



Recent Research Progress on Study of *Paragonimus westermani* in China

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Abstract

Recently, along with the development of biochemical and molecular immunological techniques, the diagnosis methods have been improved in which preparing natural parasite antigens, and genetically engineered recombinant antigens have been applied by using agglutination test, complement fixation methods to detect the human's serum antibodies for parasite identification of paragonimus infections. To improve the detection rate, some scholars have used phage peptide library technology to further improve the diagnostic effect on *Paragonimus westermani* in humans. In this paper, we summarized recent studies on diagnosis, pathogenesis and treatment of Paragonimus flukes in China.

Keywords

Paragonimus westermani, Antigen, Phage Peptide Library Technology, China

Subject Areas: Infectious Diseases, Respiratory Medicine

1. The Outline of *Paragonimus westermani*

1.1 Morphological Characters

The ovum of *Paragonimus westermani* is oval in shape and golden yellow in color and its operculum is big, conspicuous and inclined usually. Its ootheca is near papery and the other side is incrassate. There is an egg cell in the center of ovum surrounded by several yolk cells.

The imago looks like a half peanut, the reverse side of which is upheaval and the abdomen sucker is planar, while the size of oral sucker is similar to ventral sucker. Every adult has two intestinal tubes, arranging along the two sides of polypide. The ovary and uterus are tied behind the ventral sucker, meanwhile the testicular is di-

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vided into two and tied in polytide after a third position [1].

1.2. Life Cycle Characters

The life cycle of *Paragonimus westermani* includes ovum, miracidium sporocyst, redia, cercaria, metacercariae and adults. When the ovum passed into water, it would grow and mature under appropriate environment for almost three weeks, then the miracidium would be hatched from ovum. The worm would invade into the first intermediate host, then, the miracidium would live through the phase of sporocyst and redia, and finally develop into cercaria, which then escape from the snails and intrude into the body of second intermediate host, such as freshwater crab or crayfish in which it grow into a mature metacercariae. People would often be infected if they ate the raw freshwater crab or crayfish containing living metacercariae which would take off the capsule with the help of decomposing of digestive enzyme in small intestine, then enter into pulmonary across the diazoma. It would mature and form cyst in the pulmonary finally called paragonimiasis. The immunopathological effect caused by the migration and the parasitism of paragonimiasis was the primary mechanism of pathopoiesis [2].

1.3. Symptoms and Signs

Patients at acute stage would appear some symptoms such as chilly, remittent fever, bellyache, diarrhoea, feebility, night sweat and anorexia during a week of infection. Then it would appear pectoralgia, cough, asthma in two or three weeks. On the other hand, the chronic typical symptoms were cough and hemoptysis [3]. The sputum was jam-like where we can find Charcot Leyden crystals and plenty of ovums. In addition, the chronic phage could be splitted into various types according to its basic symptom and pathogenesis. For example, the basic pathology of the pulmonary type was lung abscess and hydatoncus in which we could find too many ovums. A few number of patients would appear expiratory dyspnea, asthma and pleural effusion. The clinical manifestations of abdominal type were abdominal pain, diarrhoea, vomit and hematochezia, the severe abdominal pain such as acute abdomen was rare. The intestinal type mainly demonstrates abdominal pain, diarrhoea and mucous bloody stool. The patients of cerebral type or myeloid form would appear some changes in cellular damages. The number of cells and proteins would increase slightly and the eosinophile granulocyte appeared. In addition, these patients' intracranial pressure would rise too quickly. Above all, the chronic patients have common characteristics in which the old and new lesions alternate each other due to the wandering of larvae and imago.

2. The Diagnosis and Identification of *Paragonimus westerman*

Previous morphological methods on parasitic detection with the electron microscope are used for identifications of eggs and adults of *Paragonimus westermani*, even though the specificity is high, and the sensitivity is relatively low. Cultivation of the adult worms is also effective upon time, temperature, humidity and other restrictions. In addition, the shape characteristics of worms are similar to accurate identification [3]. On the immunological aspects, the paragonimus antigens used for clinical diagnosis are basically same. It was very difficult to implement the large-scale survey of diseases only by pathogen detections. At the same time, the antigen complex will also affect the diagnosis results [4]. Some researchers also used the adult worm's cDNA library of *Paragonimus westermani* to screen out the positive antigens because of its high specificity and high sensitivity that provides the means for the diagnosis and treatment of infection with detection technology.

2.1. Antigen Detection

Antigens of lung fluke tested include circulating antigen (CAg) and circulating immune complexes (CIC). It is very helpful to clinically diagnose patients in endemic areas using CAg-dot-ELISA and CAb-ELISA methods. Some parasitologists had used CAg-dot-ELISA to detect 70 cases of patients with *Paragonimus* trematodes that has been confirmed that 29 cases were positive with sensitivity of 41.5% comparing with *Clonorchis* trematodes. Using CAb-ELISA to detect 70 cases of *Paragonimus* trematodes, 67 patients appeared positive with the sensitivity as 95.7% comparing with clonorchiasis diseases. CAg-dot-ELISA method was also used for the early diagnosis of *Paragonimus* infections because of CAg appearing earlier, at that time, a low level of antibodies had not yet been detected [5]. For CAg detection, early diagnosis can also be used as indicators of assessment activity and therapeutic effects of infections. Dogs infected with *Paragonimus westermani* metacercariae isolated from crabs would be quantitatively diagnosed with multi-resistant dot-EusA assay [6].

2.2. Antibody Detection

Using intradermal test with 1: 0.1 ml antigen to detect adults' paragonimiasis, the positive rate is up to 95%. The method is simple, but the specificity is not high, and there exists a higher cross-reactivity among schistosomiasis and chlonochiasis which would be used for epidemiological screenings [7]. After using cercariae membrane reaction to test metacercariae that were incubated in serum, the sensitivity is 98.5% and 100% with the value of early diagnosis, but there is a positive cross-react compared with schistosomiasis and fasciolopsiasis, etc. [7].

Currently, rapid detection methods for paragonimiasis include using P-IgG4-DIGFA for indication, the sensitivity and specificity were 95.2% and 100% respectively. Gold filtration assay for rapid detection of specific IgG4 of lung flukes is not only simple, fast, but also high sensitivity and specificity which is suitable for clinical laboratory and field investigation applications [8].

2.3. Cross-Immunity Reaction

Paragonimiasis and paragonimus infections could be identified by SDS-PAGE, spectrometry detection and immuno-gold labeling that showed the main components of the parasite surface membrane and secretions [9] [10].

2.4. Advances in Molecular Biology

Genetic screening and expression of recombinant antigens of *Paragonimus westermani* were screened by ELISA and cell cultures. Performed with *Paragonimus westermani*, the cDNA library was screened five times and the positive clones were amplified by PCR using *in vivo* phage of recombinant plasmids of DNA sequence determination. BLAST method (accounting sequence database search program) was used to detect DNA sequence homology that may act as a *Paragonimus westermani*'s gene fragment of positive clones [11]. Various developmental stages of *Paragonimus westermani* secrete at least six different types of cysteine proteases, in nutritional intake, invasion, and immune evasion and has played an extremely important role in pathogenicity of the host in order to design targeted at the protein corresponding anti-infective drugs. Using cDNA library of *Paragonimus westermani* to screen recombinant bacterium gene of PwAg, the homology is as high as 99% [12]. Phage screening method on *Paragonimus* antigen epitope of simulation can also be helpful for the diagnosis. The new diagnostic reagents provide a new focus from cDNA screening [13]. For patients with lung fluke, their antibody reactions with the existing detection methods could be extracted serum antigen that does not require cultured parasites [14].

2.5. The Genetic Testing of *Paragonimus westermani*

Ribosomal DNA gene and a second section (ITS2) sequences of mitochondrial cytochrome C oxidase subunit (COI) partly on *Paragonimus westermani* had been studied and colonized. COI gene belongs to the mitochondrial genome, its evolutionary rate was faster than the nuclear genome, and with maternal inheritance, it was basically not affected by genetic recombination, genetic differences within species. ITS2 gene evolution rate is relatively slow and conservative, reflecting the level of variation in parasites suitable for *Paragonimus* genetic differences between species, especially for close genetic relationship and is the ideal targets for detection and identification of parasites. In 1970s, Japanese researchers found that *Paragonimus* had two types of genotyping such as triploid ($2n = 22$ $3n = 33$) which was very similar to the metacercariae, worms and eggs. Diploid type worms have sperms, the reproductive mating lines, known as *Paragonimus westermani*. Triploid type worms have no sperms, but pathogenic impacts are significantly stronger than diploid of *Paragonimus* [15]. According to specific gene sequences (ITS2) of *Paragonimus westermani*, parasitologists had designed specific PCR primers and probes suitable for the detection of paragonimiasis in order to distinguish *Paragonimus westermani*. from *Paragonimus westermani*, *Paragonimus Okazaki*, Mexico *Paragonimus*, Fasciolopsis, liver flukes and Schistosomas using the second rRNA and the internal transcribed spacer District ITS2 gene sequences. In specific PCR testing method, only *Paragonimus westermani* significant DNA samples have amplified bands, and in the specific real-time PCR testing method, it is only *Paragonimus westermani* that appear fluorescent amplification curves.

3. Prospect

Excluding diagnosis on lung flukes and identification of patients experienced more than parasites with electron

microscopy and optical microscopy, identifications of antigen-antibody agglutination test and other immunological methods were used and developed. Due to limitations of parasites' antigens, a large-scale preparation of recombinant antigens and improving its purity would enter a new field conducted in-depth research. Positive clones have been obtained with immunological method for *Paragonimus* cDNA library by PCR amplification which addressed the questions of quantity and improved the specificity and accuracy of detections. At the genetic levels, specific primers and probes designed by PCR or real-time PCR methods for identification of similar *paragonimus* species are made as possible.

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