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BRIEF COMMUNICATION

EVALUATION OF WGA AND CONCANAVALIN A (CON A) LECTIN AS BIOMARKERS OF HEPATOSPLENIC SCHISTOSOMIASIS IN HUMAN BIOPSIES WITH NO EVIDENCE OF EGG-GRANULOMA SYSTEM

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SUMMARY

Introduction: Colonic lesions are predominant in patients with schistosomiasis. However, carbohydrate alterations in colonic schistosomiasis remain unclear. Lectin-ligands allow us to identify changes in the saccharide patterns of cells. **Methods**: Biopsies of descending and rectosigmoid colon of patients were submitted to WGA and Con A lectin histochemistry. **Results**: WGA stained stroma and gland cells of descending colon and rectosigmoid tissues in a granular strong cytoplasmatic pattern in schistosomiasis specimens differing from normal control and Con A failing to recognize all samples analyzed. **Conclusions**: WGA ligands are expressed differently in patients with hepatosplenic schistosomiasis and no evidence of egg-granuloma system.

KEYWORDS: Lectin histochemistry; Colonic lesions; Schistosomiasis.

Schistosomiasis causes considerable morbidity and mortality all over the world. The *Schistosoma mansoni* worm is endemic in 52 countries and territories, spreading across South America, the Caribbean, Africa and the Eastern Mediterranean. This severe public health problem is linked to poverty and low economical development, which propitiates the use of contaminated water in agricultural practice, domestic use and leisure activities[17]. Rises in the prevalence rates and the spread of this endemic disease to new areas show that schistosomiasis is assuming its cruelest expression, less lethal but more incapacitating in terms of irreversible physical damage to human beings[5]. The pathogenic stage of the Schistosomiasis is the ova, which initiate an immunologically delayed hypersensitivity cell-mediated reaction in the organs in which they are deposited. The liver, colon, urinary bladder and ureter are the main organs affected; however, any organ can be affected, even the skin and the brain[3].

Several vascular alterations of the mucosa including sudden vessel interruption, scattered petechial spots and coiled vessels have been described in detail, suggesting that these stromal lesions may help to establish a correct diagnosis[13]. GEBOES *et al*.[4] considered the diagnostic value of these alterations to be limited, since their exact meaning is not clear. MIRANDA *et al.*[12] proposed an endoscopic classification for portal colopathy in Schistosomiasis mansoni based upon the vascular alterations in the intestinal mucosa. On the other hand, no investigation into a histological stromal compounds field shows such proposition.

Carbohydrates of cell surface are involved in several normal and pathologic events, and the parasite-host recognition generates modifications in that host cell's sugars[6]. Thus, membrane carbohydrates represent a key component in cell-cell and cell-extracellular matrix as well as parasite and host interaction, acting as signal transducers and serving as lectin ligands[14,16]. Lectins are structurally diverse carbohydrate-binding proteins or glycoproteins that by virtue of their binding specificity have been used in medical and biological areas[1,14]. Lectin histochemistry with different carbohydrate specificities can provide a sensitive detection system linked to different labels, such as peroxidase and acridinium ster[15] for changes in glycosylation and carbohydrate expression that may occur during embryogenesis, growth and disease[2]. Among lectins, we can highlight Con A, since the schistosoma egg-granuloma surface system was evidenced by this lectin[11] and the high pattern of WGA staining in pathologic disorders[16]. Besides this, lectin probe analyses on *Schistosoma mansoni* tegumental surface glycopeptides showed high levels of surface polypeptides reacting with horseradish peroxidase-labelled Canavalia ensiformis (Con A), Erythrina corallodendron (ECA), Glycine max (SBA) and Triticum vulgaris (WGA), indicating that carbohydrates associated with the tegumental primary sporocyst may act as receptors, mediating recognition by the internal defense system of the molluscan host, *Biomphalaria glabrata*[8]. *In vitro* studies show that the addition of carbohydrates such as N-acetyl-glucosamine to the culture medium can significantly inhibit sporocyts cell adhesion to parasite tegument[9].

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Furthermore, lectin-carbohydrate interaction is also evidenced in this disease when increasing evidence is observed that the interplay between egg glycoproteins and host C-type lectins plays an important role in shaping immune responses during schistosomiasis[10]. Thus, this study aims to investigate disorders of Con A ligands (D-glucose and/or D-mannose), and WGA ligands (N-acetylglucosamine and sialic acid) as Hepatosplenic Schistosomiasis (HS) biomarkers in the colon and rectum biopsies of patients with no evidence of egg-granuloma system but with the presentation of eggs of *S. mansoni* in the feces by Hoffman method[7]. Briefly, the fecal material is homogenized and after final sedimentation the samples are placed on slides and analyzed in duplicates. Were adopted as exclusion criteria the presence of other intestinal parasitic infections.

Four µm sections of descending colon (n = 25) and rectosigmoid (n = 25) biopsies of patients with HS were deparaffinized in xylene and hydrated in graded alcohol (100 - 70%) and then treated with 0.1% (w/v) trypsin solution for two minutes at 37 °C, followed by a 0.3M (v/v) methanol-H_2O_2 solution for 15 minutes at 25 °C. Afterwards tissues were incubated with lectins conjugated to horseradish peroxidase (*Wheat germ* agglutinin and Concavalina A agglutinin, 25 µg/mL) for two hours at 4 °C, peroxidase reaction was performed with diaminobenzidine-H_2O_2 Then, tissues were hematoxylin counter-stained and evaluated in an Olympus microscope optic Eclipse 50i (Olympus, Tokyo, Japan). For image acquisition, an Image Analyses System (software NIS—Elements F version 2.30—Nikon, USA) was used. Normal colonic biopsies (n = 20) were used as control and submitted to the same assay. Inhibition assays of lectin-carbohydrate recognition were carried out with α-D-glucose for Con A, and N-acetylglucosamine for WGA, at 300 mM. Negative control was performed by replacing the lectins with PBS. Hematoxylin and eosin was performed to confirm the absence of egg-granuloma system.

In HS patients, WGA lectin, stained extracellular matrix, endothelial cells and gland cells of descending colon mucosa (Fig. 1b) and rectosigmoid (Fig.1d) in a cytoplasmatic and membrane pattern differing from normal biopsies that show a weak staining pattern in both analyzed sites (Fig. 1a, 1c). Con A failed to recognize D-glucose and/or D-mannose residues in cells of all normal (Fig. 1e) and HS colonic biopsies (Fig. 1f). These findings do not indicate an absence of carbohydrate residues, but that if they are present, the Con A was unable to access them, even after treatment with trypsin. This lack of recognition can be explained by the addition of N-acatyl-Lactosamine biantennary and polilactosamine structures, preventing access of Con A with the residues of D-glucose and/or D-mannose, differing from N-acetylglucosamine and sialic acid residues often located in terminal positions of glycoconjugated, thus facilitating their recognition by the WGA viewed in our study[16].

Our results showed an increased expression of WGA ligands in accordance with studies of *Schistosoma* and other helminthes derived glycoconjugates, where the increase of sialic acid expression by the host is an attempt to increase the immune response against the pathogen, because the sialylated antigens are often highly immunogenic. In addition to this, there is also an increase in the amount of polylactosamine sites containing N-acetylglucosamine, which function as WGA ligands[10,16]. The results indicate that residues of N-acetylglucosamine and sialic acid are differentially expressed and present a modified expression pattern in patients infected by HS, indicating that WGA is a potential biopsies biomarker for patients with no evidence of egg-granuloma system.

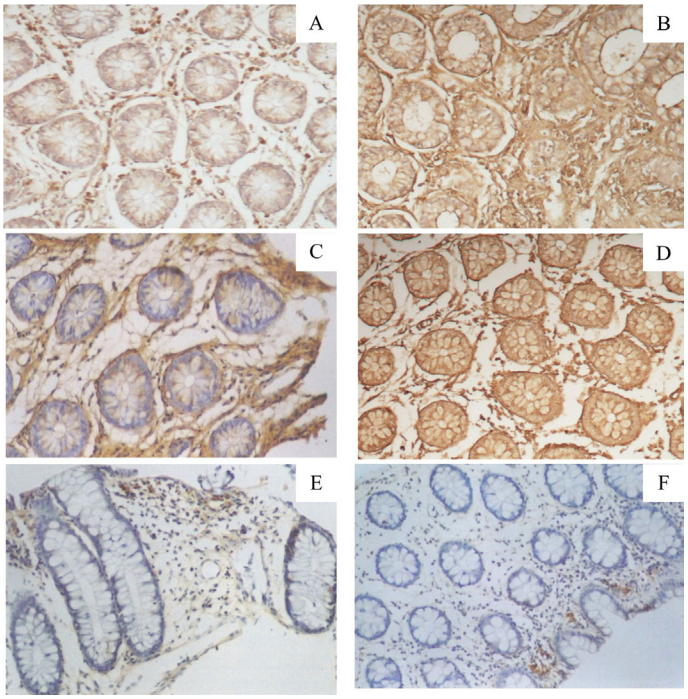

Fig. 1 - WGA staining for normal colonic mucosa (A) reveals a weak affinity of the lectin and their ligands in gland cells and stroma, differing from biopsies of HS patients, which show a strong staining pattern for this lectin (B). The same difference could be observed in rectosigmoide biopsies (C,D). On the other hand, Con A failed to recognize both biopsies normal(E) and HS one(F). For best viewing, Figures A, B, C and D had 100x magnification and E and F, 50X.

RESUMO

Avaliação das lectinas WGA e Con A como biomarcadoras de esquistossomose hepatoesplênica em biopsias humanas sem evidência do sistema ovo-granuloma

Introdução: Lesões do cólon são predominantes em pacientes com esquistossomose, entretanto alterações dos carboidratos no cólon com esquistossomose permanecem desconhecidas. Ligantes de lectinas permitem a identificação das mudanças no padrão dos carboidratos celulares. **Métodos:** Biópsias do cólon descendente e sigmóide dos pacientes foram submetidas a histoquímica com as lectinas WGA e Con A. **Resultados**: WGA marcou o estroma dos tecidos das células glandulares do cólon descendente e sigmóide com um padrão citoplasmático intenso e granular em espécimes com esquistossomose diferindo do controle normal e da Con A, a qual não reconheceu nenhum tecido estudado. Cólon sem esquistossomose não apresentou marcação. **Conclusões**: ligantes de WGA são diferentemente expressos em pacientes com esquistossomose hepatoesplênica sem evidência de sistema ovo-granuloma.

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AUTHOR CONTRIBUTIONS

Moacyr Jesus Barreto de Melo Rêgo and Gabriela Souto Vieira-de-Mello contributed to data acquisition and analysis, interpretation and manuscript drafting. Cláudio Witaker Araújo contributed with data acquisition and interpretation. Maria do Socorro de Mendonça Cavalcanti and Eduardo Isidoro Carneiro Beltrão contributed with study concept and design, critical review and final approval of the manuscript.

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