Diagnosis of Breast Sparganosis by Ultrasonography Combine with Molecular Biological Methods

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Case Report

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Abstract

Background: Sparganosis is a parasitic zoonosis caused by the plerocercoid larvae of the genus *Spirometra*. The sites affected by the plerocercoid infection include subcutaneous tissues, brain, eyes, the abdominal viscera, and urogenital organs. In clinical practice, due to the limited laboratory approaches for the specific detection of sparganum, breast Sparganosis was often initially suspected to be breast tumor.

Case presentation: In the present report, a 65-year-old woman was admitted to the Sun Yat-sen University Cancer Center, Guangzhou, China, because of a mass in her left breast. Breast ultrasonography showed multiple bands with low echo and uneven echo, and color doppler flow imaging showed no blood flow signal in the lesion. Therefore, the possibility of tumor was excluded and a preliminary diagnosis of Sparganosis was made. Subsequently, a lumpectomy was carried out and revealed a 30 cm worm. Then histopathological examination diagnosed this case was sparganosis and the worm was *Sparganum proliferum*. To further verify our diagnosis, the cytochrome oxidase subunit 1 (*COX1*) gene was amplified by PCR, sequence analysis of PCR products made us sure that it was the *Sparganum proliferum*.

Conclusion: We present Sparganosis could be preliminarily distinguished from breast tumor by ultrasonography, and then confirm Sparganosis by molecular biological methods, which provided a more efficient method for the detection of Sparganosis.

Background

Sparganosis is an important parasitic zoonosis caused by the plerocercoid larvae of the genus *Spirometra*. Typically, humans are infected by the parasite through eating raw meat of snakes, frogs, drinking water contaminated with procercoid-infected cyclops, or using poultices made of raw infected frogs or snakes on open wounds[1]. The majority of sites affected by the plerocercoid infection are subcutaneous tissues, brain, eyes, the abdominal viscera, and urogenital organs.

The traditional methods for detecting Sparganosis include serological examination using ELISA and surgical observation[2]. Breast Sparganosis presenting as a breast mass. Because eosinophils are normal in many cases of Sparganosis, breast Sparganosis was initially suspected as breast tumor, thus adding to the mental burden of patients. In this report, we found Sparganosis could be preliminarily distinguished from tumors by ultrasonography and color doppler flow imaging, and then confirm Sparganosis by molecular biological methods, which provided a more efficient method for the detection of Sparganosis.

Case Presentation

We present a case of a 65-year-old woman who lives in rural areas presented with a 1.5×2cm mass in her left breast three months ago. Three months later, the mass increased to 3×3cm and she was admitted to the Sun Yat-sen University Cancer Center. The patient's spirit, appetite and sleep were normal since the discovery of the mass, and there was no significant change in body weight. On initial presentation, her

vital signs were as follows: temperature 36.5°C, heart rate 82 beats/min, respiratory rate 18 breaths/min, blood pressure 143/84 mmHg, breathing is stable, lung breathing is clear, the chest was symmetrical, the breasts were symmetrical and there was no local uplift or depression, the skin was not redness, edema and orange skin changes, and the superficial veins of the breast were not dilated, both nipples are at the same level, no nipple invagination observed. Her initial laboratory tests demonstrated a white blood cell (WBC) count of 6.91×10^9/L with normal eosinophils, a hemoglobin concentration of 126 g/L, and a platelet count of 296×10^9/L.

A palpable mass was present 8cm from the nipple in the inner quadrant of the patient's left nipple, bout 3×3cm in size, with hard texture, uneven surface, unclear boundary and poor mobility. There was an enlarged lymph node palpable in the left axilla, about 1.5cm in diameter. Breast ultrasonography showed multiple bands with low echo and uneven echo (Fig. 1A). Color doppler flow imaging showed no blood flow signal in the lesion (Fig. 1B). Subsequently, a lumpectomy was carried out and revealed a 30 cm worm.

Histopathological examination of the worm showed tegmental layer, muscle fiber bundles, and the parenchymal tissue (Fig. 2A). Therefore, we diagnosed this case was sparganosis and the worm was *Sparganum proliferum*. To further verify our diagnosis, we used PCR to identify the cytochrome oxidase subunit 1 (*COX1*) gene of the worm. Briefly, the genomic DNA of the worm was extracted by HiPure Tissue DNA Mini Kit (Magen, China). The following protocol was conducted to amplify *COX1* gene: 95˚C for 5 min, 35 cycles at 95˚C for 50 s, 52 for 50 s, and 72˚C for 50 s, with a final extension at 70˚C for 10 min. The primers were *COX1* gene of *Spirometra erinaceieuropaei*: forward primer: 5'-TTTTTTGGGCATCCTGAGGTTTAT-3'; reverse primer: 5'- TAAAGAAAGAACATAATGAAAATG-3'. Fig. 2B shows the result of the PCR. Genetic sequencing was used to analyze PCR products. After homology searching with the Nucleotide BLAST in the National Center for Biotechnology Information (NCBI) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence exhibited 99% homology with *Spirometra erinaceieuropaei COX1* gene (Sequence ID: KJ599680.1). The alignments displayed the following: score = 739 bits (400), expect = 0.0, identities = 408/411 (99%), gaps = 3/417 (0%), and strand = Plus/Minus. These data indicated that the worm was *Sparganum proliferum*.

Discussion

The traditional methods for detecting Sparganosis were serological examination and surgical identification of sparganum in tissues. Serological tests using anti-sparganum antibodies and crude antigen extracts of sparganum, including ELISA, multidot ELISA and immunoblot[3]. Serological tests of crude antigen extracts of sparganum showed high sensitivity (85.7%)[4]. However, serological tests may produce false negatives during the early stage of infection and may cross reactions with other parasites[5]. Furthermore, in late stages of infection when the worm was surrounded by fibrosis, the level of the serum antibody was decreased[6]. For the surgical identification, because of the time-consuming and suspected tumor may cause a great psychological burden of patients.

In the present case, we confirmed that a 65-year-old woman contracted breast Sparganosis. We found that ultrasonography can initially distinguish Sparganosis from tumors. Ultrasonography examination of the tumor was round or oval, the tumor showed a low echo area, homogeneous, and with a capsule. However, in this case, breast ultrasonography showed multiple bands with low echo and uneven echo, suggesting that it may be the plerocercoid larvae of genus *Spirometra*. In addition, color doppler flow imaging showed no blood flow signal in the lesion, the possibility of tumor was further excluded. Therefore, it reduces the psychological burden of patient. Subsequently, a lumpectomy was carried out and revealed a 30 cm worm. Then histopathological examination and molecular biological methods of sequence analysis made us sure that it was the *Sparganum proliferum*.

In summary, we present an ultrasonography combine with molecular biological methods for diagnosing breast Sparganosis, which uses ultrasonography to distinguish breast Sparganosis from breast tumor as early as possible, and then molecular biological methods to determine it.

Declarations

Ethics statement

The acquisition of samples was approved by the medical research ethics committee of Sun Yat-sen University. Informed consent was obtained from the subject, and the experiments were conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from the patient for publication of this Case Report and any accompanying images.

Competing interests

There are no any financial or non-financial competing interests in this manuscript.

Author Contributions

Data curation: Lifu Wang, Ping Yang, Wei Zheng

Investigation: Lifu Wang, Ping Yang, Wei Zheng

Methodology: Lifu Wang, Ping Yang

Writing–original draft: Lifu Wang, Ping Yang

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Figures

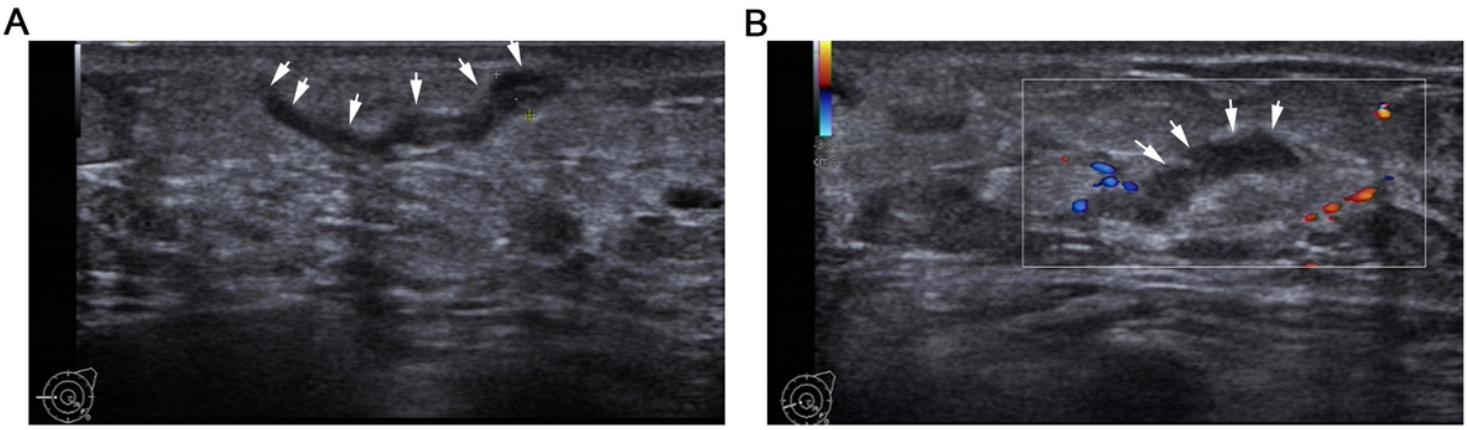

Figure 1

Breast ultrasonography and color doppler flow imaging examination. (A) Breast ultrasonography showed multiple bands with low echo and uneven echo (Arrows). Color doppler flow imaging showed no blood flow signal in the lesion (Arrows show the lesion, and red and blue dots are blood flow signals).

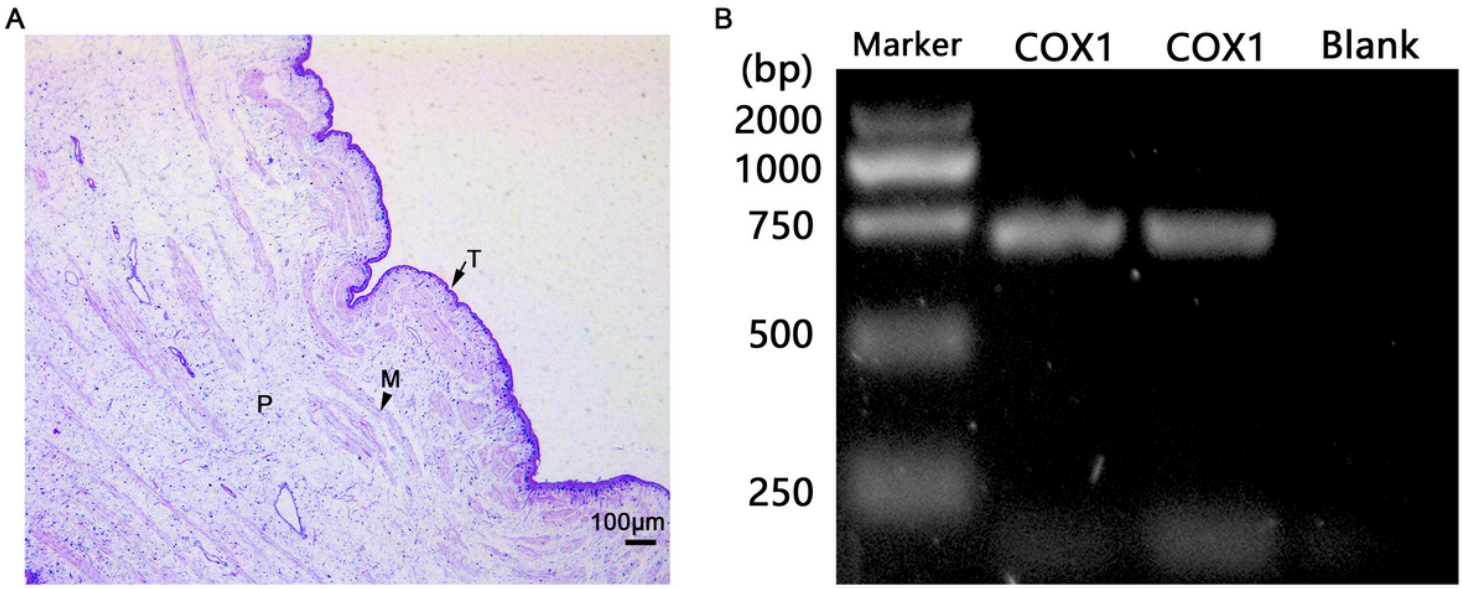

Figure 2

H&E staining of worm and electrophoresis result of COX1 gene. (A) Histopathological finding of the plerocercoid. Arrow: T, tegmental layer. Arrowheads: M, muscle fiber bundles; P, parenchymal tissue. (B) Representative electrophoresis result of COX1 gene.

Supplementary Files

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