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EVIDENCE OF ANTI-DOUBLE STRANDED DNA ANTIBODIES AND C-REACTIVE PROTEIN IN EGYPTIAN PATIENTS WITH HIGH ANTI-*SCHISTOSOMA MANSONI* ANTIBODY TITER

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Abstract

Schistosomiasis is one of the major public health diseases that induced a diversity of immunologic reactions. Significant association between autoreactive antibodies and autoimmune disorders was suggested. This study investigated the presence of anti-double stranded DNA (anti-ds DNA) antibodies and inflammatory biomarker, C-reactive protein (CRP) in 89 Egyptian patients, with *Schistosoma mansoni* clinical evidence and high anti-*Schistosoma* antibody titer. The results showed positive serum anti-ds DNA antibodies in 11/89(12.4%) of them, without significance to age, sex or anti-*Schistosoma* antibody titers. CRP was positive in 20/89(22.5%) of patients, with significant correlation with anti-ds DNA antibodies (P=0.000).

Keywords: Egypt, *Schistosoma mansoni*-anti-ds DNA- CRP- autoimmune disease.

Introduction

The schistosomiasis affected worldwide population for all forms was 230 million, with an estimated risk of 700 million due to *Schistosoma japonicum, S. haematobium, & S. mansoni* (Lackey and Horrall, 2021). In Egypt, schistosomiasis *haematobium* and *mansoni* a snail-transmitted infections were still endemic (Abou-El-Naga, 2018).

A recent epidemiological survey revealed that urinary Schistosomiasis is still prevailing among village residents in the basin of the White Nile River (Lee *et al,* 2019). Also, Mulero *et al.* (2019) reported the preadaptation and establishment of schistosomiasis in several European Countries depended on the presence of locally adapted lineages of the snail host in these temperate countries.

Autoimmune and allergy occurred when an individual mounts an inappropriate immune response to a self-antigen or an innocuous environmental antigen (Smith and Peakman, 2018). Several factors, e.g. genetic and environmental triggers (particularly, viruses, bacteria, parasites and other infectious pathogens) played a role in autoimmune diseases development (Seyyed *et al,* 2017).

The chemical messenger cross talk occurred between certain parasites as (schistosomiasis) and their specific hosts, including the human ones (Stefano and Kream, 2007).

In Egypt, Abdel Wahab *et al.* (1989) found that concomitant infection of Swiss Albino mice with *Toxoplasma gondii* and *Leishmania major* showed clinical and histopathological pictures differed in concomitant infection from that given by infection with either parasite alone. Hammouda *et al.* (1994) reported that *Toxoplasma gondii* caused specific humoral and cellular immunosuppression to *Schistosoma mansoni* infection.

Nowadays, organ transplantation is becoming an increasingly routine to treat diverse populations, but the danger of transplantation-mediated parasites, exacerbated by coincident immunosuppression, must be considered (Li *et al,* 2020). Besides, some parasi-tes (*Cryptosporidium, Cyclospora, Entamoeba histolytica, Toxoplasma* and *Giardia*) acquired from the donor allograft, from reactivation, or from de novo acquisition post-transplantation (Cooper *et al,* 2017). Carrai *et al.* (2018) in the United Kingdom reported a case of post-transplant liver graft infection with *Schistosoma* spp. in a migrant from the Sub-Saharan Africa transplanted for HBV-related cirrhosis and with undiagnosed schistosomiasis pre-transplantation.

However, it was proposed that infections might by guard from the autoimmune disorders or even nullifies an on-going autoimmune process reliant on interplay between

pathogenic agents and host as immune system modulatory agents (Rook, 2012). Therefore, infectious agents can be considered. On the other hand, several evidences have supported the higher vulnerability of autoimmune subjects to infections, perhaps as a consequence of the immunosuppressive therapies (Sfriso *et al,* 2010).

Butcher (1996) speculated that systemic lupus erythematosus (SLE) and sarcoidosis were not prevalent in Africans residents in West Africa; nevertheless their incidence is higher in white people descending from USA and UK. Interestingly, malaria precluded these autoimmune disorders in West Africa by influencing macrophage functions (Fox, 1996). Jones (1977) reported high serum levels of anti-ssDNA antibodies in rabbits either in intense and light exposures to schistosomal infections soon after the onset of ova-deposition by female worms. Rahima *et al.* (1994) reported the high anti-nuclear (ANA) antibodies in acute infected mice with *schistosoma,* associated with the elevated anti-*Schistosoma* antibodies titers in patients with SLE.

Swaak and Smeenk (1985) and Förger *et al.* (2004) reported that anti-dsDNA antibodies in particular could be utilized as a prognostic tool and the diagnostic biomarker for the SLE. Alba *et al.* (2003) reported anti-dsDNA as significant markers in patients with lupus nephritis. The anti-dsDNA population was produced against deoxyribonucleoprotein (DNP) during disease development.

C-reactive protein (CRP) is an effective inflammatory biomarker compared with erythrocyte sedimentation rate (Osei-Bimpong *et al*, 2007). C-reactive protein plays an essential role during the inflammatory mechanism being involved in the innate immunity by attaching the pathogenic agents, disrupting their cellular composition, and activating the complement system and phagocytosis process (Reeves, 2007). CRP was an acute phase reaction indicator by reacted quickly to inflammation, whether due to an infection or autoimmune disorder (Feldman *et al*, 2013).

The present study aimed to evaluate the serum anti-dsDNA autoantibodies population and CRP in Egyptian patients clinically evidenced with *Schistosoma mansoni* infection and developed high anti-*Schistosoma* antibody titers.

Materials and Methods

All procedures fulfilled the ethical standards declared by Helsinki 1964, and accepted by Cairo University. The study aim was explained to patients, with informed consent, before data and sera collections.

Medical sheets were filled out on each participant including demographic data, medical history, residence, profession, and clinical history. A total of 89 patients with manifestations suggestive schistosomaisis attended the Internal Medicine Clinics; Cairo University Hospitals from January 2017 to December 2020 were included. Exclusion criteria included pregnant women and children as well as patients on chemotherapy or isoniazid, procainamide, hydralazine, or anti-convulsing drugs. Also, patients with periarteritis nodosa, rheumatoid arthritis, scleroderma, or chronic hepatitis were excluded.

The IHAT was performed to show exposure to *Schistosoma* antigens; only patients with high anti-*Schistosoma* antibody titer (≥320) were involved in the current study.

Quantification of anti-*Schistosoma* antibody levels: Using serum samples, anti-*Schistosoma* antibody was quantified by using IHAT as given by the manufacturers' instructions (Bilharziosis Fumouze®, # 514000, Allée d′Athènes, France). For each patient, 1:40 stock dilution of the tested sera was prepared where 50µl was delivered in sterile tube and mixed promptly with 1.95mL of phosphate buffer saline (PBS). Test execution on microplate involved delivery of 50µl PBS in 8 consecutive wells of the microplate. Then 50µl of the serum stock dilution was added in the 1st well and mixed well. Then 50µl was delivered to the 2nd well, from the 2nd well to the third and so on till the last well. Positive and negative controls were tested prior to the whole procedure.

Characterization of serum anti-dsDNA autoantibody populations and CRP: Latex test was used for the qualitative evaluation of anti-dsDNA autoantibody and CRP**.** A drop of 50µl of patient undiluted serum was put on to a circle of a test slide. A drop of latex reagent was mixed gently with sera over the test circle entire area. Slides were gently tilted for two minutes at regular interval. Any visible agglutination was regarded as positive. Test was interpreted as negative when no difference is detected between the tested sera and the negative control. In CRP, agglutination gave a sera level ≥ 6mg/L, but in dsDNA autoantibodies test gave a titer ≥200.

Statistical analysis: Data was analyzed using the package SPSS version 25. Data were expressed as mean, standard deviation and range. Chi-square test ($\chi 2$) compared between groups and differences were significant at $P<0.005$.

Results

Positive cases were 71/89(79.8%) &18/ 89 (19.2%) males & females respectively. Antibody levels showed titers of 320, 1280 & 2560 in 29.2%, 30.3% & 40.4% of patients, respectively, without significant association between antibodies levels and patients, sex or age. Serum anti-ds DNA antibodies were positive in 11/89 (12.4%) of the infected patients. Positive rates in male and female patients were 10/71(14%) & 1/17(5%) respectively, without significant difference between them, and in different positive anti-ds DNA antibodies titers.

The inflammatory biomarker CRP was positive in 22.5% (20/89) of all sera. Positive CRP was revealed in 63.3% (7/11) of patients with positive anti-DNA antibodies. There was statistical significant correlation between CRP and anti-ds DNA, (P=0.000) as positive CRP was revealed in 63.3% (7/11) of patients with positive anti-DNA antibodies, whereas it was negative in 16.6% (13/78) of patients with negative anti-DNA antibodies (Tab. 1).

Table 1: Relation of Anti DNA antibodies to sex and CRP of study population

Anti- dsDNA	Sex (n=89)		CRP (n=89)	
	Male	Female	Positive	Negative
Positive	10	1	7	4
Negative	61	17	13	65
Total	71	18	20	69
P value	0.3		0.000*	

*Statistically significant

Discussion

Autoreactive antibodies were previously recorded in human and experimental studies conducted on schistosomal infection (Osada *et al, 2015*). Autoantibodies identified were rheumatoid factor, anti-DNA, anti-lymphocyte, anti-cardiolipin and anti-collagen antibodies (Rahima *et al,* 1994).

An autoimmune disorder can be triggered by infectious agents, which can also determine its clinical presentations. Most pathogens, such as bacteria, viruses, and parasites, can induce autoimmunity through various mechanisms (Kivity *et al,* 2009). The autoimmune diseases have been associated with circulating auto-reactive antibodies, and their detection in serum has been suggested to have high predictive value as they can develop earlier than the clinical illness (Scofield, 2004; Youinou *et al*, 2010). They play a vital role in the diagnosis as well as classification of auto-immune disorders. However, their functions in the biological process remains unclear (Scofield, 2004; Lleo *et al,* 2010).

In the present work, sera of patients with *S. mansoni* infection were screened for the presence of anti-ds DNA autoantibodies. Besides, the study investigated the association between the autoreactive antibodies and the inflammatory biomarker CRP in those patients. The serum anti-ds DNA antibodies were detected in 12.4% of the patients. A positive association between schistosomiasis and auto antibodies levels was previously reported. Rahima *et al. (*1994) experimentally found that circulating antinuclear antibodies detected in acute infected mice with *S.*

mansoni. They added that schistosomiasis can trigger SLE, considering suitable hormonal and immunogenic factors. Also, Osada *et al*. (2015) found elevated serum anti-dsDNA anti-bodies & IgG rheumatoid factor in experimental schistosomiasis with high autoreactive antibodies that diminished severity of spontaneous autoimmune arthritis. Hence, the authors suggested the reciprocal influence of intestinal schistosomiasis infection on autoimmune arthritis in animals. Elevated levels of autoreactive antibodies were reported in *S. japonicum* and *S. haematobium (*Arinola, 2002; Youinou *et al,* 2010*).*

The possible explanation suggested for the association between infections and autoreactive antibodies is the molecular mimicry hypothesis, i.e. antigenic determinants of pathogens may resemble those of their host, and so induce auto-immune reactions that harm the host (Blank *et al,* 2007; Mutapi *et al*, 2011).

Besides, an inverse correlation between ANA levels and schistosomiasis was recorded (Mutapi *et al*, 2011; Chimponda and Mduluza, 2020). Moreover, other studies reported that infections with helminthes including *S. mansoni,* can prevent autoimmune diseases such as, multiple sclerosis, type 1 diabetes and inflammatory bowel disease (Cooke *et al*, 1999; La Flamme *et al,* 2003; Smith *et al*, 2007; Cleenewerk *et al*, 2020). This was explained by the so-called 'hygiene hypothesis' which propose that low exposure to pathogens led to more reactive immune system, which can result in autoimmune reactivity (Yazdanb-akhsh and Matricardi, 2003; Briggs *et al,* 2015).

In the present study, the results showed that 22.5% of the screened *Schistosoma* serum samples showed positive CRP.

In fact, inflammatory biomarkers such as CRP have been used to detect acute inflammation, which can be indicative of a particular disease or possibly predictive of a disease outcome (Watson *et al,* 2012; Chimponda *et al,* 2019). Previous studies on schistosomiasis have shown a positive correlation between CRP and *Schistosome* infection (Coutinho *et al,* 2006; Garza, 2010; Amin *et al*, 2019). Also, schistosomiasis showed positive relationship between other serum inflammatory biomarkers and autoreactive antibodies, as Chitinase 3-Like 1 protein (YKL-40-25) and IL17 (Wang *et al,* 2018). However, Sinkala *et al.* (2016) did not find difference in CRP levels between schistosomiasis patients and healthy control.

The present study showed significant association between CRP and anti-DNA antibody response (P=0.000), as positive CRP was in 63.3% (7/11) of patients with positive anti-DNA antibodies, whereas it was negative in 16.6% (13/78) of patients with negative anti-DNA antibodies. This agreed with Chimponda *et al.* (2019) who reported that CRP showed no significant difference with infection intensity. But, they indicated that CRP in schistosomiasis might be used as potential biomarker to identify inflammatory environment rather than diagnosis. Besides, Pêgo *et al.* (2019) found that *S. mansoni* co-infection attenuated *T. gondii*-induced ileitis by preserving mucosal integrity and down-regulating the local inflammatory response based on the activation of NF-kappa B and MAPK. They added that protective function of prior *S. mansoni* infection caused involvement of innate immune mechanisms and supported a new approach to treat the chronic inflammatory diseases, including CD.

Conclusion

Sera autoreactive antibodies and CRP in schistosomiasis proved to be valuable marker in patient diagnosis with future autoimmune or inflammatory conditions.

None correlated between autoreactive antibodies and CRP in *S. mansoni* infection so far. The anti-ds DNA, and CRP positivity were in high anti-*S. mansoni* antibodies patients denoted potential inflammatory or autoimmune disorder, as useful clinical marker

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