# Research Journal of Parasitology

ISSN 1816-4943

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Parasitology

ISSN 1816-4943
DOI: 10.3923/jp.2018.47.56

# Research Article
# Comparative Serodiagnostic Evaluation of Tegumental Antigen and its 16.5 KDa Subunit to Crude Antigens in Human Fascioliasis

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## Abstract

**Background and Objective:** Enzyme-linked Immunosorbent Assay (ELISA) was considered the most sensitive and specific adjuvant to fecal analysis for diagnosis of fascioliasis. Since specificity of ELISA depended mainly on the used antigens and history of tested sera, so the present study was designed to comparatively evaluate the diagnostic performance of Tegumental (T) Antigen (Ag) and its 16.5 KDa subunit to Somatic (S) and Excretory-Secretory (E-S) Ags of *F. gigantica* in an indirect total IgG-ELISA for serological diagnosis of human infection. **Materials and Methods:** The 16.5 KDa T Ag subunit was prepared by SDS polyacrylamide gel electrophoresis from T Ag followed by purification and elution of the selected band by Pall Nanosep microcentrifugal device also the S, E-S and T Ags were prepared. Their immunodiagnostic performance was compared in an indirect total IgG ELISA. **Results:** The ROC analysis revealed the highest sensitivity from the T Ag (100%) followed by SAg (86.6%), E-S Ag (73.3%) and the least value was shown from the 16.5 KDa subunit (53.3%). Again, as regard specificity, the highest value was obtained from T Ag (100%) followed by E-S Ag (79.2%) then the 16.5 KDa subunit (75%) and least value were obtained from SAg (70.8%). **Conclusion:** The *F. gigantica* T Ag was superior to S, E-S and the 16.5 KDa subunit in the serological diagnosis. This performance was followed by S and E-S Ags. Unexpectedly, the purified 16.5 KDa T subunit showed a poor diagnostic outcome.

Key words: Fascioliasis, *F. gigantica*, ELISA, Tegument, 16.5 KDa, subunits, somatic, excretory-secretory, antigen

Citation: I.M. Abdelsalam, R.M. Sarhan, A.F. Badawy and G.H. Shinkar, 2018. Comparative serodiagnostic evaluation of tegumental antigen and its 16.5 kda subunit to crude antigens in human fascioliasis. Res. J. Parasitol., 13: 47-56.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Fascioliasis was recognized as a serious public health problem in human[1] with an estimated 17 million people infected worldwide[2]. It is a major parasitic disease with a worldwide losses in agriculture estimated at over 2 billion dollars per year due to increased animal mortality and reduction in productivity[3,4].

Immunodiagnosis is considered a sensitive and reliable means for diagnosing acute infections and also it can be used as an adjuvant to fecal analysis for the diagnosis of latent and chronic infections[5].

It is commonly understood that enzyme-linked Immunosorbent Assay (ELISA) is a sensitive and simple method for quantitative and qualitative determination of antibodies[6]. Specificity of ELISA depended mainly on the purity of the used antigen as well as history of the tested sera[7]. Most of the immunodiagnostic assays used for diagnosis of human fascioliasis rely on antibody detection using crude worm antigens of which the Somatic (S) and Excretory-secretory (E-S) ones constitute a major component[7,8].

Previously, ELISA using E-S products gave better sensitivity and specificity than other crude extracts. The E-S antigens have more contact with the host immune system since the parasite excretes the content of the intestine with cytolytic activities that degrade tissues and facilitate the invasion and migration of the parasite and induce a stronger humoral immune response, useful for diagnostic purposes and protection against future infections[9]. However, the use of complex antigenic preparations from crude Ags of E-S and S can result in a reduced specificity of the assay since *Fasciola* share cross-reactive antigens with many parasites, namely *Schistosoma* spp. and *Echinococcus* spp[10,11].

Tegumental proteins are another important source of immunodiagnostic antigens[12]. One major interface between the parasite and the host is the tegument[13,14]. Anuracpreeda *et al.*[15] reported that the tegumental proteins from adult *F. gigantica* were easily released to stimulate the host immune response and therefore were considered potent diagnostic antigens. Furthermore, T Ag preparation is simpler and faster and does not require particular experience or advanced technology in comparison to the E-S Ags which are prepared using *in vitro* techniques that require a degree of sterility or the recombinant proteins which are costly and need complicated protocols in equipped laboratories[15].

Several *F. gigantica* antigens were purified from crude, E-S or S antigens to enhance the specificity of the diagnostic assays. Some of these antigens were found to be excellent immunogens[16-22]. Gaudier *et al.*[23] proved that anti-*Fasciola hepatica* (Fh) T Ag 16.5 KDa IgG antibody could be a useful diagnostic reagent. Caban-Hernandez *et al.*[24] demonstrated that *F. gigantica* 16.5 KDa T Ag was an excellent antigen for the serodiagnosis of chronic fascioliasis with a sensitivity reaching 91.4%. Immunohistochemical studies demonstrated that it was strongly expressed at the outer surface of the tegument as well as in parenchymal tissue and moderately expressed in the syncytium. It is not actually a part of the architecture of the tegument, but it is a protein produced in parenchymal cells then delivered to the tegument and accumulates there[25].

The present study aimed to evaluate comparatively the diagnostic performance of *F. gigantica* T Ag and its 16.5 KDa subunit to S and E-S Ags, in an indirect total IgG ELISA in human infection.

## MATERIALS and METHODS

This study was conducted from February 1st, 2017 to June 30th, 2018 at the Diagnostic and Research unit of medical parasitology, Faculty of Medicine, Ain Shams University. All chemicals were purchased from Sigma Chemicals Co., USA unless otherwise listed.

**Collection of worms and preparation of antigens:** Adult *F. gigantica* worms were collected from bile ducts of naturally infected liver of cattle obtained from a local abattoir at Cairo, Egypt.

**Preparation of Somatic (S) Ag and Excretory-secretory (E-S) Ag:** The somatic antigen[26,27] and excretory secretory antigen[28] were prepared and kept in aliquots at -20°C until used.

**Preparation of tegumental antigen (Tag):** Liver flukes were incubated in PBS containing 1% of the non-ionic detergent (NP-40; Sigma) for 1 h at 4°C with gentle shaking. The supernatant was collected. The NP-40 was removed from the supernatant by high protein and peptide recovery detergent removal resin kit detergent removal resin kit (HiPPR) (Thermo Scientific, USA)[23].

**Preparation of 16.5 KDa tegumental subunit involved 2 steps:** Separation of T Ag by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

The prepared sample buffer was added to *F. gigantica* T Ag samples in labeled aliquots, then titration trials were done using different Ag concentrations, the final loaded volume was (25 µL) Ag added to (15 µL) reducing sample buffer to make a final volume of 40 µL. The aliquots were put in boiling water bath for 4 min for denaturation of proteins[29,30].

**Elution of the purified T Ag subunit (16.5 KDa) from the SDS-PAGE gel by ultrafiltration:** The SDS-PAGE was carried using *F. gigantica* T Ag then the electrophoretic run took place to detect precisely the desired 16.5 KDa subunit. The excised gel containing the specific band from the unstained gel was then minced, homogenized then introduced to the Nanosep Centrifugal Devices (0.45 µL) (Pall Life Sciences, USA). The protein content of the sample was then measured[31]. The sample was stored at -20°C till use.

**Indirect total IgG ELISA:** The ELISA for detection of antibodies in human sera against *F. gigantica* Ags; S, E-S, T and 16.5 KDa subunit was performed[32]. The optimum concentrations of antigens were adjusted as 5, 2, 2.5 and 0.01 µg $mL^{-1}$ for S, E-S, T and 16.5 KDa subunit, respectively. Sera dilutions of 1:50 was used for all Ags and conjugate dilutions of 1:10000, 1:1000, 1:6000 and 1:6000 were adjusted for S, E-S, T and 16.5 KDa subunit, respectively. The ELISA optical densities were read at 450 nm.

**Selection and grouping of individuals included in the study:** Cases required for the present study were selected from patients referred to the Diagnostic and Research Unit, Parasitology Department, Faculty of Medicine, Ain Shams University. Group I: It included 15 fascioliasis patients diagnosed by detection of eggs in stool and proved serologically positive by IHAT (Fumouze, Distomatose, France). The selected cases were negative for other parasitic diseases. Group II (Positive control): It included 20 patients negative for fascioliasis and positive for other parasitic infections by IHAT (Fumouze, Distomatose, France) specific for each disease (8 cases of hydatidosis, 7 cases of intestinal schistosomiasis, 3 cases of amoebiasis and 2 cases of toxoplasmosis). Group III (negative control): It included 15 healthy individuals, confirmed to be negative for fascioliasis and parasitic diseases by stool examination and IHAT specific for each disease.

**Statistical analysis:** The data were collected, coded, tabulated and introduced to a PC using the Statistical Package for Social Science (SPSS version 20.0.). Quantitative data were expressed as Mean±SD. Qualitative data were expressed as frequency and percentage. The Z-test[33], ANOVA[34] and ROC Curve[33] were used and probability value (p-value) was calculated for the level of significance ($p>0.05$: Non-significant (NS), $p<0.05$: Significant (S), $p<0.001$: Highly significant (HS).

**Ethical consideration:** An informed consent was taken from the patients after explaining the aim of the study. The study was approved by the Ethical Committee of Scientific Research, Faculty of Medicine, Ain Shams University.

## RESULTS

The present study was conducted to comparatively evaluate the diagnostic performance of T Ag and its 16.5 KDa subunit prepared from *F. gigantica* adult fluke to S and E-S Ags, in a standard total indirect IgG ELISA for serological evaluation of human infection.

To prepare the 16.5 KDa tegumental subunit, SDS-PAGE was carried using *F. gigantica* T Ag with a concentration of (70 µg $mL^{-1}$) (the clearest outcome resolution from the electrophoresis trials) (Fig. 1) and then the electrophoretic run took place to detect precisely the desired subunit (Fig. 2). An antigen amount of 300 µL was added to 300 µL sample buffer and only 40 µL were added to the 3rd well and the rest to the 2nd well. Guided by the marker, the gel in the 3rd lane was cut stained in Coomassie then destained to be used as a reference to locate the desired band from the 2nd lane. The16.5 KDa subunit from the 2nd lane with unstained gel was cut guided by the marker and the destained gel from the 3rd lane.

As regard S Ag, Group I showed a positive outcome of 86.6%. Among Group II, only sera of hydatidosis exhibited a cross-reactivity of a 37.5% and among Group III, a cross-reactivity of 26.6% was revealed. As regard E-S Ag, Group I showed a positive outcome of 73.3%. Among Group II, schistosomiasis exhibited cross-reactivity of 14.3% while hydatidosis exhibited cross-reactivity of 12.5%. There was a cross-reactivity of 13.3% among Group III. As regard tegumental Ag, Group I showed a positive outcome of 100%. No cross-reactivity was exhibited among all other groups of non-*Fasciola* patients (Groups II and III). Using T Ag 16.5 KDa subunit, 53.3% were proved to be positive from Group I. Among Group II, schistosomiasis exhibited cross-reactivity of 14.2% while Group III showed a cross-reactivity of 6.6% (Table 1).

The possible values of AUC range from 0.5 (no diagnostic ability) to 1.0 (perfect diagnostic ability). The highest value was revealed from the T Ag (1.0) while the least was revealed by the 16.5 KDa subunit (0.71) (Table 2).

The comparative analysis between optical densities revealed a significant outcome of results between infected and non-infected groups of patients as regards the S, E-S Ag and T Ags, while a non-significant outcome resulted from the 16.5 KDa subunit (Table 3).

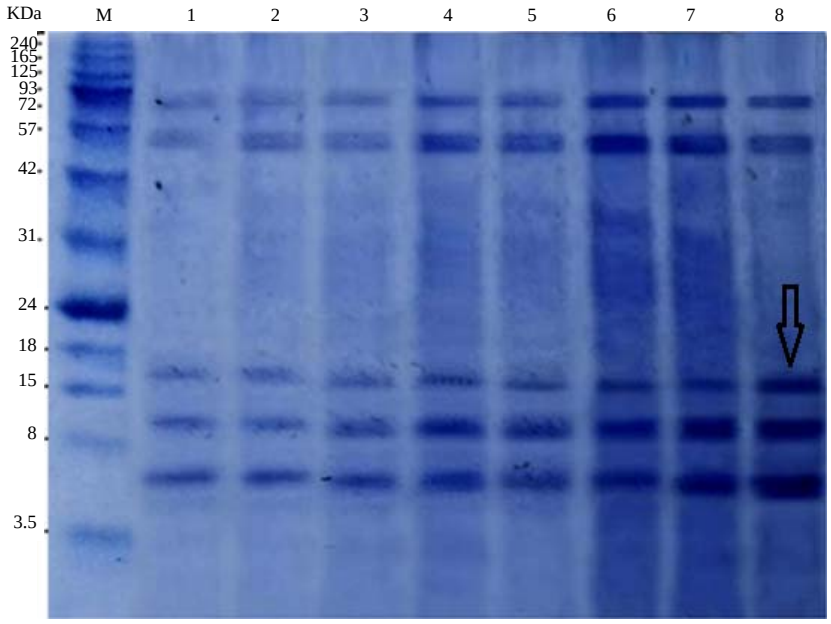


Fig. 1: T Ag separated by SDS-PAGE (12%) gel stained by coomassie blue stain. The arrow points to the desired subunit (16.5 KDa). M: Wide range prestained marker (BLUelf Prestained Protein Ladder, Genedirex, USA) of molecular weights of : 3.5, 8, 15,18,24, 31, 42, 57, 72, 93, 125, 165 and 240 KDa. Lane (1-8): Different concentrations of T Ag; 20, 30, 40, 50, 60, 70, 80 and 90 μg $mL^{-1}$

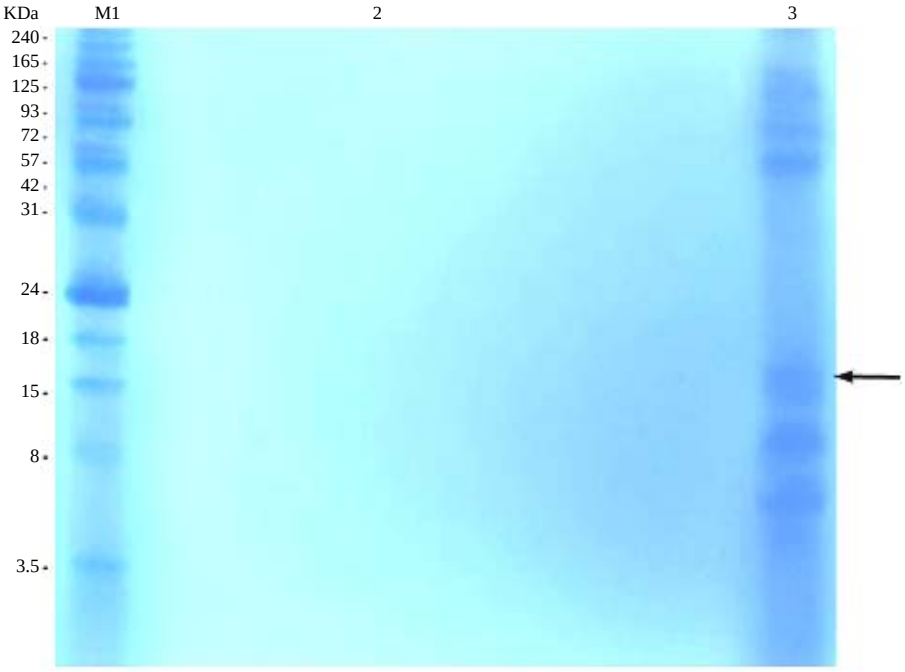


Fig. 2: Characterization of 16.5 KDa subunit from *F. gigantica* T Ag. Lane 1: Marker, Lane 2: Unstained gel containing Ag from which the desired protein band will be sliced and Lane 3: Stained gel containing Ag acting as a reference lane to locate the position of the desired protein band

Table 1: Serological reactivity of *Fasciola gigantica* antigens in TIgG-ELISA

| Groups (G) | Number of sera examined | Number (%) of ELISA positive sera for TigG |
|---|---|---|
| **S Ag** | | |
| G I: Patients suffering fascioliasis | 15 | 13 (86.6) |
| **G` II: patients suffering** | | |
| Schistosomiasis | 7 | 0 (0) |
| Hydatidosis | 8 | 3 (37.5) |
| Amoebiasis | 3 | 0 (0) |
| Toxoplasmosis | 2 | 0 (0) |
| G III: Healthy control | 15 | 4 (26.6) |
| **E-S Ag** | | |
| G I: Patients suffering fascioliasis | 15 | 11 (73.3) |
| **G II: Patients suffering** | | |
| Schistosomiasis | 7 | 1(14.3) |
| Hydatidosis | 8 | 1(12.5) |
| Amoebiasis | 3 | 0(0) |
| Toxoplasmosis | 2 | 0(0) |
| G III: Healthy control | 15 | 2(13.3) |
| **T Ag** | | |
| G I: Patients suffering Fascioliasis | 15 | 15(100) |
| **G II: patients suffering** | | |
| Schistosomiasis | 7 | 0(0) |
| Hydatidosis | 8 | 0(0) |
| Amoebiasis | 3 | 0(0) |
| Toxoplasmosis | 2 | 0(0) |
| G III: Healthy control | 15 | 0(0) |
| **16.5 KDa subunit** | | |
| G I: Patients suffering fascioliasis | 15 | 8 (53.3) |
| **G II: patients suffering** | | |
| Schistosomiasis | 7 | 1(14.2) |
| Hydatidosis | 8 | 0(0) |
| Amoebiasis | 3 | 0(0) |
| Toxoplasmosis | 2 | 0(0) |
| G III: Healthy control | 15 | 1(6.6) |

Table 2: ROC analysis of *Fasciola gigantica* Ags tested in TIgG-ELISA

| Antigen | Cut-off | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Diagnostic accuracy (%) | AUC |
|---|---|---|---|---|---|---|---|
| S Ag | >0.145 | 86.6 | 70.8 | 58.8 | 89.5 | 79.2 | 0.79 |
| E-S Ag | >0.071 | 73.3 | 79.2 | 61.5 | 82.6 | 78.0 | 0.78 |
| T Ag | >0.060 | 100 | 100.0 | 100 | 100.0 | 100.0 | 1.00 |
| 16.5 KDa subunit | <0.105 | 53.3 | 75.0 | 72.7 | 69.0 | 71.5 | 0.71 |

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve

Table 3: Comparative analysis of the mean optical densities of TIgG-ELISA using S, E-S, T Ag and 16.5 KDa subunit

| Sera | G I | G II | | | | GIII | | |
|---|---|---|---|---|---|---|---|---|
| Ag | F | S | H | A | T | Neg. | ANOVA | p-value |
| **OD/ Mean±SD** | | | | | | | | |
| S | 0.200±0.117 | 0.101±0.041 | 0.13±0.05 | 0.12±0.05 | 0.01±0.06 | 0.12±0.04 | 2.913 | 0.049** |
| **Range** | | | | | | | | |
| | 0.09-0.54 | 0.04-0.14 | 0.06-0.18 | 0.08-0.28 | 0.07-0.38 | 0.06-0.19 | | |
| **OD/ Mean±SD** | | | | | | | | |
| E-S | 0.09±0.04 | 0.05±0.01 | 0.05±0.03 | 0.05±0.01 | 0.07±0.05 | 0.06±0.03 | 3.095 | 0.041** |
| **Range** | | | | | | | | |
| | 0.04-0.18 | 0.04-0.07 | 0.01-0.10 | 0.01-0.17 | 0.01-0.21 | 0.01-0.14 | | |
| **OD/ Mean±SD** | | | | | | | | |
| T | 0.36±0.13 | 0.03±0.01 | 0.03±0.01 | 0.02±0.06 | 0.03±0.1 | 0.03±0.01 | 46.12 | <0.001*** |
| **Range** | | | | | | | | |
| | 0.12-0.57 | 0.02-0.04 | 0.02-0.04 | 0.07-0.97 | 0.06-0.1 | 0.01-0.06 | | |
| **OD/ Mean±SD** | | | | | | | | |
| 16.5 KDa subunit | 0.10±0.02 | 0.153±0.055 | 0.107±0.02 | 0.11±0.04 | 0.1±0.04 | 0.1±0.04 | 2.185 | 0.121* |
| **Range** | | | | | | | | |
| 0.06-0.15 | 0.11-0.23 | 0.08-0.13 | 0.09-0.30 | 0.08-0.2 | 0.09-0.20 | | | |

SD: Standard deviation, OD: Optical densities, ANOVA: A one-way analysis of variance, p: Probability value, Ag: Antigen, Neg: Negative, F: *Fasciola*, S: *Schistosoma*, A: *Amoeba*, T: *Toxoplasma,* H: Hydatid. *p-value: Non significant, **p-value: Significant, ***p-value: Highly significant

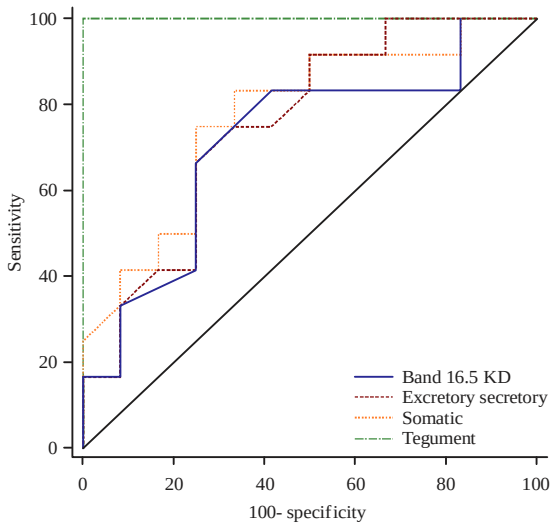


Fig. 3: ROC curves for the comparative analysis between all *Fasciola gigantica* prepared antigens (S, E-S, T and 16.5 KDa subunit) in TigG-ELISA

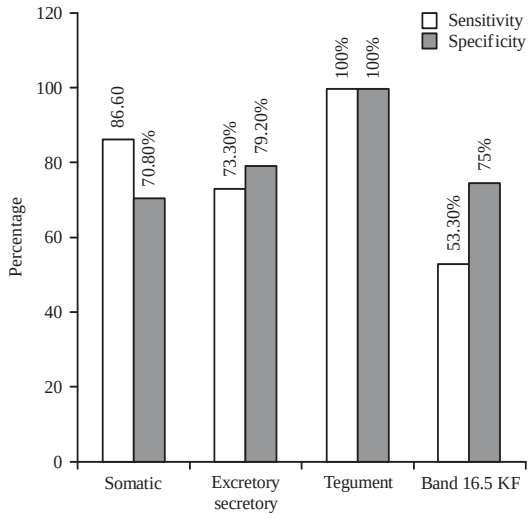


Fig. 4: Comparative results of sensitivity and specificity obtained from TIgG- ELISA calculated from ROC test on using the 4 antigens (S, E-S, T and 16.5 KDa subunit)

Table 4: Comparative analysis between the diagnostic accuracy of S, E-S Ag, T Ag and 16.5 KDa subunit in TIgG-ELISA

| Antigen | AUC | Z | p-value | Accuracy (%) |
|---|---|---|---|---|
| Tegument | 1.0 | - | - | 100.0 |
| 16.5 KDa Subunit | 0.71 | 3.179 | <0.001*** | 71.5 |
| Excretory Secretory | 0.78 | 2.248 | 0.003** | 78.0 |
| Somatic | 0.79 | 2.111 | 0.004** | 79.2 |

Ag: Antigen, AUC: Area under the curve, P: Probability value, Z: Score for the Z ratio

From ROC curve, the highest sensitivity was obtained from the T Ag (100%) followed by SAg (86.6%) then E-S (73.3%) and least value from 16.5 KDa subunit (53.3%). Concerning specificity, the highest value was obtained from T Ag (100%) followed by E-S Ag (79.2%) then the 16.5 KDa subunit (75%) and least value was obtained from SAg (70.8%) (Fig. 3, 4). The highest AUC value (1.0) was that of T Ag which was used as a reference to calculate the results, followed by SAg (0.79) then E-S Ag (0.78), while the least value was that of the 16.5 KDa subunit (0.71). There was a significant outcome between the AUC values of T Ag compared to S and E-S Ags. While a highly significant outcome was found when compared to the 16.5 KDa subunit (Table 4).

For each of the prepared antigens, a dot diagram was plotted where the reference line represented the cut-off value, any serum value above it was considered positive and below it considered negative. The diagrams show the results of both the cases (GI) and the controls (G II and G III). The Sag (Fig. 5a), 13 out of 15 of the cases were above the cut-off value, while 28 out of 35 of the controls were below the cut-off value. For the E-S Ag (Fig. 5b) 11 out of 15 of the cases were above the cut-off value, while 31 out of 35 of the controls were below the cut-off value. As for T Ag (Fig. 5c) showed the highest sensitivity and specificity, as all the cases were above the cut-off value (true positive) and all the controls were below the cut-off value (true negative). The 16.5 KDa subunit showed 8 out of 15 cases above the cut-off value and 33 out of 35 controls below the cut-off value (Fig. 5d).

## DISCUSSION

Concerning the SAg, the study showed a sensitivity of 86.6% and a specificity of 70.8% with a diagnostic accuracy of 79.2%, PPV 58.8% and NPV 89.5%. These were different from Hassan *et al.*[10], who reported a sensitivity of 100% and specificity of 63% and Rokni *et al.*[35] who reported a sensitivity of 100% and specificity of 96.9% with a PPV of 96% and NPV of 100%. Furthermore, Rahimi *et al.*[36], who evaluated *F. hepatica* SAg and recorded sensitivity of 97.2%, specificity of 100%. The PPV was 94.6% and NPV was 95.6%. This difference could be attributed to the complexity of the SAg which may include other constituents shared with other parasites that affect its specificity e.g., the tegument spines[32], it can also be referred to the method of Ag preparation used that can give rise to more purified contents and also the different species used[35,36].

The E-S Ag revealed a sensitivity of 73.3%, specificity of 79.2% with a diagnostic accuracy of 78%, PPV 61.5% and NPV 82.6%. This diagnostic performance was in contrast to Noureldin *et al.*[37] who reported a sensitivity of 96% and a specificity of 94.6%, Hillyer *et al.*[5] using *F. hepatica* and recorded sensitivity of 95% and specificity of 90% and again,

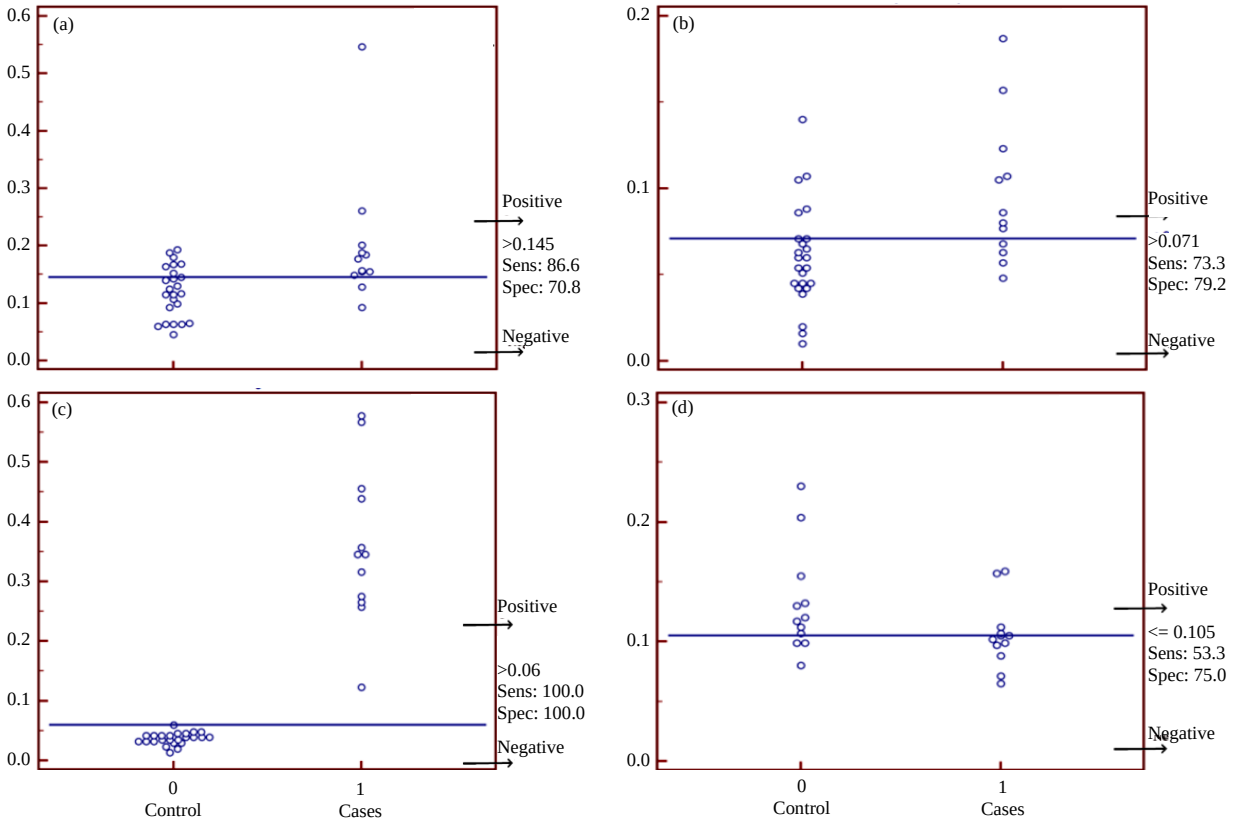


Fig. 5(a-d): Dot diagram plotting the detection of anti *F. gigantica* antibodies in sera from cases and control groups of the study. Each dot represents the absorbance of a single patient. The horizontal line depicts the cut-off value. (a) S Ag, (b) E-S Ag, (c) T Ag and (d) 16.5 KDa subunit

Hillyer *et al.*[38], who reported sensitivity of 100% using *F. hepatica* E-S antigen using FAST-ELISA and Cornejo *et al.*[39] who reported sensitivity of 97% and specificity of 96.6% in a sandwich ELISA. Also, this was in contrast to Ali[40], who used a dip stick and recorded sensitivity of 100%, specificity of 96.7%, PPV was 96.7% and NPV was 100% using *F. gigantica* E-S Ag with cross-reaction with a case of schistosomiasis. Previously, a dot-blot ELISA[41] using *F. gigantica* E-S Ag revealed a sensitivity, specificity, positive and negative predictive values of 80, 90, 94.1 and 69.2%, respectively. That difference in the sensitivity and specificity primarily confirmed that different response was attributed from different species and that the different techniques applied had varied responses[37] with the presence of cross-reactive epitopes[42].

The S and E-S Ags, showed nearly the same outcome as regards sensitivity and specificity. They both showed a cross-reaction with each other since they share common antigenic epitopes[43]. While, in a previous study the E-S Ag showed better performance as regards; sensitivity, accuracy, PPV and NPV in both dot ELISA and indirect ELISA when compared to crude Ag[14] attributed to their further purification and concentration in the processing of the Ag.

In the current study, T Ag revealed sensitivity, specificity, PPV and NPV of 100%. The tegument had always proved to be a valuable source of antigens. Many studies showed that T Ag from trematodes was highly species-specific and represent good molecules in immunodiagnosis[23]. Similar results were previously documented by Morales and Espino[30] on *F. gigantica* T Ag- ELISA who showed a sensitivity of 91.1%, a specificity of 97.3% and an accuracy of 95.0% and also Figueroa-Santiago *et al.*[8], who used *F. gigantica* T Ag- ELISA and showed a sensitivity of 100% and a specificity of 95%.

As regards the 16.5 KDa T subunit it showed a sensitivity of 53.3%, specificity of 75% with a diagnostic accuracy of 71.5%, PPV 72.2% and NPV of 69%. Sabry *et al.*[44] used a different electric elution unit in its separation from the E-S Ag and showed 95% sensitivity and 90% specificity. Again Caban-Hernandez *et al.*[24] demonstrated a sensitivity of 91.4%. The method used for purification of the desired subunit from the SDS gel electrophoresis could alter the sensitivity potential of the subunit; this could explain the difference in outcomes. Also, the presence of different levels of antibody titer in diseased serum samples which is usually associated with chronic infection may alter the results[45]. Attallah *et al.*[46]

supported the idea that immune complex formation in the samples may show false-negative ELISA results. The absence of the target subunit antigen in sera of infected individuals by ELISA may be due to undetectable levels of circulating antigen in these serum samples as a result of the immune complex formation with host antibodies that tend to decrease the potential rate of circulating antigen.

Diagnosis of *Fasciola* is challenging and though, there have been some improvement in the development of immuno-diagnostic tests, yet these tests still suffer from insufficiency in sensitivity or/and specificity[47].

## CONCLUSION

Results showed that *F. gigantica* TAg is superior to S, E-S and the 16.5 KDa T subunit in the diagnosis of human fascioliasis, it seems to remain the most promising antigen as regards sensitivity, specificity and diagnostic accuracy in conventional total IgG ELISA. This performance was followed by S and E-S Ags which showed a nearly equivocal result. Unexpectedly, there was a poor diagnostic outcome from the 16.5 KDa T subunit.

## SIGNIFICANCE STATEMENT

The ELISA was chosen to compare between the selected Ags. The E-S Ags have more contact with host immune system, thus, induces a more specific and stronger humoral immune response than somatic antigens. However, because of the high complexity of these antigenic extracts, cross-reactions with other parasites have been reported, diminishing the specificity of the used techniques. So, studies shifted to the T Ag which is a major interface between the parasite and the host in trials for a better performance. Then the purified subunits from crude antigens have been introduced as useful immunodiagnostic molecules for fascioliasis, one of them was the 16.5 KDa subunit, highlighted for its potential surpassing crude and T Ag in diagnosing *F. hepatica* and *F. gigantica*, yet not sufficiently experimented. Results highlighted the potential of T Ag over S, E-S and the purified 16.5 KDa T subunit; it seems to remain the most promising antigen as regards: sensitivity, specificity and diagnostic accuracy in total IgG ELISA. This performance was followed by S and E-S Ags which showed nearly equivocal results. Unexpectedly, there was a poor diagnostic outcome from the selected 16.5 KDa T subunit.

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