HISTOLOGICAL CHANGES FOLLOWING URINARY BLADDER AUGMENTATION PERFORMED WITH A GASTRIC OR INTESTINAL SEGMENT EXCLUSIVELY OR SIMULTANEOUSLY IN CHILDHOOD: HUMAN AND ANIMAL STUDIES

ZOLTÁN KISPÁL M.D.

PH.D. THESIS

PROGRAMME LEADER

PROF. PÉTER HORVÁTH ÖRS

TOPIC LEADER

PÉTER VAJDA, M.D., PH.D., MED. HABIL.

UNIVERSITY OF PÉCS

CLINICAL CENTER

PEDIATRICS' CLINIC

2014.

INTRODUCTION

Partial or total childhood urinary incontinence (i.u) is one of those lifelong deficiencies which may seriously damage quality of life and render fitting into society impossible. For 80% of the cases congenital disturbances of the bladder innervation (neuropathic bladder) is responsible. This is most often caused by meningomyelocele, an innate failure of the closure of the spinal tube (MMC). The other 20% are caused by other developmental disorders (bladder extrophy, total epispadias, cloacal extrophy) and, rarely, damage to the nerves exiting the spinal cord and/or to the nerves of the bladder (Guillain Barré's syndrome, spinal cord injury, tumors).

At the pathophysiologic background of i.u. in childhood the following causes may stand (single or combined):

1. Urine storage deficiency: decreased bladder capacity is not able to store urine for the appropriate time (3-4 hours),
2. Urine containment deficiency: due to the deficient closure function of the bladder neck urine is leaking continuously despite appropriate bladder capacity
3. Failure of urine emptying: the patient cannot empty his/her bladder without residual urine.

The above listed disorders usually present in combination with each other, but symptomatic may be dominated by either one of them alone.

The aim of treatment is to decrease high bladder pressure while increasing bladder compliance and capacity.

It is an important criteria that besides abolishing the above mentioned pathophysiological cause(s), the morphology and function of the upper urinary tract and the kidneys should be preserved. Furthermore primary therapy should be conservative as operative treatment can have irreversible consequences.

In 80% of patients i.u. can be well treated with conservative methods, pharmaceutical bladder augmentation, clean intermittent catheterisation (CIC), pelvic floor training and the combination of these.

If childhood urinary incontinence is a result of small bladder capacity and/or inadequate bladder compliance and it cannot be treated (semi-) conservatively, surgical augmentation is needed.

In certain cases when the bladder is extremely small (e.g. bladder extrophy) or it was removed due to malignancy (rhabdomyosarcoma), bladder substitution (formation of an orthotropic bladder) can be performed.

To augment the bladder- besides several less often used techniques (e.g. autoaugmentation, ureterocystoplasty, tissue engineering) - nowadays a full thickness segment of the gastrointestinal tract (stomach small or large intestine) is used.

To surgically augment (substitute) the bladder small intestine is recommended as a first choice by the literature, but there is no evidence based procedure. The uncertainty is caused by the fact that following the introduction of these procedures, the complications which depend on the type of the gastrointestinal segment used were discovered in the last three decades.

The complications arising after the operation can be divided into two groups (metabolic and non-metabolic complications):

I. Metabolic complications and histological alterations

- metabolic acidosis/alkalosis
- electrolyte disturbances (hypo/hyper -chloraemia, -kalemia -natremia)
- growth disturbance
- changes in bone metabolism (bone demineralisation, osteopenia)
- vitamin deficiency
- histological alterations: inflammation, metaplasia, dysplasia, carcinoma formation

II. Non metabolic, surgical complications

- abdominal wall stoma complications
- ileus
- perforation of the bladder enlarged with intestinal or gastric segment
- persisting reflux after ureter neoimplantation
- ureter obstruction following ureter neoimplantation
- complications after bladder neck closure (vesicourethral fistula, orchidoepidydimitis)
- hematuria-dysuria syndrome
- reaugmentation
- small bowel bacterial overgrowth
- other surgical complications

From the complications listed above I wished to study histological alterations and tumor formation in my thesis. This is potentially the complication that is the most serious, most hard to prevent and often late detected. Long term follow-up is of extreme importance in the case of urinary bladder augmentation or substitution performed in childhood and adolescence because of the risk of malignant tumor formation.

Partly due to the earlier study of our group and partly due to the reports constantly appearing in the literature it is internationally accepted that following augmentation of the bladder with a gastrointestinal segment, regular histological biopsies should be taken (surveillance).

It is imperative during the care of these patients to prevent and detect malignancy in time, and to treat complications should they appear.

The risk of malignancy increases in itself with ageing, which is possibly aggravated further by augmentation.

The following factors may be held responsible for malignancy following bladder augmentation:

1. Chronic bacteriuria

Bacteria living in patients' urine can change nitrates into nitrites and finally into N-nitroso amines. Besides that, they may produce oxygen free radicals. Both groups may damage the DNA of the reservoir's epithelial cells.

2. The Urine's direct toxic effect on the gastrointestinal mucosa

Urothelial cells in the wall of the bladder accumulate organic osmolites and form an apical barrier which consists of a crystalline uroplakin network (asymmetric unit membrane). This barrier presents a formidable transepithelial resistance and low permeability for water and urea. Without this barrier osmotic stress may damage the genom's integrity which may lead to tumor formation

3. Enteral epithelium

The gastrointestinal segment used for bladder augmentation does not perform any absorption of nutrients in the classic meaning following augmentation. It loses this function and it may come to a chronic state of inflammation ("famine" enteritis) at the mucosa.

4. The interaction of two different tissue types

During bladder augmentation at the junction of the gastrointestinal and the uroepithelial mucosa (anastomosis line) aberrant signal transmission mechanisms occur.

In order to prevent tumor formation, the gravest complication following bladder augmentation and substitution, we need to look for such tumor markers which may signal a praecancerous state even before a macroscopic tumor can be detected. Mucines may be such markers in the case of adenocarcinomes.

Every epithelium in the body has its own mucin expression pattern, which changes in the case of malignancy. Mucins are high molecular weight glycoproteins which contain up to 50% carbohydrates. To date there are at least 20 types of mucins known. The main mucin protein of the urothelium is MUC1. On the surface of the enterocytes of the small and large intestine mainly MUC2 can be detected. In case of malignancy these expression patterns change. In adults it was observed that these changes in mucin expression pattern occur in the early stages of carcinogenesis. They may even precede macroscopically detectable changes. In the case of colonic adenocarcinoma the expression of MUC2 decreases on the cellular surface whereas the expression of MUC1 increases. In case of bladder adenocarcinoma the expression of MUC2 protein increases and MUC1's decreases.

AIMS

1. *Prospectively study the histological changes in patients following urinary bladder augmentation performed with a gastric-, small- or large bowel segment.*

2. *To study MUC1 and MUC2 gene expression in patients after bladder augmentation performed with a gastric-, small- or large bowel segment.*

3. *To study histological alterations, blood and urine samples in dogs following bladder augmentation performed simultaneously with a gastric and intestinal segment, or with a gastric (gastrocystoplasty) or intestinal segment (colocystoplasty) exclusively.*

MATERIALS AND METHODS

1. Human Studies

Our investigations were performed on the patients who underwent surgery at the University of Pécs Department of Pediatrics Surgical Unit between 1988 and 2008 (Medical Scientific Council Scientific and Research Ethics Licence number: 5011-0/2010-1018EKU). During this period urinary bladder augmentation or subsitution was performed in 86 patients. Ileocystoplasty was performed in 32 patients (pts), colocystoplasty in 30 pts and gastrocystoplasty in 18 pts. Bladder substitution with a large intestinal segment was performed in 6 pts.

Mean age at the time of surgery was 12.5 (4.3-20.9) years. Mean follow-up time was 8.3 (0.25-16.5) years.

Altogether 56 patients took part in the study, 8 from the gastrocystoplasty patients, 22 from the ileocystoplasty patients and 26 from the colocystoplasty patients.

Group A: In case of augmentation performed with small intestine (ileocystoplasty-32 patients) mean age at the time of surgery was 132 months (11 years 51-213 months), mean follow-up time was 42.5 months (3.9 years 3-90 months).

Group B: In case of augmentation performed with large intestine (colocystoplasty-30 patients), mean age at the time of surgery was 160 months (13.3 year 3-198 months), mean follow-up time was 100.5 months (8.4 years 3-198 months).

Group C: In case of augmentation performed with a gastric segment (gastrocystoplasty-18 patients), mean age at the time of surgery was 160 months (13.3 years, 79-241 months), mean follow-up time was 92.5 months (7.7 years 12-173 months).

Group D: In case of bladder substitution with large intestine (6 patients), mean age at the time of surgery was 123 months (10.3 years 83-195 months), mean follow-up time was 91.7 months (7.6 years 83-136 months).

Before surgery physical examination, imaging and instrumental examinations (cystography, cystomanometry), blood and urinalysis are performed. During bladder augmentation histological samples are taken from the bladder and from the gastrointestinal segment used for augmentation.

Following surgery patients took part in a regular follow-up, during which isntrumental examinations (abdominal and pelvic ultrasound scan, cystography, scintigraphy, cystomanometry, cystoscopy with biopsy), blood and urinalysis are performed.

1.1 Histological Studies

During bladder augmentation mucosal- submucosal biopsies were harvested from the original bladder and from the gastrointestinal segment used for augmentation.

In case of augmentation performed with a gastric segment, biopsies were also tested for H. pylori. After the 4th postoperative year biopsy was performed yearly, later on biannually including patients reaching adulthood. This was performed during cystoscopy of the augmented or substituted bladder. In younger and shyer patients interventions were performed in general anaesthesia, in older and cooperative patients under midazolam. If the original urethra was available (if it was not closed earlier), cystoscopy was performed through it. In other patients (in whom the urethra was closed and a catheterisable abdominal wall stoma was installed), cystoscopy and biopsy were performed through the stoma. During these interventions four biopsies were taken from the mucosa of the gastrointestinal segment used for augmentation, four from the mucosa of the original bladder, and four from the anastomotic line between these two.

Samples were either fixed in formaldehyde or were stored frozen. Biopsies stored in formaldehyde underwent routine histological examination (hematoxillin-eosine staining). The other half of the biopsies were embedded into OCT (Optimal Cutting Temperature) immediately at the time of cystoscopy and were stored at - 80°C afterwards. Further studies (immunofluorescent staining and submicroscopic examinations) were performed in The Evanston Hospital, Chicago Il, USA.

1.2. Immunohistological studies

Seven millimetre thick sections were cut in cryostat, then following fixation in 4% formaldehyde, blocking of aldehydes were performed in phosphate buffered saline solution with glycine (glycine-PBS). Antigen blocking was performed with 5% goat serum, after washing with phosphate buffered saline and a half hour incubation with phosphate buffered saline +Tween (PBST). Materials were incubated with the primary mouse anti MUC1 and MUC2 antibodies (Invitrogen) on 4 °C overnight. On the next day following repeated washes with PBST, secondary, rabbit- anti- mouse -antibodies conjugated with Alexa Fluor 488 (green) and Alexa Fluor 594 (red) were applied to the slides. At the end of the staining process Diammino-phenil-indol (DAPI) and mounting agent

(Invitrogen Slowfade Gold with DAPI) were used to stain nuclei. The immunostaining detailed above is suitable for qualitative analysis of MUC1 and MUC2 protein levels.

Stained samples were illuminated with green, red and blue light during fluorescent microscopy (quantitative analysis). When illuminated with green light, we detected intense green fluorescence at the sites where MUC1 protein was found. In the case of MUC2 protein, when the slide was illuminated with red light, an intense red fluorescence was detected at the sites where the protein was present. Nuclei were stained blue and were transilluminated with blue light. Images were taken with a charged coupled device (CCD) camera. With the help of the software attached to the microscope the intensity of green (MUC1), red (MUC2) and blue (nuclei) were measured and the data was statistically evaluated. Changes were regarded as significant at $p<0.05$.

Evaluation of data was performed with a thorough survey of all patients' documentation. For statistical calculations SPSS software, Fisher test, least squares method, linear correction was used with the help of a statistician ($p<0.05$).

1.3. MUC1, MUC2 gene expression studies

Submicroscopic studies were performed on such patients' samples in whom augmentation was performed more than 15 years ago (14 patients). On the samples of the above mentioned patients, which were stored at - 80°C, Laser Capture Microdissection (LCM) was performed.

During LCM with the help of a thin laser beam arbitrary cells (mucosal epithelial cells) of a histological sample can be derived without any damage. This procedure increases sensitivity of submicroscopic studies (protein, DNA, RNA analysis) greatly.

For RNA extraction first 50 μL extraction buffer was put into a 0.5 ml micro centrifuge tube (Applied BioSystems Catalog #N8010611). Afterwards using the LCM Cap Insertion Tool the Capsure Macro LCM cap was applied to the micro centrifuge tube. This setup was turned upside down and was incubated for half an hour at 42 degrees Celsius. To collect the cell extract to the micro centrifuge tube, it was centrifuged for 2 minutes at 800xG.

Following centrifuge the micro centrifuge tube contained the cell extract from which RNA was isolated.

The RNA purification column was conditioned as a first step to RNA isolation: 250 μL condition buffers was pipetted on the filter membrane of the column and was incubated for 5 minutes at room temperature. The column was centrifuged to the given collecting tube at 16000xG for a minute then 50 μL 70% ethyl alcohol was pipetted to the cell extract.

The mix of ethylene and cell extract were pipetted on to the preconditioned purification column. It was centrifuged for 2 minutes at 100xG till the binding was formed then immediately at 16000xG for 30 seconds.

Washout from the column was performed as follows: 100μL Wash Buffer 1 was pipetted to the column and was centrifuged for one minute at 800xG. Then 100 μL Wash Buffer 2 was pipetted on the purification column and underwent centrifugation for 2 minutes at 16000xG.

The column was put into a new micro centrifuge tube. Elution buffer was pipetted onto it, the amount of which was determined by the table included in the kit, and it was incubated for one minute at room temperature. The column was centrifuged for a minute at 1000xG to dissolve the elution buffer then at 16000xG for a minute to release RNA. At the end of the process we had the purified RNA at our disposal and ran Real Time PCR with MUC1 and MUC2 primer. The method described above is suitable for quantitative measuring of MUC1 and MUC2.

2. Animal experimental model: creating a composite bladder in dogs

Animal experiments were carried out at University of Pecs, Department of Surgical Research and Techniques with licence from the University Ethical Committee (Licence no: 3793.316-4612/2010). Operations were carried out on 12 female, six-month-old beagle dogs in development. The animals weighed approximately 10 kg. Females were chosen because of easier catheterisation and repeated cystoscopic biopsies. The animals were divided into three groups:

Group A. In two dogs cystoplasty was performed with a gastric segment only (gastrocystoplasty).

Group B: In two dogs cystoplasty was performed with an isolated colonic segment only, opened along the anti-mesenteric side (colocystoplasty).

Group C: In eight dogs cystoplasty was performed with a gastric and an intestinal segment simultaneously (composite bladder).

The animals in group A and B served as control groups.

The animals were not fed for twenty-four hours before surgery, but they were allowed to drink as much as they wished. Parenteral antibiotics calculated for their body mass (2.5 mg/kg gentamycin and 2g penicillin) were administered preoperatively. In the case of gastrocystoplasty and creation of composite bladder H2 receptor blockers (150 mg ranitidine) was also administered. Urine and blood samples were also gathered preoperatively.

The operations and the repeated biopsies (cystoscopies) were performed in intra-tracheal narcosis (halothane, N2O) following thiopental induction. After narcosis induction a 10 Ch Foley catheter was introduced to protect the anastomosis.

Group A (gastrocystoplastny): Operations were performed from long median laparotomy. A 7x7cm approximately even sided triangle was incised from the corpus of the stomach which blood supply was provided through the left gastroepiploic artery. The integrity of the stomach was restored with a double layered suture. The gastric flap with its blood vessel was brought intra-peritoneally to the original urinary bladder. The cranial half of the bladder was resected, and then the gastric segment was sutured to the remaining caudal bladder half with a one-layered running suture.

Group B (colocystoplasty): Operations were performed from lower median laparotomy. An approximately 10-12 cm long segment was isolated from the descending colon with its blood supply intact. Continuity was restored by using end-to-end anastomosis. The isolated segment were opened longitudinally on its anti mesocolic side, by which a quadrangle segment was formed. This was sutured in a U shape (detubularisation) and was sutured with one layered running suture to the remaining caudal bladder half.

Group C (composite bladder): Operations were performed from long median laparotomy. Gastric and intestinal segments were isolated as previously described in group A and B. The cranial bladder half was resected and the isolated gastric and intestinal segments were anastomosed to the original bladder half and to each other. Thus a urinary reservoir was created which consisted of the gastric segment in one quarter, the colonic segment in another quarter and the remaining caudal (original) bladder half.

Following surgery urinary drainage was provided by a suprapubic 20 Ch Pezzer and a transurethral (Foley) catheter. The animals received parenteral (i.m) pain medication (10 mg/kg Algopyrin), and antibiotics (gentamycin and penicillin) till the 7th postop day, which were continued orally for two weeks (50 mg/kg amoxicillin and clavulanic acid). The animals from group A and C received parenteral (i.m.) H2 receptor blockers till the 5th postop day. The animals received pulpy food from the 3rd postop day and solid food from the 5th postop day. The transurethral catheter left in the bladder was removed on the 7th postop day, the suprapubic catheter on the 14th postop day.

2.1. Histological studies

During cystoplasty and at the end of the twelve-month-follow up after sacrificing the animals 4-4 biopsies were taken from the gastrointestinal segment used for augmentation (stomach and large intestine), from the original bladder and from the anastomosis between these two. In case of the composite reservoirs, samples were also taken from the stomach-bladder, stomach-large intestine, and the large intestine-bladder anastomosis too. Following the operations 4 and 8 months postoperatively biopsies were harvested during cystoscopy in narcosis.

2.2. Immunohistological studies

Part of the biopsies were formalin fixed, the sections were examined by two independent pathologists following hematoxillin-eosine and PCNA staining. Mitotic index was also calculated in the sections. In ten large magnification (400x) randomly selected field of view PCNA positive (brown stained) cells were counted and the percentage was given to 1000 cells. In the large field of view following pathological consensus 1000 cells were evaluated. We did not include necrotic or haemorraghic tissue areas. The other half of the samples were embedded in OCT and were processed with the method described at the human samples. Data was evaluated with a statistician.

RESULTS

1. Human studies

1.1 Histological studies

We detected metaplastic changes in 3 patients 8, 10 and 14 years after augmentation. In one patient it was detected in the colonic segment used for augmentation, in another one in the original bladder part and in one in the anastomotic line between the original bladder part and the intestinal segment used for augmentation.

Dysplasia was detected in 6 cases after 4 years. Malignancy was seen once in our patient material (polyp- like in situ adenocarcinoma), which was detected 11 years after colocystoplasty at the colon-original bladder anastomosis line as a polyp- like neoplasm. No H. pylori infection was detected after gastrocystoplasty.

1.2. Immunohistological studies

We found no significant changes in protein levels in the tissues used for augmentation and in the urothelium of the original bladder following gastrocystoplasty, ileocystoplasty and bladder substitution (Group A,C, D).

Following colocystoplasty (Group B) Muc 1 protein expression increased significantly ($p<0,001$), Muc 2 protein expression decreased significantly ($p<0.05$) in the mucosa of the colon used for augmentation. That is to say that the amount of MUC 1 protein increased in the mucosa of the colon used for augmentation, whereas the amount of MUC2 protein decreased.

We found strong MUC1 expression and weak MUC2 expression at the double immunostaining of the polyp found in a twenty-eight-year-old female patient 11 years after augmentation. We detected the same changes when staining the biopsies taken from the surrounding mucosa of the polyp- like adenocarcinoma.

1.3. MUC1, MUC2 gene expression studies

The expression of MUC1 gene increased with time whereas the expression of MUC2 decreased ($p<0,05$) during RT PCR examinations of the samples of those 14 patients who underwent colocystoplasty. An increased expression of MUC 1 gene expression and a decreased expression of MUC 2 gene expression was detected in the samples of the patient in whom 11 years post augmentation an in situ carcinoma was found. RT PCR examination detected maximal MUC1 gene expression and minimal MUC2 gene expression in the samples of this patient 11 years after augmentation.

In the original samples of these patients no significant MUC1 and MUC2 gene expression changes were found.

2. Animal experiments

2.1 Histological studies

All samples taken from the gastric and intestinal segments and from the original bladder showed normal morphology at the time of augmentation.

Group A (gastrocystoplasty): Both augmented bladders showed normal morphology 4 months after augmentation. Eight and 12 months following surgery chronic inflammation was detected in the original bladder parts of both animals. Dysplasia was seen in one animal's original bladder part. PCNA Index was 38-40%

Group B (colocystoplasty): Both augmented bladders showed normal morphology 4 months post augmentation. Eight and 12 months following surgery chronic inflammation was detected in the original bladder parts of both animals. Dysplasia was detected in one of the colonic segments used for augmentation. PCNA Index was 35-40%.

Group C (composite bladder): In the gastric part of one of the composite bladders an ulcer was found 4 months postoperatively, which healed spontaneously by the end of the follow up (12 months). Four months after augmentation 6 composite bladders showed normal morphology in the gastric as well as in the colonic segment and in the original bladder parts. Chronic inflammation was seen in one original bladder part and in a different animal in the colonic segment of the composite bladder. Eight months following augmentation there was chronic inflammation in 5 animals' bladders. In three animals it was localised to the original bladder parts and in two it was detected in the colonic segments. Chronic inflammation was detected twelve months postoperatively in 4 animals' colonic segments, and in two animals' gastric segments and in one animal's original bladder part. Dysplasia was detected in 3 animals' colonic segments of the neocyst and in one animal's gastric segment. One adenoma was found in the colonic segment and

one low grade dysplasia was found in another animal's gastric segment. PCNA Index on the mucosa of the gastric segment was 85-90%, on the colonic segment 70%

2.2. Immunohistological studies

Statistical evaluation showed a significant decrease in the expression of MUC1 proteins in both the samples of group B (colocystoplasty) and C (composite bladder) 4.8 and 12 months after augmentation ($p<0,0001$).

No significant changes were detected in MUC2 protein expression of the colonic samples. The expression of MUC2 decreased less descriptively but significantly ($p=0.03$). No changes were detected in the expression of MUC1 protein after gastrocystoplasty.

SUMMARY OF RESULTS

1. *Based on routine histological examinations and a nearly fifteen-year-long follow up it can be stated that malignancy is less likely to form in the first ten years following augmentation performed with a gastric-, small- or large- intestinal segment. However metaplasia or premalignant changes (dysplasia) can be expected in the first decade.*

2. *Based on histological studies performed on human samples the expression of MUC1 protein increases, the expression of MUC2 decreases in the colonic segment used for augmentation.*

3. *A prominent MUC1 protein level and an extremely low MUC2 protein level could be measured in the samples of the patient with the polypoid in situ adenocarcinoma. Earlier samples from the same patient from before the appearance of the tumor showed a reduced MUC2 protein level and an increased MUC1 protein level, which may herald the formation or an increased chance of malignancy.*

4. *Based on immunohistological studies on samples harvested from patients and dogs, a follow up of MUC1 and MUC2 protein levels following augmentation may help early diagnosis of malignancy.*

5. *Histological changes in the reservoir and the risk for possible malignancy are not reduced after bladder augmentation performed simultaneously with a colonic and a gastric segment (composite bladder) in dogs.*

6. *In beagles the levels of MUC1 and MUC2 proteins decrease with time in the original bladder part following cystoplasty performed with a gastric- or colonic segment. They also decrease in the colonic segment in the case of composite bladder formation.*

LIST OF PAPERS AND PRESENTATIONS

PAPERS FROM THE TOPIC OF THE THESIS

1) Kispál Z, Vajda P, Vástyán A, Juhász Zs, Pintér A: A húgyhólyag gyomorral és béllel történő egyidejű megnagyobbítása – kompozit hólyag (állatkísérletes modell). Magyar Urológia. 18:(4) pp. 230-6, 2006. **IF: -**

2) Vajda P, Kispal Z, Lenart I, Farkas A, Vastyan AM, Pinter AB: Quality of life: Urinary bladder augmentation or substitution in children. Pediatr Surg Int, 2009, 25(2); 195-201. **IF_{2009}: 0,945**

3) Kispal Z, Balogh D, Erdei O, Kehl D, Juhasz Z, Vastyan AM, Farkas A, Pinter AB, Vajda P: Complications after bladder augmentation or substitution in children: a prospective study of 86 patients. BJU Int. 108:(2) pp. 282-9, 2011. **IF_{2011}: 2,844**

4) Kispal Z, Vajda P, Kereskai L, Jakab CS, Vastyan A.M., Juhasz ZS, Pinter A.B.: Composite urinary reservoire in dogs: histological indings. J Urol. 187:(3) pp. 1110-5, 2012. **IF_{2012}: 3,696**

5) Kispál Z, Vástyán A, Pintér A, Vajda P, Jilling T: MUC-1 és -2-fehérje expressziójának változása kutyákban gyomorral és vastagbéllel egyidejűleg megnagyobbított húgyhólyagban. Gyermekgyógyászat. 63:(1) pp. 24-8, 2012.**IF: -**

Total impact factor: 7,485

PRESENTATIONS IN THE TOPIC OF THE THESIS

1) Kispal Z, Vajda P: Az életminőség hólyagmegnagyobbító és pótló műtétek után. Házi TDK konferencia. Pécs, 2006.

2) Vajda P, Kispál Z, Farkas A, Vástyán A, Lénárt I, Kappéter B: Quality of Life in Patients and Parents following Urinary Bladder Augmentation and Substitution. National Congress of the Hungarian Association of Pediatric Surgeons. Kőszeg, 2006.

3) Kispál Z, Vajda P: Urinary bladder augmentation performed simultaneously with gastric and intestinal segment-An experimental model in dogs. International Student Congress on Medical Sciences. Groningen, The Netherlands, 2007.

4) Kispál Z, Vajda P: A húgyhólyag gyomor- és bél-szegmentummal egyidejűleg történő megnagyobbítása - új állatkísérletes model. Házi TDK konferencia. Pécs, 2007.

5) Kispál Z, Vajda P: A húgyhólyag gyomor- és bél-szegmentummal egyidejűleg történő megnagyobbítása - új állatkísérletes modell. Országos TDK konferencia. Budapest, 2007.

6) Kispál Z, Vajda P: Húgyhólyag gyermek- és serdülőkorban végzett megnagyob-bításának sebészi szövődményei. Spring session of Hungarian Association of Pediatric Surgeons, (Young investigators' Forum). Göd, 2009.

7) Kispal Z, Balogh D, Juhasz Zs, Vastyan AM, Farkas A, Pinter AB, Vajda P: Complications Following Bladder Augmentation or Substitution In Children - A Prospective Study of 86 Cases. 11th European Congress of Pediatric Surgery. Bern, Switzerland, 2010.

8) Kispal Z, Jilling T, Balogh D, Lovasz M, Vastyan AM, Farkas A, Pinter AB, Vajda P: Altered expression of muc 1 and 2 genes following urinary bladder augmentation or substitution in children as a predictive sign of malignancy. Spring session of Hungarian Association of Pediatric Surgeons (Young investigators' Forum). Pécs, 2011.

9) Kispal Z, Balogh D, Jilling T, Vastyan AM, Pinter AB, Vajda P: Changes of mucin gene expression (altered expression of mucin genes) in gastrointestinal mucosa used for creating a composite reservoir in dogs. 12th European Congress of Pediatric Surgery. Barcelona, 2011.

OTHER PAPERS NOT RELATED TO THE THESIS

1) Widhalm K, Dietrich S, Prager G, Silberhummer G, Orth D, Kispal Z:Bariatric surgery in morbidly obese adolescents: a 4-year follow-up of ten patients. Int J of Ped Obesity. 2008; 3 Suppl 1:78-82. **IF_{2008}: 2.276**

OTHER PRESENTATIONS AND POSTERS NOT RELATED TO THE THESIS

1) Kispál Z, Vástyán A, Kelemen D Sebészi beavatkozást igénylő pancreassérülés. Háziorvos továbbképzés. Pécs, 2008.

2) Kispál Z, Vástyán A, Kelemen D: Sebészi beavatkozást igénylő pancreassérülés. (Poszter) Fiatal Gyermekgyógyászok Kongresszusa. Kőszeg, 2009.