DIAGNOSIS OF HUMAN TRICHINELLOSIS: PITFALLS IN THE USE OF A UNIQUE IMMUNOSEROLOGICAL TECHNIQUE

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Summary :

Serum samples belonging to three outbreaks in Argentina (47 patients) taken at different times post-ingestion were analysed employing IIF and ELISA simultaneously. Results show that: a) the number of patients diagnosed by a unique technique, especially by ELISA (31 patients), was lower than the one obtained by the simultaneous use of both assays (38 patients); b) four patients out of the seven diagnosed by a unique technique were negative by the other assay over the period of time evaluated. Therefore, it can be concluded that the use of a sole immunoserological technique can not only lead to the delay in the detection but also to the misdiagnosis of this parasitic infection.

KEY WORDS : immunodiagnosis, human trichinellosis, ELISA, indirect immunofluorescense.

Although the diagnosis of human trichinellosis depends on a combination of epidemiological anamnesis, clinical signs and symptoms and laboratory tests, the detection of specific host antibodies (Abs) are commonly the only reliable diagnostic procedure. The most widely employed techniques for detection of human trichinellosis in epidemic outbreaks are the indirect immunofluorescense test (IIF) (Ljungström, 1974; Ruitenberg, 1975; Coltorti, 1981) and ELISA (Ljungström, 1983; Gamble, 1983; Chapa-Ruiz, 1990). However, the possibility that serology detects the early phase of infection, time at which an antiparasite treatment would be most effective, is not always possible due in part to inherent characteristics of the techniques employed. Taking this into account, the aim of this work was to assess the possibility of achieving an early diagnosis of the infection employing two techniques simultaneously which use different antigens (Ags).

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MATERIALS AND METHODS

HUMAN SERA

Serum samples from 47 patients belonging to three outbreaks, arisen in different areas in Argentina, were taken at 14, 21, 30 and 40 days post-ingestion (dpi) and analyzed.

INDIRECT IMMUNOFLUORESCENCE TEST

Human sera were analyzed by IIF using cryostat sections of *Trichinella spiralis* muscle larvae (ML) as Ag (Coltorti, 1981). Briefly, sections were incubated for 30 min at 37° C with 30 µl of sera diluted 1/64 in phosphate-buffered saline solution (PBS). After washing three times with PBS plus 0.05 % Tween 20 (PBS-T), samples were incubated for 30 min at 37° C with 30 µl of anti-human gammaglobulin rabbit serum conjugated to fluorescein isothiocyanate (Diagnostic Pasteur) diluted 1/50 in Evans blue. After washing, sections were mounted in buffered glycerine solution and examined by fluorescence microscopy (Zeiss). Fluorescence in the larval cuticle and internal structures was considered a positive reaction.

ELISA

Serum samples were also analyzed by indirect ELISA. Briefly, flat-bottomed PolySorp polyvinyl microtiter plates (Nunc) were coated with 50 µl/well of ML excretory-secretory products (ML-ESP), obtained as previously described (Gamble, 1983), at a concentration of 5 µg/ml in carbonate buffer pH 9.6 during one hour at 37° C and overnight at 4° C. After coating, and between each step, plates were washed three times with PBS-T. Plates were then blocked using 3 % non-fat dry milk in PBS for one hour at 37° C. Serum samples were tested diluted 1/200 in PBS-T plus 0.3 % non-fat dry milk (dilution buffer). After incubating one hour at 37° C a horseradish peroxidase-conjugated anti-human IgG serum (Diagnostic Pasteur) diluted 1/2000 in dilution buffer was added and incubated for another hour at 37° C. Colour reaction was developed

Outbreak N°	Technique	Number of diagnosed patients				
		14 dpi	21 dpi	30 dpi	40 dpi	Total
I	IIF	3	7	0	0	10
(n: 17)	ELISA	2	6	0	0	8
	IIF and/or ELISA	4	7	0	0	11
II	IIF	NA	8	7	0	15
(n: 17)	ELISA	NA	8	4	0	12
	IIF and/or ELISA	NA	8	7	0	15
III	IIF	NA	11	0	0	11
(n: 17)	ELISA	NA	10	0	1	11
	IIF and/or ELISA	NA	11	0	1	12
I + II + III (n: 47)	IIF and/or ELISA	4	26	7	1	38

NA: sera not available.

Table I. – Number of patients diagnosed by a unique and/or both immunoserological assays at different days post-ingestion.

using 0.004 % w/v *o*-phenilendiamine (Sigma) and 0.00 4 % v/v H_2O_2 in citrate buffer pH 5.0. Reaction was stopped by addition of 50 µl 4N H_2SO_4 and optical densities (OD) read at 490 nm in an ELISA reader (Metertech Σ960). The cut-off OD, previously calculated for this reaction, was 0.400.

RESULTS

Thirty-eight out of the 47 analyzed patients could be diagnosed during the period of evaluation. Four out of the 17 patients evaluated at 14 dpi rendered positive results at least by one immunoserological technique (one by ELISA, two by IIF, one by ELISA and IIF). Out of the remaining 43 individuals evaluated, 26 rendered positive results at 21 dpi (one by IIF, 25 by ELISA and IIF), seven were diagnosed at 30 dpi (three by IIF, four by ELISA and IIF), one was diagnosed at 40 dpi (by ELISA) and nine were negative over the period of time evaluated.

Seven patients were diagnosed by a unique technique (two by ELISA, five by IIF): three of them rendered positive results by the other assay while the remaining four individuals persisted seronegative over the follow-up period.

The number of patients diagnosed by a unique technique, especially by ELISA (31 patients), was lower than the one obtained by the simultaneous use of both assays (38 patients).

These results are shown in Table I.

DISCUSSION

Our results show that the simultaneous use of two immunoserological techniques which use different Ags allows the achievement of an earlier diagnosis than the one obtained by the use of a unique technique, allowing the establishment of a more effective antiparasite treatment. Besides these results reinforce the importance of a serological follow-up in those individuals whose clinical and/or epidemiological histories make them suspicious of being infected by this parasite.

The advantage of achieving an early detection of the infection could not be ascribed to a particular technique, showing individual features in the immune response generated in each host-parasite relationship mainly at the onset of the infection, since specific Abs can be detected by both techniques by 30 dpi in most of the patients. However in some individuals, Abs could be detected by only one technique meaning that the simultaneous use of these two methodologies has the additional advantage of a reduced probability of misdiagnosis of this parasite infection.

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