GASTROINTESTINAL PATHOLOGY IN AUTISM SPECTRUM DISORDERS: THE VENEZUELAN EXPERIENCE

By Lenny G. González, MD

Lenny González, MD, specializes in pediatric gastroenterology and nutrition and is a member of the staff of SOVENIA (Venezuelan Society of Autistic Children). Her focus is on the diagnosis and treatment of GI pathology in children with autism and children with developmental delays. Dr. Gonzalez sees children from all over South America and has performed intestinal endospcopies on more than 956 children with autism. She has collaborated with Thoughtful House and Dr. Andrew Wakefield in Austin, Texas, where she investigated the histological findings of gastrointestinal mucosa of patients and diagnosed infections and immune system disorders. She is actively involved in the Defeat Autism Now! Conference. In addition, she has presented her findings in most Latin American countries. She is dedicated to education, research, and support for families with children who have autism and/or gastrointestinal diseases.

Recent studies in the medical literature have confirmed that gastrointestinal (GI) symptoms are common in patients with autism spectrum disorders (ASD). In two prospective studies, GI symptoms were present in 80% and 70% of autistic children, respectively.[1] In contrast with the ASD group in the latter study, Valicenti-McDermott et al. reported GI symptoms in only 28% of neurotypical controls.[1,2,10] Retrospective studies that rely only upon review of the children's existing clinical records are likely to underestimate the true size of the problem since these records rarely document GI symptoms. The inadequacy of this approach means that it is impossible to determine whether symptoms were not present or, more likely, that the clinician just failed to document them. On the other hand, prospective studies that systematically ask about the presence or absence of specific symptoms provide a much more accurate picture of the size of the problem.

Clinical manifestations of GI disease in ASD children

Physical symptoms in ASD children are often misinterpreted as just autistic behaviors. In our experience, symptoms of what turns out to be GI distress often present as inexplicable irritability, aggressive or auto-aggressive (self-injurious) behaviors, discomfort, sleep disorders, and other behavioral disturbances. The problem of physical symptoms such as abdominal pain being interpreted simply as aberrant behaviors is particularly problematic in children who are nonverbal and who have serious difficulties expressing themselves.[10]

Detailed case histories often provide evidence of abdominal colic and sleep disorders during the nursing stage and frequent infections of the upper respiratory tract (such as otitis and tonsillitis) and GI tract caused by bacterial, viral, parasitic, or yeast infections. Affected children are often hypersensitive to sounds, light, flavors, smells, and clothing labels. In addition, there is often a history of intolerance to certain foods containing gluten and casein as well as indicators of food allergies.[5-7]

Children with autism often present with GI and extra-intestinal symptoms. The digestive symptoms include abdominal pain, pyrosis (heartburn), chronic diarrhea, flatulence, drooling or excessive salivation, vomiting, regurgitations, weight loss, rumination, bruxism (teeth grinding), irritability, dysentery, constipation, and fecal impaction. During symptomatic episodes, periods of irritability, insomnia, and auto-aggressive behaviors are observed. In ASD children, it is common to observe abnormal toileting patterns. Diarrhea and constipation are common, and constipation can coexist

One explanation might be that part of the neurological disability in children with autism results from absorption across an inflamed intestinal lining of molecules that are toxic to the developing brain.

with episodes of diarrhea. In the case of diarrhea, the stools are semi-liquid, very fetid with mucus and undigested food; sometimes they can have a sandy/grainy consistency and other times show blood. Diarrhea is one of the most common symptoms as reported in the studies of D'Eufemia, Torrente, Horvath, Wakefield, Furlano, and Sabrá. This has been our experience in Venezuela, also.[14]

The extra-intestinal problems experienced by our ASD children with GI symptoms include respiratory, neurological, and dermatological disorders. These include frequent coughing (often dry), upper respiratory tract infections, skin rashes, eczema, atopic dermatitis, seborrheic dermatitis, and itching.

The most common clinical signs are Dennie Morgan infraorbital skin folds (caused by edema or fluid collecting in areas of inflammation), dark circles under the eyes, long eyelashes, abdominal distension, halitosis, perianal erythema (diaper rash), anal fissures, dry skin, angular cheilosis (sore cracks at the corners of the lips), and greenish anterior rinorrhea (runny nose). There are also alterations in the stool consistency, color, and smell (excessively offensive) as well as the presence of mucus or blood, food remains, and visible fat (often semi-liquid, acidic, excessively fetid, greasy feces, with mucus and/or blood).

Etiopathogenesis

Recent studies of ASD children report chronic inflammation of the gastrointestinal tract that may be present anywhere from the esophagus down to the rectum: this inflammation may well explain the GI symptoms and at least some of the behaviors.[18-15, 35-40] Several theories have been proposed for how deterioration in gastrointestinal function might influence neurological functioning. The epithelial cell layer that lines the GI mucosa forms a barrier that restricts the contents of the gut from getting into the blood stream. It it is composed of cells with absorptive surfaces (the brush border) that interact with the contents of the lumen. Between these cells are gates called *tight junctions*, the integrity of which is important in preventing noxious substances from entering the bloodstream without passing directly through the cells.[10]

One explanation might be that part of the neurological disability in children with autism results from absorption across an inflamed intestinal lining of molecules that are toxic to the developing brain.[10-14] Inflammation of the intestinal wall can be induced by diverse causes such as food allergy, use of antibiotics and non-steroidal anti-inflammatory drugs, infection, or by enzymatic insufficiency, mycotoxins from yeast/fungi, gluten, casein, chemical additives, colorings, preservatives, malabsorption of proteins, heavy metal intoxications, and pesticides.[3-6]

The integrity of the intestinal wall also plays an important role in the adequate absorption of nutrients and the exclusion of potentially harmful toxins, bacteria, allergens, and peptides coming from certain foods. In our experience, food components such as gluten and casein can provoke the behavioral abnormalities characteristic of autism[4], possibly when they enter the systemic circulation. Increased intestinal permeability (leaky gut) may be the link that explains the association of autism with an abnormal intestinal immune response, multiple food allergies, dysbiosis, fungal overgrowth (*Candida albicans*), as well as with micronutrient deficiencies.[4,5] Probably due to GI inflammation and abnormal immune function, children with autism may have increased levels of harmful bowel organisms. Frequent antibiotic use in the first years of life can also contribute to the chronic imbalance, referred to as intestinal dysbiosis. Several investigators have found evidence of this imbalance in autistic children. A good example of a pathogenic (disease-causing) bacterium is *Clostridium difficile*. This organism is a common cause of severe colitis that occurs when broad-spectrum oral antibiotics have killed off the beneficial gut bacteria and have allowed this antibiotic-resistant opportunistic organism to overgrow and cause inflammation.[10]

The gut-brain connection is recognized as playing a role in the neurological complications of a number of gastrointestinal diseases. Symptoms like constipation, pain, or abdominal distension are reported by adults with degenerative disorders of the central nervous system like Parkinson's disease,[4] while parents of autistic children report similar symptoms, although the precise nature of any link between the gut and the brain is unknown.

Ileo-colonoscopy and autistic enterocolitis

In 1998, a team of doctors at the Royal Free Hospital in London reported the results of ileocolonoscopies on 12 children who presented with autism and GI symptoms. In a series of papers, Wakefield and colleagues described a new variant of intestinal inflammatory disease, which was named *autistic enterocolitis*. The disease is characterized by mild-to-moderate chronic patchy inflammation of the mucosa and lymphoid nodular hyperplasia (LNH) (swelling of the lymph glands) in the bowel lining. Visible features suggestive of inflammatory bowel disease included the red halo sign – an expression of pre-ulcerative reddening around the swollen lymphoid tissues – typically located at the terminal ileum, potentially extending to involve the whole colon, loss of vascular pattern, and mucosal granularity, erythema (redness), and ulceration. When compared with

neurotypical children, including those with ulcerative colitis (a well described inflammatory bowel disease), the findings suggested a novel disease process.[14]

In the UK study and in our own experience, these abnormal findings are more frequent in autistic children than in developmentally normal children with GI symptoms. The only exception was ulceration, which was uncommon in both groups. The biopsies from the children with autism showed reactive LNH in 88.5% of the children compared with only 29% of children with ulcerative colitis, and 0.0% in the control group without IBD. In many cases, the researchers also saw infiltration of inflammatory cells like neutrophils (pus cells) and lymphocytes (chronic inflammatory cells) in the epithelium of the bowel mucosa. Active neutrophilic inflammation in the ileum was present in 8% of the children with autism and in none of the non-inflammatory bowel disease controls. Chronic lymphocytic inflammation in the colon was present in 88% of the autistic cases, 4% of the controls, and 100% of ulcerative colitis cases.

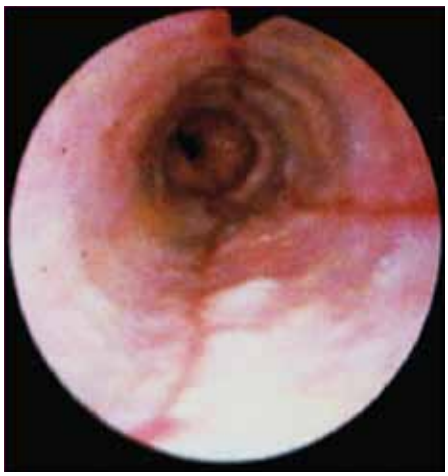
In our published study of 45 ASD children and 57 developmentally normal controls presenting for GI assessment, chronic inflammation and LNH in the colon and ileum was present in 100% of the autistic cases compared with 66.66% of the controls, reflecting a high background rate of infectious enterocolitis in Venezuelan children (see below.)[13] Since then, other studies carried out in the United States, Brazil, Italy, and Venezuela have confirmed the finding of inflammation and LNH in ASD.[10-14, 29]

Immune profiling of the intestinal disease in ASD

Furlano (2001), Torrente (2002), and Ashwood (2004) have described immune abnormalities and abnormal cytokine profiles in the intestine of ASD children with GI symptoms. Cytokines are chemical messengers that communicate between cells to increase or decrease the activity of the immune system. In ASD children, upper and lower gastrointestinal inflammation have been found to different degrees of severity;[6] the relationship, if any, between the severity of the bowel inflammation and the autistic features is not yet known. Using techniques that identify specific immune cell types, under the microscope these investigators have shown characteristic patchy inflammation in many ASD children including a pronounced infiltrate of CD4, CD8, gamma delta T cells and antibody producing plasma cells. The inflammatory response is different from that seen in IBD (inflammatory bowel disease) patients and in the normal controls. The inflammation was more localized to the epithelium and basement membranes (superficial layers) of the gut mucosa than that which is commonly seen in classic IBD.[34-38]

Intestinal symptoms are not necessary for the presence of intestinal disease. An increase in intestinal permeability in patients with ASD who were not symptomatic from the GI perspective and who had no other evidence of GI illness was described by D'Eufemia in 1996[8] and in 1998-2000. Wakefield and colleagues suggest the association of inflammatory intestinal chronic disease and autism from an analysis of ileal and colonic biopsies where the frequent presence of LNH and unspecific colitis are evidenced.[9-15] Currently, the structure and function of the digestive mucosa in autistic children is being studied in the search for an association between the histological changes and the etiopathogenesis of autism.

Reflux Esophagitis (Endoscopy)



Reflux Esophagitis (Biopsy)

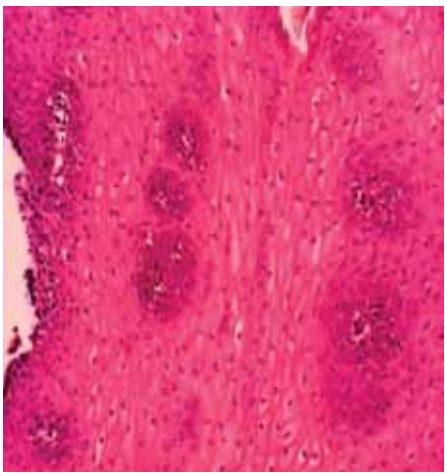


Figure 1: (a) endoscopic image typical of reflux esophagitis (b) matches the 4 histological criteria for diagnosis of reflux esophagitis.

Upper gastrointestinal endoscopy

Based upon our experience, we stress the importance of a full examination of the GI tract in symptomatic children with any degree or type of ASD, to include upper GI endoscopy (esophagus, stomach and, duodenum). The literature supports the existence of upper gastrointestinal disease in these children.[3-15] In our Venezuelan series,[13] we found that 88% of the patients had reflux esophagitis, 55% had nonspecific gastritis due to *Helicobacter pylori*, 52% of ASD children had *Giardia intestinalis* infection, and 37% had chronic unspecific inflammation of the small intestine. This was in addition to the findings of colitis with LNH (69%), of which 11% had eosinophilic colitis, and 100% of the children presented chronic inflammation and lymphoid nodular hyperplasia (LNH) of the terminal ileum.

Esophagus

Over the past decade, the expanding body of literature on esophageal physiology and pathology has led to the perception that the esophagus is an immunologically active organ. It can respond to a variety of stimuli by augmenting certain immunological

defenses, including recruitment and activation of eosinophils – immune cells involved in allergic responses. Esophageal pathology is common in Venezuelan children with ASD (in our study, degrees of esophagitis were described according to the guidelines of the European and American Society of Pediatric Gastroenterology and Nutrition. [11,12]). One of the most common visual findings in eosinophilic esophagitis is linear streaks of nodular mucosa. Eosinophilic esophagitis is a common pathology in ASD children and is defined as an increase in the number of eosinophils seen microscopically per high-power field (HPF). For eosinophils, the normal range in the esophagus is between 0-2 cells/HPF. In eosinophilic esophagitis associated with various food allergic disorders, we often see 20 cells/HPF or more.[14, 24, 40, 41]

Reflux esophagitis, in which stomach acid moves back up into the lower esophagus, is also common. Visually this is indicated by esophageal rings, mucosal erythema, linear ulcers, and erosive esophagitis when damage from the acid reflux is extreme. The finding is confirmed by biopsy. *(Figure 1)* In our series, the most frequent diagnosis was reflux esophagitis that was present in 89% of the children with autism compared with 49% in the control group, with a significant difference between the groups of $p<0.001$.[14] A neurotypical child with esophageal pathology as described above would complain bitterly; for the nonverbal child, drawing attention to the source of their distress is particularly challenging, and doctors should retain a high index of suspicion.[40]

Stomach

Our experience with gastric disease in Venezuela is somewhat different from the experience of others since we have a high rate of infection with *H. pylori* in children. *H. pylori* can be a painful bacterial infection, and children who have it in their stomach characteristically have a very obvious nodular gastritis. *(Figure 4)* We observed chronic *H. pylori*-associated gastritis in 55.6% of the children with autism and 33.3% in the controls, compared with active chronic gastritis without *H. pylori* in 33.33% of the children with autism and 2.56% of the controls. This compares with a finding of gastritis in 15 of 36 ASD children by Horvath et al. in US children.[14]

(When assessing stomach pathology, we used the Sydney System for classification and grading of gastritis[14] and a raised eosinophil count as one greater than 20 cells per HPF).

One of the characteristic lesions we see in the stomach is reactive gastropathy, which can be associated with the reflux of bile into the stomach from the duodenum. Endoscopic findings include congestion, enanthema (an eruption on the mucus membrane of the stomach), erythema (reddening), micro- and macro-nodularity in the body and antrum, ulcers, and biliary reflux as a result of hypomotility (reduced peristalsis) moving food from the antrum of the stomach into the duodenum. One observation in affected children is that they have very distended bellies.

Small Bowel

The small bowel is the duodenum, the jejunum, and the ileum. It is the longest part of the bowel, and it is important because it is where the body absorbs calories and nutrients. Currently, the small bowel can be visualized using capsule endoscopy, but, other than the very first and last parts of the small intestine it is not routinely accessible to biopsy. We sometimes find celiac disease in children with autism, but it is surprisingly rare, considering that this group is exceedingly sensitive to gluten. Because celiac disease can only be diagnosed with certainty while the child is ingesting gluten, it is critical to obtain baseline celiac antibodies before beginning a gluten-free diet in order to confidently exclude celiac disease.

Endoscopic Image of Duodenum

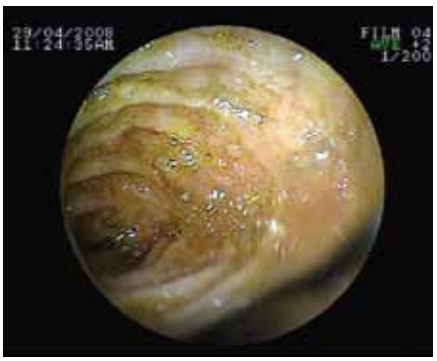


Figure 2: nodular duodenal bulb

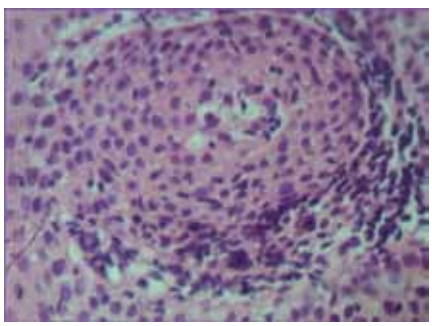


Figure 3: lymphoid accumulations cumulus in the esophagical mucosa of a 4-year-old child with diagnosis of severe autism.

Stomach

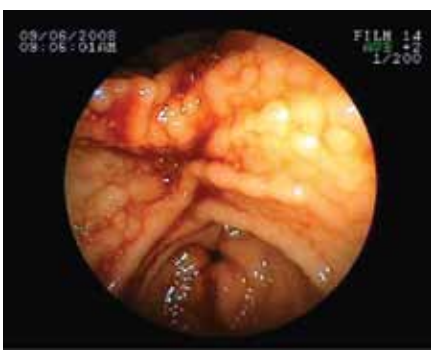


Figure 4: corresponds to image of gastric antrum where nodularity (stony antrum) can be seen, characteristic of the infection by *Helicobacter pylori*.

Since then, other studies carried out in the United States, Brazil, Italy, and Venezuela have confirmed the finding of inflammation and LNH in ASD.

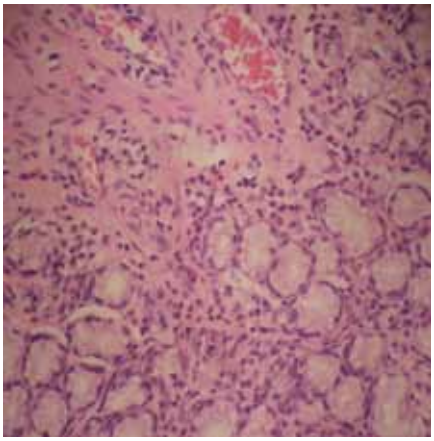


Figure 5: histologic specimen with eosinophilic gastritis

Colitis with LNH

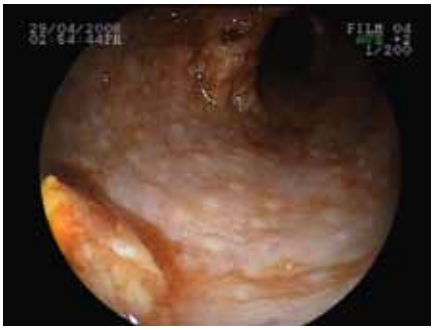


Figure 6: image where total loss of normal characteristics of the colon can be observed.

Histological image of Ileum

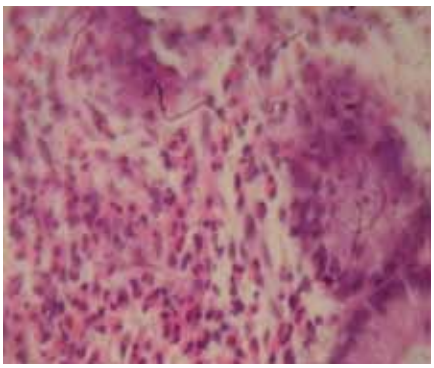


Figure 7: eosinophilic colitis at 10X in a child with severe autism.

Treatment

The diagnosis and treatment of gastrointestinal disease in children with autism involves an exhaustive assessment that includes a detailed case history and physical examination, investigation of the macroscopic characteristics of the stool, laboratory testing, a complete stool analysis, a search for intestinal infections to include parasites, *Campylobacter jejuni* and *Clostridium difficile*, and a fungal culture.

Treatment is designed to reduce damage and inflammation of the gastrointestinal mucosa, diminish intestinal permeability, improve nutrient intake and micronutrient absorption, and treat any associated infection(s). In addition, the children should avoid proteins in the diet such as gluten and casein, among others (because these may result in an immune/allergic response that may exacerbate any intestinal inflammation) and strengthen their immune system throughout with the treatment of any IgA secretory deficiency.

All of the intestinal lesions, damage, or abnormalities are potentially treatable.Many children respond very well to combinations of the following: of a restricted diet, anti-inflammatory medication, probiotics, antibiotics, antifungals, and digestive enzymes, among other things. There is correlation between the treatment of GI disease and improved cognitive functions, decreased self-aggressiveness, and improvements in attention, visual contact, and sleep disorders.

In summary, particular attention should be paid to:

1. Nutritional intervention (e.g., gluten-free/casein-free diet and appropriate supplementation)
2. Treatment of gastroesophageal reflux disease (GERD)
3. Treatment of eosinophilic esophagitis
4. Management of gastritis with or without *Helycobacter pylori* infection
5. Treatment of constipation[29]
6. Management of pancreatic insufficiency[30]
7. Antifungal treatment
8. Probiotics, prebiotics (foods that support beneficial bacteria), fermented foods[31, 32]
9. Treatment of other infections including *Clostridium difficile*, intestinal parasites such as protozoarians and helminths, and other bacteria including *ECEP, Klebsiella pneumoniae, Citrobacter feundii, Enterobacter cloacae, Pseudomona aeruginosa, Proteus mirabilis, Aspergillus, Trichosporum* and *Geotrichum sp*, among others.

Conclusions

Our experience of Venezuelan children with ASD is that most have GI symptoms that may not be immediately evident or may not be obviously related to intestinal distress. The absence of obvious GI symptoms does not mean an absence of the disease. There may be chronic inflammation anywhere from the esophagus down to the rectum that may be seen even in asymptomatic patients; the GI evaluation is an essential part of the investigation protocol in ASD. In our experience, treating GI disease is consistently associated with improved cognitive functions, decreased self-aggressiveness, better attention, improved eye contact, and decreased sleep disorders.

In our experience, treating GI disease is consistently associated with improved cognitive functions, decreased self-aggressiveness, better attention, improved eye contact, and decreased sleep disorders.

References

[1] Valicenti-McDermott M, McVicar et al. Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. *J Dev Behav Pediatr* 2006 Apr 27 (Suppl 2):S18-36.

[2] American Psychiatric Association *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition.* Washington (DC): American Psychiatric Association: 1994.

[3] White JF. Intestinal Pathophysiology in autism. *Exp Biol Med* (Maywoo).2003 Jun;228(6):639-49.

[4] Hovarth K, Papadimitiou J, Rabsztyn A, Drachemberg C., Tildon T, et al. Gastrointestinal abnormalities in children with autisitic disorders. *J of Pediatr* 1999; 135:559-63.

[5] Brudnack M., Bucholz I., Hoener S., Newman L., Pangborn J., *Guide to Intestinal Health In Autism Spectrum Disorders*. 2001.

[6] Quigley E, Hurley D. Autism and the gastrointestinal tract. *AJG* 2000; 9:2154-56.

[7] Hviid A, Stellfeld M, Wohlfahrt J, Melbye M. Association between thimerosal-containing vaccines and autism. *JAMA* 2003; 290(13):1763-6.

[8] Hovarth K, Perman JA. Autism and Gastrointestinal Symptoms. *Curr Gastroenterol Rep* 2002;4(3):251-8.

[9] Pangborn J, Baker S. Consensus report of The Defeat Autism Now! (DAN!). October 2002. San Diego California.

[10] Bryan Jepson, Jane Johnson. Is Autism a Gut Disease? *Changing the Course of Autism First Edition*, 2007 (9):87-97.

[11] Wakefield AJ, Puleston JM, Montgomery S, Anthony A, O'leary J, Murch S, Review article: The concept of entero-colonic encephalopathy, austim and opioid receptor ligands. *Aliment Pharmacol Ther.* 2002 Apr;16(4):663-74.

[12] D´Eufemia P, Celli M, Finocchiaro R, Pacífico L, Viozzi L, Zaccagnini M, et al. Abnormal intestinal permeability in children with autism. *Acta Paediatr* 1996;85:1076-9.

[13] Wakefield AJ., Anthony A., Murch SH., Thomson M., Montgomery SM., Davies S., et al. Entero-colitis and inmunodeficiency in children with developmental disorders. *Am J Gastroenterol* 2000;95:2285-95.

[14] González L, López K, Navarro D, Negrón L, Martínez M, Sabrá A. Características Endoscópicas, Histológicas e Inmunológicas de la Mucosa Digestiva en Niños Autistas con Síntomas Gastrointestinales. *Arch Venez Pueril Pediatr* 2006; Vol 69 (1): 19-25.

[15] Wakefield AJ., Murch SH., Anthony A., et al. Ileal lymphoid nodular hyperplasia, non-specific colitis and pervasive disorder in children. *The Lancet* 1998;351:637-41.

[16] Vandesplas Y. Reflux esophagitis in infants and children: A report from the working group on gastro-oesophaggeal reflux disease of the European Society of Gastroenrerology and Nutrition. *J Pediatr Gastroenterol Nutr* 1994;18:413-22.

[17] Rudolph C, Mazur L, Lipton G, Baker R. Pediatric GE reflux clinical practice guidelines. North American Society for Pediatric Gastroenterology and Nutrition. *J Pediatr Gastroenterol Nutr* 2001;32(suppl 2):S5-7.

[18] Dixon M, Genta R, Yardley, Correa P. Classification and grading of gastritis: The updated sydney system. *Am J Surg Pathol* 1996; 20(10):1161-1181.

[19] Murch S, Phillips A. Small intestinal biopsy. In: Walker A, Durie P. *Pediatric Gastrointestinal Disease Third Edition*. 1999.

[20] Justinich C. Enfermedades alérgicas del intestino y gastroenteritis eosinofilica. En: Wyllie R, Hyams *J. Gastroenterologia Pediatrica 2nd edition*. 2001. Editorial McGraw Hill Iteramericana editores, 372-393.

[21] Rapin I. The autistic spectrum disorders. *N Engl J Med* 2002;347(5):302-3.

[22] Black C, Kaye J, Jic H. Relation of childhood gastrointestinal disorders to autism: Nested case-control study using data from the UK General Practice Research Database. *BMJ* 2002;325(21):419-421.

[23] Siafakas C, Ryan Ch, Bronw M, Miller T. Multiple esophageal Rings: An association with eosinophilic esophagitis. *Am J Gastroenterol* 2000;95:1572-1575.

[24] Orenstein S, Shalaby T, Di Lorenzo C, Putnam P, Sigurdsson L, Kocoshis S. The spectrum of pediatric eosinophilic esophagitis beyond infancy: A clinical series of 30 Children. *Am J Gastroenterol* 2000;95(6):1422-1430.

[25] Torrente F, Anthony A, Henschkel R, Thompson M, Ashwood P, Murch F. Focal-enhanced gastritis in regressive autism with features distinct from Crohn's and *Helicobacter pylori* Gastritis. *Am J Gastroenterol* 2004;99(4):598-605.

[26] Piñero R, Plascensio A, Avila M, Urrestarazu M, Serrano N, Corrente M, Cavaza M. Helicobacter pylori e niños de El Clavo, una población rural venezolana. *G.E.N.* 2000;54(1):12-17.

[27] Navarro D, López K, Vásquez M, Martínez M, Daoud N, col. Gastritis por Helicobacter pylori en niños con síntomas gastrointestinales. *Arch Venez Puer Pediatr*, 1996;59(3):124-28.

[28] Senior K. Possible autoimmune enteropathy found in autistic children. *The Lancet* 2002;359:1674.

[29] Furlano R, Anthony A, Day R, Bronw A, Mc Garvey L, Thonsomp M, et al. Colonic CD8 and γδ T cell filtration with epitelial damage in children with autism. *J Pediatr* 2001;138:366-72.

[30] Sabra S, Bellanti JA, Colon AR. Ileal lymphoid hyperplasia, non-specific colitis and pervasive developmental disorder in children. *The Lancet* 1998;352:234-5.

[31] *Journal Pediatr Gastroenterol & Nutr.* 2006 Sep:43 (3)405-47, NASPGHAN.

[32] Brudnak MA, Beneficial effects of enzyme-based therapy for autistic spectrum disorders **www.list.feat.org**

[33] Isolauri *E. Pediatr Res.* 1992, Aug 32(2): 141-4.

[34] Defeat Autism Now! 2007.

[36] Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. *J Neuroimmunol.* 2006 Apr;173(1-2):126-34. Epub 2006 Feb 21.

[36] Ashwood P, Wakefield AJ. Department of Medical Microbiology and Immunology, University of California at Davis, M.I.N.D. Institute, Wet Lab building, 50th Street, Sacramento, CA USA.

[37] Comment in: *Eur J Gastroenterol Hepatol.* 2005 Aug;17(8):821-2. Eur J Gastroenterol Hepatol. 2006 May;18(5):569-71; author reply 571-3. The significance of ileo-colonic lymphoid nodular hyperplasia in children with autistic spectrum disorder. *Eur J Gastroenterol Hepatol.* 2005 Aug;17(8):827-36. Links

[38] Jepson B, Johnson J, *Changing the Course of Autism*, 2007, Chapter 9.

[39] Wakefield AJ, Ashwood P, Limb K, Anthony A. Thoughtful House Center for Children, Austin, Texas USA. Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: Mucosal immune activation and reduced counter regulatory interleukin-10. *J Clin Immunol.* 2004 Nov;24(6):664-73.

[40] Ashwood P, Anthony A, Torrente F, Wakefield AJ. Centre for Paediatric Gastroenterology, Royal Free and University College Medical School, London, United Kingdom.

[41] Krigsman A. Gastrointestinal pathology in autism: Description and treatment. *Medical Veritas* 4 (52007)1522-1530.

[42] Balzola F, Barbon V. Panenteric IBD-like disease in a patient with regressive autism shown for the first time by the wireless capsule enteroscopy: Another piece in the jigsaw of this gut-brain syndrome. *AMJ Gatroenterol* 2005 Letters to the editor.