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ORIGINAL ARTICLE

Evaluation of Ultrasonography and Mammography in Diagnosis of Mammary Gland Tumor in Bitches: Based on Tumor Markers

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Abstract

Objective- This study refers to the role of ultrasonography in the diagnosis of mammary gland tumor in bitches as a complementary diagnostic method and its ultimate goal is to evaluate the results of mammography with the positive results of ultrasonography.

Design- Prospective study.

Animals- 12 German Shepherd bitches with swollen mass in the mammary gland region (group I) and 12 healthy German shepherd bitches without any swollen mass (group II-healthy group).

Procedures- these bitches were evaluated by ultrasonography and assessment of axillary lymph nodes was performed simultaneously. Also, mammography was performed in these dogs and the results were reported by another radiologist. Finally, all suspected cases were referred for biopsy or surgery, and definite results were announced by the pathologist. In addition to, tumor markers such as carcino emberionic antigen (CEA) and cancer antigen 15.3 (CA 15.3) were detected in all samples (group I and group II).

Results- Based on the results of the 12 cases of suspicious masses evaluated by ultrasonography, 9 cases of tumors (definitive diagnosis with pathological tests) and 3 cases of abscess were reported in the cases of group I. Moreover, tumor markers remarkably increased in the all sera samples of group I compared group II. The average diameter of the mass was 13 mm and the mean diameter of the lymph nodes was 5 mm. In mammography findings due to presence of dense mammary tissue, 18.3% of the cases had negative or only one asymmetric density and the remaining cases (81.7%) were positive.

Conclusion and Clinical Relevance- Based on the results of this study, ultrasonography in diagnosis of mammary gland tumors especially in young bitches can be effective with high sensitivity.

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Introduction

Mammary gland cancer is one of the most common cancers and is a cause of death in bitches.^{1,2,19} Mammary gland tissue in young bitches is dense and with increasing age, fat tissue gradually replaces it. In spite of bad prediction, high mortality and morbidity, the patient's prognosis will be better if it is diagnosed early. Early diagnosis of mammary cancer is the ultimate goal of radiology and the role of radiologist at this stage is very vital.^{3,15} Mammography screening has led to 22% reduction in the mortality of dogs over 9 years old and 15% reduction in the mortality of dogs under 9 years old.^{4,5} Considering the incidence of mammary tumor in young bitches in recent years and the presence of dense mammary tissue in this group and the possibility of hiding the lesion in this type of tissue, the presence of a diagnostic supplementary method to increase the sensitivity of diagnosis seems necessary.^{6,7}

The sensitivity of mammography is heavily influenced by mammary tissue compression and with increasing mammary density, the sensitivity of mammography decreases, so that the sensitivity of mammography in dogs with dense mammary may be reduced by 30 to 48 percent.⁸ In more than half of dogs less than 8 years old and at least one third of dogs older than 8 years, mammary tissue is dense, while this group is at a high risk in the longer period and have worse clinical symptoms.^{9,16} In addition, a dense mammary gland with constricted tissue is a risk factor for mammary tumor.^{10,11} Therefore ultrasonography in addition to mammography it seems necessary especially in high risk dogs and also the younger ages.

1. Materials and Methods

In this prospective study, - 12 German Shepherd bitches (9-15 years old) with swollen mass in the mammary gland region (group I) and 12 healthy German shepherd bitches (9-14 years old) without any swollen mass in the mammary gland region (group II-healthy group) were evaluated by ultrasonography and assessment of axillary lymph nodes was performed simultaneously. All cases of group II (healthy) had been clinically and para-clinically examined prior study. In this study the ultrasound device model (EUB-8500 XP, Hitachi Medical Corporation, Tokyo, Japan) and an 8-12 MHz linear probe (EUP-L54M, length: 53 mm) were used. The ultrasonography reports were stored, then this information was recorded in table A. After

the end of ultrasonography in cases where mammography was performed on the patient and its information was reported by another radiologist it was recorded in table B. Of course, if the patient did not have mammography and in ultrasonography, there was a suspicious finding such as hypoechoic mass with poorly defined, microcalcification, hypoechoic axillary adenopathy the patient was referenced for mammography and its information according to other radiologist's reports was recorded in Table (B). Those dogs that were suspected to be malignant in ultrasonography or mammography were referred for biopsy or surgery and then pathological information was recorded in Table C. After collecting the data, the results were analyzed by SPSS software and descriptive and inferential statistics.

Analysis of tumor markers

All blood samples were taken from 12 dogs with swollen mass (Group I) and same number without swollen mass in the breast region (Group II) in 5-mL tubes containing separating gel for sera preparation and after centrifugation at 5000 rpm for 10 minutes, sera were collected.

The LDH activity was measured through ultraviolet kinetic method (Spectrophotometer, Cecil, Italy) as U/L.

In respect of CEA (Carcino Embryonic Antigen) and CA 15.3 (Cancer Antigen 15.3) concentrations in sera, noncompetitive ELISA kits (CanAg). CEA unit was ng/ml and CA 15.3 unit was U/ml. All data of Group I were compared with Group II. P value was set as 0.01.

3. Statistical Analysis

Statistical analysis was accomplished in all analyses. The data were denoted as Mean± SD. The statistical package of SAS v9.1 (SAS Institute Inc., Cary, NC, USA) was allocated and significant level was set at $p \leq 0.01$.

4. Results

The age distribution of patients was 5-16 years old and the mean age of patients was 8 years old. The highest prevalence was at the age of 9 years old (19%) and the most common symptom of the patients was the feeling of a mass in the mammary region (in 66.6% of cases). There was no blood secretion in any of the patients (Table 1).

Of the 12 cases of suspicious mass that was characterized by ultrasonography 9 cases of tumors (definitive diagnosis with pathological tests) and 3 cases of abscess were

reported. The pathologic lymph node was seen in about 60% of the patients, with a diameter of the lymph nodes between 5-10 mm. In mammography findings due to presence of dense mammary tissue, 18.3% of the cases had negative or only one asymmetric density and the remaining cases (81.7%) were positive. In mammography, lymphadenopathy was reported in 30% of cases.

Table 1. Abundance of Symptoms in Patients with mammary gland tumor

Symptoms	Positive (%)
Feel the masses	66.6
Nipple dipping	23.8
Skin redness	4.8
Thickness of the skin	4.8
Blood secretion	0

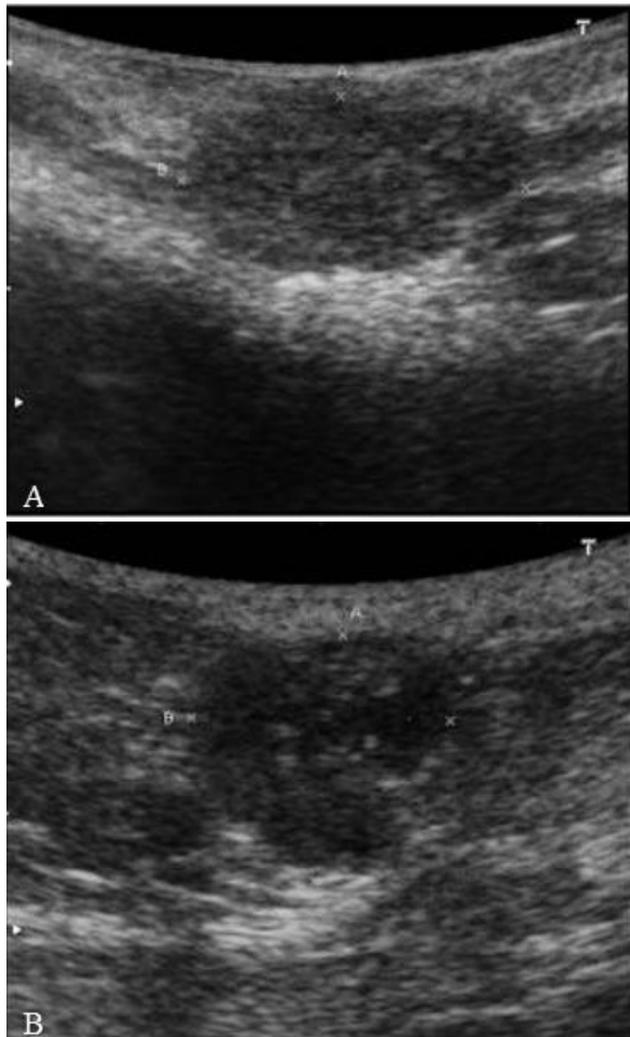


Figure 1. Ultrasonography images of the benign (A; adenoma) and malignant (B; carcinoma) mammary gland tumors.

Results of tumor markers

Based on Table 2 results, considerable increase of tumor markers in Group I (Swollen mass) were determined as 6 and 9 times than Non-Swollen mass group.

Table 2. Serum CEA and CA 15.3 of female dogs (Groups I and II). Mean \pm SD

Parameters	Group I (Swollen Mass)	Group II (Non-Swollen Mass)	p Value
CEA (ng/ml)	6.29 \pm 0.15 [†]	0.13 \pm 0.02	p \leq 0.001
CA 15.3 (U/ml)	9.71 \pm 1.43 [†]	0.06 \pm 0.01	p \leq 0.000
LDH (U/L)	391.57 \pm 58.19	182.64 \pm 47.12	p \leq 0.000

Note: Data are expressed as mean \pm SD. † as superscript denotes significant difference in comparison with Non- Swollen group. (P<0.01).

5. Discussion

Generally, early diagnosis of mammary gland tumor is very important. It should be noted that dense mammary tissue is one of the limitations of mammography. In this study, the age of half the patients was 8 years old or less, which is a lower age range, and in these younger years, the density of mammary tissue is high. Based on the results of 12 suspicious masses evaluated by ultrasonography, 9 cases of tumor and 3 cases of abscess were reported. The average diameter of the mass was 13 mm and the mean diameter of the lymph nodes was 5 mm. In mammography findings due to presence of dense mammary tissue, 18.3% of the cases had negative or only one asymmetric density and the remaining cases (81.7%) were positive. In another study, a comparison between mammography and ultrasonography was performed to evaluate the masses of the mammary gland that it was argued the application of these two methods together has a higher sensitivity to performing a single method, especially in lactating, pregnant and young dogs. Also, they announced that density is the limiting factor for mammography therefore, ultrasonography increases the diagnostic sensitivity.^{12,17} Another study was performed on 12 patients that have at least one increase in mammary tissue density, accuracy of mammography was 78% and ultrasonography was 90% and it was suggested that supplementary screening method is more useful in increasing the diagnosis of mammary

gland tumor.^{13,18} In a recent study, in one of the cases examined multifocal multicentric cancer revealed that only one asymmetric density was observed in mammography but in complement ultrasonography, at least 3 hypoechoic centers were observed. In a study by Langenbach et al, 2001,¹⁴ lymphadenopathy was observed in mammography 45% and in ultrasonography 88%. In the present study, regional lymph nodes, 30% in mammography and 60% in ultrasonography were found to be consistent with mentioned studies.

The biochemical tumor markers are molecules present in both healthy and sick individuals. However, they are present in higher concentrations in those patients with malignant neoplasms. The increase in the concentration of these markers is associated to a variety of causes, including intense cell exchange, necrosis or increased secretion of certain proteins. Thus, the biochemical tumor markers are widely used as tools for diagnosis and prognosis of cancer patients.²⁰⁻²² The serum tumor marker CA15.3 has been broadly studied in human medicine since 1980s; however, in veterinary medicine, there are few references about tumor markers in the literature. In this study, the results demonstrated a significant difference in the CA15.3 concentrations in cancer group compared with healthy one.²³ The role of the CEA tumor marker in animals with neoplasms was studied and a significant increase in its serum level was observed.²⁴ Levels of serum CEA could be influenced by the rate and amount of its production by carcinoma cells, the existence of tumor necrosis, and perivascular infiltration.²⁵ The LDH levels showed to be high in present study.

These data agree with previous studies that the bloodstream concentration of this enzyme is increased with disease evolution, indicating a poorer prognosis and affirmed that neoplastic cells observed experimentally have an increased glycolysis rate and proposed an increase in LDH activity. This is because of up-regulation of LDH in cancer cells ensures an efficient anaerobic/glycolytic metabolism for tumors and reduced dependence on oxygen.^{26,27,28} According to this important observation, the enzyme demonstrated statistically significant differences between healthy dogs and the group of dogs with mammary cancer. The results of this study indicate that the incidence of mammary gland tumor in patients has decreased. Most of the patients who have mammary tumor are referred to the clinic at the stage of mass occurrence. Our patients were referred to the clinic with a large mass (average size 13 mm) however, before the formation of the masses, progress has been made towards cancer. Also, despite a lot of

education about blood secretion as a sign of breast cancer, none of our patients had blood secretion.

Conclusion

Based on the results of this study, ultrasonography in diagnosis of mammary gland tumors especially in young bitches can be effective with high sensitivity.

Acknowledgment

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Conflicts of interest

None

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چکیده

ارزیابی اولتراسونوگرافی و ماموگرافی در تشخیص تومور پستان سگ‌های ماده: بر اساس نشانگرهای توموری

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هدف - این مطالعه به نقش اولتراسونوگرافی در تشخیص سرطان پستان سگ‌ها به‌عنوان روش مکمل تشخیصی اشاره می‌کند و هدف نهایی آن همخوانی نتایج ماموگرافی با نتایج مثبت اولتراسونوگرافی است.

طرح - مطالعه آینده‌نگر.

حیوانات - ۱۲ سگ ماده با توده متورم در ناحیه پستان (به‌عنوان گروه یک) و ۱۲ سگ بدون توده متورم (به‌عنوان گروه دو-گروه سالم).
روش کار - این سگ‌های ماده تحت ماموگرافی و سونوگرافی قرار گرفتند و ارزیابی عقده‌های لنفاوی آگزیلاری نیز به‌صورت هم‌زمان به عمل آمد. در این سگ‌ها ماموگرافی نیز انجام شد و نتایج آن توسط رادیولوژیست دیگری گزارش گردید. در نهایت کلیه موارد مشکوک جهت بیوپسی یا جراحی ارجاع شدند و نتایج قطعی توسط پاتولوژیست اعلام شد. ضمناً تومورمارکرهاى CEA و CA 15.3 در نمونه‌های سرمی هر دو گروه مورد سنجش و ارزیابی قرار گرفت.

نتایج - بر اساس نتایج حاصله از ۱۲ مورد توده مشکوک گزارش شده با اولتراسونوگرافی در گروه یک، در ۹ مورد تومور (تشخیص قطعی با آزمایش‌های پاتولوژی) و در ۳ مورد آبسه گزارش گردید. به‌علاوه اینکه، افزایش شدید و قابل توجه تومور مارکرهاى فوق در گروه یک در مقایسه با گروه دو (سالم) مشاهده شد. متوسط قطر توده ۱۳ میلی‌متر و متوسط قطر گره‌های لنفاوی مبتلا ۵ میلی‌متر بود. یافته‌های ماموگرافی به دلیل بافت متراکم پستان، ۱۸/۳ درصد موارد منفی و یا تنها یک دانسیته آسیمتریک را گزارش کرده بود و بقیه موارد (۸۱/۷ درصد) مثبت بود.

نتیجه‌گیری و کاربرد بالینی - بر اساس نتایج این تحقیق، اولتراسونوگرافی در تشخیص تومورهای پستانی به‌خصوص در سگ‌های ماده با سن کم می‌تواند با حساسیت بالا مؤثر باشد.

کلمات کلیدی - تومور غده پستان، سگ‌های ماده، اولتراسونوگرافی، ماموگرافی.