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Correlation of hypoechogenic lesions with lactic dehydrogenase isoenzymes, culture and cytological findings of prostatic fluid and biopsied tissue in dogs

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개에서 전립선액과 생검조직에 대한 젖산탈수소 효소치, 배양 및 세포학적 검사와 초음파상의 저에코 영역과의 연관성

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초 록 : 초음파학적 검사에 따라 미만성 저에코 영역, 낭포, 다병소성 낭포 및 비후된 피막 등 비정상적 소견을 보이는 전립선에서 추출한 전립선액과 생검조직에서의 LDH 분획비(LDH I/V)의 평균치는 각각 0.92±0.55, 2.69±0.82를 나타냈으며, 정상군(각각 0.23±0.20, 0.57±0.36)에 비하여 유의성($p<0.01$) 있게 높았다.

세포병리학적 검사에서 전립선 비대증 및 전립선염으로 나타난 비정상군(2.76±0.77)의 생검조직 LDH 평균분획비는 정상군(1.38±1.19)에 비해 유의성 있게 높았으나 전립선액에서는 분획비의 유의차가 인정되지 않았다. 배양결과에 따른 전립선액 및 조직시료의 LDH 분획비는 정상군과 비정상군 사이에서의 유의성은 인정되지 않았다.

결론적으로 저에코 영역 및 낭포부위는 생검조직과 전립선액의 배양 및 세포병리학적 검사에 따른 비정상성 및 높은 LDH 분획비와 밀접한 연관성을 보였다. 특히 경직장 초음파상에 나타나는 미만성 저에코 영역은 전립선 비대증 및 만성 전립선염 등과 같은 병적 관련성을 내포하고 있는 것으로 사료된다.

Key words : dogs, prostate gland, transrectal ultrasonography, lactic dehydrogenase, electrophoresis.

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Introduction

Transrectal ultrasonography(TRUS) of the prostate gland can clearly show the prostate parenchymal echo patterns. Furthermore, this method offers direct and objective information on the prostate without hazard and pain[1,2].

Rifkin *et al*, divided the TRUS echo patterns of prostate lesions into 3 ways such as hypoechoic or echopenic, isoechoic and hyperechoic or echodense[2]. In human literatures, there are some arguments against the TRUS image of prostatic echogenicity which have a hypoechogenic or hyperechogenic[3~9].

Both these signs are shown as results from pathological changes of the prostate tissue. For that reasons, the definitive diagnosis of prostatic disease have been detected with histological examination of biopsy specimens[10]. The degree of echogenicity is not accurately proportion to the pathological condition of the prostate, however, hypoechogenic signs of the prostate suggests a possibility and degrees of prostatic parenchymal abnormality[3,6].

One of the diagnostic methods for prostatic disease is a isoenzyme level test such as lactic dehydrogenase(LDH) of prostatic tissue or fluid, which has been effectively applicated and reflected for assessment of prostate diseasese[11~17]. Clinical application of the determination of LDH and its isoenzymes in serum, tissue, and body fluids includes the diagnosis of benign prostatic hyperplasia[14], prostatitis[15], and cancer[16~18] in human literature. LDH-V isoenzyme subunit predominates in the presence of inflammation, since human granulocytes and lymphocytes have an LDH-V. Elevated LDH-V isoenzyme can also be associated with epithelial cellular damage[15].

None of these TRUS echogenisity has been compared with isoenzyme level of LDH from the extracted specimens of prostatic fluid and biopsy tissue in veterinary and human medicine. The objective of the present study is to compare TRUS echogenic appearance of the canine prostate gland with the analysis of LDH isoenzymes, cytopathological findings, and microbiological culture findings of prostate fluid and tissue.

Materials and Methods

Experimental animals : Thirty three dogs, aged from 5 to 9 years(mean 7.2 years), without having prostatic disease on the basis of clinical signs, CBC, or physical examination including rectal palpation, and urinalysi were selected randomly.

Weight of the dogs ranged from 9.2 to 22.3kg(mean 16. 6kg) and the breeds of dogs included mongrel dogs(n=16), German shepherd dogs(n=3), Labrador retrievers(n=6), and Beagle dogs(n=8).

Transrectal ultrasonography : Dogs were positioned in stand or sternal recumbency. Wrapped probe with surgical glove was inserted into the rectum after defecation of itself as far as possible and tried to enema. The intraoperative typed convex probe equipped with 7.5MHz scan head was used in transrectal ultrasonographic examination(Fig 1-D). In each dog, serial longitudinal sections of the prostate were

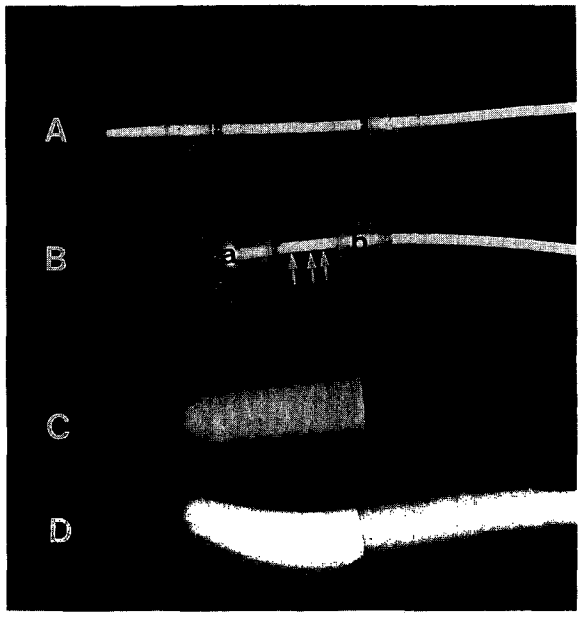


Fig 1. Euipment for cellection of prostatic fluid and transrectal probe.
A. Shihata dual balloon catheter set®.
B. Modified double balloon catheter with collecting holes(arrows), proximal(a) and distal balloon(b).
C. O-ring kit balloon®.

obtained by continuous scanning from the level of the left lobe to the right lobe of the prostate gland. Ultrasonographic images were recorded on Polaroid film. Scanning time was not exceeded 3 minute to prevent any irritations to the prostatic parenchyma.

Double balloon catheter : A Shihata dual balloon catheter set®(Cook Urological Inc., USA, 6.5Fr.), originally designed for the treatment of urethral calculus problems in humans, was used. It consists of 2 tips each for proximal and distal balloon inflation, a guide wire tip and an injection tip for 2 small holes between the proximal and distal balloon(Fig 1-A).

It was modified in accordance with the dog's prostate for this experiment(Fig 1-B). This redesigned catheter was cut at the end portion of the proximal balloon and the outlet of the guide wire hole was closed. The proximal balloon was newly placed at the top of the catheter with another balloon (O-ring kit®, 32 UA. Asahi Optical Co., Japan) for endoscopic use(Fig 1-C). Then the proximal balloon was modified so that it could expand to 5-7 times more than its prior size and the distance between two balloons was shortened from 3cm to 1.5cm(Fig 1-D). Three new holes for collecting the prostatic fluid were made on the guide wire canal connecting the proximal and distal balloons. The diameter of each remade hole was approximately 3mm. The 2 original small(0.05mm) holes were used for the irrigation of the prostatic urethra before prostatic massage.

Catheterization and prostatic fluid collection(Fig. 2) : Each dog was placed in lateral recumbency and sedated with only acepromazine maleate(0.2mg/kg) administered intravenously. Afterwards the penis and prepuce were washed with saline and sterilized with 3% betadine and then dried. A urinary catheter(5Fr) was inserted about 10cm into the urethra, and then withdrawn, after which a swab obtained from the tip of urethral catheter was cultured to identify urethral flora. For cleaning and flushing, the urinary catheter was advanced from the opening of the penis to the end of the prostatic urethra under fluoroscopy for guidance, at the same time flushed it with saline solution(1ml/kg). Flushing continued while retracting the urinary catheter. During the retraction, the flushed fluid was collected in test tubes for the creatinine concentration test.

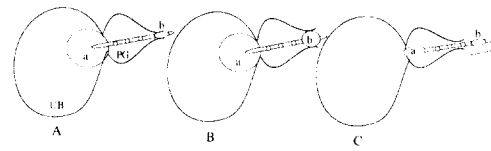


Fig 2. A schematic presentation of the double balloon catheterization. UB : urinary bladder, PG : prostate gland, a : proximal balloon and b : distal balloon.
A. Double balloon catheter was positioned in prostatic urethra with inflated proximal balloon(a) pulled back against bladder neck and then catheter holes were appropriately placed.
B. After flushing the urethra with saline, the distal balloon was inflated and negative pressure applied by the fluid being secreted at this time.
C. After the distal balloon was totally inflated, the catheter was retracted back whil gradually deflating the proximal balloon.
D. Intraoperative type 7.5MHz convex probe.

The penis was sterilized again, after which a sterile modified double balloon catheter was inserted into the bladder. Five milliliters of air were injected into the proximal balloon, inflating it to a diameter of about 2~3cm.

The inflated balloon catheter was pulled out gently until resistance was felt at the bladder neck and cranial margin of the prostate gland. The inflated balloon catheter was maintained without only sliding during the second prostatic urethral flushing with saline through the injection holes. Expelled flushing fluid was collected in sterilized test tubes for culture. Approximately 0.5ml of air was instilled into the distal balloon, changing it to a cylindrical shape with a diameter of about 0.3~0.5cm. The cranial to the caudal prostatic urethra was blocked from the bladder and urethra by this double balloon catheter.

Prostatic massage was performed as previously described[10]. After a transrectal massage for 1 minute, negative pressure for retrieving the prostatic fluid was applied by a syringe connected to the 3 remade holes on the guide wire canal, which were then utilized as collecting holes for this modified procedure. The same amount of negative pressure was maintained while turning the catheter both left and right slightly to collect the secreted fluid.

After the fluid was collected in the syringe, the specimen

was dripped into a test tube before removing the catheter. To remove the catheter, the distal balloon was first totally deflated and then the proximal balloon was deflated while maintaining that it was retracted caudally. This ensured the removal of the catheter without any damage to the urethra.

Fluid Culture : Culture was performed using blood and Mckonkey agar as soon as was possible using specimens collected in the syringe. Specimens for impression smear slides were prepared using drops from the catheter tip. The remaining fluid was collected into test tubes and centrifuged at 3000rpm for 10 minutes. The supernatant was then evaluated for the creatinine concentration level.

Core biopsy : The ultrasound-guided biopsy procedure was tailored to type of echo pattern identified on the pre-biopsy ultrasound examination. Tru-cut biopsy procedure were carried out in sedated animal. General anesthesia was done with ketamine HCl and acepromazine maleate combination. The animal was placed in ventral recumbency for percutaneous perirectal prostatic biopsy. The perianal area was clipped and the accumulants in anal sacs were excreted for sterile preparations. Biopsy site were prepared in a sterile manner, and a local anesthetic skin block was performed with lidocaine. An automated biopsy gun was used with a 18G×20cm(for homogenization) or 16G×20 cm(for pathological examination) Tru-cut biopsy needles(Bard, Radiplast, Sweden, Fig 3-A) under TRUS-guide(Hitachi EUB 450, Hitachi Medico, Japan). After selection of a lesion which was more cystic and/or diffuse hypoechoic lesions than those of its surrounding, a Tru-cut biopsy needle was inserted into those lesions through stab incised skin at the portion between anal sphincter and internal obturator muscle while determining a needle pathway under TRUS-guide. Two or three times biopsies were conducted on lesions which had abnormal TRUS signs. Afterward biopsy of parenchymal areas were ascertained on TRUS image whether bleeding was. Obtained tissue specimens were stored at −80℃ for isoenzyme analysis and pathological examination as previous studies.

Preparation of specimens for isoenzyme analysis : Tissue specimen which had been stored at freezer was homogenized with using a glass teflon homogenizer. Tissue was mixed with 4℃ sucrose solution (300μl, 250 nmol/L) and

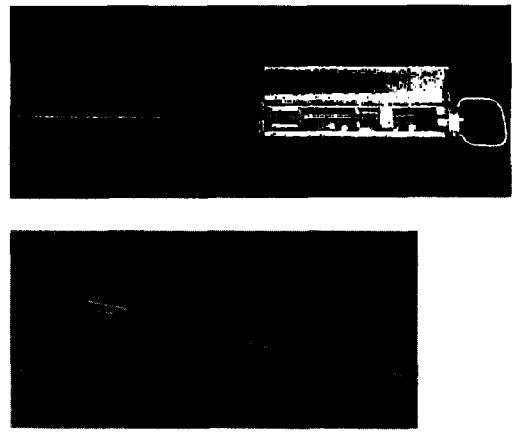


Fig 3. A. Equipment for prostatic biopsy. This Tru-Cut biopsy needle is stored in a biopsy gun. B. After prostate biopsy, a biopsied tissue is located in groove of the needle.

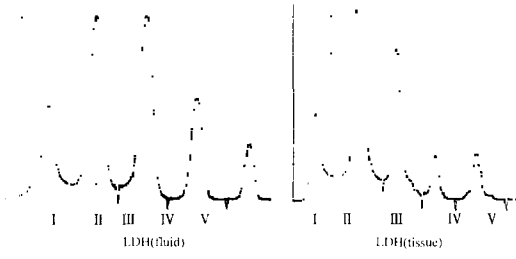


Fig 4. Curves represent density of LDH isoenzyme bands I to V as seen in films. LDH-V/I ratios were calculated from area under curves. Curves represents LDH-V/I ratio of 0.46, 0.29 in prostatic fluid and tissue, respectively. in normal dog.

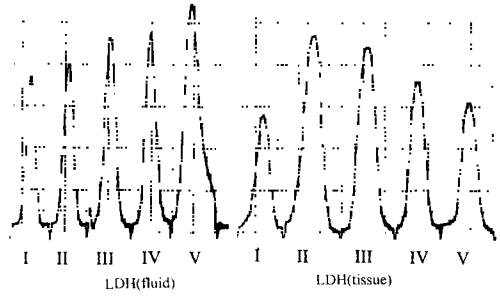


Fig 5. Curves represent density of LDH isoenzyme bands I to V as seen in films. LDH-V/I ratios were calculated from area under curves. Curves A represents LDH-V/I ratio of 2.1, 1.28 in benign hyperplastic prostatic fluid and tissue, respectively.

homogenization was performed for 30 seconds in ice con-

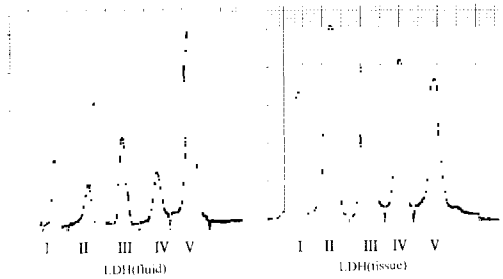


Fig 6. Curves represent density of LDH isoenzyme bands I to Vsa seen in films. LDH-V/I ratios were calculated from area under vurves. Curve A represents LDH-V/I ratio of 6.8, 1.34 in the fluid and tissue, respectively, in the prostatitis dog.

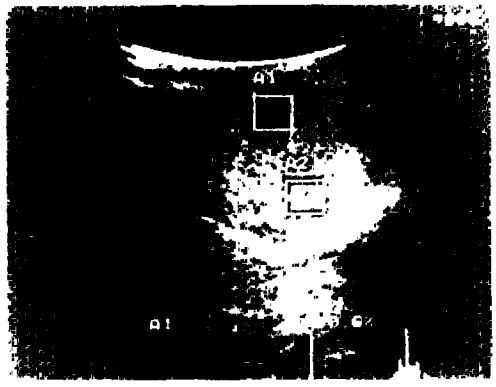


Fig 7. Longitudinal scanning of prostate. Diffuse hypoechoic lesions(square A1) seen at upper part and hyperechoic lesion (square A2) in lower part are present in longitudinal scan surface.

tained box. After centrifugation in temperature-set equipment

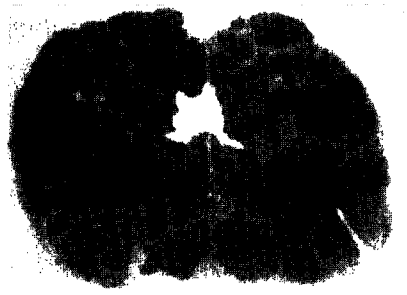
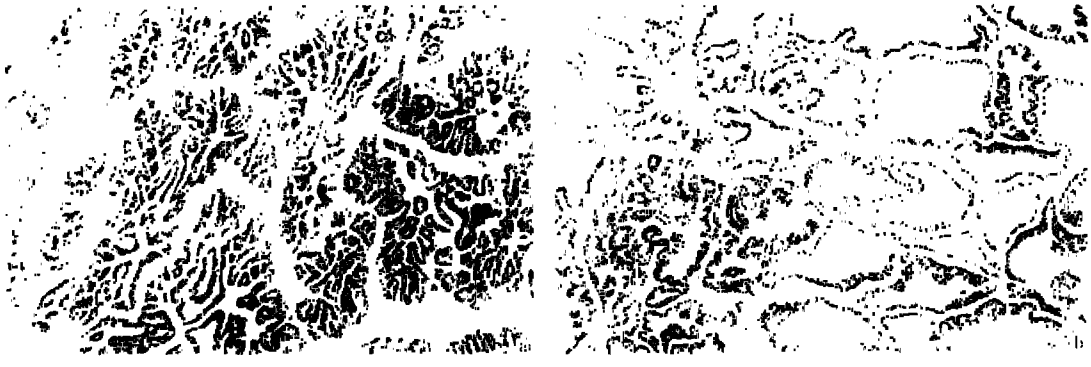


Fig 8. Histological section of prostate from dogs with diffuse hypoechoic lesions (thin arrow) and hyperechoic area(thick arrow) that were founded in Figure 7 (HE staning. 40×).

at 4℃, supernatant was concentrated in 50 times with using dialytic concentrator(Amicon B-15, Amicon Co.). Stored prostate fluid was thawed in ice contained box, analysed and concentrated as the same way with tissue. All specimens for isoenzyme analysis were maintained at level of 4℃ set at room temperature.

Electrophoresis of LDH : LDH isoenzymse were analyzed by electrophoresis on cellulose acetate plates(Titan Ⅲ-Lipo, Helena Lab., Beaumount, Texas). Tri-barbital-sodium was used for buffer solution. Electrophoresis was conducted at 160 voltage for 40 minutes. After finished the electrophoresis, plate was mounted with reagents for titan LDH isoenzyme staining. Separated bands were made visible by incubation at 37℃ for 20 minutes. Plate was entered into acetic acid



a b

Fig 9. Microscopic examination of normal(a) and diffuse hypoechoic lesions(b). Grandula alveoi are enlarged with foci of atrophy. Many acini are cystically dilated. Stroma is prominent and relatively dense(HE staining, 40×)

solution for stopping the reaction. The isoenzyme fractions were quantitative by means of densitometer by scanning the plate at 570nm wave length. Each specimen was applied 3 times for electrophoresis and graphically calculated from each determination and was averaged to obtain a ratio of LDH isoenzyme fraction LDH V/I.

Data analysis : The animals were divided into negative and positive groups based upon the TRUS, culture, and histological findings. The data of the mean percentage ratio of LDH V/I were compared with these findings. Statical analysis was completed with the aid of the ANOVA test.

Results

Table 1. Results of percentage mean ratio of LDH-V/I subunit transrectal ultrasonography, culture and cytopathological findings

No. of dog	Ratio of LDH-V/I		TRUS findings	Culture findings (no./ml)	Cytopathological findings
	fluid	tissue			
1	0.30	0.59	Ns	Nf	Ns
2	0.01	0.87	Ns	*E coli* (4×10^3)	Ns
3	0.14	0.67	Ns	Nf	Ns
4	0.37	0.53	Ns	*E coli* (5×10^3)	Ns
5	0.06	0.43	Ns	Nf	Ns
6	0.16	0.37	Ns	*E coli* (2.7×10^6)	Ns
7	0.63	0.31	Ns	Nf	Ns
8	0.08	0.29	Ns	Nf	Ns
9	0.13	0.21	Ns	Nf	Ns
10	0.68	1.03	Mf	*E coli* (5.2×102)	Ns
11	1.31	3.24	Dh	*E coli* (5.6×10^4)	Ns
12	2.13	2.53	Mf/Dh	*E coli* (5×10^3)	Ns
13	0.38	3.20	Mf/Dh	*E coli*(9×10^3	Ns
14	1.57	3.23	Mf	*E coli* (1.6×10^4)	Ns
15	0.76	2.97	Mf	Nf	Ns
16	0.69	2.17	Dh	*E coli* (7×10^5)	Ns
17	0.89	0.88	Dh	*E coli* (2×10^5)	Ns
18	0.84	3.17	Mf/Ct/Dh	*E coli* ( ) 10^7)	prostatitis
19	0.89	3.10	Mf/Dh/Cyst(3mm)	*P areu* (8×10^6)	prostatitis
20	0.63	1.82	Mf	*E coli* (4×10^5)	prostatitis
21	2.29	2.08	Ct/Cyst(8mm)	*E coli* (2×10^4)	prostatitis/hyperplasia
22	0.98	3.53	Mf	*E coli* (3×10^4)	hyperplasia
23	1.16	3.31	Ct/Cyst(18mm)	Nf	hyperplasia
24	0.87	3.29	Mf/Dh	Nf	hyperplasia
25	0.56	2.13	Ct/Dh	Nf	hyperplasia
26	0.91	3.40	Ct/Dh	*E coli* (3.5×10^2)	hyperplasia
27	1.44	3.74	Cyst(6mm)	*E coli* (6×10^5)	hyperplasia
28	0.49	3.94	Dh	Nf	hyperplasia
29	0.46	1.46	Ns	*E coli* (7×10^4)	hyperplasia
30	0.14	2.90	Mf/Dh	Nf	hyperplasia
31	0.82	2.16	Dh/Cyst(9mm)	*E coli* (7.4×10^5)	hyperplasia
32	0.64	1.90	Mf/Dh	Nf	hyperplasia
33	0.05	2.31	Dh	Nf	hyperplasia

Prostatic specimen were successfully collected in all animals. Complications were not observed as a result of collection of double balloon catheter or tissue biopsy procedure with Tru-cut biopsy needle. Results of TRUS, culture, cytological findings and analysis of lactic dehydrogenase isoenzymes are listed in Table 1.

In this study of 33 dogs, 23 dogs had abnormal TRUS signs with either focal anechoic area(11dogs) or diffuse hypoechogenicity(13 dogs) and 7 of 23 dogs had both cystic and hypoechogenic TRUS signs. Cysts were averaged ranged from 3 to 8mm in diameter. Multifocal cysts were counted from 2 to 7 with average ranged from 2 to 4.7mm diameter. Ten dogs were allotted into normal group on the basis of TRUS signs and their mean percentage ratio of LDH V/I(MPL) value in the collected fluid and biopsy tissue were 0.45 ± 0.30 and 0.57 ± 0.36, respectively. In the 23 dogs, they had focal cystic or diffuse hypoechogenic lesions, the MPL of both fluid and tissue were significantly($p<0.01$) elevated like as 0.92 ± 0.55 and 2.69 ± 0.82, respectively (Table 2).

Table 2. Results of percentage ratio of isoenzymes LDH-V/I on the basis of transrectal ultrasonographic(TRUS) findings(mean±SD)

	TRUS findings	
	prostatic fluid	prostatic tissue
Negative(n = 10)	0.23±0.20	0.57±0.36
Postive(n = 23)	0.92±0.55	2.69±0.82
Statistical difference	p < 0.01	p < 0.01

Fourteen of 33 dogs were presented as positively cultured from the specimen of tissue or fluid. Cultured agents were *E coli* and *Pseudomonas aureus*. *E coli* was predominantly cultured(Table 1). The MPL in the negatively cultured fluid and tissue specimen was 0.45 ± 0.30 and 1.75 ± 1.32, respectively.

Each of the MPL in the positively cultured group was elevated as level of 0.90 ± 0.64 in fluid specimen and 2.27 ± 1.11 in biopsy tissue. However, the MPL was increased in cultured specimen group compared with normal group. Significance was not recognized fluid and tissue specimens (Table 3).

Table 3. Results of percentage ratio of isoenzymes LDH-V/I on the basis of culture findings(mean±SD)

	Culture findings	
	prostatic fluid	prostatic tissue
Negative(n = 14)	0.45±0.30	1.75±1.32
Postive(n = 19)	0.90±0.64	2.27±1.11
Statistical difference	Not significant	Not significant

Prostatic fluid were conducted into cytological findings. Sixteen dogs were founded in a cytological abnormal. Nonseptic inflammation demonstrated in 4 dogs, and benign hyperplasia or cystic hyperplasia in 13 dogs. Of these 13dogs, one dog(dogs No. 19) had both inflammatory and hyperplastic signs. The MPL was recorded in the fluid(0.60 ± 0.59) and tissue(1.38 ± 1.19) in normal group. The MPL of fluid specimen in abnormal group was 0.82 ± 0.52, but significance could not be recognized. The MPL of tissue specimen was 2.76 ± 0.77 with significance($p<0.01$). These results are listed in Table 4.

Table 4. Results of percentage ratio of isoenzymes LDH-V/I on the basis of cytological findings

	Cytopathological findings	
	prostatic fluid	prostatic tissue
Negative(n = 17)	0.60±0.59	1.38±1.19
Postive(n = 16)	0.82±0.52	2.76±0.77
Statistical difference	Not significant	p = 0.01

Discussion

Five LDH isoenzymes have been demonstrated in the tissue and fluid of the prostate gland in 33 dogs in this study.

Six dogs(Dog No. 1, 3, 5, 7, 8, 9) without any problems resulting from the test which includes TRUS findings, culture and cytological findings of prostatic fluid and tissue had a low level of MPL in prostatic fluid(average of MPL = 0.22) and tissue(average of MPL = 0.42) of less than 1(Table 1). LDH isoenzymes distribution in canine serum, prostatic fluid and tissue, found that the average of the mean percentage ratio of LDH-V/I(MPL) subunits is 0.66 in normal prostatic tissue and 6.16 in prostatic fluid11. The normal MPL of prostatic tissue was 0.42 in this study and has a similarity with this previous study. However, the MPL of the prostatic fluid which was collected by the modified double balloon catheter is significantly different from previous study which using the installed catheter in the prostate gland. This method is more invasive to the prostate itself, and the LDH-V subunits could be increased resulting from affected prostatic parenchymal tissue and associated with epithelial cellular damage[19~21]. From these results, modified double balloon catheterization is effective and useful method to collect a prostatic fluid with the least invasion to the prosate gland.

In this study, TRUS findings were focused on the characteristics of the dog's prostate ultrasonographic echo-patterns: isoechogenic as normal prostate; diffuse hypoechoic, small multifocal cysts, a large cyst and thickness of prostatic capsule as abnormal prostate, and refers to previous literature[22~25]. Core biopsy was sited at these lesions and the biopsy under ultrasound guided.

The abnormalities include diffuse hypoechoic, cysts, capsular thickness compared with a normal prostatic echogenicity. Multifocal cysts and a large solitary cyst also presented. Diffuse hypoechoic lesions were corresponded with areas of glandular hyperplasia admixed with foci of atrophy in the pathological findings. Acini were presented and cystically dilated. Stroma was prominent and relatively dense(Fig 9). The exact representation of correlation of prostatic disease with ultrasonographic echo patterns and its mechanism is controversial[26,27]. Focal to multifocal areas of increased echogenicity were seen only in dogs with bacterial prostatitis and neoplasia[28]. However, a diffuse increase in prostatic echogenicity is nonspecific and may indicate fibrosis, stromal proliferation or possibly just glandular aging. Prostatic echo-patterns have been widely described and noted in cancer in human patients but not in dogs. TRUS findings, in this study, coincided with cytological and culture results in diffuse hypoechoic prostate. Considering from the discussion, this coincidence was compared with the LDH isoenzymes which can be a reply of a prostatic parenchymal condition. Abnormal TRUS groups without distinction of echogenicity had a significantly($p<0.01$) increased MPL compared with the normal group in prostatic fluid and tissue specimens. From these results, the ratio permitting reasonable discrimination between normal or abnormal ultrasonography in the prostate gland.

The ratio of LDH-V/I subunits reported in patients with prostatic cancer support the suggestion that a diffuse cellular change of apparently benign tissue accompanies or precedes the development of definite histological evidence of malignancy. Though, there seems little question that clinical evidence of prostatic pathological process is associated with an alteration in the normal LDH isoenzymes pattern. When body tissue is affected by pathological procedure such as inflammation, hyperplasia, or cancer, it has an increased the level of LDH-V subunits in excretory body fluids and in tissue itself[14]. The MPL reported on the basis of culture findings that culture results were not accompanying prostatic abnormalities. It can be explained that inflammatory cells related with cultured bacteria may have been absent caused by small bacteria numbers, or improper technique in obtaining or preparing the sample[29].

Cytological findings results support the suggestion that biopsy tissue had a more significantly increased MPL value than that of the prostatic fluid. Heine *et al* [14] has described that prostatic fluid provides a unique opportunity to sample metabolic activity of the prostatic tissue element, the epithelial cell, and to see it undergoing major pathologic change[14]. Though the MPL of prostatic fluid, in this study, was presented with an increased level, it can not be discriminated from cytological findings. This presents a question that prostate glands diagnosed with abnormalities according to culture and cytological findings do not correlate with the MPL values. These results are recognized by the

following explanation. Though mixed or distilled, pathological lesions may be possible to identify with cytological or culture findings. If prostatic fluid suppose to be secreted from the entire prostate gland through massage, then prostatic fluid derived from local or solitary pathological lesions may be mixed or distilled with normal fluid. Biopsy procedure can be possible to obtain a pathological or abnormal tissue selectively from the organ.

Considering from the results of this study suggest that analysis of the LDH isoenzymes in biopsy tissue and TRUS findings is reliable in differentiating prostatic abnormalities in dogs and analysis of the LDH isoenzymes of prostatic tissue specimens more can be sensitively presented and accompanied with results of culture or cytological findings than that of prostatic fluid specimens.

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