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Evaluation of the Exposure of Autoimmune Bullous Diseases Patients to Parasites

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Abstract

Introduction: Autoimmune blistering diseases are characterized by the presence of autantibodies. Based on clinical, histopathological, and immunopathological criteria; autoimmune bullous diseases are categorized into 4 fundamental groups: pemphigus and pemphigoid diseases, epidermolysis bullosa acquisita, and dermatitis herpetiformis.
Aim of the work: The purpose of this study is to evaluate the exposure of autoimmune bullous disease patients to three parasites; Schistosoma, Fasciola and Toxoplasma.
Patients and Methods: Forty-four autoimmune bullous disease patients and 45 controls included in this cross-sectional, case control study. Patients and controls were recruited from the dermatology out-patient clinic in Kasr El-Ainy, Cairo University. Five milliliter blood sample was withdrawn from each subject. The blood samples were centrifuged to extract the serum. Serum samples were tested on immunoglobulin G ELISAs of Schistosoma, Fasciola and Toxoplasma antigens.
Results: As regards *Toxoplasma* IgG, seventeen of patients were positive (38.6%), and only 4 of controls were positive (8.9%). There was a statistically significant difference between patients and controls (p value: 0.001) in immunoglobulin G antibodies against *Toxoplasma*.
There was no statistically significant difference between patients and controls as regards *Schistosoma* and *Fasciola* in immunoglobulin G antibodies.
Key words: Autoimmune Bullous Diseases- *Toxoplasma – Schistosoma – Fasciola*.

Introduction

Autoimmune blistering diseases are acquired persistent heterogeneous skin diseases with autoantibodies directed against structural proteins in the skin. It is categorized into 4 groups: pemphigus and pemphigoid diseases, epidermolysis bullosa acquisita, and dermatitis herpetiformis. The first group of diseases includes serious blistering disorders characterized by blister formation in the skin epidermis. The rest of these diseases are characterized by blister formation sub epidermally. Target antigens of auto antibodies have been recognized for almost all of the autoimmune blistering diseases **[1].**
Infectious agents are important ecological factors involved in the pathogenesis of autoimmune bullous diseases **[2, 3& 4].** The most important planned mechanisms for deviation of the immune

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response are the participation of molecular mimicry, where a foreign antigen shares sequence or structural similarities with self-antigens **[5]**.

Different parasites were related to autoimmune bullous diseases in different populations. Pemphigus vulgaris (PV) patients had significantly higher rates of seropositivity of IgG antibodies against *Strongyloides stercoralis* compared to the control group in Iran **[6].**

In Brazil, data acquired from many studies indicated a significant connection between pemphigus, known as Fogo Selvagem (FS), and contact to black flies **[7].** Accordingly, recent studies confirmed that anti-desmoglein 1 (Dsg1) antibodies are present in endemic areas of Brazilian pemphigus foliaceus, not only in pemphigus subjects but also in patients with parasitic diseases transmitted by hematophagous vectors as onchocerciasis (83%), Chagas disease (58%), and leishmaniasis (43%) **[8 & 9].**

In Tunis the zoonotic cutaneous leishmaniasis (ZCL) due to leishmania major is transmitted by a hematophagous vector *Phlebotomus papatasi* is more prevalent in rural areas where a higher incidence of endemic pemphigus has been also reported **[10].** Also, hydatidosis is endemic in rural areas where a higher incidence of Tunisian endemic pemphigus has also been reported **[11].**

In Egypt there is high rate of sero-prevalence of *Toxoplasma gondii (T. gondii*) which was 155 among 260 asymptomatic blood donors (59.6%) as determined by enzyme-linked immunosorbent assay (ELISA) **[12].**

Schistosomiasis is an endemic disease in Egypt**.** Its prevalence differs in different regions. For example, the prevalence of *S. mansoni* in five governorates in Lower Egypt; was 36.4%, while the prevalence of *S. haematobium* in four governorates in Upper Egypt; was 7.8% **[13].**

Fascioliasis is a growing problem in Egypt with a prevalence of 12.8% **[14].** The two most important species are *Fasciola hepatica* and *Fasciola gigantica* **[15 & 16].**

Patients and methods

This case control study was conducted on 44 patients (more than 18 years and having good general conditions) having autoimmune bullous diseases and 45 healthy volunteers as controls.

All subjects were exposed to:

1. Obtaining a written consent for participation in the study after detailed explanation of the study.
2. Complete history taking.
3. Collecting 3 ml venous blood samples for assessment of serum level of immunoglobulin G targeting Schistosoma, Fasciola and Toxoplasma antigens.

Three ml of venous blood were aseptically collected from each patient and control. Samples were dispensed in tubes and left to clot for 30 minutes at 37 ºC, then they were centrifuged at 1000 RPM for 15 minutes. The collected sera were finally stored at -20 ºC until analysis.

Three Enzyme linked immunosorbent assay (ELISA) kits were used for detection of *Toxoplasma gondii*, *Schistosoma* spp. and *Fasciola* spp.

1- *Toxoplasma* antibody detection:

All reagents were allowed to reach room temperature (18-25ºC) before use. The wash buffer was Diluted 1 volume of (20x) with 19 volumes of distilled water and was mixed well before use. All samples were diluted 1:40 adding 5 µl of the sample to 200 µl of Sample Diluent.

100 µl of diluted sera, calibrators, and controls were dispensed into the appropriate wells. For the reagent blank, 100 µl Sample Diluent was dispensed in 1A well position. Then the plate was Incubated at 37ºC for 30 minutes. At the end of incubation period, liquid was removed from all wells. The microtiter wells were rinsed and flicked 5 times with diluted Wash Buffer (1x).

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100 µl of Enzyme Conjugate was dispensed to each well and mixed gently for 10 seconds. Then the plate was incubated at 37ºC for 30 minutes. Enzyme Conjugate was removed from all wells by rinsing and flicking the microtiter wells 5 times with diluted Wash Buffer (1x). 100 µl of TMB Reagent was dispensed into each well and mixed gently for 10 seconds. Then the plate was incubated at 37ºC for 15 minutes.

100 µl of Stop Solution (1N HCl) was added to stop reaction and mixed gently for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely. And, there are no air bubbles in each well before reading. Optical density (OD) was read at 450 nm within 15 minutes with a microwell reader.

Calculation of the results:

1. The mean of duplicate calibrator 2 was calculated, calibrator value xc.
2. The mean of duplicate high control (xh), low control (xl) and samples (Xs) were calculated.
3. *Toxoplasma* IgG Index of each determination was calculated by dividing the mean values of each sample by the mean value of calibrator 2, xc.

Negative: *Toxo* G index less than 0.90 is negative for IgG antibody to *T. gondii.*

Equivocal: *Toxo* G index between 0.91- 0.99 is equivocal.

Positive: *Toxo* G index of 1.00 or greater is positive for IgG antibody to *T. gondii.*

2- *Schistosoma* antibody detection:

All reagents were allowed to reach room temperature (18-25ºC) before use.The content of the wash buffer bottle (25ml) was added to 475 ml distilled water. Diluted wash buffer was put into a squeeze bottle.

100 µl of Negative Control was added to well #1, 100 µl of Positive Control to well #2, and 100 µl of the diluted (1:40) test samples to the remaining wells.The plate was incubated at room temperature for 10 minutes. The contents were shacked out and washed 3 times with diluted wash buffer. 2 drops of Enzyme Conjugate were added to each well.The plate was incubated at room temperature for 10 minutes. The contents were shacked out and washed 3 times with diluted wash buffer.2 drops of Chromogen were added to every well.

The plate was incubated at room temperature for 5 minutes. 2 drops of Stop Solution were added to each well. O.D. was read at 450 nm with a reference filter at 620-650 within 15 minutes with a microwell reader.

Calculation of results:

The Wells were read using a bichromatic reading with filters at 450 nm and 620-650 nm.

Positive - Absorbance reading greater or equal to 0.2 OD units.

Negative - Absorbance reading less than 0.2 OD units.

3- *Fasciola* antibody detection:

All reagents were allowed to reach room temperature (18-25ºC) before use. The Content of the wash buffer bottle (25ml) was added to 475 ml distilled water. Diluted wash buffer was put into a squeeze bottle.

100 µl of Negative Control was added to well #1, 100 µl of Positive Control to well #2, and 100 µl of the diluted (1:100) test samples to the remaining wells. The plate was incubated at room temperature for 10 minutes.

The contents were shacked out and washed 3 times with diluted wash buffer.2 drops of Enzyme Conjugate were added to each well. The plate was incubated at room temperature for 5 minutes.

The contents were shacked out and washed 3 times with diluted wash buffer. The plate was slapped against paper towels to remove all the wash buffer.2 drops of Chromogen were added to

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every well.The plate was incubated at room temperature for 5 minutes.2 drops of Stop Solution were added to each well and mixed by tapping strip holder.
Wells were read by ELISA reader at 450/650 to 620 nm.
Positive - Absorbance reading greater than 0.1 OD units.
Negative - Absorbance reading less than 0.1 OD units.

Statistical Analysis Methodology:
Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples. For comparing categorical data, Chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

Results
The patients having autoimmune bullous diseases included 9 males (20.5%) and 35 females (79.5%), their ages ranged from 21 to 80 years with a mean of 48.1 years±14.7 (SD). Their disease duration ranged from one month to 15 years. Thirty-nine patients were pemphigus vulgaris, two patients were superficial pemphigus and 3 patients were ocular cicatricial pemphigoid (mucous membrane pemphigoid). Forty-one patients were receiving systemic steroids and 3 patients didn't start treatment yet.
The control group included 13 males (28.9%) and 32 females (71.1%), their ages ranged from 21 to 77 years with a mean of 41.33years ± 16.96 (SD).

IgG antibodies against *Toxoplasma* in patients and controls:

As regards *Toxoplasma* IgG, seventeen of patients were positive (38.6%), twenty-five were negative (56.8%) and only 2 patients were equivocal (4.5%). Four of controls were positive (8.9%) and 41 were negative (91.1%). There was a statistically significant difference between patients and controls (p value: 0.001).

Out of 17 positive patients one patient was superficial pemphigus, one patient was ocular cicaticial pemphigoid (OCP) and 15 patients were pemphigus vulgaris (PV). (Table 1)

Table 1: IgG antibodies against *Toxoplasma* in patients and controls

	Patients	Controls	P value
Positive *Toxoplasma* IgG	17	4	0.001
	38.6%	8.9%	

IgG antibodies against *Schistosoma* in patients and controls:
As regards *Schistosoma* IgG, thirty-seven of patients were positive (84.1%) and 7 patients were negative (15.9%). Thirty-nine of controls were positive (86.7%) and 6 controls were negative

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(13.3%). There was no statistically significant difference between patients and controls (p value: 0.731).

Out of 37 positive patients 2 patients were superficial pemphigus, three patients were ocular cicaticial pemphigoid and 32 patients were pemphigus vulgaris.

Table 2: IgG antibodies against *Schistosoma* in patients and controls

	Patients	Controls	P value
Positive *Schistosoma* IgG	37	39	0.731
	84.1%	86.7%	

<u>IgG antibodies against *Fasciola* in patients and controls:</u>

As regards *Fasciola* IgG, two of patients were positive (4.5%) and 42 patients were negative (95.5%). None of controls were positive (0.0%) and 45 controls were negative (100%). There was no statistically significant difference between patients and controls (p value: 0.242).
The 2 positive patients were pemphigus vulgaris. (Table 3)

Table 3: IgG antibodies against *Fasciola* in patients and controls

	Patients	Controls	P value
Positive *Fasciola* IgG	2	0	0.242
	4.5%	0.0%	

Interestingly none of the patients possessed autoantibodies against the 3 parasites. Eighteen patients possessed autoantibodies against the 2 parasites. Sixteen patients possessed autoantibodies against *Toxoplasma* and *Schistosoma*. Two patients possessed autoantibodies against *Fasciola* and *Schistosoma*.

Discussion

The pathogenesis of autoimmune blistering diseases is not fully understood. The mechanisms that break the tolerance and trigger those autoimmune bullous diseases have been thoroughly studied in different populations [1].
Although host genetic heritage contributes to induction of immune reaction to self, epidemiological and molecular evidence implicates infectious agents (viral and bacterial) as the principal environmental insults responsible for the induction of autoimmune diseases **[17].**
There are a variety of mechanisms including molecular mimicry, bystander activation, exposure of cryptic antigens, and superantigens by which pathogens can aid in the expression of an autoimmune disease. Several environmental factors such as wasp, bee and spider stings have been associated with pemphigus. Few investigators tried to relate parasites and pemphigus **[18].**
Toxoplasma gondii contributes to the pathogenesis of many autoimmune diseases **[19].** Our results showed that seventeen of patients were positive (38.6%) and four of controls were positive (8.9%) for IgG against toxoplasma. There was a statistically significant difference between patients and controls regarding exposure to *Toxopasma gondii*. This suggests that *Toxoplasma gondii* might contribute to the pathogenesis of autoimmune bullous diseases in the Egyptian population. Our results agree with the results of ***Sagi et al.*** who showed that the

prevalence of IgG antibodies against *Toxoplasma gondii* in autoimmune bullous diseases was 65% which was significantly higher than the controls (40%) **[20].** Our results contradict the results of ***Mortazavi et al.*** who reported that in Iran, there was no significant difference between patients and controls in IgG antibodies against *Toxoplasma gondii* (28% in patients, 27.5% in controls) **[6].**

Since the exposure of the autoimmune bullous disease patients to *Toxoplasma gondii* was higher than the controls thus it is better to advise the patients to ensure that food is thoroughly cooked and ensure that food is handled hygienically **[21].**

Schistosomiasis is the third most devastating tropical disease globally **[22]**. Acute Schistosoma infection is associated with T helper-1 response while chronic infection is associated with T helper-2 response **[23].** Our results showed that 37 (84.1%) of patients and 39 (86.7%) of the controls had IgG against *Schistosoma.* There was no statistically significant difference between patients and controls. Immunoblot is needed to confirm these results.

Two (4.5%) of patients and none of controls had IgG against *Fasciola*. This suggests that *Fasciola* might not contribute to the pathogenesis of autoimmune bullous diseases in the Egyptian population. On the other hand, it was reported that the lower incidence of autoimmune diseases in developing countries was associated with higher prevalence of parasites. It was suggested that parasites immunosuppress pathogenic T cells that mediate autoimmune diseases through production of interferon gamma and interleukin 17 **[24].** Thus, one can postulate that less exposure of the autoimmune bullous disease patients to the *Fasciola* might contribute to the pathogenesis of the autoimmune bullous diseases.

Limitations of this study include that we did not correlate the IgG antibodies against *Toxoplasma, Schistosoma* and *Fasciola* to the disease related autoantibodies of the patients (example: anti-desmogleins, anti-bullous pemphigoid), we did not measure the time course of the IgG antibodies against toxoplasma, Schistosoma and Fasciola to detect whether the titre is rising or not.

Conclusion:

In conclusion there is association between autoimmune bullous disease and *Toxoplasma* in the Egyptian population which suggests that *Toxoplasma* may contribute to the pathogenesis of these diseases in the Egyptian population. There is no association between neither Schistosoma nor fasciola and autoimmune bullous diseases.

Declarations:

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Conflict of Interest: The authors declare that they have no competing interests.
Availability of data and material: All data and specimens used are available.
Code availability: Not applicable.
Author contribution: All manuscript authors contributed to every activity of it; idea of paper, study design, collection of materials, methodology, writing the paper and revising it.
Ethical approval: The research was approved from the Scientific Research Ethical Committee, Faculty of Medicine Cairo University.
Consent to participate: All procedures were explained to patients and informed consents were obtained.
Ethical statement: The manuscript is not submitted in any other journal. Work is original and haven't been published elsewhere in any form or language. It's a single study and not splitted into several parts

to increase quantity of submission. Results are presented clearly, honestly and without fabrication. No data, text, or theories are presented as if they were the author's own

The entire manuscript is an unpublished original article.

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