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Three-dimensional Power Doppler Ultrasonographic Evaluation of Induced Cystitis in Dogs

N. Ramezani[1]
Sarang Soroori[*2], DVSc
Shahram Jamshidi[2], DVSc
Mohammad Molazem[2], DVSc

[1] Undergraduate Student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
[2]Department of Clinical Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Abstract

Objectives: The aim of this study was to investigate if three-dimensional power Doppler (3DPD) ultrasound (US) can detect bacterial cystitis in dogs better than two-dimensional (2D) US.

Animals: Six mixed breed dogs

Procedures: Ten ml of 0.1% alcoholic solution of salicylic acid was entered into the urinary bladder of six male mixed-breed dogs by catheter. Twenty four hours later, culture solution of *Proteus mirabilis* (10^3CFU /ml) was inoculated into the urinary bladder. The dogs were examined with complete blood and urine analysis and bacteriological culture on specified days during the infection and the treatment period. The urinary bladders of dogs were assessed by both 3DPD and 2D US techniques. All dogs were treated with therapeutic doses of Ceftriaxone for two weeks, 14 days after the infection.

Results: results showed that 3DPD can better define the morphological and vascular characteristics of cystitis; [1] however, the main lesions were identified by both 2D and 3DPD US.

Clinical Significance: The data showed that, the indicators of 3DPD can differentiate the disease acuteness and treatment response better than 2D US.

Key words: Three-dimensional Power Doppler Ultrasonography, Two-dimensional Ultrasonography, Cystitis, Dog, Proteus Mirabilis.

[*] **Corresponding author:**
Sarang Soroori, DVM, DVSc,
Department of Clinical Science, Faculty of Veterinary Science, University of Tehran, Tehran, Iran;
Email: Soroori@ut.ac.ir

Introduction

Urinary tract infection refers to a bacterial invasion to any or all parts of the urinary tract but most commonly the urinary bladder called *bacterial cystitis*. Urinary tract infection is common in dogs; especially in females is most often happening the result of ascending fecal bacterial contamination of the vulva, perivulvar skin, vestibule, or prepuce. The most common organisms causing bacterial cystitis include *Escherichia coli* (40–50% of cases) and *Staphylococcus, Proteu*s, *Streptococcus,* and *Enterobacter* species. Diseases that cause polyuria and polydipsia or those that affect host immunity may predispose an animal to develop urinary tract infection. [3]

The methods for diagnosing cystitis include: physical examination, urinalysis, complete blood cell and serum biochemical analysis, diagnostic imaging and urine culture (gold standard).[4] All methods have advantages and disadvantages, so it would be better choose the one which is less time-consuming and semi-aggressive. Additionally, in some cases there is no clinical sign in physical examination; Complete Blood Cell, biochemistry test and urinalysis are not sensitive methods neither. Conventional ultrasonography merges three-dimensional data into two-dimensional tomographic images. These data have made mental reforming process easier and more accurate. In two-dimensional power Doppler ultrasonography, there are multiple sample volumes within a circumscribed region shown as a color box on the B-mode tomogram. By stacking up the acquired images, in conventional ultrasonography and power Doppler ultrasonography, imaging can be displayed in three-dimensional power Doppler ultrasonography superimposed within the surrounding tissue. A major advantage of three-dimensional power Doppler ultrasonography is that not only the flow in a selected vessel can be detected, but also the anatomic pattern can be appreciated without any invasiveness, therefore accuracy and sensitivity of this method is more. [5] Three-dimensional power Doppler ultrasonography also allow to detect the vascularization of the organ in quantitative values.[6]

Although there are a few studies on the potential applications in veterinary practice and it seems to be numerous in near future. Recent publications have shown increasing usage of the methods in human medicine in diagnostic and treating indications such as breast, ovarian and prostate cancer, liver tumors, fetal vascular anomalies, intrahepatic portosystemic venous shunt ,and ischemic nephropathy.[7-8-9-10-11-12-13]

In cystitis, increasing perfusion in the wall of urinary bladder is expected; therefore, three-dimensional power Doppler ultrasonography can measure the quantity of the vascularization. Urinary bladder is ideally suited for the ultrasonographic examination because of the excellent acoustic properties of the fluid nature of urine and the superficial location. Three-dimensional ultrasonography can provide information relative to the capacity of the bladder, change in bladder outline, and changes in the thickness and structure of the wall, identification of luminal structures and mural masses, and identification of extrinsic may displace the bladder or distort the wall. In two-dimensional ultrasonography, acute cystitis produces no distinctive changes, unless blood clots are present. Even in chronic cystitis detective are rare and limited to increase wall thickness.[14]

The purpose of this study was to compare three-dimensional power Doppler ultrasonography and two-dimensional ultrasonography for diagnosing acute and chronic bacterial cystitis in dog.

Material and Methods

Animals

A total six 1-2 year-old, male and mixed breed dogs (20-30 kg weight) were selected which had been candidates for euthanasia due to incurable diseases. Two days before inoculation of bacteria, the dogs were examined with complete blood cell, biochemical test (BUN, Serum Creatinine) and urinalysis. The samples were evaluated by high-resolution microscope (WBC: HPF (High-Pass Filter) 3<, RBC: HPF 5 <, Epithelial Cell: HPF 5< and existing Bacteria) for lower urinary track infection.[15]

The dog's urinary bladders were examined by both two-dimensional and three-dimensional power Doppler ultrasonography. All dogs were healthy in clinical and hematological side. The dogs were all taken 10 mg Morphine Sulfate every 8 hours orally during the examination period for reducing any suffering.

Induction of experimental infection:

Cystitis was induced in each dog by introducing 10 ML of 0.1% alcoholic solution of salicylic acid (the equal proportion of each) in to urinary bladder after catheterization and drainage of residual urine. Twenty four hours later, 5 ml of 24 hour culture solution of *Proteus mirabilis* (10^3 Colony-Forming Units/ml) was entered into urinary bladder via urethral catheter.[16-17]

Examinations:

Ultrasonogarphic Methods

The urinary bladder was assessed by two ultrasonographic techniques (two-dimensional and three-dimensional power Doppler ultrasonography) in days (2, 8, 10, 12, 14, 17, 19, 21, 23, 26 and 28) after inoculation. The ultrasonographic examination of urinary bladder was established in all dogs in the same days (2, 8, 10, 12, 14, 17, 19, 21, 23, 26 and 28). The animals were placed in dorsal recumbency. All the abdominal hair was clipped ventral to the sublumbar muscle. The skin was prepared by cleaning (70% alcoholic solution) and plenty of acoustic gel. Appropriate setting for two-dimensional and three-dimensional power Doppler ultrasonography was done. Exactly after the urination of dogs, the measurement was performed by these techniques. The data were processed by 4D view [*1]. The three-dimensional power Doppler ultrasonography vascularization indexes were considered (Vascularization Index, Flow Index, and Vascularization and Flow Index) evaluated by the software itself.[18]

Urine Culture and Urinalysis

Urinalysis was recorded on specific days (Day 2, 8, 10, 12, 14, 17, 19, 21, 23, 26 and 28). Sampling for urine culture was performed in 2 days before and 2, 14, 21 and 28 days after inoculation, also of the survey. All urine sampling for urine analysis and culture were

*-Version 5.0 software designed by GE Medical Systems Kretztechnik GmbH & Co OHG Company

obtained by cauterization. Criterion of infection in the urine culture test was based on the number of bacteria per CFU/Ml (10^3 CFU/Ml < indicate infection).[15]

Blood Tests

Two days after inoculation, Complete Blood Cell and biochemical tests were done on the specific days (Day 2, 8, 10 and 14) for ensuring the process of infection. During the treatment period, these tests were also repeated again (Day 17, 19, 21, 23, 26 and 28) for following up the results.

Treatment

After 14 days of inoculation and performing all tests, the dogs were treated for 2 weeks with Ceftriaxone 1 gr after a antibiogram was done with 9 antibiotics including: Cotrimoxazole, Trimetoprim Solfametoksazol, Oxytetracyclin, Ceftriaxon, Enrofloxacin, Gentamicin, Ciprofloxacin, Ceftiofore.[4] During the treatment period, all tests described above were repeated for assessment of the effect of the treatment on dogs and all indexes were recorded. Eventually, the results of both methods of ultrasonogarphy were compared.

Statistical analyses

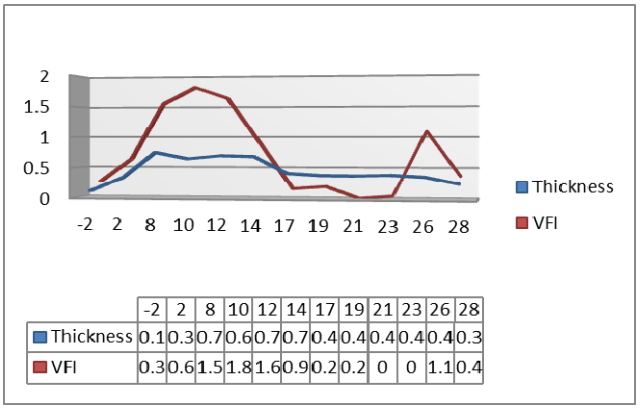
The Repeated Measures Define Factor Test (SPSS software) was used for the statistical analysis of the results. A $p<0.05$ was considered significant.[19]

Results

The results were considered in the three groups as follow:

Blood Test Result (CBC, Biochemistry)

The complete blood cell counts (White Blood Cell, Granulocyte and Band cell) increased in the early stage of infection period and then there were steady decreasing in the late infection and treatment periods until they reached to the normal ranges. BUN and Serum Creatinine were in the normal ranges throughout the whole study (Table 1).

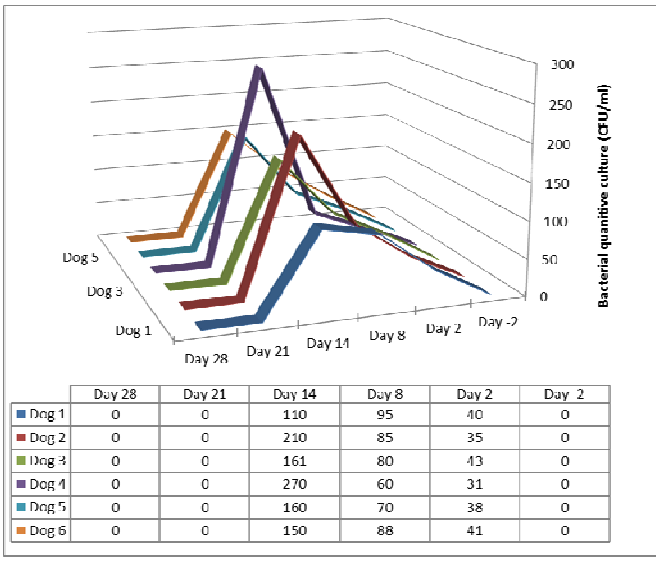


	-2	2	8	10	12	14	17	19	21	23	26	28
Thickness	0.1	0.3	0.7	0.6	0.7	0.7	0.4	0.4	0.4	0.4	0.4	0.3
VFI	0.3	0.6	1.5	1.8	1.6	0.9	0.2	0.2	0	0	1.1	0.4

Table 1- Comparision between urinary bladder wall thickness detected by two-dimensional ultrasonogarphy and VFI indexes by three-dimnetional ultrasonography (VFI: Vascularization Flow Index) (X Axis: Days Y Axis: VFI index (0-100); wall Thickness in Cm).

Urinalysis and Urine Culture Test Result

The urinalysis and urine culture tests showed no signs of white blood cell, red blood cell, epithelial cell and bacteria before the induction of infection (day-2 to day 0) but these tests depicted an increase in the number of white blood cell, red blood cell, epithelial cell and bacteria during the infection period (day 0 – day 14). Continuing decreased in the treatment period until bacteria counts reached to zero at the end of study (day 14- day 28) (Table 2).



	Day 28	Day 21	Day 14	Day 8	Day 2	Day -2
Dog 1	0	0	110	95	40	0
Dog 2	0	0	210	85	35	0
Dog 3	0	0	161	80	43	0
Dog 4	0	0	270	60	31	0
Dog 5	0	0	160	70	38	0
Dog 6	0	0	150	88	41	0

Table 2. Bacterial quantitative culture on the specific days in dogs

Ultrasonography

The utrasonographic results showed that the bladder wall thickness, which was measured by Two-dimensional ultrasonography, was in the normal range before infectious period (day -2 to day 0), thickened in the infection period (day 0 – day 14); and thinned gradually until reaching the normal range again at the end of treatment period (day 14 - day 28). Three-dimensional power Doppler ultrasonography results illustrated that all the indexes (VI, FI and VFI) were in the normal ranges before infectious period (day-2 to day 0), increased significantly in the infection period (day 0 – day 14) and decreased steadily in the treatment period (day 14 - day 28) (Table1, Figure 1-2).

According to all of the tests and statistical analysis, both two-dimensional and three-dimensional power Doppler ultrasonography can diagnose cystitis in same days whereas there was a significant difference in vascular indexes of three-dimensional power Doppler ultrasonography in the treatment period; Therefore, three-dimensional power Doppler ultrasonography can be more accurate technique than two-dimensional ultrasonography by measuring the urinary bladder vascularization and quantitative assessment of blood flow in tissue instead of assessing the wall thickness of urinary bladder only.

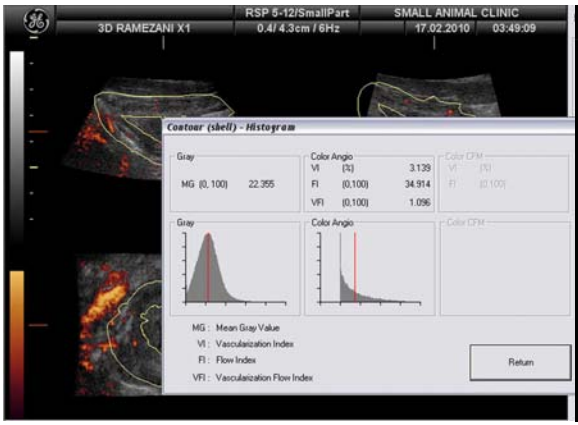


Figure1. Bladder wall thickness by two-dimensional ultrasound during the infection period

Figure2. Three-dimentional color histogram during the infection period

Discussion

This survey demonstrates that three-dimensional power Doppler ultrasonography can assesse urinary bladder vascularization and its healthy aspect. In the light of our foundlings, we believe that three-dimensional power Doppler ultrasonography can be performed in clinical cases in human and animal and is a simple method technically. In addition to vascular pattern, three-dimensional power Doppler ultrasonography can show quantitative assessment of blood flow in tissue achieved by histogram factors. Beside the mentioned factors, this technique can be used for the diagnosis of lower urinary tract infection and following up the treatment process.

Previous researches showed that three-dimensional power Doppler ultrasonography can be an appropriate method for imaging, assessing, measuring the volume and pattern of vascular organs, angiography, evaluation and diagnosis of malignant tumors from benign tumors in the body, detection of fetal chromosome anomalies and follow up the patient is responding to treatment.[20-21-22-23]

The results showed that both two-dimensional and three-dimensional power Doppler ultrasonography techniques were able to detect cystitis at the same time and both techniques were shown the recovery process in the treatment period.; furthermore, there was no significant different between the day of full recovery and the day before inoculation in three-dimensional power Doppler ultrasonography vascularization indexes which can be a good sign for recovery diagnosis in contrast with two-dimensional wall thickness data which were significantly different with pre-inoculation period.

The blood results illustrated that the dogs were got systemic infection in the first days of infection period. This infection was controlled by dominance of immune system and over the time; therefore, the blood factors returned to normal. Besides, urinalysis testing factors (white blood cell, red blood cell, epithelial cell and bacteria) were negative before infection period (day-2 to day 0), got surged in the infection period (day 0 - day 14) decreased and got normal until end of the treatment period (day 14 - day 28). Drawing on the data collected, laboratory results confirmed the infection process in the course of disease (day 0 - day14), the patient recovered in the treatment period and full recorded. We utilize urine culture test as golden was shown that amount of bacteria increased in the infection period and there was steep fall to zero until end of the treatment period.[3-4]

Systemic infection usually is not happened in cystitis; therefore, blood indexes cannot be appropriate factors for diagnosis of lower urinary tract infection although during our survey (day 2- day 19) systemic infection happened which may caused by injection of virulent bacteria and was controlled by dominance of immune system and injection of antibiotic over the time. Besides, urine culture is another diagnostic method for cystitis which is expensive and time-consuming and due to the dilution of bacteria during the treatment period, the measurement errors may occur.

According to the pervious researches, bladder wall thickness in two-dimensional has been only diagnostic index for cystitis, but this survey showed that increased wall thickness has remained even after treatment; therefore it cannot be an accurate diagnostic factor for following up the response to treatment. On the other hand, vascularization indexes obtained from three-dimensional power Doppler reduced and got to normal during treatment period.

In addition, there was increasing in VFI index in 26 day which may be related to our mistake in evaluating the urinary bladder by ultrasound or because of the recurrent of cystitis in dogs. It should be mentioned that this increasing controlled and got normal at the end of treatment period.

In this research, for better assessment of significance of data, Repeated Measures Define Factor was used as statistical method in SPSS software due to few number of studding samples and short interval of sampling.[19]

Finally, we believe that using three-dimensional power Doppler ultrasound and histogram factors can be useful for diagnosis and assessment of responding to treatment. The present study demonstrates three-dimensional power doppler ultrasonography as new applied trend in faster diagnosis and evaluation of recovery from diseases in comparison with two-dimensional ultrasonography.

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چکیده

ارزیابی اولتراسونوگرافی داپلر قدرتی ۳ بعدی سیستیت ایجاد شده در سگ

رمضانی، سارنگ سروری، شهرام جمشیدی، محمد ملازم

گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

هدف- بررسی اینکه آیا الوتراسونوگرتافی داپلر قدرتی سه بعدی می تواند سیستیت باکترایی در سگ را بهتر از اولتراسونوگرافی دو بعدی تشخیص دهد.

حیوانات- شش قلاده سگ نژاد مخلوط

روش-۱۰ میلی لیتر محلول الکلی ۰/۱ درصد اسید سالیسیلیک به داخل مثانه ۶ قلاده سگ نژاد مخلوط نر بوسیله کاتتر وارد شد. ۲۴ ساعت بعد محلول کشت باکتری پروتئوس میرابیلیس به داخل مثانه وارد گشت. سگ‌ها از نظر آنالیز خونی و اداری و کشت باکتریایی در روزهای خاص در طی دوره عفونت و درمان مورد ارزیابی قرار گرفتند. مثانه سگ‌ها با استفاده از سونوگرافی دوبعدی و سه بعدی ارزیابی شدند. همه سگ‌ها با دوز درمانی سفتریاکسون برای دو هفته، ۱۴ روز بعد از عفونت درمان شدند.

نتایج- نتایج نشان داد که روش داپلرقدری سه بعدی حدت و پاسخ به درمان را نسبت به روش دوبعدی بهتر نشان داد.

نتیجه‌گیری-: اندیکاتورهای اولنتراسونوگرافی قدرتی سه‌بعدی می‌تواند حدت و پاسخ به درمان را بهتر از اولتراسونوگرافی دوبعدی تفریق دهد.

کلید واژگان- اولنتراسونوگرافی قدرتی سه‌بعدی، اولتراسونوگرافی دوبعدی، سیستیت، سگ، پروتئوس میرابیلیس