Article/Artigo

Immunohistochemical expression of oestrogen and progesterone receptors during experimental acute and chronic murine Schistosomiasis mansoni

Expressão imunohistoquímica de receptores para estrogênio e progesterona nas fases aguda e crônica da esquistossomose mansônica experimental em camundongos

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ABSTRACT

Introduction: The responsibility of *Schistosoma mansoni* in female infertility is still controversial. This study was conducted to evaluate the effect of acute and chronic schistosomiasis mansoni infection on the endometrium using immunohistochemical analysis of uterine hormone receptor expression. **Methods**: Twenty-four nonpregnant swiss albino mice were divided into three groups: control, noninfected; acute; and chronic *Schistosoma mansoni* infection. Histological sections of uterine specimens were examined by light microscope with an image analyzing system to detect structural histological, estrogen receptor (ER) and progesterone receptor (PR) expression in the endometrium. **Results**: No secretory phase was detected in the endometrium in acute and chronic *Schistosoma* infection. Hormone receptor expression (ER and PR) showed statistically significant differences among the groups ($p< 0.05$), with significant low ER hormone expression in chronic infection, compared to control proliferative, control secretory and acute infection cases, and statistically significant high PR expression in both acute and chronic infection cases compared to the control secretory cases ($p< 0.05$). **Conclusions**: *Schistosomiasis mansoni* seems to have an important impact on the hormone expression of affected women. Further studies to explore the mechanism of such changes are recommended.

Key-words: *Schistosoma mansoni.* Endometrium. Hormone receptors. Oestrogen. Progesterone-immunohistochemistry.

RESUMO

Introdução: A responsabilidade do *Schistosoma mansoni* em esterilidade feminina é ainda controversa. Este estudo é conduzido para avaliar o efeito da esquistossomose mansoni aguda e crônica no endométrio usando análise de imuno-histoquímica da expressão de receptor hormonal uterina. **Métodos**: Vinte e quatro camundongos fêmeas albinas suíças não grávidas foram divididas em 3 grupos (controle não-infectado, grupos agudos e crônicos infeccionados com *Schistosoma mansoni*). As seções histológicas de espécimes uterinos foram examinadas por microscópio leve com imagem, analisando sistema para detectar no endométrio expressões histológicas estruturais, receptor de estrogênio (ER) e receptor de progesterona (PR). **Resultados**: Nenhuma fase secretora foi detectada no endométrio com infecção aguda e crônica de *Schistosoma*. A expressão hormonal de receptor (ER e PR) mostrou diferenças estatisticamente significantes entre grupos diferentes ($p<0,05$) com baixa significativa hormonal de ER com infecção crônica (comparado com controle proliferativo, controle secretório e casos agudos de infecção) e alta expressão de receptor de PR estatisticamente significativa em casos tanto agudos e crônicos de infecção como comparado com os casos de controle secretório ($P <0,05$). **Conclusões**: A esquistossomose mansoni parece ter um maior impacto em expressão hormonal das mulheres afetadas. Mais estudos para explorar o mecanismo de tais mudanças são recomendados.

Palavras-chaves: *Schistosoma mansoni.* Endométrio. Receptores hormonais. Oestrogen. Progesterona-imuno-histoquímica

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INTRODUCTION

Schistosomiasis is one of the most serious parasitic diseases. More than 250 million people are infected with schistosomes in tropical or subtropical regions[1] and about 40 million women of child-bearing age are infected[2]. The parasitic flukes have some unique biological features; complex life cycles, mechanisms to avoid host immune responses and an apparent reliance on host endocrine and immune signals to complete their development, maturation and egg production[1].

Despite the complex pathophysiological reactions during schistosomiasis, female genital infection is an almost entirely neglected disease entity that may give rise to considerable suffering among women in areas where schistosomiasis is prevalent. Several disorders in the female genital system due to both *Schistosoma haematobium* and *mansoni* have been reported, such as complications during pregnancy and related outcomes, menstrual disorders and other problems related to the female genital system[2-4]. Although infertility is determined to be the result of *Schistosoma* infection[5-7], the responsibility of schistosomiasis in female infertility is controversial.

Little is known regarding the hormonal changes associated with such infection in women. Parasites can alter hormone concentrations in their hosts, including estrogen and progesterone. Therefore, studies concerning host hormones responses to infection and the extent to which changes occur in endocrine-immune interactions following infection, mediated by the host or the parasite, are still recommended[8].

Estrogen and progesterone receptors are members of the steroid receptor super family that binds the corresponding hormones that are expressed in the uterus, ovary, vagina and fallopian tubes in the female genital system. These receptors play a central role in women's fertility cycles and

diverse reproductive events associated with the establishment and maintenance of pregnancy[8].

The objective of this work was to study the effect of acute and chronic *Schistosoma mansoni* infection on the mouse endometrium through immunohistochemical analysis of uterine hormone receptor expression.

METHODS

Experimental animals infection

Swiss albino nonpregnant female mice weighing 20-25 grams were housed in breeding cages, with a 12-hr light/dark cycle and free access to food and water. The mice were divided into 3 groups, each consisting of 8 mice: 1) control noninfected; 2) acute infection by *S. mansoni*; and 3) chronic infection by *S. mansoni*. For infection, the mice received a dose of 70-80 *S. mansoni* cercariae by tail immersion. Cercariae were obtained from the Tudor Bilharz Research Institute, Cairo, Egypt. For acute infection, the mice were maintained for 8 weeks, whereas for chronic infection, the mice were maintained for 16 weeks before sacrification[9].

Light microscopy and Immunohistochemical examination

At the expected date, the mice were sacrificed by cervical dislocation and dissected. The uteri were extracted and fixed in 10% buffered formalin and prepared for embedding in paraffin blocks. Preparations for structural histological examination were performed by cutting serial sections and staining with hematoxylin and eosin[10]. Tissue blocks were cut into 6µm thick sections, deparaffinised and rehydrated. Epitope retrieval was performed using the Tris-EDTA buffer epitope retrieval method. To reduce nonspecific background staining due to endogenous peroxidase, the slides were incubated in hydrogen peroxide block for 10-15 min. They were then washed twice in a buffer. Anti-ER and anti-PR antibodies (mouse anti-human estrogen and progesterone, Lab Vision Corporation, USA), were applied and incubated overnight in a humidity chamber. Streptavidin peroxidase was applied and incubated for 10 min at room temperature. Biotinylated goat anti-polyvalent was then applied and incubated for 10 min at room temperature. After each step the sections were washed four times in phosphate-buffered saline. One or two drops of DAB Chromagen were added to 1ml of DAB substrate, mixed by swirling and applied to the tissues, which were then incubated for 5-15 min, depending on the desired stain intensity. Lastly, the slides were counterstained with Mayer's haematoxylin and cover-slipped using a permanent mounting media[11]. Estrogen and progesterone receptor expressions were evaluated as the percentage of positive nuclei in epithelial cells of the observed field in control, acute and chronic schistosomiasis specimens, observed at high magnification (400x). Cases were classified as ER and PR positive when more than 10% of cells exhibited positive nuclear epithelial staining. The mean of three fields were chosen from each slide, so as to best reflect the overall immunostaining of the hormone receptors contained on the entire slide. The two observers who examined the slides were blinded regarding the clinicopathological data[12].

Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Turkey's HSD (honestly significant difference) pair wise comparisons. The level of significance was set at $p < 0.05$ throughout the study. Current SPSS (version 13) statistical package was used in all statistical analysis. For each hormone receptor expression, Paired-Samples t test was used to detect significant differences between different groups[13].

RESULTS

Sections of control mice uterus showed either the proliferative or the secretory phases of endometrium. The proliferative endometrium was relatively thin, consisting of stratum basalis and stratum functionalis (stratum spongiosum and stratum compactum). The glandular epithelium exhibited basally located nuclei and prominent nucleoli, while the stroma was highly cellular and devoid of collagen fibers. Some sections showed the convoluted tubular glands of the late proliferative phase with the pseudo stratified appearance. The secretory endometrium showed irregular configuration of the glands that were lined by tall columnar epithelium with basal vacuolation. The glands of the late seretory phase appeared tortuous, coiled and contained copious thick glycogen secretion. The stroma appeared highly vascular with the interstitial fluid accumulated between the cells **(Figure 1, A and B)**.

After acute and chronic *S. mansoni* infection, although no granulomas were observed, the endometrium showed only the late proliferative phase with the highly cellular stroma and the convoluted tubules that showed no secretion in their lumina **(Figure 1, C and D)**. Nuclear epithelial expression for ERs showed negative reaction in all the groups studied, whereas PR expression was negative only in the control secretory group and positive in all the other groups **(Figure 2, 3)**.

Hormone receptor expression (ER and PR) showed statistically significant differences among the groups ($p < 0.05$) **(Table 1)**. Statistically significant low ER hormone expression occurred in chronic infection cases compared to that of control

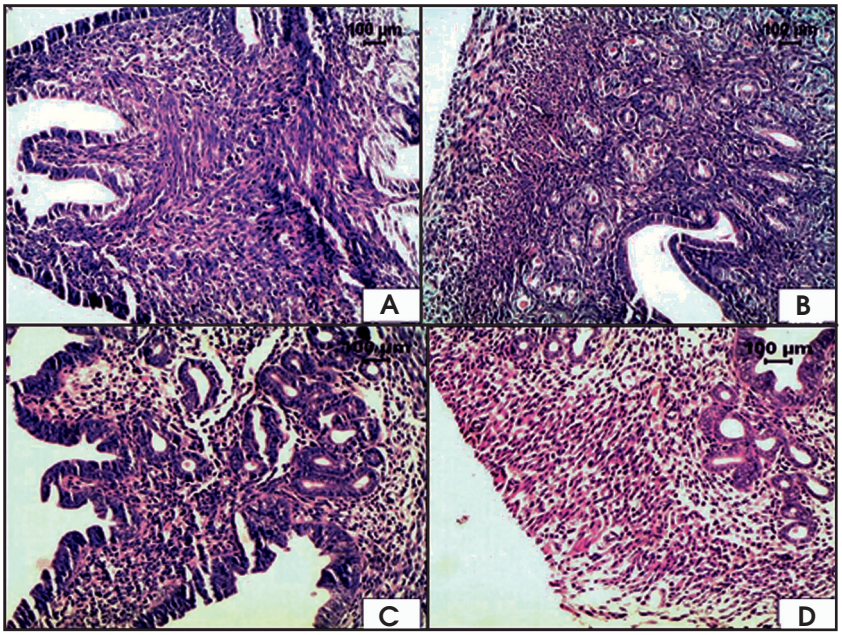

FIGURE 1 - Photographs of histopathological sections from the uterus of control and infected groups: A) Proliferative endometrium with highly cellular stroma, some convoluted tubular glands of the late proliferative phase can be observed, B) The secretory endometrium with highly vascular stroma and coiled irregular glands that contain glycogen secretion, C) Acute and D) Chronic *Schistosoma* infection showing the late proliferative endometrium with the highly cellular stroma and the nonsecretory convoluted tubules (HE, 200x).

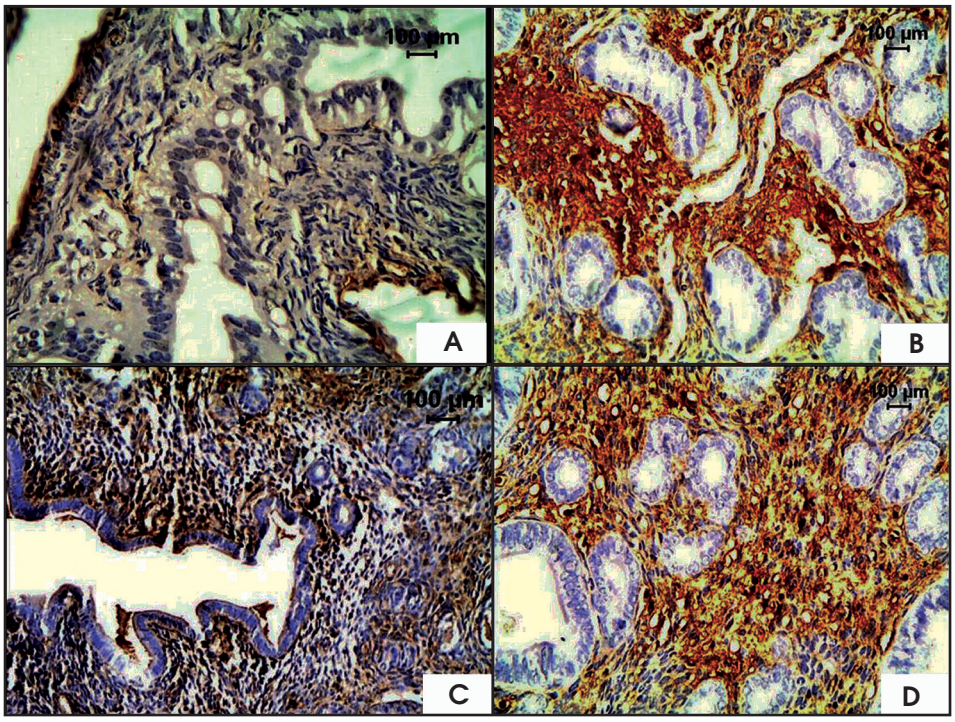

FIGURE 2 - Photographs showing estrogen receptor expression by the nuclei of the uterine epithelial cells in all the groups studied: A) Control proliferative, B) Control secretory, C) Acute *Schistosoma* infection and D) Chronic *Schistosoma* infection.

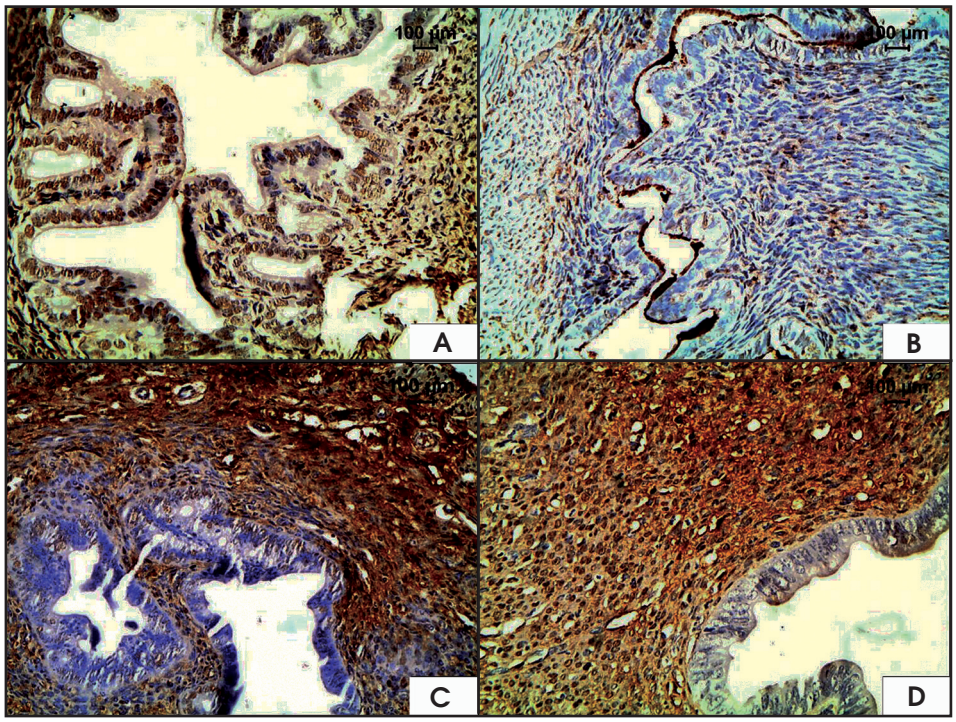

FIGURE 3 - Photographs showing progesterone receptor expression by the nuclei of the epithelial cells in all the groups studied: A) Control proliferative, B) Control secretory, C) Acute *Schistosoma* infection, and D) Chronic *Schistosoma* infection.

TABLE 1 - Hormonal receptor expression* in control, acute, and chronic schistosomiasis using anti- estrogen (ER) and anti-progesterone (PR) antibodies.

	Control proliferative (n=6)	Control secretory (n=6)	Acute schistosomiasis (n=8)	Chronic schistosomiasis (n=8)	Significance
ER receptors	3.98 ± 0.28	4.37 ± 0.34	4.32 ± 0.56	0.09 ± 0.05	0.00
PR receptors	84.95 ± 2.84	7.89 ± 0.44	83.61 ± 1.51	84.63 ± 2.44	0.00

*Values are presented as means ± standard error of mean. **ER:** estrogen receptor, **PR:** progesterone receptor.

proliferative, control secretory and acute infection cases (P= 0.000). No statistically significant differences were was observed between acute infection and any of the control cases (**Table 2**). In contrast, statistically significant higher PR expression occurred in both acute and chronic infection cases compared to the control secretory cases (P= 0.000) (**Table 2**).

TABLE 2 - Statistical analysis of hormonal receptor expression in control, acute, and chronic shistosomiasis using Paired-Samples t test.

Pairs	ER (t- test)	PR (t- test)
Control proliferative - acute infection	0.817	0.765
Control proliferative-chronic infection	0.000*	0.361
Acute infection - chronic infection	0.000*	0.715
Control secretory- acute infection	0.718	0.000*
Control secretory-chronic infection	0.000*	0.000*

*significant at p< 0.05

ER: estrogen receptor, **PR:** progesterone receptor.

DISCUSSION

Schistosomiasis is a serious and highly prevalent helminthic infection related to water contact and poverty and affects approximately 250 million people living in tropical and subtropical areas[14]. The number of women with schistosomiasis-related signs and symptoms is likely to be high and could easily involve millions of cases. Numerous published case reports have presented clinical features, often described as unusual, of genital schistosomiasis in female patients. Yet, it is surprising how scarce and unsatisfactory the literature is on this subject[15]. Incidental schistosomiasis of various lesions has been cited in the literature due to both *S. mansoni* and *haematobium*; however, insufficient data is available regarding the clinical picture of genital schistosomiasis. Systematic research concerning comorbidities caused by genital involvement during the course of *Schistosoma* infection is currently sketchy and research on the possible impact of schistosomiasis on different genital problems is still unsatisfactory[15].

The genital system is usually affected by urinary schistosomiasis caused by haematobium species, with complications including ectopic pregnancies or infertility[16-18]. Concurrently, genital problems due to *S. mansoni* have also been reported in the form of atrophy of the corpus luteum cells and nuclear alterations of the interstitial cells in the ovaries of mice[19]. Farah et al[20] reported *S. mansoni* to be a contributing factor in diminished fecundity among women in endemic regions. Numerous authors have emphasized the relation between parasitic and hormonal status in female mice, concluding that not only can host hormones affect responses to infection, but parasites can have pronounced effects on hormone signaling within the host[8]. Moreover, after studying endocrine-immune interactions during parasitic infection, Klein et al[8] confirmed the importance of the relation between the endocrine system and parasitic infection. The lack of data concerning hormonal changes associated with *Schistosoma* infection prompted our group to study the effect of *S. mansoni* on the hormonal profile of endometrium using immunohistochemical technique.

In the present study, although no direct evidence of *S. mansoni* infection was detected (no granulomas were observed), alterations in uterine hormone receptor expression were observed. Hormone receptors in the endometrial epithelial cells showed statistically significant higher PR expression in both acute and chronic infection cases as compared to the control secretory cases. This corroborated the histopathological results of the present study, where no secretory glands were observed in the endometrium in either acute or chronic cases, and reflected the expected low progesterone level in both cases.

Regarding ER expression, chronic cases showed statistically significant low ER hormone expression compared to that of both control proliferative and secretory cases, whereas no significant changes occurred in ER expression in acute infection cases compared to any of the control cases. This also coincides with the detection of late proliferative endometrium, which was observed in this study, and reflects the expected high estrogen level in chronic infection cases. These results explore the negative impact of *S. mansoni* on the female genital system, even in the absence of direct lesional infectious stages. Such observations might explain the infertility that has been attributed to Schistosomiasis infection by some authors[5, 20].

Hormonal alteration is also recorded in males during *S. mansoni* infection, since it suppresses testosterone production in male mice[21]. Alterations in hormone receptors in the present study might be due to the toxic effect of such hemoparasites on female hormones. These results are in agreement with those obtained by Klein et al[8], who reported increased concentration of serum oestradiol and progesterone levels and serious complications in genital system during hemoparasite *Plasmodium* infection. To our knowledge, no data exists concerning the findings described in the present work and this study seems to be the first report regarding uterine estrogen and progesterone hormone receptors in cases of *Shistosoma mansoni* infection.

From the data obtained, it is clear that genital infection by schistosomiasis worsens the disease burden of women in the child bearing period. Pathological consequences can be damaging for the affected women. A lack of clinical awareness of genital schistosomiasis can lead to misdiagnosis and therefore false and ineffective therapy. Therefore, future studies concerning the effect of different species of schistosomes are recommended to current understanding and to explore the possible mechanisms of such changes during both acute and chronic infection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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