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SERODIAGNOSIS OF STRONGYLOIDIASIS WITH THE HELP OF ELISA

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Abstract:

Objective: *Evaluation of an enzyme- related immunosorbent assay using filariform larvae of Strongyloidesstercoralis is to identify particular antibodies in patients with strongyloidiasis.*

Design: *Source of Serum samples collection was people infected, with strongyloidiasis, with parasitic diseases and common people. By using crude antigen of filariform larvae, its analysis was conducted with the help of IgG-ELISA.*

Materials and Methods: *The study was conducted at the Nishtar Hospital Multan.*

Subjects: *46 individuals infected with strongyloidiasis is were selected for serum sample collection. 379 cases infected with different parasitic infections and 37 ordinary people were also part of sample collection.*

Main outcome measures: *Positive and negative predictive values, specificity and the sensitivity of the test.*

Result: *The cut-off point value was noted as 0.537. The specificity of the test was recorded as 96.15% while test sensitivity was calculated as 93.47%. The positive predictive value was 72.88% however; negative predictive value was 99.25%. Twelve individuals with toxocariasis, three individuals with hydatidos is and one individual with ascariasis, had antibodies which were reactive in nature against larval antigen.*

Conclusion: *A specific and sensitive diagnostic assay when ELISA method is used with filariform larval antigen.*

Key Words: *Strongyloidesstercoralis, Strongyloidiasis, IgG-ELISA.*

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INTRODUCTION:

Nematode Strongyloidiasis stercoral is are responsible for the human disease, Strongyloidiasis, which is an intestinal parasitic malady. Thirty million people in seventy countries are affected by it. Free living filariform larvae which is present in the ground, pierce in the skin of people and after entering the venous or lymphatic channel reaches the small intestine. The presence of S. stercoralis larvae in duodenal fluid or human feces leads to the ultimate diagnosis of strongyloidiasis [1]. On the contrary, the intestinal worm load and output of larvae are minimal in a number of simple cases of strongyloidiasis. Since various benchmarks are required for accurate diagnosis, it has been observed in almost up to seventy percent of the cases, a single benchmark to differentiate larvae, is not sufficient. It is pertinent to mention that unsuccessful detection of larvae in benchmark tests will not essentially show the clear absence of the particular infection. So, it is highly recommended to use a particular and result oriented test for S. stercoral is, having ability to be utilized in numerous helminthes infection. Multiple immunodiagnostic assays were examined in the past without adequate results [2]. The performance of Serologic tests, which were developed to identify antibodies of S. stercoral is by ELISA test, was questioned and they are available at some specific locations. In previously conducted researches, the sensitivity of ELISA test was testified as 88%10, 97%11 and 95%12. The aforementioned researchers noted the specificity of the test as 99%,99% and 94.6% consequently. Identification of particular antibodies in human strongyloidiasis by the ELISA method is the objective of the study at hand.

MATERIALS AND METHODS:

Clinical samples

Blood samples were obtained from the people who were infected with S. stercoral is. Formel-ether method was used for the diagnoses founded on coprological analysis for S. stercoralisrhabditi form larvae. The study at hand includes those 46 people who were having disease history and were coprologically positive. As far as Serum samples are concerned, they were collected from the individuals infected with toxocariasis(n=50), entrobiasis (n=35), hydatidosis (n=50), ascariasis (n = 13), amoebiasis (n=10), toxoplasmosis (n=47), trichuriasis (n=44), fascioliasis (n=40), hymenolepiasis (39) and giardiasis(n=37). The study was conducted at the Nishtar Hospital Multan

Preparation of antigens

Filariform larvae of S. stercoralis were collected from human feces cultures composed of rhabditid form larvae of the parasite. A variety of media consisting of agar plate, activated charcoal and charcoal were used in varying temperatures i.e. 20, 25, 30 and 33 degrees centigrade. At 25-degree centigrade, conventional charcoal media was used for preparing the larvae. The mixture of infected feces, distilled water and charcoal was used to create crude antigen offilari form larvae. Before segregation of larvae, it was incubated for seven to ten days at temperature 30 ° degrees centigrade. By utilizing of Baermann method, only third staged filariform larvae were derived from fecal cultures. Segregated Larvae were concentrated by centrifugation at 600 g for fifteen minutes under at 4degree centigrade. To eliminate supplementary bacteria, six-time washing was carried out in sterile phosphate-buffered saline, pH 7.2, by centrifugation at 150 g. for three minutes at 4degree centigrade. An electrical homogenization (Edmund Buhler Co., modelHomo 4/A mituhr) was used for homogenization of larvae in a volume of 0.045M PBS/pH 7.2 consisting 1.7mM of phenylmethylsulphonyl fluoride (PMSF); 5mM pepstatin15, 5mMEDTA; and 5mM EGTA. Sonication, via Tomy Seiko model UP-200P, Tokyo, was followed by it. Thirty minutes were allocated for centrifugation at 16000 × g at 4º centigrade. Afterwards, collection of supernatant was delipidized with ether. At 4ºcentigrade overnight temperature, dialysis against distilled water was performed, output of which, was utilized as final antigen. Lowery method was adopted to determine the protein content. These antigens were, then, aliquoted and storage was completed at 20 degrees centigrade.

ELISA test

After a few modifications, the immunodiagnostic assay was carried out. Two procedures were adopted in its execution. These two procedures were performed with or without preincubation of sera. Extracts of various parasites i.e Toxocara and, hydatidprotoscolices and Ascaris in concentration of 50 µg/ml for each were also included. Mixture of extracts and serum samples was done with the ratio of 1:50 at 37 degrees centigrade for 01 hours. The dispensation of 100micro-liters of S. stercoralis crude antigen (2µg/ml) was performed into the wells of microtiter plates (Denmark, Nuclon, Kamstrup, Roskilde). Its incubation was executed overnight at 4degree centigrade. 200µl bovine serum albumin (2% diluted in PBS/0.1% Tween 20) was utilized in blocking excess binding sites and 30 minutes' incubation was performed at 37 degrees centigrade. Addition of 100µl of a serum sample, diluted with the ratio 1:200, was carried out to each plate when the wells were washed thrice. Incubation continued for sixty minutes at 37 degrees centigrade.100µl of

peroxidase conjugated goat anti-human IgG, diluted with the ratio of 1:400, was mixed to all wells after an additional washing step. Incubation of plates was extended to 60 mins at 37 degrees centigrade. 100µl of O-phenylenediamine dihydrochloride (OPD) substrate (from Sigma Chemical Co., Poole, Dorset, UK) was added to all wells. After five minutes, the reaction stopped when 50µl of 12.5% H2S04 was added. Titerteck (Helsinki, Finland) multi-scan ELISA plate reader was utilized to acquire measurement as 492 nm of optical density (OD) of the samples. Testing of all assays was carried out in triplicate and was repeated two times.

Statistical analysis:

Mean plus 3.0 standard deviation OD value was used for healthy group sera. It was considered as the lower limit of positivity. Method of Galen was employed to calculate specificity, sensitivity and predictive values. SPSS for Windows (version 10) was used to verify statistical analysis.

RESULTS:

Coprologically positive for strongyloidiasis, serum samples were collected from 46 people. Their analysis was further carried out by ELISA to identify all antibody responses against crude antigen of S. stercoralis. Cut-off point value noted as 0.537. Absorbance readings having greater than the cut off value were recorded as seropositive for strongyloidiasis. In the same way, 42 people whose clinical demonstration was strongyloidiasis before the absorption of sera were noted as seropositive. When absorption was over, false negative of three individuals was observed. Sensitivity of this test was recorded as 91.3% and 93.47% before and after pre-incubation of sera. No reasonable variation was noted in absorbance readings in no age group and genders within the sample population showed an inclination to get a greater incidence of infection. Standard deviation and the mean absorbance for every group of people infected with maladies except strongyloidiasis were determined and demonstrated to be not considerably dissimilar from the ones collected for the negative control sera. Obtained absorbance readings from the samples of Stercoralis seropositive individuals were observed greater than the patients who were either infected with other parasites or seronegative ($P<0.001$). Nonetheless, before absorption, reactive antibodies against S. stercoralis crude antigen were of four people with ascariasis, nineteen with toxocariasis and six with hydatidosis, . After absorption, these readings reduced to 01, 12 and 3 cases respectively (figure.01). Current data records the specificity of crude antigen as 93.02 percent and 96.15 percent, before and after absorption. Positive predictive value of the test, before and after absorption, was measured as 59.15 percent and 72.88 percent, while obtained reading for negative predictive value was calculated as 98.97percent and 99.25percent likewise.

DISCUSSION:

Majority of parasitic infection to diagnose is Strongyloidesstercoralis. It is pertinent to note that to detect an indicative, chronic and low-level of strongyloidiasis infection in men, extremely rapid and sensitive immunodiagnostic procedure is required [3]. Apparently, moderation of infection with the help of parasite, parasitological proof of the light is problematic. Numerous endeavors were made to establish an immunological test for the diagnostic purpose of strongyloidiasis [4]. It consisted of complement fixation test, skin test, radio allergosorbent testing for specific IgE, gelatin particle agglutination and indirect immune fluorescence analysis of fixed larvae [5].

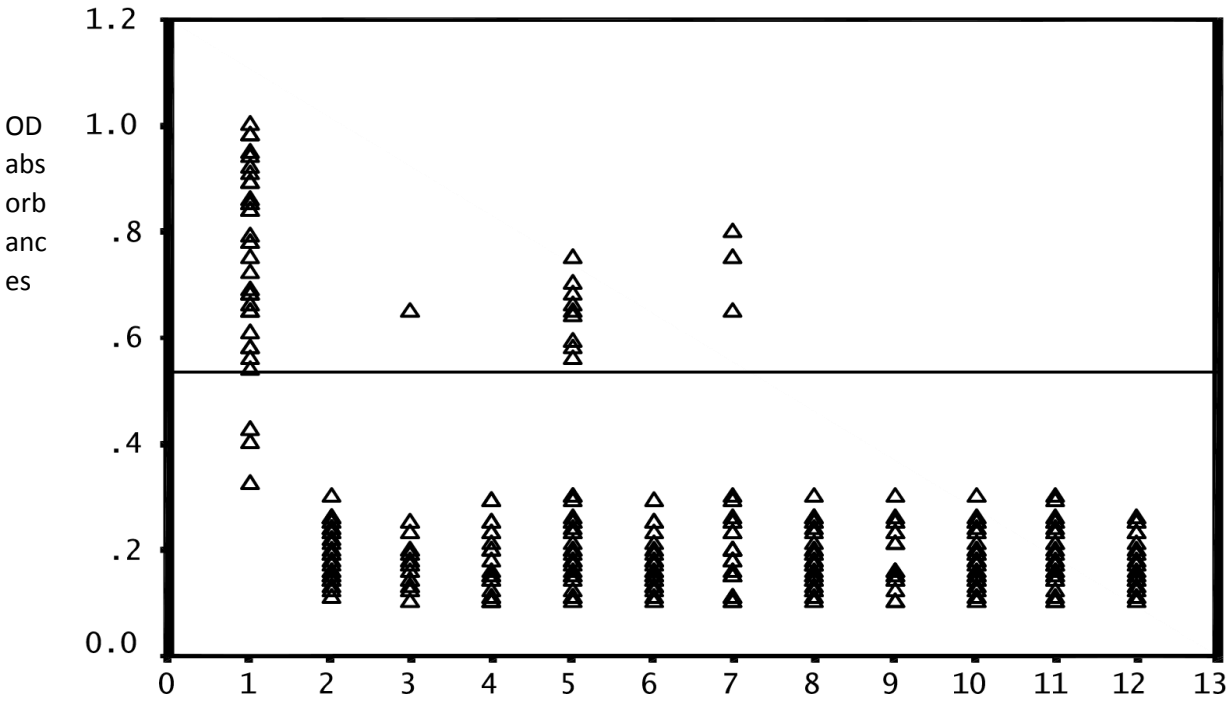

Fig. 1: Investigation of sera from those patients infected with different infections by IgG-ELISA using S. stercoralis filiform larvae crude antigen. Samples of serum collected from patients infected with strongyloidiasis (46, lanes 01), entrobiasis (35, lanes 02), ascariasis (13, lanes 03), trichuriasis (44, lanes 04), toxocariasis (50, lanes 05), hymenolepiasis (39, lanes 06), hydatidosis (50, lanes 07), fascioliasis (40, lanes 08), amoebiasis (24, lanes 09), giardiasis (37, lanes 10), toxoplasmosis (47, lanes 11) and control human sera (37, lanes 12).

Immune diagnosis of strongyloidiasis by ELISA, was noted by various investigators. These reports determine the sensitivity of the as 88-97% approximately [6]. Specificity and the sensitivity of the test can be enhanced as samples of serum are pre-incubated with aforementioned parasites antigens. The sensitivity and specificity of ELISA can be improved with the help of Lindo et al8and Conway15 when presoaking of sera with Onchocerca antigen is used [7]. As demonstrated in figure 1, antibodies in the sera of several cases infected with, andtoxocariasis, ascariasis and hydatidosis were reactive in nature against S. stercoralis crude antigen after pre-incubation of sera. These cases may have been revealed to cross-reactive antigen of S. stercoralis [8]. It is noted that helminthes having cross-reactive antigens, retains the capability to continue longer in the host and propensity to create circulating antibodies which can be identified for numerous years after being exposed [9]. Data also indicates that Strongyloidiasis antibody demonstrates cross-reactivity with another helminth infection. It includes ascariasis, filariasis and acute schistosomiasis. To conclude, ELISA is found to be an outstanding assay to diagnose the disseminated and hidden strongyloidiasis [10].

CONCLUSION:

A specific and sensitive diagnostic assay when ELISA method is used with filiform larval antigen.

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