operator evaluated all patients.

CONCLUSION

Ovarian reserve based on AFC, ovarian volume, and ovarian stromal flow seems to be negatively affected in patients treated with detorsion because of ovarian torsion. These results showed that ovarian reserve is compromised in patients with ovarian torsion. Clinical studies with larger cohorts should be performed to clarify these results and also serial measurements of these ovarian reserve parameters before and after torsion might highlight the pathophysiological mechanisms.

ETHICS

Ethics Committee Approval: The study was approved by the Local Ethics Committee of University of Health Sciences Türkiye, Taksim Training and Research Hospital (date: 07.02.2018, no: 101).

Informed Consent: Informed consent was obtained from all participants included in the study.

Contributions

Concept: M.K., N.K.; Design: M.K., B.A.Ç.; Data Collection or Processing: M.K.; Analysis or Interpretation: G.Y.; Literature Search: Gö.Y.; Writing: M.K., N.K.

DISCLOSURES

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Urethral ligament plication operation (ULP) for minimal invasive cure of SUI without tapes

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ABSTRACT

Objectives: To present 12-month data of the urethral ligament plication (ULP) operation for cure of stress urinary incontinence (SUI).

Materials and Methods: Thirty-six women, mean age 58.6 years from two centres underwent the ULP operation. Operation: Two full thickness parallel incisions in the vaginal sulcus were made, extending from bladder neck to urethral meatus. The incision was opened out to reveal external urethral ligament and both branches of pubourethral ligaments, which were plicated with no 2 or 3 polyester sutures. The vaginal incisions were closed with polyglactin sutures. The 36 women constitute the learning curve of the surgeons. Inclusion criterion: Demonstrated urine loss on coughing controlled by a hemostat at midurethra. Exclusion criteria: None. Criterion for cure: No urine on coughing with a full bladder.

Results: All women were discharged the of day of surgery. Six-month results 31/36 women (86%) were cured of SUI. Twelve-month results 29/35 women (83%) remained cured of SUI.

Conclusion: The 12-month data is encouraging, especially as it included the learning curve of the authors. Minimum deterioration in SUI cure from 6 to 12 months suggested ongoing new collagen formation in response to the polyester sutures. Absence of tapes and tape related complications has much to recommend this new direction for SUI surgery. The ULP is a low-cost, low-resource operation which requires only moderate skills, and is especially suitable for the developing world. However, further well-monitored studies by other surgeons will be required before this operation can be recommended for general use.

Keywords: ULP operation for SUI; polyester repair of pubourethral ligament; collagen; PUL neoligament

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INTRODUCTION

The midurethral sling (MUS) operation for cure of stress urinary incontinence (SUI),[1] is a day-care surgery procedure, the gold standard for SUI for some 20 years, with some 10 million operations to date, and reported cure rates of 80-90%, with acceptable complications.

Despite its effectiveness, the MUS has become one of the most litigated operations in the history of surgery. The medicolegal issues seem to be based on tissue reactions from tapes, about 2-3%. Yet that is how the MUS works![2,3] The MUS harnesses the wound reaction from an implanted tape to create new collagen, to reinforce collagen-deficient ligaments, a very different modus operandi from mesh sheets, which block organ descent, but do not address root causes of the prolapse.[4]

Serious complications from vaginal implantation of large mesh sheets[4] caused massive condemnations in social media, leading to banning of mesh sheets and in some places, MUS also. Where not banned, increasing numbers of women are refusing even the well-tried MUS.

The two research questions which led to the conception of the urethral ligament plication (ULP),[5] Figures 1-3, were 1. "Would wide-bore No 2 or 3 polyester sutures create sufficient collagen tissue reaction to repair PUL and cure SUI?. 2. "How to do it?" With reference to 1. Review of collagen from a rejected polyester aortic graft as part of a Doctor of Surgery thesis[6] indicated No 2 polyester sutures could produce new collagen two orders of magnitude (X100) beyond normal ligament strength. The answers to the 2nd question, how to do it?, came from a live anatomy study of the pubourethral ligament,[7] Figure 2, during a 1997 MUS operation which was performed via two parallel incisions in the distal vaginal sulci.[8] The anatomical and biomechanical basis of the ULP operation as shown in Figure 1, and "very very early results" were reported previously.[5]

Our aim in this work was to test the ULP operation as proposed, for safety and efficacy, then, based on our experience, to critically examine the possible advantages previously quoted:[5]

1. No tape. Little cost: A polyester suture.

2. Simplicity: Less surgical skill required; local anaesthetic methodology makes the operation widely scalable especially in countries with few facilities.

3. Built-in safety: No tape to compress urethra; no applicators to damage bladder, nerves, blood vessels, bowel.

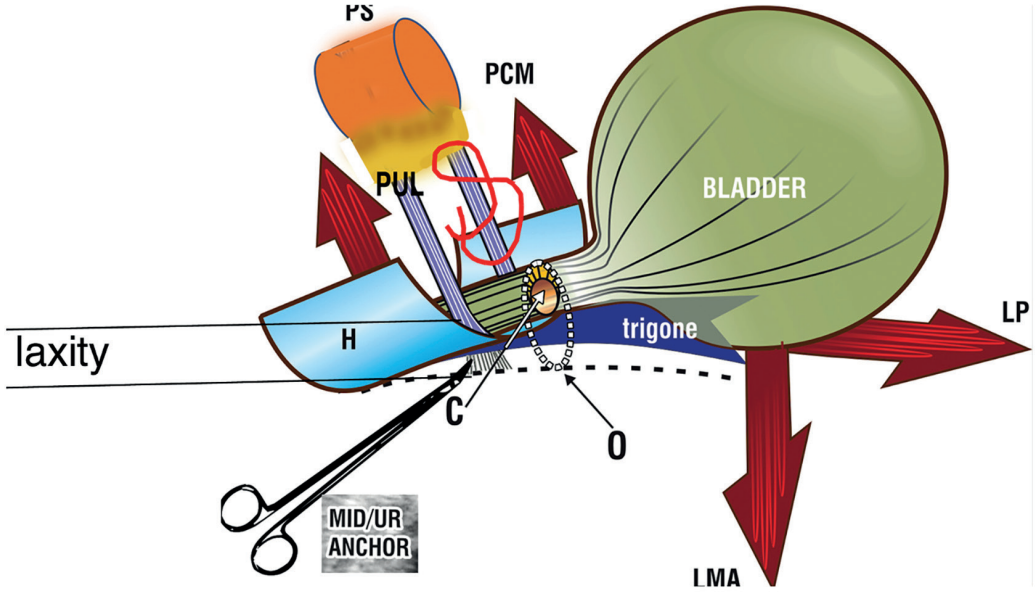

Figure 1A. Sagittal schematic view, woman with stress urinary incontinence (SUI)
At REST (unbroken lines). Pubourethral ligament (PUL) extends from pubic symphysis (PS) to attach to midurethra. STRAIN (broken lines) on effort, LMA pulls down the trigone. A weak or loose PUL is stretched by LMA, opening posterior urethral wall from "C" closed to "O" open; urine is lost (SUI). Hemostat test hemostat inserted behind the symphysis as in the Video 1 mechanically supports PUL. Urethral closure at bladder neck and distally by pelvic muscles (arrows) is restored (white arrow, right ultrasound frame). No 2 or 3 polyester suturing (red) of both PULs would prevent PUL elongating on stress in the same manner as a sling below the urethra
PS=symphysis; C=closed urethra; O=open urethra; H=distal vagina "hammock"; PCM=pubococcygeus muscle; LP=levator plate; LMA=conjoint longitudinal muscle of the anus

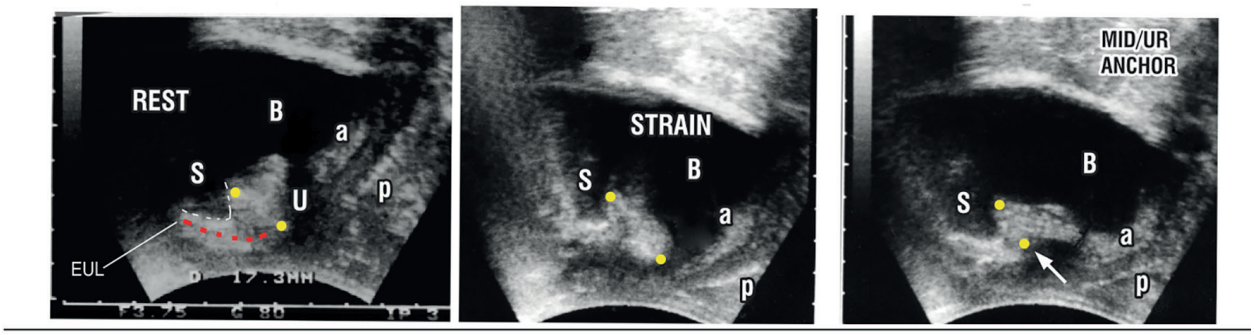

Figure 1B. Transperineal ultrasound of a woman with SUI4
At rest (left frame) S=symphysis; U=urethra;B=bladder; a&p are the anterior and posterior walls of the vagina; the two yellow circles mark length of pubourethral ligament (PUL) extending from behind lower border of the symphysis to the midurethra. Strain (middle frame) PUL extends. a&p are stretched backwards/downwards to open out the posterior wall of the urethra along its length. Mid/urethral anchor (right frame). A hemostat (white arrow) inserted behind the symphysis creates closure at the bladder neck and distally; a&p are obviously tensioned. EUL marks position of the external urethral ligament as it attaches the external meatus to the anterior part of the symphysis; the red broken lines below "S" indicate the position of the distal urethra

METHODS

The ULP surgical study was approved by the Ethical Committee of Muğla Sıtkı Koçman University (date and number: 8th June 2022 & 11/VII). Patient permission was obtained to publish de-identified surgical results and videos. Trial registration details: ClinicalTrials ID: ID05733052.

ULP surgery was performed in 36 women with SUI from two different locations, Türkiye (n=30, using No 3 polyester sutures), and Australia (n=6, using No 2 polyester sutures). Surgery was performed under spinal anesthesia, following the same surgical protocol described.[5] The main inclusion criterion was urine loss on coughing controlled by a hemostat behind the symphysis, Figures 1A, B. There were no exclusion criteria. The criterion for cure was no urine loss on coughing with a full bladder.

Technique

After vulvar and vaginal cleansing, under spinal anaesthesia, with patients in lithotomy position, full thickness vaginal incisions were made in the periurethral sulci extending from bladder neck to the urethral meatus. The incision was opened with dissecting scissors to reveal descending and midurethral branches of pubourethral ligament, Figure 2. Local anesthetic (LA) infiltration of the sulcus was used only in the Australia cohort (n=6).

With reference to Figure 2, the operation was performed in a space of approximately 2.5 cm^2. A No 3 polyester suture (AAS) or No 2 (RH) was inserted into the urethral part of pubourethral ligament, then pubic part of pubourethral ligament, then external urethral ligament (EUL), then laterally into the pubococcygeus muscle, then tied, but not tightly (Figure 3). Incisions were closed

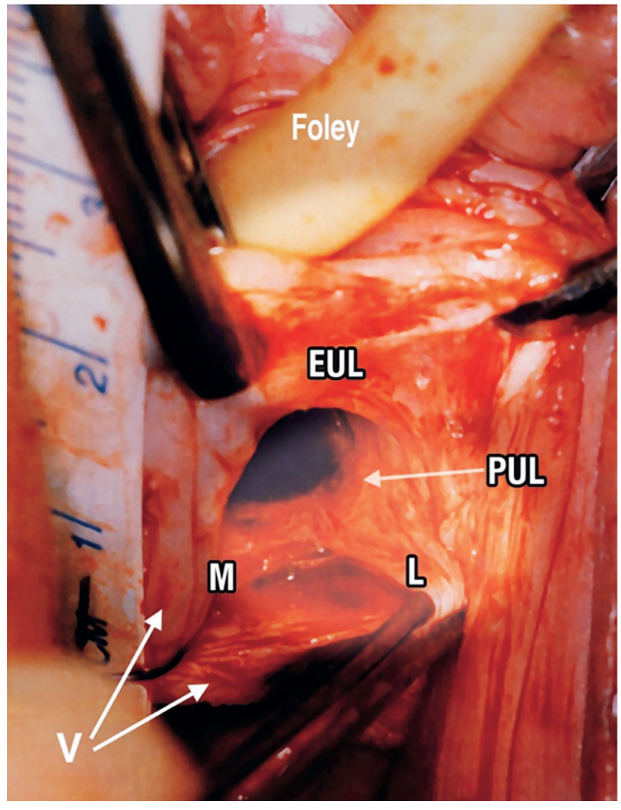

Figure 2. Live anatomy-surgical binding of loose PULs
Original live anatomical dissection of PUL from the two incision IVS operation.[8] The tape measure overlies the urethra. The left paraurethral sulcus has been incised along its length and opened out laterally with forceps. EUL is the external urethral ligament which sits in front of the pubic symphysis (PS) and is attached to the external urethral meatus. The pubourethral ligament (PUL), originates behind PS, 1.5 cm from its lower border. Coming down from PS, PUL splits into two parts, medial (M) to insert into the side of the midurethra and L (lateral). "L" attaches laterally to pubococcygeus muscle (not seen) then down to attach to the vagina (V)

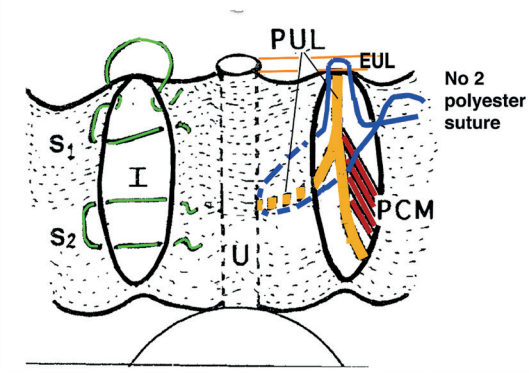

Figure 3. ULP operation. Perspective: Looking into the anterior vaginal wall. Two full thickness parallel incisions "I" are made in each sulcus to the level of bladder neck, and are opened out. The midurethral and descending branches of the pubourethral ligament (PUL) are located and sutured with no 2 or no 3 wide-bore polyester sutures as indicated, into the midurethral part of PUL, then its upper part, then into the external urethral ligament (EUL), then into the medial part of pubococcygeus muscle PCM and tied, but not too tightly.
The vaginal skin is sutured with two vicryl sutures S1 and S2.
U=urethra; PCM=pubococcygeus muscle. EUL is the external urethral ligament inserting into the urethral meatus "U" on the anterior surface of the pubic symphysis Copyright Peter Petros published by permission

with vicryl sutures. In 6 women, the ULP operation was carried out with 300 mL in the bladder. In these 6 women, the No 18 Foley catheter was inserted and removed as required, so cough tests could be carried out for continence at relevant stages of the operation, before starting, after the polyester suture on one side, then after suturing was completed on both sides.

Statistical Analysis

The SPSS.22 program was used for the analysis of the dataset. Statistical analysis was conducted using standard descriptive statistical methods for continuous quantitative variables (mean, standard deviation). Categorical variables (frequency of occurrence) were presented with frequencies and percentages of the total. The evaluation of quantitative measurements was performed using the "Student's t-test". *P*-value was set at <0.05 for statistical significance.

RESULTS

The 36 cases constitute the learning curve for both surgeons (AAS and RH).

Age: 58.6±16.4 years

BMI: 27.3±14.2 kg/m^2

Parity: 2.6±3.4

Preoperative UDI-6 score: 64.58±9.87;

Postoperative (6 months) at 6 months post-operatively, 31/36 (86%) women with leakage on cough test were cured of SUI There were 5 surgical failures, 4 immediately post-operatively, and one after 3 months. UDI-6 score (6 months): 18.75±15.50 (p=0.001). Though classified as failure at 3 months because of a positive cough test, the self-assessed 75% improvement in her SUI, was, for her, a satisfactory outcome. No post-operative retention problems were reported.

Postoperative (12 months) at 12 months post-operatively, 29/35 (83%) remained cured of their SUI. There were 6 surgical failures, 4 immediately post-operatively, one at 3rd months, and one at 7th month. One patient was not able to be contacted. UDI-6 score: (12 months) 16.66±13.40 (Student's t-test p=0.001).

With intra-operative testing 300 mL in the bladder (n=6) prior to surgery commencing, a hemostat behind the symphysis as in Video 1) confirmed urine loss on coughing. Testing after the polyester suture was applied on one side showed restoration of continence in the 3 women in whom the test was able to be successfully completed. Urine leakage on coughing ceased after the sutures were tied on one side.

DISCUSSION

We have demonstrated that cure of SUI by native tissue repair of pubourethral ligaments (PUL), using wide-bore polyester sutures to structurally reinforce weakened PULs, is possible, without the need for a tape. Minimum deterioration in SUI cure from 6 to 12 months is encouraging. It isuggests ongoing new collagen formation in response to the polyester sutures has lasted well beyond the 2-6 month post-operative period, where 40% SUI failure was seen in women where the collagenopoietic stimulus (tape) had been removed.[2]

The TVT data indicate, that once collagen 1 has formed, the restoration of continence is likely to continue.[9] However, this has to be proven by longer-term studies in the ULP operations.

The MUS and ULP are based on the same anatomical principle, surgical repair of the pubourethral ligament "PUL",[5] but with one important difference. The MUS, creates a completely new, U-shaped neocollagenous PUL, whereas the ULP uses a large bore polyester suture to repair PUL and to prevent it from lengthening, Figures 1A, B (Frame 2).

The ULP operation avoids serious problems reported with MUS, such as urethral perforations by tapes, urinary retention, nerve and vascular damage by instruments. Instead, it relies on anatomically accurate confinement of ligaments (PUL, EUL), to restore the muscle forces important for continence,[2] (3 large arrows, Figure 1A). The ULP operation can fail if the polyester

suture is too loose, as seems to have occurred in 4 women in this study who were discharged still leaking on coughing. The suture needs to secure the PUL close to its origin and insertion points, midurethra, symphysis, external ligament, Figure 2.

Our results seem to (cautiously!) answer the key research question, "Do wide bore polyester sutures create sufficient collagen for longer-term efficacy of the ULP?". The original animal experimental, biomechanical and anatomical data on which the MUS is based,[2,3] showed a progression from wound reaction, to initial collagen 3, and conversion to collagen 1 within 12 weeks. Based on these data, the 86% SUI cure at 6 months in 31/36 operations, and 83% at 12 months in 29/35 ULP operations indicates collagen 1 had formed, at least for these 29 women.

The results to date appear to confirm statements from:[5] "Built-in safety", "no tape to compress urethra; no applicators to damage bladder, nerves, blood vessels, bowel", day surgery with no significant intra-operative complications, and virtual absence of post-operative urinary retention. We do not necessarily agree with the concept paper's comment:[5] "Simplicity: less surgical skill required." The paraurethral sulci can be deep, and may pose some difficulties in dissection in some women. However, injection of LA elevates the sulcus and facilitates the dissection.

CONCLUSION

The ULP operation is essentially a native tissue repair of the PUL, but using collagen creating wide-bore polyester sutures. Its anatomical pathway for cure is very similar to that of the MUS; it reinforces PUL and prevents its lengthening during stress. Our past studies in neocollagen production from implanted material makes us cautiously optimistic about the long-term effectiveness of the ULP operation, but uncertainties remain. Well monitored studies by other surgeons under EC supervision will be required before the ULP operation can become more widely recommended. If longer-term cure for SUI can be maintained, the ULP methodology could, at least theoretically, be applied to repair pelvic organ prolapse caused by any ligament in the pelvis.

ETHICS

Ethics Committee Approval: The ULP surgical study was approved by the Ethical Committee of Muğla Sıtkı Koçman University (date and number: 8th June 2022 & 11/VII). Patient permission was obtained to publish de-identified surgical results and videos. Trial registration details: ClinicalTrials ID: ID05733052.

Informed Consent: Written consent from each patient was obtained to publish de-identified surgical results and videos.

Contributions

Surgical and Medical Practices: A.A.S., F.M., R.H.; Concept: A.A.S., F.M.; Design: A.A.S., F.M.; Data Collection or Processing: A.A.S., F.M., R.H.; Analysis or Interpretation: A.A.S., P.P.; Literature Search: A.A.S., F.M., R.H., P.P.; Figures and Video: P.P., A.A.S.; Writing: A.A.S., R.H., P.P.

DISCLOSURES

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Video 1 Link: https://youtu.be/SJxrult0Xko

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A critical analysis of integral theory statements concerning pathogenesis of LUTS and chronic pelvic pain

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ABSTRACT

Lower urinary tract symptoms (LUTS) and pelvic pain are highly prevalent in women and often poorly understood. The aim was to critically analyse the integral theory paradigm's (ITP) statements concerning anatomical pathogenesis of LUTS, in particular, chronic primary pelvic pain syndrome (CPPPS) and LUTS as defined by the 2002 ICS report. The ITP explains pelvic floor function is determined by three directional muscle forces: forward, backwards and downward-acting muscle vector forces supported by ligaments which result in an anterior and posterior resultant keeping the balance needed for optimal sphincter closing and voiding. Muscular weakness caused by loose ligaments may result in LUTS and pain in some patients. Loose uterosacral and pubourethral ligaments provoke dysfunctional bladder filling and evacuation. Weakened uterosacral ligaments (USL), often accompanied by pelvic organ prolapse, weaken the posterior resultant, as seen in the posterior fornix syndrome and may explain chronic pelvic pain, LUTS, and dysfunctional defecation in some. Further studies are needed to investigate the importance of the ITP when treating CPPPSs and LUTS, in particular, possible non-surgical options to support USLs, considering the possible complications of reconstructive surgery.

Keywords: Biomechanics; chronic pelvic pain; interstitial cystitis; neuroanatomy; painful bladder syndrome; posterior fornix syndrome; urinary stress incontinence

INTRODUCTION

Lower urinary tract symptoms (LUTS) and pelvic pain are highly prevalent in women of all ages and often poorly understood.[1] In women, 59.2% have storage symptoms, 19.5% voiding symptoms, and 14.2% reported post-micturition symptoms.[1,2] Lower urinary tract pain, appears to be more common in women than men.[1]

The integral theory (IT) suggests a comprehensive approach to pelvic floor sphincter function and dysfunction, possibly explaining the malfunction of the pelvic organs in some.[3]

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The IT postulates three directional muscle forces forming an anterior and posterior resultant to be responsible for normal bladder function. The pubococcygeus muscle (PCM) forms a vector in forward direction, the levator plate (LP) in backwards direction, and the conjoint longitudinal muscle of the anus (LMA) contracts in a downward direction. The back and downwards directed vector forces form a backward resultant. LUTS occur when the balance between these resultants is disturbed by loose ligaments and/or muscular weakness.[4] Tension in the pelvic floor fascia and bladder neck triggers proprioceptive afferents that may disrupt the micturition reflex perceived by patients as urgency.[4] The IT suggests tension in the vaginal wall and disturbed proprioceptive stimuli in the bladder base resulting from weakened muscular vector forces to be responsible for LUTS in some (e.g., OAB, urine retention, weak flow, urgency, nocturia) and pelvic pain.[4-6]

Testing with a haemostat gently supporting the pubourethral ligament (PUL) or speculum blade supporting the posterior fornix can show a possible indication for reconstructive surgery in some.[4,7,8] The IT suggests the use of an anterior, middle or posterior mesh to suspend regional weakness when treating incontinence, bladder or anorectal dysfunction and pain.[7-10] The IT in pelvic pain (e.g., vulvodynia) may show that uterosacral ligament (USL) weakness exists by injecting Xylocaine into the USL at the posterior fornix of the vagina (Bornstein test).[11,12]

We explain the possible anatomical pathogenesis of disturbed bladder and sphincter function using the 2002 standardization report from the ICS on LUTS[13] and discuss CPPPS and pelvic organ dysfunction from a critical point of view. Chronic primary pain is used unless another diagnosis could better explain the pain and symptoms present.[14] Such other diagnoses are referred to as “chronic secondary pain”, where pain may initially be assigned as a symptom secondary to an underlying disease.[14]

OBJECTIVE

The aim was to critically analyse the Integral Theory Paradigm’s (ITP) statements concerning anatomical pathogenesis of lower urinary tract symptoms, in particular, chronic primary pelvic pain syndrome (CPPPS) and LUTS as defined by the 2002 ICS report.[4,13]

METHODS

Explain normal bladder function and LUTS as postulated by the Integral Theory, according to the 2002 ICS report.[4,13]

1. Function and Dysfunction of the Lower Urinary Tract

Normal bladder function

Afferent and efferent stimuli controlled by higher centres determine the normal function of the lower urinary tract.[15] The control system regulates the continence and emptying of the bladder and is determined by cortical and peripheral factors. Sensory stimuli from stretch receptors in the bladder wall initiate the sensation of a full bladder and the need to void. Muscular and ligament proprioceptive afferent stimuli from the small pelvis also trigger the higher centres and periaqueductal grey matter, the pelvic organ stimulation centre and the pelvic floor stimulation centre in the brainstem.[15] There is a balance between the muscular open and closing forces and tension in ligaments, that control the proprioception around the bladder in a normal situation. Three oppositely acting directional muscles determine the tension in the vaginal wall supporting the bladder. Figure 1 shows the ligaments and muscular vector forces of the pelvic floor. The PCM contraction pulls on the PULs in a forward direction, the LP on the PUL in a backwards direction, and the conjoint LMA contracts in a downward direction on the USL. The back and downwards directed vector forces form a backward resultant (Figure 1).[4]

Dysfunction

Any anatomical damage in the afferent or efferent system or higher centres may cause bladder continence or voiding

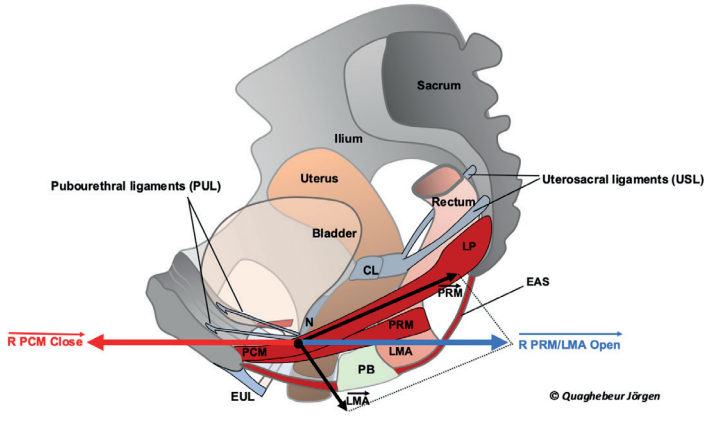

Figure 1. The three directional muscle forces of the pelvic floor and the anterior and posterior resultant

CL: cardinal ligament; EAS: external anal sphincter; EUL: external urethral ligament; LMA: longitudinal muscle anus; LP: levator plate; N: bladder stretch receptors; PB: perineal body; PUL: pubourethral ligaments; USL: uterosacral ligaments

The figure shows zero resultant force at the level of the bladder neck. Weak PUL decrease forward muscle forces, so the stronger backwards-pulling muscles move the zero force backwards. Weak USLs decrease the backward vector forces, so the stronger forward muscles pull the zero-force forwards

dysfunction and disturb the control of the micturition reflex, as seen in overactive bladders. Damage in the central nervous system can be in the brain, spinal cord (e.g., tumor), peripheral nerves (multiple sclerosis), and disturb the micturition reflex. Tension or damage of the pelvic muscles, ligaments, and urothelial stretch receptors "N" (e.g., cancer or external pressure by fibroid, scarring by iatrogenic or obstetric fistula), can disturb the peripheral control. Disturbance of the binary control of the bladder (continence, evacuation) results in bladder dysfunction, such as deficiencies in closure (incontinence), evacuation (urinary retention) or premature activation of the micturition reflex (OAB).[4] Figure 2: The afferent and efferent nervous system innervating the bladder wall, ligaments and muscular pelvic floor.

With reference to Figures 1 and 2, control of the bladder is binary, with only two modes, EITHER "closed" (closure reflex) OR "open" (micturition reflex).[13,16] "Closed" is the dominant mode. "Open" is activated when the afferent signals of fullness from the bladder base stretch receptors "N" signal a need to empty.[16] Anatomical damage in some part of the control system, Figure 2, in particular collagen-based weakness in the suspensory ligaments, Figure 1, can disturb the closed/open binary balance, to cause premature activation of an otherwise normal micturition reflex,

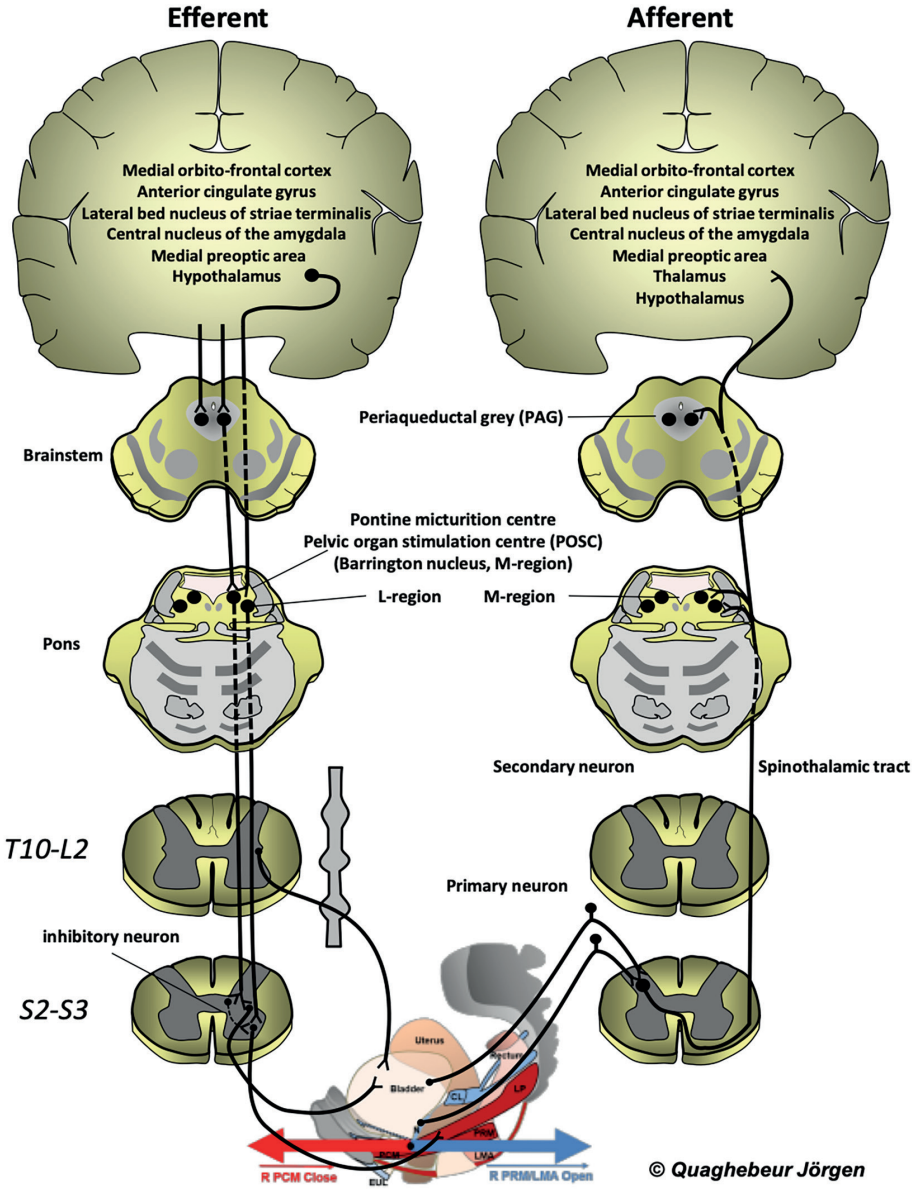

Figure 2. The afferent and efferent nervous system innervating the ligament and muscular pelvic floor
CL: cardinal ligament; EAS: external anal sphincter; EUL: external urethral ligament; LMA: longitudinal muscle anus; LP: levator plate; N: bladder stretch receptors Figure shows the higher centres, afferent and efferent innervation of the bladder and pelvic floor sphincter

which has been shown to be equivalent to urodynamic "detrusor instability".[17]

The International Continence Society (ICS) standardization committee classified the terminology of LUTS as storage symptoms and voiding dysfunction (e.g., obstructed micturition, urinary retention).[13] This ICS report includes the description of LUTS, urodynamic observations, symptoms associated with sexual intercourse, pelvic organ prolapse, and chronic pelvic pain syndromes.[13]

We summarized how the IT may play a role in some patients the anatomopathological explanation of LUTS, based on the IT in Table 1.

Storage symptoms include overactive bladder symptoms (OAB), nocturnal enuresis, stress and mixed urinary incontinence, and disturbed bladder sensation. Deficient contractility of the bladder is a storage problem accompanied with urine residue.

The IT explains OAB symptoms (e.g., frequency, urgency, and nocturia) as manifestations of damage in some part of the

Table 1. Integral theory and LUTS
Storage symptoms
Overactive bladder symptoms (OAB) and nocturnal enuresis
The three directional forces fail to stretch the vagina sufficiently, continuously triggering the proprioceptors at the bladder base (N) with urgency and frequency at a low bladder volume. When the closure reflex cannot control the micturition reflex, urge incontinence can occur. Nocturia occurs when the urgency wakes the patient to go to the toilet at night. Nocturnal enuresis occurs when a child with an immature bladder does not wake up to void.[1]
Stress urinary incontinence (SUI)
When a weak pubourethral ligament (PUL) cannot structurally support the middle part of the urethra and the LP/LMA muscles pull down the distal vagina, trigone and posterior urethral wall in an open position, SUI occurs. A haemostat placed immediately behind the pubic symphysis (PS) prevents the elongation of a weak PUL and decreases the incontinence confirming the SUI mechanism.[1-3]
Mixed urinary incontinence
Loose PUL weaken the PCM force triggering the stretch receptors (N) at the bladder base, which sends uncontrolled afferent impulses to higher centres. This is perceived as urgency. A haemostat positioning behind the pubis diminishes urge in 50% of cases and indicates further treatment.[1,3,4]
Bladder sensations
The balance of forward (PCM) and backwards (LP/LMA) muscle forces, stretching the vagina to support "N" from below, is disturbed when loose uterosacral ligaments (USLs) weaken LP/LMA posterior resultant. Higher hydrostatic pressure is required to activate the urothelial stretch receptors for evacuation because autonomic stress responses from higher centres let the PCM stretch the vagina further forwards. Urinary retention occurs as seen in under active bladder.[5]
Voiding, obstructed micturition, and urinary retention
The voiding mechanism is similar to SUI, except during micturition, the PCM relaxes, and LP/LMA opens out the urethra to decrease resistance to urine expulsion. Weak USLs reduce the opening force of the LMA, and the posterior urethral wall opens inadequately. Symptoms of obstructed micturition occur.[5,6]
Slow stream
Loose USLs weaken the LP/LMA posteriorly directed opening forces, and the urethra cannot open adequately. Therefore, the detrusor must push urine out through a narrower tube with a slow stream.[1]
Splitting of spraying
Dislocation of one of the pubococcygeus muscles causes "PCM" to unbalance the forces against the symphysis. The opposite healthy muscle pulls the urethra towards it, causing a divergent stream or spraying.[1,7]
Intermittent stream
The EMG must decrease during normal voiding, although in women with an intermittent stream, it repeatedly rises, indicating that the muscles cannot sufficiently open the urethra.[1]
Hesitance
The LP/LMA muscles externally open the urethra insufficiently due to weak USLs causing hesitance.[1]
Straining
Straining increases the abdominal pressure and helps the detrusor to evacuate once the urethra has been pulled open during the flow.[1]

Table 1. Continued
Feeling of incomplete emptying
Incomplete emptying
The striated LP/LMA muscles tire with repeated efforts to evacuate. The EMG is disturbed, incomplete evacuation follows, and further emptying is needed in 20 min.[1]
Postmicturition dribble
Repeated efforts in the EMG occur when the LP/LMA striated muscles tire. The urethra closes under the influence of its elastic tissues, exponentially raising resistance to detrusor during emptying. The detrusor can only expel urine at a prolonged flow.[1]
Incontinence during sexual intercourse
The lateral part of the PUL inserts into the lateral part of PCM and directly into the vagina.[1,4] A penis thrusting backwards from the introitus may stretch the vagina sufficiently to pull open the urethra. If the PUL cannot sustain the backward stretching from the penis, the PUL elongates. The elongation allows the posterior forces to stretch open the urethra. Such cases respond to mid-urethral sling surgery.[1]
Symptoms associated with pelvic organ prolapse
In uterus prolapse, the uterus descends into the vagina by intraabdominal pressure and gravity pulling on USLs, which contain nerves causing backache and dragging.[1] Digitally supporting the perineum prevents the diversion of faeces into the rectocele during defecation.

control system, disturbing reflex control of the micturition reflex.[4] Disturbed tension of the vaginal wall caused by a weak sphincter mechanism triggers the proprioceptive stretch receptors "N" which the cortex perceives as urgency and lower bladder volume develop as a consequence. Urge symptoms at night wake the patient to void "nocturia".[4] Urge urinary incontinence occurs when the closure reflex cannot sufficiently control the afferent impulses from "N", and the micturition reflex is continuously activated. In children sleeping, nocturnal enuresis manifests when afferent impulses activate the micturition centre without waking up.[4]

The IT explains stress urinary incontinence as a result of a weak PUL that cannot structurally support the middle part of the urethra. The anterior resultant decreases, and the LP/LMA muscles pull down the distal vagina, trigone and posterior urethral wall to open the urethra.

Three directional forces close the distal urethra and bladder neck. The PCM stretches the suburethral vaginal hammock against a competent PUL to close the distal urethra from behind.[4,10] The LP pulls backwards against PUL to stretch the vagina, proximal urethra, trigone, and bladder base backwards to render the tissues semi-rigid.[4,10] The LP also tensions the pubovesical ligament (PVL), which inserts into a smooth thickening on the anterior wall of the bladder[4,10] (arc of Gil-Vernet).[18] Normally, the pubovesical ligament (PVL) and the arc hold the anterior bladder wall steady, while the conjoint LMA (downward vector) contracts against the USL rotating the now semi-rigid bladder around the arc to close the urethra at the bladder neck.[4,10] A weak PUL stretches down on effort and does not support the distal vagina into which it inserts.[19] A haemostat or finger gently placed behind the pubic symphysis (PS) and above the urogenital diaphragm supports the PUL preventing the elongation of a weak PUL and decreasing the loss of urine, showing the need for anterior pelvic floor support.

The vagina requires stretching from both ends and supports the bladder neck from below. Mixed urinary incontinence occurs if loose PUL weaken the PCM force and accompanies uncontrolled afferent impulses interpreted by the cortex as urgency. A haemostat test gives support just behind the pubis, diminishes urge in 50% of cases, and indicates SUI and urge cure.[4,19,20]

The balance of the forward and backward resultant muscle forces stretches the vagina to support "N" from below and is essential for optimal bladder sensation. LP/LMA vector forces weaken when the USLs are loose. Autonomic impulses from higher centres in the brain activate the PCM and stretch the vagina further forwards. This requires a higher hydrostatic pressure to activate the urothelial stretch receptors for evacuation. High preoperative mean bladder volumes (598 mL) in women with under active bladder symptoms and retention decreased to a mean of 301 mL following USL repair, which cured/improved the emptying symptoms and residual urine.[21]

Voiding, obstructed micturition, and urinary retention can also be caused by a disturbed peripheral muscular and ligament mechanism. The SUI and micturition mechanisms are similar, except that during micturition, the PCM relaxes, and LP/LMA opens out the urethra to decrease resistance to urine expulsion. The LMA opening force weakens when the USLs are loose. Obstructed micturition is experienced when the posterior urethral wall opens inadequately.[22] Lax PUL weaken forward

muscle forces, and the posteriorly directed resultant pulls the equilibrium point backwards, broadening the urethra and causing SUI.[21,22] Lax USLs weaken the backward vector forces, and the stronger forward muscles pull the vagina and equilibrium point forward.[21,22] Imbalanced muscle forces may over-tension the distal vagina and close the distal urethra more tightly. Imbalanced muscle forces may create much firmer support below the urothelial stretch receptors "N".[21,22] To activate the micturition reflex, a greater bladder volume is necessary to activate the afferent impulses required and explains the more significant resting bladder volumes and retention as reported in "underactive bladder" and Fowler's syndrome.[21,22]

Symptoms such as slow stream and hesitance can be caused by a weak posterior resultant (LP/LMA opening forces) due to loose USL. The urethra cannot adequately open, and the detrusor pushes the urine out against resistance with a slow stream.[4]

Splitting or spraying can be caused by the dislocation of one of the PCMs provoking unbalance of the "PCM". In divergent streams or spraying, the opposite healthy muscle pulls the urethra towards it.[4,23] During normal voiding, the EMG repeatedly rises in women with an intermittent stream, although it must decrease. The muscles cannot "grip" sufficiently to open the urethra.[4] An increased abdominal pressure by straining helps the detrusor to empty, and once the urethra has been pulled open and urine is flowing.[4]

The lateral part of PUL inserts into the lateral part of PCM and directly into the vagina.[4,20] Coital incontinence may occur when a penis thrusting backwards from the introitus stretches the vagina sufficiently to pull open the urethra. If the PUL cannot sustain the backward stretching from the penis, the PUL elongates. The elongation allows the posterior forces to stretch open the urethra provoking urinary leakage during sexual intercourse. Such cases respond to mid-urethral sling surgery.[4]

In uterus prolapse, the uterus descends into the vagina pulling on USLs, which contain myelinated and unmyelinated nerves and causing backache and dragging.[4] Digitally supporting the perineum prevents the diversion of faeces into the rectocele during defecation and repair may improve the prolapse and symptoms.[24,25]

Chronic pelvic pain and the posterior fornix syndrome

Damage to the posterior suspension system in the pelvis (e.g., USLs) is mainly responsible for prolapse-induced visceral plexus, chronic lumbosacral, and pelvic pain often accompanied by LUTS and anorectal dysfunction (e.g., urgency, frequency, nocturia, and obstructive miction and defecation).[26,27] The USLs connect the sacral vertebrae (S2-S4) with the fornix and contain autonomic nerve fibres and nociceptive afferents.[26,28] The posterior fornix syndrome (PFS) is caused by laxity or injury of the USLs and causes LUTS, chronic pain, and obstructive defecation that can be relieved by the suspension.[25,29] Gentle support using a lower blade of a bivalve speculum generally relieves the pain, and excessive caudal traction will exacerbate it.[4,11,29,30] Some cases of vulvar pain syndrome also will determine the pain's origin by the posterior fornix's support.[12,24,31,32] Permanent traction on the hypogastric or sacral plexus and irritation of the Frankenhäuser's ganglion caused by USLs laxity and POP may also be the reason for chronic neuropathic pain mechanisms and gynaecological low back pain.[26]

DISCUSSION

Although the IT suggests an all-encompassing explanation for bladder dysfunction and bowel evacuation problems, CPPPS and LUTS comprise a more complex pathophysiology.

The CPPPS are determined by exclusion of infection, inflammation, or obvious pathology and defined as "primary pelvic pain syndrome" if there is adequate evidence for its use.[33] When the pain can be localised to an organ, the subclassification must be attributed to a more specific term adding "primary" in front of the organ attained.[14] When chronic pelvic pain or LUTS are accompanied with USL-related POP, as suggested in IT, the pain and symptoms are secondary to prolapse and must not be classified as a "primary" pain syndrome. However, USL-caused chronic pelvic pains usually co-occur in multiple sites. These disparately-sited pains are generally relieved simultaneously either by the speculum test, by local anaesthetic injection into the lower end of the USL (Bornstein test)[11] or by USL surgery.[22] These data would seem to require revision of the "regional pain syndrome" classification of multiple organ involvement. It is considered a regional pain syndrome if multiple organs are involved.[33] Determining a specific end organ phenotype also involves the evaluation of significant emotional stress, functional disability, sexual and behavioural problems.[14,33]

Functional urological (e.g., CPPPS, BPS/IC, OAB) and gastrointestinal disorders (IBS) are interrelated, and the stress response mediates the severity of symptoms via the sympathetic-adreno-medullar axis (SAM), the hypothalamus-pituitary-adrenal axis (HPA), and the immune system.[15,34-36] Any psychological or physical stimulus that disturbs the homeostasis of the human body causes an autonomic stress response.[37-40] This stress response is also determined by the phylogenetic development of the nervous system as described in the Polyvagal Theory.[15,41,42] Comorbid conditions often accompany chronic pelvic pain and LUTS, and cross-organ sensitization.[43-48] The pelvic organs share

the same innervation, making them susceptible to cross-organ sensitization.[36,46,49] Cross-sensitization at a neuronal level and the gut microbiome explain the co-occurrence of LUTS and bowel dysfunction.[50-52] Determining the origin of pelvic pain syndromes or LUTS is difficult because of the overlap of symptoms, emotional factors, comorbidity and associated conditions.

The IT postulates ligament weakness with pelvic floor dysfunction as an explanatory model for a variety of often poorly understood bladder dysfunctions, genitourinary pain (e.g., vulvodynia) and anorectal dysfunction. The IT model explains pelvic pain and LUTS in women, although chronic pelvic pain conditions and genitourinary pain exist in men as well, which suggests that loose ligaments and disturbed muscular vector forces in the pelvic floor seem not always the reason for pelvic organ dysfunction and unexplained pelvic pain. The symptoms in patients with CPPPS appear to be equal between gender.[53] The symptoms in men are challenging to explain with IT, even taking into account recent discoveries of male analogues for USL and PUL.[54,55]

Not all LUTS and pelvic pain syndromes can be explained by IT, and testing the support with a haemostat or speculum blade might indicate some cases with specific regional weakness. LUTS and pain accompany POP, but also minor prolapse must be seen as a pathological condition. In POP, symptoms such as urinary stress incontinence, urinary urge incontinence, abnormal bowel, bladder emptying and faecal incontinence may occur, with or without pain.[56] Loose USL may cause PFS, often accompanied by faecal urgency, pelvic pain, nycturia, and anorectal evacuation problems.[56] The IT suggests the Bornstein test to detect chronic pain due to loose ligaments; the Xylocaine injection works by its local analgesic (LA) effect on the visceral nerve plexuses at the lower end of the USL. A valid criticism of this LA test is it cannot *per se* directly diagnose USL weakness. The extra link to causation is the speculum test which mechanically supports USL and (when it works) immediately relieves several CPP sites,[29] and the results of surgical ligament strengthening by plication[25] or by slings.[26,27,32] It may indicate pain due to constant traction on nervous structures (visceral plexus). The IT suggests that pelvic organ dysfunction is provoked by disturbed muscular vector forces in the pelvic floor caused by ligament weakness and pelvic organ prolapse (POP) affecting the binary innervation and disturbing the pontine micturition reflex.[56]

LUTS and pelvic pain syndromes are often unexplained, but when in POP, they result from a disorder and are the consequence or "secondary to an underlying disorder".[14]

Neurodynamic testing showed significant mechanosensitivity in at least one lumbosacral plexus nerve in 88% of the patients with CPPPS, suggesting minor nerve injuries. Eighty-five per cent showed palpatory-provoked pudendal nerve mechanosensitivity. Neurodynamic testing also showed the involvement of multiple lumbosacral plexus nerves in 42%, suggesting abnormal impulse generation sites not caused by traction on the visceral plexus, as suggested by IT.[57] Compression and continuous traction are traumatic for nervous structures. Inside nerves, we find blood circulation, primo vascular channels, and lymphatic circulation in the perineurium. It remains unclear if a nerve injury provokes chronic pain and circulatory disturbances in the bladder wall and mucosa resulting in fibrosis and Hunner's lesions at a later stage.

Scheffler et al.[58] suggested a cure for BPS/IC by repairing the ligaments outside of the bladder (cardinal, uterosacral) according to the PFS protocols[24] of the 1993 Integral Theory.[59] Goeschen and Gold[8] revisited clinical data (n=198) of women who had presented with chronic pelvic pain (CPP) plus varying degrees of uterine/apical prolapse. Posterior intravaginal slingplasty repair of the USLs using a collagen-producing tape variously cured several bladder symptoms and pain.[8,60] The cure rate after USL sling repair was CPP 74%, urge incontinence 80%, frequency 79.6%, abnormal emptying 53%, nocturia 79%, and obstructive defecation 80%. Goeschen et al.[60] suggested that the PFS is similar to BPS/IC but still further rigorous scientific investigation, preferably by RCT, is required. It remains unclear whether lesser visceral plexus problems or only a part of the plexus provokes BPS/IC or causes different effects in the bladder wall. CPPPS co-occurs in multiple sites. It would be interesting when looking at the data from the studies, if it becomes possible to link the loss of support grade or side to plexus problems limited or global to the symptoms. It remains unclear whether plexus problems explain why the impact of bladder inflammation can be broad or restricted. Probably the pain response on visceral plexus traction is global because Wu et al.[29] reported relief of several sites with the speculum test, and a Bornstein test carried out in 3 women with BPS/IC relieved several pain sites simultaneously.[11] Research on the lack of pelvic floor support and BPS/IC is needed but can only be considered a start based on the limited data in the literature. The actual findings indeed show the need for testing the support system, excluding POP, when assessing CPPPS and LUTS during the patient's physical examination.

The IT in its formal statement states pelvic organ dysfunctions are mainly caused by collagen-induced ligament weakness; the IT predicts "strengthening the ligament will restore the function".[61] The strengthening can be non-surgical (as demonstrated by the speculum test for USL weakness), but to date, treatment for ligament weakness has been overwhelmingly surgical: Midurethral slings for SUI, and USL slings for LUTS and POP, with

high cure rates and low direct tape reaction.[26,27,32]

However, multiple publications describe mesh complications (e.g., pain, erosion, voiding dysfunction, infection, recurrent urinary tract infections, fistulae, organ perforation, bleeding vaginal scarring, neuromuscular alterations, LUTS, bowel complications, and immune disorders).[62-65] A study showed that 42% of women had at least one adverse event, and 12% had at least one serious adverse event.[66] A systematic review showed that using polypropylene pelvic mesh becomes highly controversial because of serious complications provoked by an essential mismatch between its viscoelastic properties and the structure of the surrounding tissue. In multiple countries, polypropylene mesh for treating POP has been banned, with no available alternative.[67] An important distinction was drawn between mesh usage for POP and tapes mesh sheets block descent of the prolapse, but do not cure it; tapes repair the prolapse by creating new collagen for its structural supports, the ligaments.[68]

CONCLUSION

As a theory, the ITP describes how ligament and muscular factors under peripheral and central nervous control determine how the bladder functions, how in specific cases, anatomical damage to pelvic muscles, ligaments or their nervous control can disturb the equilibrium between anterior and posterior resultant muscular forces and to explain LUTS and chronic pelvic pain dysfunctions, collagen being the weakest link. Also as a theory, the ITP is obliged to make firm statements that are falsifiable, which never cover every possible cause. Though surgical repairs such as the midurethral sling have dominated the ITP to date, equally important is intravaginal mechanical support of pubourethral and USL described, which has been shown to immediately reduce SUI, urge, pain and nocturia on application. This is a promising non-surgical future way forward for the ITP, as it avoids the complications inevitably encountered with reconstructive surgery. Looking ahead, the main task now, as we see it, are further studies, non-surgical, to assess the impact of ligament weakness on POP, LUTS and chronic pelvic pain.

ETHICS

Contributions

Surgical and Medical Practices: P.P., J-J.W., S.D.W.; Concept: J.Q., P.P.; Design: J.Q.; Data Collection or Processing: J.Q.; Analysis or Interpretation: J.Q., P.P., J-J.W., S.D.W.; Literature Search: J.Q., P.P.; Writing: J.Q.

DISCLOSURES

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Catheter treatment as a low-cost power tool in 2.302 patients with a fresh obstetric urine fistula; a call for mass campaign

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ABSTRACT

To show the impact of early indwelling bladder catheterization with high oral fluid intake as an important tool in the immediate management of the obstetric urine fistula. A total of 2.302 patients with an obstetric urine fistula of less than 3-month duration had an indwelling bladder inserted for 4 weeks together with high oral fluid intake upon arrival in the referral center. Several relevant patient and fistula date were given. Out of the total of 2.302 patients the result was healing of the fistula in 1.398 or 61% by this immediate management. The patents not healed by catheter were operated the day after catheter removal. Final overall healing by catheter and/or early closure was achieved in 2.212 patients or 96% with total incontinence in 24 or 1% and milder incontinence but social continence in 20 or 1%. A failure was noted in 23 patients or 1% including mortality in 6 or 0.3%. The outcome was unknown in 67 patients or 3% who did not report for follow-up after catheter insertion. The results were further analyzed as to duration of leaking, as to fistula size, as to Kees classification and as to fistulas related to cesarean section. The early indwelling bladder catheterization with high oral fluid intake as part of the immediate management is highly effective in the treatment of the obstetric urine fistula. If this management could be implemented in any patient with an obstetric urine fistula it would drastically reduce the caseload of patients in the world waiting for an operation, especially since a catheter can be inserted in any setting by a nurse, a midwife or a doctor under low cost.

Keywords: Ostetric fistula; immediate management; indwelling bladder catheterization; high oral fluid intake; low-cost power tool

INTRODUCTION

The obstetric fistula is as old as mankind and has been a source of misery to the women affected. With an estimated prevalence of 2,000,000 women in the world, it continues to be a challenge as a major public health problem, especially in the low- and middle-income countries.[1]

The standard treatment as practiced by most surgeons is closure of the fistula after at least 3 months after delivery when all signs of necrosis and inflammation have subsided.

However, the management has to start the moment the urine leaking is manifest whilst spontaneous healing is possible and can be promoted by an indwelling bladder catheter with high

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oral fluid intake during the first 3 months as start of an active immediate management.[2]

Unfortunately, there are no reports on the results of early catheterization on a mass or any other scale.

Rationale for Early Catheterization and High Oral Fluid Intake

Decompression of the bladder for a sufficiently long time so that the eventual fistula edge may be adapted as the necrosis and inflammation subside and the fistula may heal, whilst the high oral fluid intake ensures high urine production to prevent ascending urinary tract infection and blocking of the catheter. The combination works as flushing drainage of the distal urinary tract.

MATERIALS AND METHODS

Materials

During a 40-year period 1983-2022 a total of 9.681 patients with postpartum involuntary urine loss of a duration of less than 3 months have been treated by the author as an immediate management, 3.930 by an indwelling bladder catheter with high oral fluid intake and 5.751 by surgical repair within the first 3 months after childbirth.

Almost all patients coming during the first month after delivery had a catheter inserted irrespective of size and type whilst few could be operated within a few days, the patients coming during the second month after delivery either had a catheter inserted or were operated within a few days, whilst the majority of the patients coming during the third month after delivery were operated within a few days and few had a catheter inserted.

Out of the 3.930 patients treated by catheter 2.302 had an obstetric fistula and the rest had either total urine stress incontinence or overflow incontinence due to a hypotonic bladder.

The obstetric urine fistula was combined with an obstetric stool fistula or stool and/or flatus incontinence in 499 patients or 22%.

The index parity varied from 1 to 16 with the majority of 1.245 patients or 54% being para I indicating the first delivery as a test case.

There were 27 twin deliveries and out of the 2329 infants born 1611 (69%) were male and 718 (31%) were female whilst 406 were born alive out of whom 73 died within the first week remaining only 332 infants alive (10%); the 2:1 ratio of male to female infants cannot be explained by the author though this was found in all obstetric fistula patients as treated by him.

A foot drop was found in 1.660 patients or 72% being bilateral in 1.260, only right side in 235 and only left side in 165, with no difference between right and left.

The Kees classification was used and 458 fistulas were type Kees I, 1.422 were Kees IIAa, 213 were Kees IIAb, 102 were Kees IIBa, 57 were Kees IIBb and 57 were Kees III.

The Kees III fistulas were all vesicocutaneous fistulas, 55 after caesarean section, 1 due to re-opening of urachus fistula after home delivery and 1 due to ruptured uterus/bladder with abscess formation after home delivery.

The fistula size varied from small to extensive and was small <2 cm in 839 patients, medium ≥2 to 3 cm in 937 patients, large ≥4 to 5 cm in 373 patients and extensive ≥6 cm in 153 patients.

Duration of urine leakage was 1-30 days in 1.742 patients, 31-60 days in 448 patients and 61 days to 3 months in 112 patients.

Methods

After history taking and careful examination of all obstetric trauma including longitudinal bladder diameter, urethra length, foot drop, necrosis, anal reflex and rectovaginal fistula, a decision was taken either to operate or to insert a catheter.

If the decision was catheterization, a Foley Ch 18 catheter was inserted and the balloon inflated with 5 mL of normal saline, functioning of the catheter checked and the patient instructed on high oral fluid intake of at least 4-6 liters per 24 hours.

Antibiotics were not prescribed since the fistula is caused by pressure necrosis and not by specific infection and the high urine production will act as a kind of flushing drainage of the bladder preventing ascending urinary tract infection.

Drainage was open into a plastic pot, and the patient instructed not to kink or block the catheter otherwise and to report immediately if there was any problem.

Most patients were kept for the 4-week catheterization in the preoperative ward but many left the hospital to fulfil their domestic duties in their household.

If the catheter got blocked it was either flushed to re-open it or if that failed it was replaced by a new one; if it fell out it was replaced by a new one.

If here was still necrosis or slough as was found in 1.226 patients or 53%, the patients were instructed to use sitz baths 3 times daily inside warm water plus a detergent.

If during the course of catheterization the balloon of the catheter entered the fistula preventing healing or if the prospect of healing was nihil, the catheter was removed and the patients prepared for surgery as soon as the fistula was clean and the condition of the patient in order.

After 4 weeks a vaginal speculum examination was performed to determine if the fistula had healed or not and the catheter removed.[3]

If it had healed the patient was instructed to continue a high oral fluid intake and to pass urine regularly at least every hour to train the bladder.

The following day the patient was seen again and asked about leaking or not, miction and incontinence.

If she complained of urine leaking or incontinence, a speculum examination was performed to check if it had really healed or if stress incontinence.

If stress incontinence was found, it was explained to the patient that she should continue the bladder drill and it might heal.

Then the patient was discharged from the hospital and instructed to continue the high oral fluid intake, to report back once a month for at least 3-6 months and to refrain from sexual intercourse for a minimum of 6 months.

If it had not healed the patient was operated within 2 days if the fistula was clean and her condition in order.

Fresh obstetric fistulas with catheter are documented in Figures 1, 2.

RESULTS

For a comprehensive understanding the results were analyzed as to overall, as to duration of the fistula, as to fistula size, as to classification and as to caesarean section related fistulas without vesicocutaneous fistulas.

Overall Results

Out of the 2.302 obstetric fistulas, a total of 1.398 or 61% had healed by catheter only out of which 17 had a recurrence after early sex; there was mortality in 5 patients or 0.2% due to gastroenteritis in 2, eclampsia in 2 and found dead in bed in the morning in 1 at day 10, 11, 25, 28 and 42 post delivery.

After an additional 1.071 operations after failed catheter treatment a total of 2.212 patients (96%) had healed with total incontinence in 24 (1%) and milder incontinence but social continence in 20 (1%); with failure in 23 (1%) out of whom 6 had developed a ureter fistula and unknown in 67 (3%) since they did not return for follow-up after catheter insertion; and

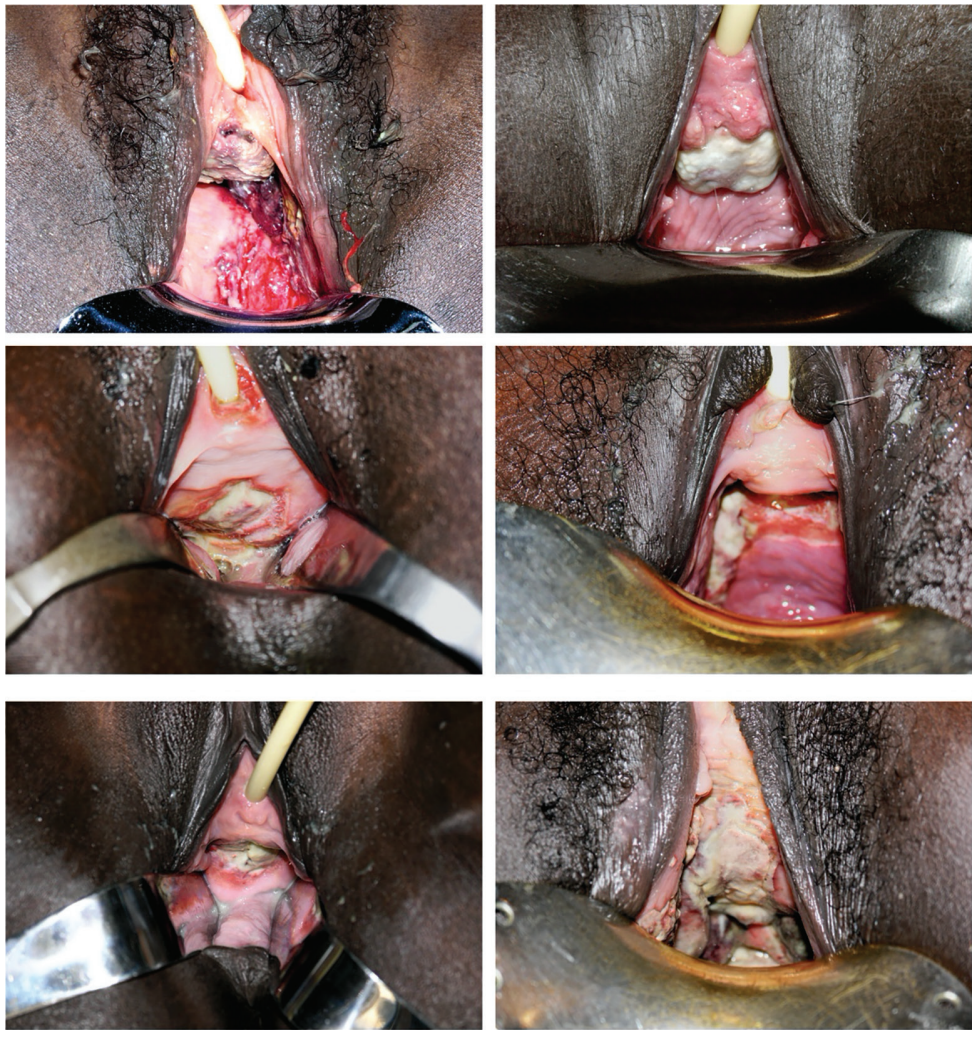

Figure 1. Necrotic fistula variety 01

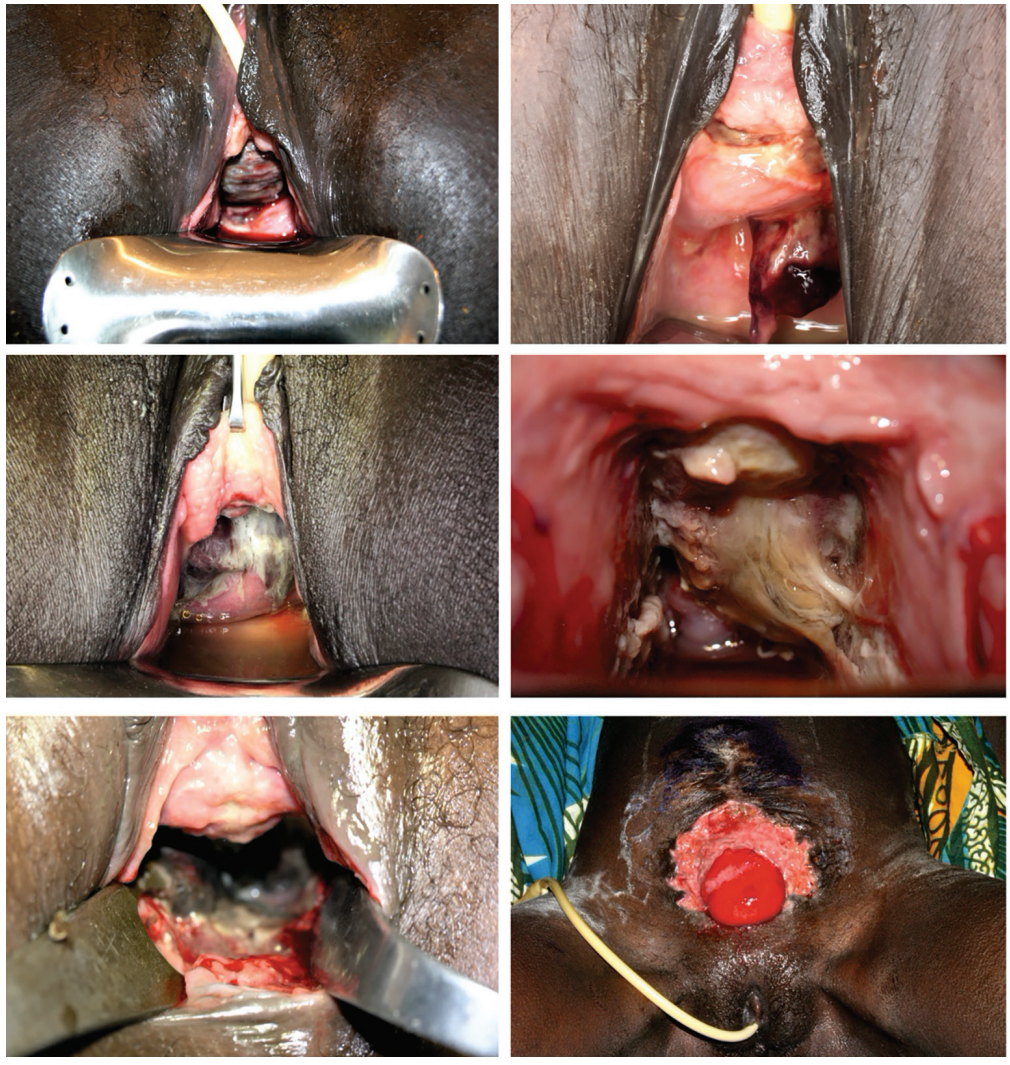

Figure 2. Necrotic fistula variety 02

with a mortality in 6 (0.3%) since 1 additional patient died from hepatorenal failure due to native medicine 15 days postoperatively.

Results as to Duration of Leaking

Out of the 1.742 patients leaking less than 31 days, 991 or 57% had healed by catheter treatment only. Out of the 448 patients leaking 31 to 60 days, 326 or 73% had healed by catheter treatment only. Out of the 112 patients leaking 61 days to 3 months, 81 or 72% had healed by catheter treatment only though the number is too small for any statistics.

Results as to Fistula Size

Out of the 839 patients with a small fistula, 647 or 77% had healed by catheter treatment only. Out of the 937 patients with a medium fistula, 529 or 56% had healed by catheter treatment only. Out of the 373 patients with a large fistula, 170 or 45% had healed by catheter treatment only. Out of the 153 patients with an extensive fistula, 52 or 34% had healed by catheter treatment only.

Results as to Kees Classification

Out of the 458 patients with a Kees I fistula, 267 or 58% had healed by catheter treatment only. Out of the 1,422 patients with a Kees IIAa fistula, 977 or 69% had healed by catheter treatment only. Out of the 213 patients with a Kees IIAb fistula, only 37 or 17% had healed by catheter treatment only. Out of the 102 patients with a Kees IIBa fistula, 65 or 64% had healed by catheter treatment only. Out of the 50 patients with a Kees IIBb fistula, only 2 or 4% had healed by catheter treatment only. Out of the 57 patients with a Kees III vesicocutaneous fistula of which 55 were caesarean section related, 51 or 89% had healed by catheter treatment only.

Then since the caesarean section fistula is on the rise, out of the 304 patients with a caesarean section related fistula but not

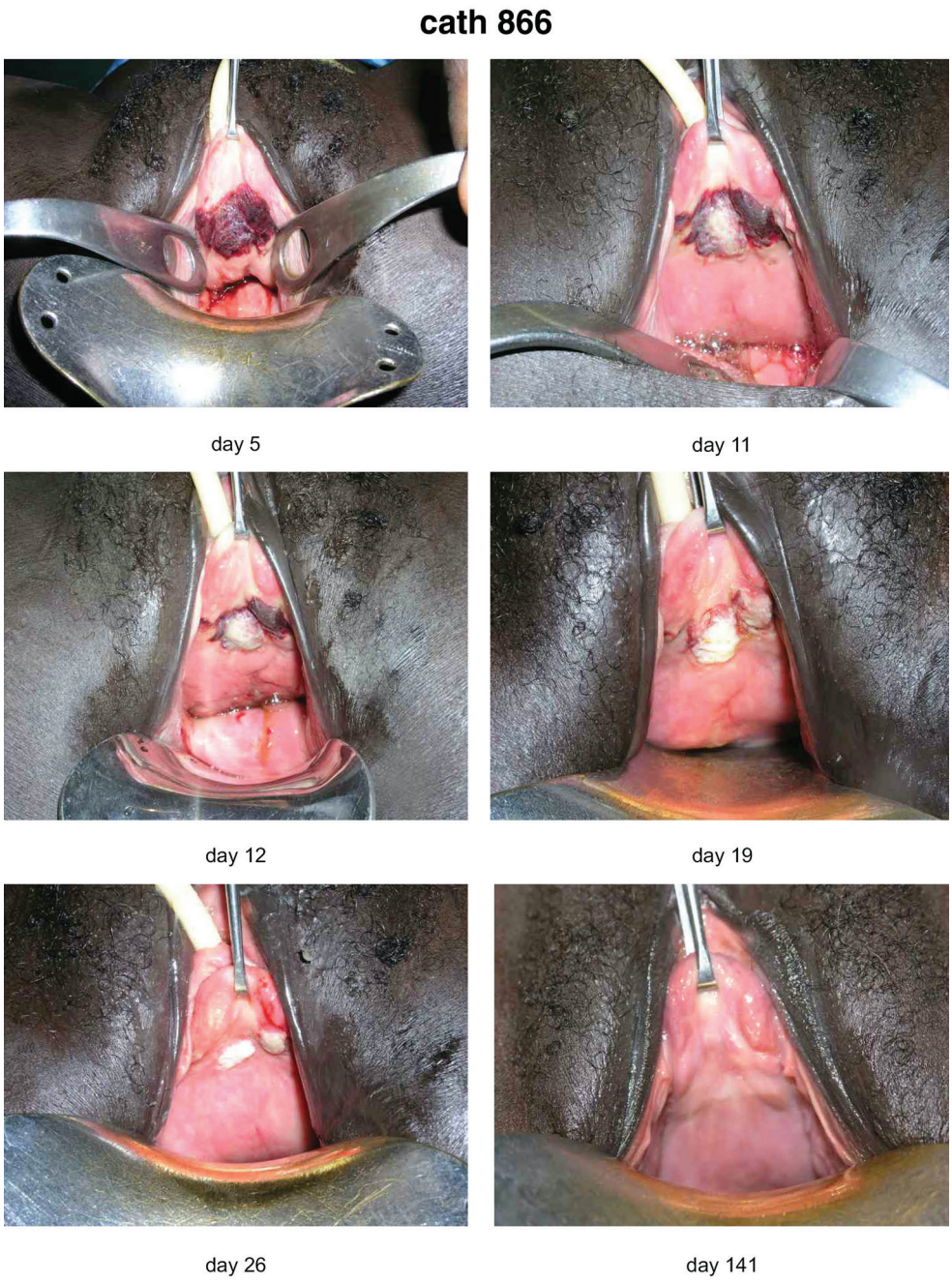

Figure 3. Healing process 01

a vesicocutaneous fistula, 167 or 55% had healed by catheter treatment only.

Based upon the theoretic insight and these results, the same was done in another 35 patients with a non-obstetric fistula due to surgery, yankan gishiri, sex or other trauma with the following results: Twenty-one had healed by catheter treatment only and after additional 15 operations 33 had healed with full continence whilst the outcome was unknown in 2 who did not report for follow-up after catheterization. The healing process has been documented in Figures 3, 4.

DISCUSSION

The immediate management was developed during the period 1983-1992 and then further perfected as standard by the author instead of waiting 3 months.

In this study only the early bladder catheterization with high oral fluid intake has been presented to show the power in the management of the obstetric fistula.

The results as to fistula size were as expected, from small to medium to large to extensive. However, the initial size of the

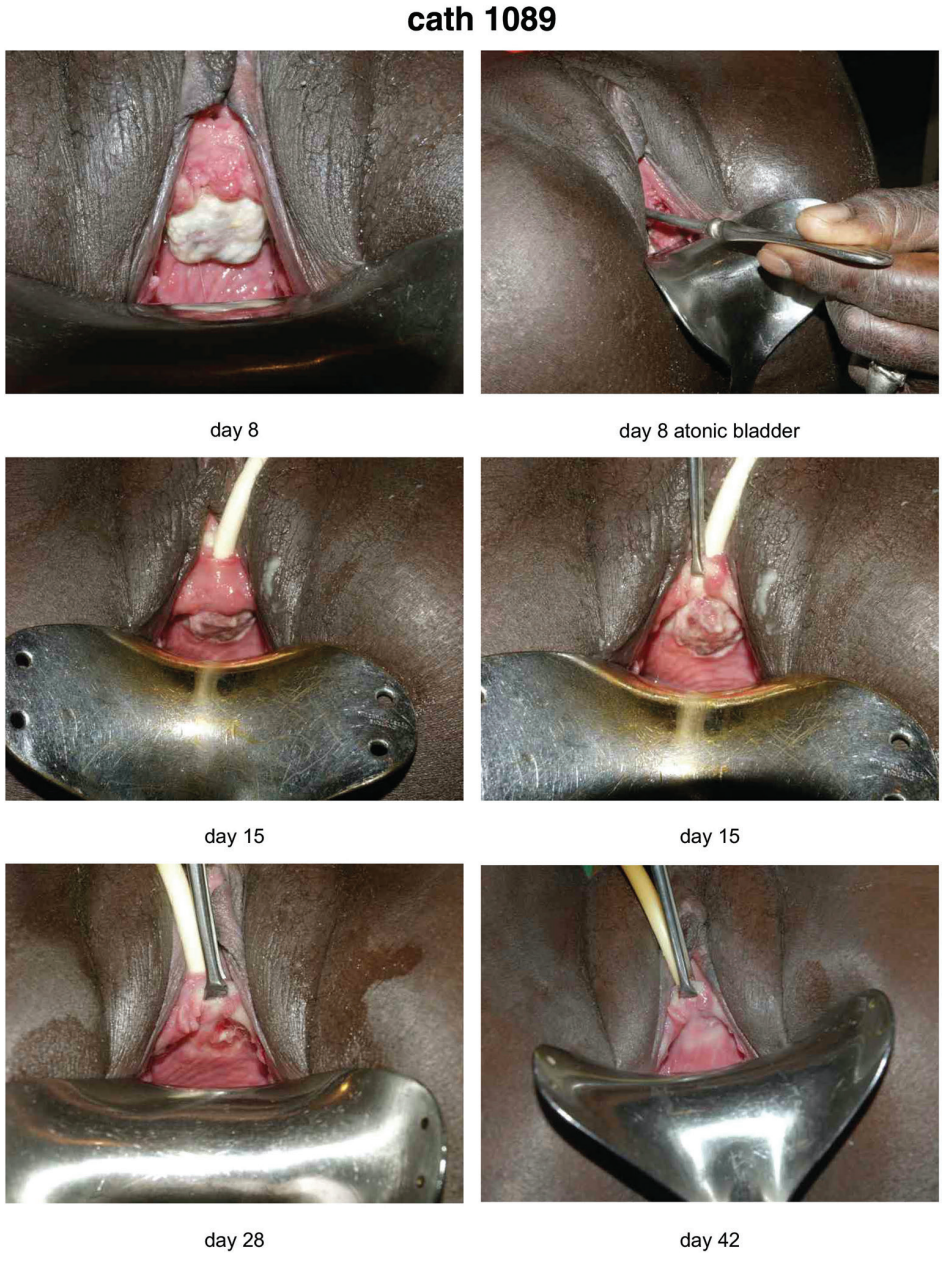

Figure 4. Healing process 02

necrosis is not so important as is the depth of the necrosis which is the decisive factor. Therefore, immediate bladder catheterization is recommended in all obstetric fistula patients irrespective of size as is shown by these results.

To interpret the results as to fistula duration one has to consider that during the first month of leaking almost all the fistula patients had a catheter inserted without any selection and only few were operated whilst for the patients leaking from 31 days to 3 months most patients were operated immediately and few selected patients had a catheter inserted.

The results as to fistula type are fine in Kees I and Kees IIAa fistulas and very low in the fistulas with a circumferential defect, whilst the numbers of Kees IIBa and Kees IIBb fistulas are too low for any reliable statistic evaluation.

The results in caesarean section related fistulas are encouraging, and early catheter treatment in these patients is highly recommended, in the low-, middle- and high-income world.

The results in the 35 patients with a non-obstetric urine fistula are comparable though the number is too small for any statistics.

Another advantage of early catheterization is that it can be done in any setting by a nurse, a midwife, a doctor or an obstetrician without additional skills under low cost as compared to surgery in a high-quality unit by a highly trained surgeon with special surgical skills under far higher cost.

CONCLUSION

The early indwelling bladder catheterization with high oral fluid intake as part of the immediate management is a power tool in the treatment of the obstetric urine fistula. It can be done in any setting by a nurse, a midwife or a doctor under low cost and would drastically reduce the number of obstetric patients in the world waiting for an operation.

Appendix Kees Classification[4]

- Kees I fistulas not involving continence mechanism
- Kees IIAa fistulas involving continence mechanism without (sub) total urethra involvement and without circumferential defect
- Kees IIAb fistulas involving continence mechanism without (sub)total urethra involvement and with circumferential defect
- Kees IIBa fistulas involving continence mechanism with (sub) total urethra involvement and without circumferential defect
- Kees IIBb fistulas involving continence mechanism with (sub) total urethra involvement and with -circumferential defect
- Kees III fistulas miscellaneous like ureter fistulas and vesicocutaneous fistulas

The classification has been documented in Figures 5, 6.

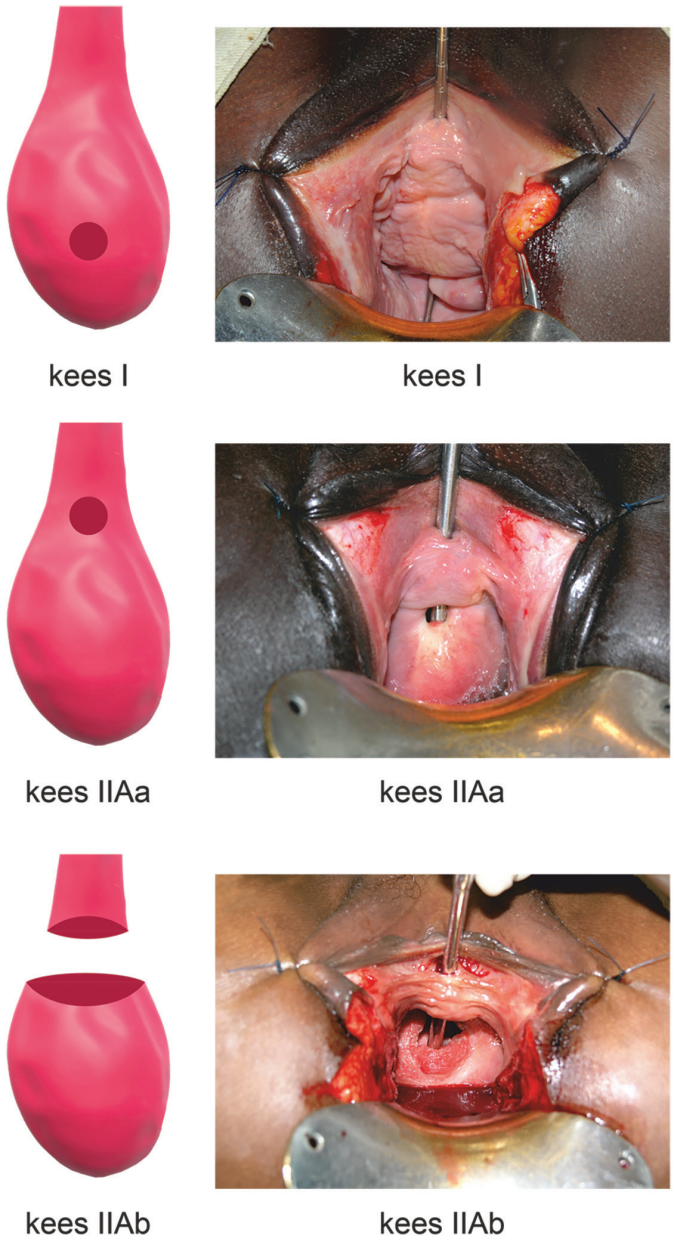

Figure 5. Fistula classification 01

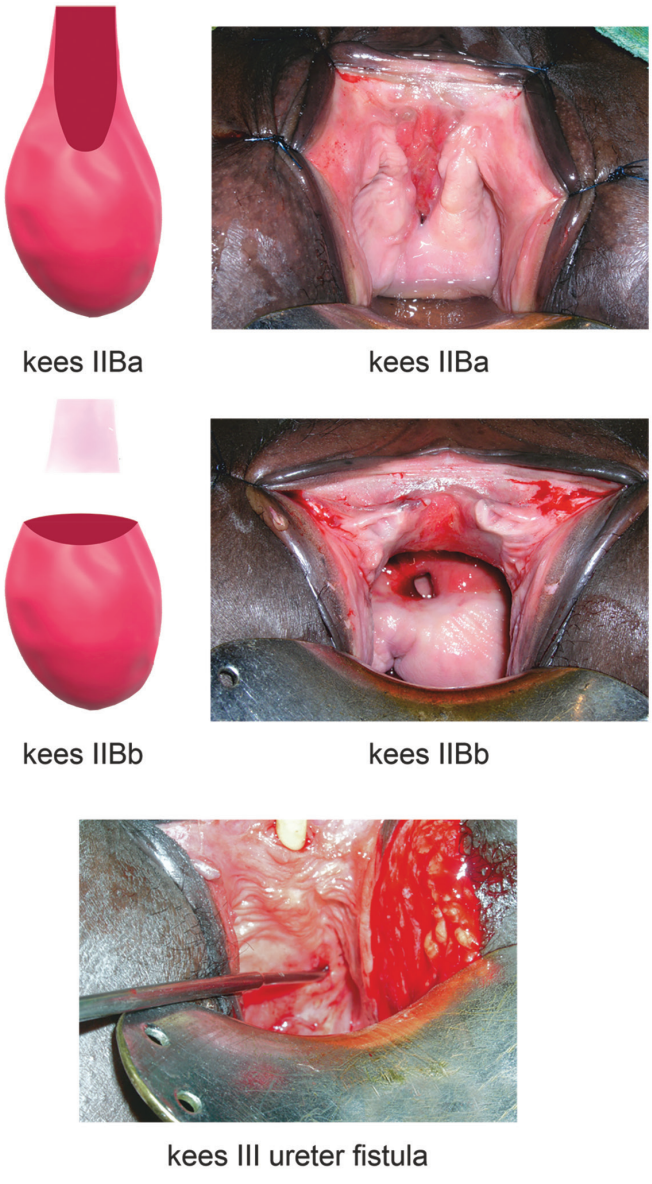

Figure 6. Fistula classification 02

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ETHICS

DISCLOSURES

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