Original research article

Clinical and serological evaluation of gastrointestinal profile by line immunoassay (LIA) in a group of patients attending tertiary care hospital of Bihar: An observational study

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Received: 03-09-2020 / Revised: 11-10-2020 / Accepted: 23-11-2020

Abstract

Objectives: the evaluation of Clinical and serological auto-antibodies against gastrointestinal tract by gastrointestinal profile using LIA in a group of patients who have attended AIIMS Patna for symptoms suggestive of gastrointestinal disorder. **Material and methods**:This was a retrospective study on all samples (130-150) received for gastrointestinal profile to be done in Biochemistry central lab for a study period of May 2019 to January 2020(9 months). A total of approximately 50 samples positive or equivocal for antibodies against IF, Gliadin, ASCA, PCA and tTg antigen were analysed further for clinical data. **Results:** In our study 56% and 44% female were participated most of the patients was 30-40 years followed by 20-30 years .The most common symptom Weight loss 48% was found in our study and followed by Chronic/intermittent diarrhea 46%, weakness 24%, Abdominal pain 20%,constipation 18%, Abdominal discomfort and vomiting.We found 32% patients had below 11micromol/L iron and 54% patients had below 12gm/100ml Hb means suffring from anaemia.The ASCA positive status was a predictor for Crohn s disease(CD). in our study out of 50 patients 20% was ASCA+,2% ASCA++,4% ASCA+++and 4% ASCAwas positive. In our study on the basis of the histological diagnosis, PCA+ patients were14%, PCA++ patients 6%, PCA+++patients 12% and PCA Equivocal 4% was found.Tissue transglutaminase (tTG) has been identified as the autoantigen in CD. Out of 50 patients 28% tTG +,2% tTG ++,12% tTG +++,2% tTG Equivocal was found. **Conclusion:** The evaluation of Clinical and serological auto-antibodies against gastrointestinal tract by gastrointestinal profile using LIA was beneficial for identification of the gastrointestinal diseases of the patients .

Keywords: gastrointestinal, immunoassay, clinical, patient.

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Introduction

Autoimmune disorders are a type of disorder in which tissue injury is caused by the immunologic reaction of the organism against its own tissues. Autoimmunity is present in all individuals; however, autoimmune disease occurs only in those individuals in whom the breakdown of one or more of the basic mechanisms regulating immune tolerance results in self-reactivity that can cause tissue damage. Autoimmune disease may affect many body organs and tissue at the same time (systemic autoimmune disease) or it may be organ specific, mainly affect a single organ or tissue, the effects frequently extend to other body systems and organs.

Examples of localised (organ specific) autoimmune diseases of gastro intestinal tract are,Celiac disease, Crohn′s disease (gastrointestinal tract), Pernicious anaemia (stomach).Atrophic gastritis.Gastrointestinal tract diseases are recognised as autoimmune based on typical histopathology, presence of auto-antibodies in serum and clinical response to immunosuppressive therapy. In this study we focus on auto antibodies of GIT. Like in other autoimmune diseases, the inducing factor is unknown; The aim of this study is to review the 5 types of auto-antibodies like ASCA, IF, Gliadin,

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PCA and TtG in a group of patients suspected with autoimmune disease of gastrointestinal tract.

Gliadin is a type of proteins present in wheat and several other cereals within the grass genus *Triticum.* Gliadins and glutenins are the two main components of the gluten fraction of the wheat seed. This gluten is found in products such as wheat flour. Gluten is split about evenly between the gliadins and glutenins .Gliadin is the water-insoluble component of gluten, and glutenin is water soluble[1]. There are three main types of gliadin (α, γ, and ω), to which the body is intolerant in celiac disease. Anti-gliadin antibodies are produced in response to gliadin, a prolamin found in wheat. In bread wheat it is encoded by three different alleles, AA, BB, and DD. These alleles can produce slightly different gliadins, which can cause the body to produce different antibodies.

There are three sub-types of anti-gliadin antibodies:(1) Anti-gliadin IgA- This antibody is present in ~80% of patients with coeliac disease[2,3]. It is directed against the alpha/beta and gamma (α,β,γ) gliadins[4]. Anti-gliadin IgG - is found at higher levels in patients with the IgA-less phenotype. It is also associated with celiac disease and non-celiac gluten sensitivity[5-7]. Anti-gliadin antibodies are frequently found with anti-transglutaminase antibodies.

Anti-gliadin IgE -The IgE antibodies are more typically found in allergy-related conditions such as urticaria, asthma,and wheat-dependent exercise-induced anaphylaxis.

The target o f the most allergenic antibodies is ω-5 gliadin[8].

Tissue transglutaminase (tTG) is a enzyme of the protein-glutamine γ-glutamyltransferases family.[9,10]It crosslinks proteins between an ε-amino group of a lysine residue and a γ-carboxamide group of glutamine residue, creating an inter- or intramolecular bond that is highly resistant to proteolysis.

Aside from its cross-linking function, tTG catalyzes other types of reactions including deamidation, GTP-binding/hydrolyzing, and isopeptidase activities[11]. tTG is expressed ubiquitously and is present in various cellular compartments, such as the cytosol, the nucleus, and the plasma membrane[12].

Antibodies to tissue transglutaminase (ATA) are found in patients with several conditions, including celiac disease, juvenile diabetes[13] inflammatory bowel disease[14] and various forms of arthritis[15,16]. In celiac disease, ATA are involved in the destruction of the villous extracellular matrix and target the destruction of intestinal villous epithelial cells by killer cells. Deposits of anti-tTG in the intestinal epithelium predict celiac disease[17].

ATA IgA are more frequently found in Celiac Disease (CD); however, ATA IgG are found in CD and at higher levels when affected individual had the IgA-less phenotype. The IgA-less phenotype is more common in CD than the normal population.

Intrinsic factor (IF) is a glycoprotein produced by the parietalcells of the stomach. It is essential for the absorption of vitamin B12 in the ileum of the small intestine. In humans, the gastric intrinsic factor protein is encoded by GIF gene[18]. In pernicious anemia which is usually an autoimmune disease auto-antibodies directed against intrinsic factor or parietal cells that lead to an intrinsic factor deficiency, malabsorption of vitamin B12, and subsequent megaloblastic anemia[19]. Atrophic gastritis can also cause intrinsic factor deficiency and anemia through damage to the parietal cells of the stomach wall[20]. Pancreatic exocrine insufficiency, gastric ulcer, gastric tumor ,excessive consumption of alcohol, all these factor leads to damage of gastric mucosa and that leads to deficiency of IF.

Anti-*Saccharomyces cerevisiae* antibodies (ASCAs) are antibodies which act against antigens presented by the cell wall of the yeast *Saccharomyces cerevisiae*. ASCAs and perinuclear antineutrophil cytoplasmic antibodies (pANCAs) are the two most useful and often discriminating biomarkers for colitis[21]. ASCA tends to recognize Crohn's disease more frequently, whereas pANCA tend to recognize ulcerative colitis[22].Diseases in which ASCA are found include the following: Behçet's disease (BD),celiac disease, crohns disease,colitis.

The target antigen of PCAs is the gastric enzyme H^+/K^+ ATPase, which consists of a catalytic 100-kDa α subunit and a 60–90-kDa β subunit. It is the main protein of the secretory canaliculi of gastric parietal cells, and it produces acid by secreting H^+ ions in exchange for K^+ ions. In pernicious anemia, immune-mediated inflammation leads to destruction of gastric parietal cells with the resultant loss of intrinsic factor production and the inability to absorb dietary vitamin B12. Diagnosis of PA involves demonstrating the presence of a macrocytic anemia in the context of vitamin B12 deficiency, as well as documenting positive autoantibody serology, specifically anti-parietal cell antibody (PCA) and intrinsic factor antibody (IFA)[23]. PCAs bind to the alpha- and beta-subunits of the membrane-bound H(+)/K(+)-ATPase. In contrast, IFAs bind directly to intrinsic factor, blocking its ability to bind vitamin B_{12}[23,24].

Symptoms seen in Classical celiac disease is observed in small children after introduction of gliadin, secalin or hordein (components of gluten) in diet. Typical symptoms include, weight loss, abdominal pain, recurrent aphthous, more often there are effects of defective absorption such as—anaemia due to iron deficiency, osteopenia/osteoporosis, enamel defects, aphths, (aphthous stomatitis), feeling of malaise chronic or intermittent diarrhoea, but also constipation, discomfort, vomitus[25]. In Crohn´s disease many patients symptoms from gastrointestinal tract are mild or unspecific, but extra-intestinal symptoms signal chronic disease. The perianal abscesses, recurrent aphthous stomatitis ,fistulas ,anal fissures, joints pain are noted before typical gastrointestinal symptoms like chronic or inter mittens diarrhoea, pain, blood in stool[25].

Clinical symptoms in typical case of Ulcerative colitis(UC) are associated with chronic diarrhoea, blood and mucin in stools, rectal bleeding, abdominal pain, cramps ,feeling of rectal urgency and many others. The extra-intestinal symptoms are rare, present in about 10% patients with UC, as arthropathy, erythemanodosum [25].

Antibodies to parietal cells (PCA) are typical for autoimmune chronic gastritis (AIG) associated with megaloblastic anaemia in consequence of wit B12 and intrinsic factor low level due to disturbances of gastric mucous function.[25]

Novelty of this study:

1. No study has been reported about evaluation of gastro profile in patients from Bihar.
2. This study will serve as a basis to undertake future research in various autoimmune diseases of gastrointestinal tract including clinical profile depending on the study outcome.
3. Cost effective: no additional funding required.

Aims and Objectives:

Aims: Clinical and serological evaluation of auto-antibodies against gastrointestinal tract by gastrointestinal profile using LIA in a group of patients who have attended AIIMS Patna for symptoms suggestive of gastrointestinal disorder.

Objective:

1. To observe the age and sex wise distribution pattern of IF , Gliadin , ASCA ,PCA and tTG antibody in patients attending AIIMS Patna using Line immunoassay platform.
2. To study the association between above mentioned antibody positivity by using LIA.
3. To study the clinical presentations of patients with above 5 mentioned antibodies positivity depending on the grades of staining

Material and Methods

This was a retrospective study on all samples (130-150) received for gastrointestinal profile to be done in Biochemistry central lab for a study period of May 2019 to January 2020(9 months). A total of approximately 50samples positive or equivocal for antibodies against IF,Gliadin, ASCA, PCA and tTg antigen were analysed further for clinical data. Patient's result data was categorised age and sex wise. The present study was conducted in the department of Biochemistry, AIIMS Patna. This research has approved by institutional ethical committee of AIIMS, Patna, Bihar. This study was extract the data from the samples tested using the following protocol.

Inclusion criteria:-All samples received in Biochemistry central lab for gastrointestinal profile.

Exclusion criteria: All lipemic, icteric and haemolysed sample excluded (repeat sample accepted).

Methodology

The protocol for gastrointestinal profiling was as follows:

Venous blood was collected under aseptic conditions in vacutainer without anticoagulant and put to centrifugation for serum separation. The separated serum was stored in -20 degree refrigerator in mini centrifuge vials till analysis. Clinical data was collected for previously admitted patients,who was positive against Gliadin,Ttg, ASCA, PCA, and IF through medical records available in MRD after due permission of competent authority and through HIS.The sample was processed by Line immunoassay (LIA)for detection of antibodies panel which includes antibodies to 5 antigens (Gliadin, ASCA, PCA, IF and tTG).LIA was an indirect membrane based enzyme immunoassay for the qualitative measurement of IgG class antibodies against the above mentioned five antigens in human serum or plasma. The kits used were provided by Human Diagnostics (IMTEC-GASTRO-LIA) and the instrument used is semi-automated analyser OZOBLOT 40M provided by Medsource Ozone Biomedicals.

Principle of the test

The test is based on the principle of line immunoassay.The membrane was blocked to prevent unspecific reactions. Antigens were applied as lines on a nitrocellulose membrane. During incubation of a strip with diluted patient serum or plasma antibodies present in the sample was bind to the antigen(s) on the strip. For the detection of the bound antibodies a secondary horseradish peroxidase (HRP)-labelled anti-human IgG antibody was used. After addition of the substrate solution the appearance of blue lines (switching to brown after stopping) indicates the existence of auto-

antibodies against respective antigens.The interpretation of the test results takes place exclusively on the basis of the respective cut-off control regarded for each strip.

Results

In our study 56% and 44% female were participated most of the patients was 30-40 years followed by 20-30 years .The most common symptom Weight loss 48% was found in our study and followed by Chronic/intermittent diarrhea 46%, weakness 24%, Abdominal pain 20%,constipation 18%, Abdominal discomfort and vomiting.We found 32% patients had below 11micromol/L iron and 54% patients had below 12gm/100ml Hb means suffring from anaemia.The ASCA positive status was a predictor for Crohn s disease(CD). in our study out of 50 patients 20% was ASCA+, 2% ASCA++,4% ASCA+++and 4% ASCA was positive. In our study on the basis of the histological diagnosis, PCA+ patients were14%, PCA+ +patients 6%, PCA+ ++patients 12% and PCA Equivocal 4% was found.Tissue transglutaminase (tTG) has been identified as the autoantigen in CD. Out of 50 patients 28% tTG +,2% tTG ++,12% tTG +++, 2% tTG Equivocal was found.

Table 1 Demographic Profile of Patients

Gender	**N=50**	**%**
Male	28	56
Female	22	44
Age (years)		
Below 20	8	16
20-30	12	24
30-40	13	26
40-50	10	20
Above 50	7	14

Table 2 Different immunoglobulin's

Parameter	**+**	**++**	**+++**	**Equivocal**
Gliadin	13	1	8	1
tTG	14	1	6	1
ASCA	10	1	2	2
PCA	7	3	6	2
IF	4	1	4	2

Table 3 clinical symptoms

clinical symptoms	**n**	**%**
Abdominal pain	10	20
Chronic/intermittent diarrhea	23	46
Weight loss	24	48
constipation	9	18
Blood in Stool	4	8
Feeling of rectal urgency	1	2
weakness	12	24
vometing	1	2
Abdominal discomfort	5	10

Table 4 Biochemical Parameter

Vit B	**n**	**%**
Below 100	13	26
100-300	16	32
300-500	5	10
Above 500	16	32

Iron (micromol/L)		
Below (11micromol/L)	16	32
Above (11micromol/L)	34	68
Hb (gm/100ml)		
Below 12gm/100ml	27	54
Above	23	46

Discussion

In our study 56% and 44% female were participated most of the patients was 30-40 years followed by 20-30 years .The most common symptom Weight loss 48% was found in our study and followed by Chronic/intermittent diarrhea 46%, weakness 24%, Abdominal pain 20%,constipation 18%, Abdominal discomfort and vomiting .

The serum IgG-tTG levels for the all the patiebnts were evaluated in this study our finding was similar to other studies .Ingrid Dahlbomet al reported that the values for IgG-tTG showed a higher correlation (correlation coefficient [**r**] = 0.91) with those for IgG-EmA for the IgA-deficient subjects than for the IgA-sufficient subjects (**r** = 0.88). The overall concordance of the positive and negative results between IgG-tTG and IgG-EmA was 97%, and the IgG-tTG assay discriminated between IgG-EmA-positive and -negative subjects with IgA deficiency at a rate of 100%. Elevated levels of IgG-tTG and IgG-EmA were measured in 70% of the IgA-sufficient subjects. IgG-tTG detection with recombinant human tTG is a good alternative to IgG-EmA detection[26]. Barbara Zanini et al found that 100% specificity and ∞ positive likelihood ratio for duodenal atrophy was observed at a cut-off value of tissue-transglutaminase (Ttg) antibody 5 times higher than the upper limit of normal. Celiac disease(CD) diagnosis was confirmed by concordance with antiendomysial antibodies, and by reduction of t-TG titre in all patients and improvement of duodenal histology in 80% during gluten-free diet[27]. Thomas Mothes was found that Tissue transglutaminase (tTG) has been identified as the autoantigen in CD. Although ultimate diagnosis is based on histological analysis of small intestinal mucosa obtained via tissue biopsy, assessment of autoantibodies can provide substantial help in the evaluation of CD. Gliadin antibodies are directed against the native disease-provoking cereal proteins. Despite their initial usefulness, these antibodies have lost diagnostic importance due to their poor specificity and sensitivity as CD markers. Recently, it was found, however, that gliadin antibodies from sera of patients with active CD preferentially recognized deamidated gliadin peptides. The use of deamidated gliadin peptides in immunoassays has significantly improved the usefulness of gliadin antibodies in diagnosis of CD to that observed with autoantibody assay methods (endomysium antibodies, antibodies against tTG[28].

We found 32% patients had below 11micromol/L iron and 54% patients had below 12gm/100ml Hb means suffring from anaemia similar results was found in other study. Sedlákova et al.Was reported that the autoantibodies against gastric parietal cell H+/K+ ATPase had a sensitivity of 68.2% with a specificity of 91.7% for the diagnosis of pernicious anaemia.The respective rates for the autoantibodies against intrinsic factor were 40.9% and 98.6%. The combined sensitivity and specificity rates for both autoantibodies were 86.36% and 90.28%, respectively, the combined positive predictive value was 73.08% and the combined negative predictive value was 95.59%. they conclude that The detection of both autoantibodies is helpful in diagnosing pernicious anaemia and the combination of the two assays increases diagnostic sensitivity[29].

The ASCA positive status was a predictor for Crohn s disease(CD). in our study out of 50 patients 20% was ASCA+, 2% ASCA++,4% ASCA+++and 4% ASCA was positive .this finding were compared with other study.

Reese, et al reported that sixty studies comprising 3,841 Ulcerative colitis (UC) and 4,019 Crohn s disease (CD) patients were included. The ASCA+ with pANCA–test offered the best sensitivity for CD (54.6%) with 92.8% specificity and an area under the ROC (receiver operating characteristic) curve (AUC) of 0.85 (LR+ = 6.5, LR– = 0.5). Sensitivity and specificity of pANCA+ tests for UC were 55.3% and 88.5%, respectively (AUC of 0.82; LR+ = 4.5, LR– = 0.5). Sensitivity and specificity were improved to 70.3% and 93.4% in a pediatric subgroup when combined with an ASCA– test. Meta-regression analysis showed decreased diagnostic precision of ASCA for isolated colonic CD (RDOR = 0.3).30Walker L J ,et al was found thatASCA-positive status was a predictor for Crohn s disease(CD) with sensitivity of 57%, specificity of 87%, positive predictive value of 78% and negative predictive value of 68%. ASCA was associated with proximal (gastroduodenal and small bowel involvement) rather than purely colonic disease (P<years (0.001) and with a

more severe disease phenotype and requirement for surgery over a median follow-up time of 9 months P<0•0001). These data implicate ASCA as a specific marker of disease location and progression in CD, emphasizing the heterogeneity within inflammatory bowel disease (IBD)[31].

In our study on the basis of the histological diagnosis, PCA+ patients were14%, PCA+ +patients 6%,PCA+++ patients 12% and PCA Equivocal 4% was found and similar results was found in other study.Antonio Anticoetal was found that 83 patients (45.8%) tested positive for PCA and underwent gastroscopy with multiple gastric biopsies. On the basis of the histological diagnosis, PCA-positive patients were divided into 4 groups:

(1) 30 patients with chronic atrophic gastritis; they had high concentrations of PCA and gastrin and no detectable IFA;

(2) 14 subjects with metaplastic gastric atrophy; they had high PCA, IFA, and gastrin;

(3) 18 patients with nonspecific lymphocytic inflammation with increased PCA, normal gastrin levels, and absence of IFA;

(4) 21 patients with multifocal atrophic gastritis with "borderline" PCA, normal gastrin, absence of IFA and presence of anti-Hp in 100% of the cases.

They Conclude that The assay of four serological markers proved particularly effective in the diagnostic classification of gastritis and highly correlated with the histological profile. As such, this laboratory diagnostic profile may be considered an authentic "serological biopsy[32].

Conclusion

The evaluation of Clinical and serological auto-antibodies against gastrointestinal tract by gastrointestinal profile using LIA was beneficial for identification of the gastrointestinal diseases of the patients.

References

1. Ribeiro, Miguel, Nunes-Miranda, Julio D et al. "One hundred years of grain omics: identifying the glutens that feed the world". Journal of Proteome Research. (2013-11-01); 12 (11): 4702–4716.
2. Volta U, Cassani F, De Franchis R, et al. "Antibodies to gliadin in adult coeliac disease and dermatitis herpetiformis". Digestion. 1984; 30(4): 263-70
3. Volta U, Lenzi M, Lazzari R, et al. "Antibodies to gliadin detected by immunofluorescence and a micro-ELISA method: markers of active childhood and adult coeliac disease". Gut. 1985;26 (7):667–71.
4. Bateman EA, Ferry BL, Hall A, et al. "IgA antibodies of coeliac disease patients recognise a dominant T cell epitope of A-gliadin". Gut. 2004;53(9):1274–1278.
5. Crabbé P, Heremans J. "Selective IgA deficiency with steatorrhea. A new syndrome". Am J Med. 1967;42 (2):319–26.
6. Tucker NT, Barghuthy FS, Prihoda TJ, et al. "Antigliadin antibodies detected by enzyme-linked immunosorbent assay as a marker of childhood celiac disease". J. Pediatr. 1988;113(2): 286–9.
7. Collin P, Mäki M, Keyriläinen O, et al. "Selective IgA deficiency and coeliac disease". Scand J Gastroenterol. 1992;27 (5): 367–71.
8. Matsuo H ,Morita E, Tatham AS, et al. "Identification of the IgE-binding Epitope in -5 Gliadin, a Major Allergen in Wheat-dependent Exercise-induced Anaphylaxis". Journal of Biological Chemistry. 2003;279 (13):12135-40.
9. Kiraly R, Demény M, Fésüs L. "Protein transamidation by transglutaminase 2 in cells: a disputed Ca2+-dependent action of a multifunctional protein". The FEBS Journal. 2011;278 (24): 4717–39.
10. Klock C, Diraimondo TR, Khosla C. "Role of transglutaminase 2 in celiac disease pathogenesis. Seminars in Immunopathology. 2012;34 (4): 513–22.
11. Facchiano F, Facchiano A, Facchiano AM. "The role of transglutaminase-2 and its substrates in human diseases". Frontiers in Bioscience. 2006;11:1758–73.
12. Kiraly R, Csosz E, Kurtán T, et al. "Functional significance of five noncanonical Ca2+-binding sites of human transglutaminase 2 characterized by site-directed mutagenesis". The FEBS Journal. 2009;276 (23): 7083–96.
13. Krause I, Anaya JM, Fraser A, et al. "Anti-infectious antibodies and autoimmune-associated auto-antibodies in patients with type I diabetes mellitus and their close family members". Annals of the New York Academy of Sciences. 2009;1173 (1): 633–9.
14. Farrace MG, Picarelli A, Di Tola M, Sabbatella L, Marchione OP, Ippolito G, Piacentini M. "Presence of anti-"tissue" transglutaminase antibodies in inflammatory intestinal diseases: an apoptosis-associated event?". Cell Death & Differentiation. 2001;8 (7): 767–70.

15. Teichmann, Joachim, Voglau, Marcus J et al. "Antibodies to human tissue transglutaminase and alterations of vitamin D metabolism in ankylosing spondylitis and psoriatic arthritis". Rheumatology International. 2009;30 (12): 1559-63.
16. Picarelli A, Di Tola, M Sabbatella, L Vetrano, S Anania, MC, Spadaro A, Sorgi ML, Taccari E. "Anti-tissue transglutaminase antibodies in arthritic patients: a disease-specific finding?". Clinical Chemistry. 2003;49 (12): 2091–4.
17. Kaukinen K, Peraaho M, Collin P, et al. "Small-bowel mucosal tranglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: A Prospective and radmonized clinical study". Scand J Gastroenterol. 2005; 40 (5):564–572
18. Alpers DH, Russell-Jones G. "Gastric intrinsic factor: the gastric and small intestinal stages of cobalamin absorption. A personal journey". Biochimie. 2013;95 (5): 989–94.
19. Osborne D, Sobczynska-Malefora A. "Autoimmune mechanisms in pernicious anaemia & thyroid disease". Autoimmunity Reviews. 2015;14(9): 763–8.
20. Neumann WL, Coss E, Rugge M, et al. Autoimmune atrophic gastritis—pathogenesis, pathology and management". Nature Reviews. Gastroenterology &Hepatology. 2013;10 (9): 529–41.
21. Desplat-Jego S, Johanet C, Escande A et al. "Update on Anti-Saccharomyces cerevisiae antibodies, anti-nuclear associated anti-neutrophil antibodies and antibodies to exocrine pancreas detected by indirect immunofluorescence as biomarkers in chronic inflammatory bowel diseases: results of a multicenter study". World J. Gastroenterol. 2007;13(16): 2312 -18
22. Joossens S, Reinisch W, Vermeire S et al. "The value of serologic markers in indeterminate colitis: a prospective follow-up study". Gastroenterology. 2002; 122 (5): 1242-47.
23. Lahner E, Annibale B: Pernicious anemia: new insights from a gastroenterological point of view. World J Gastroenterol 2009;15(41):5121-28.
24. Toh BH, Van Driel IR, Gleeson PA: Pernicious anemia. N Eng J Med 1997;337(20):1441-48
25. Anna Pituch-Noworolska and Monika Mach-Tomalska: Autoimmunity of Gastrointestinal Tract .INTECHOPEN.COM 2017 .(5) 71-95
26. Ingrid Dahlbom, Martin Olsson, NahalKazemi Forooz,et al: Immunoglobulin G (IgG) Anti-Tissue Transglutaminase Antibodies Used as Markers for IgA-Deficient Celiac Disease Patients: Clinical And Diagnostic Laboratory Immunology. 2005, p. 254–258
27. Zanini B, Magni A, Caselani F, et al. High tissue-transglutaminase antibody level predicts small intestinal villous atrophy in adult patients at high risk of celiac disease. Dig Liver Dis. 2012;44:280-85
28. Mothes T. Deamidated Gliadin Peptides as Targets for Celiac Disease Specific Antibodies dvances in Clinical Chemistry. 2007; 44: 35-63
29. Sedláková L , Dubská L, Průcha M. [Pernicious anaemia--diagnostic benefit of the detection of autoantibodies against intrinsic factor and gastric parietal cells antigen H+/K+ ATPase :EpidemiolMikrobiolImunol 2010;59(3):126-32.
30. Reese, George E. Diagnostic Precision of Anti-Saccharomyces cerevisiae Antibodies and Perinuclear Antineutrophil Cytoplasmic Antibodies in Inflammatory Bowel Disease: American Journal of Gastroenterology: 2006; 101(10):2410-22.
31. Walker LJ,Aldhous MC,Drummond HE. Anti-Saccharomyces cerevisiae antibodies (ASCA) in Crohn's disease are associated with disease severity but not NOD2/CARD15 mutations: clinical and experimental immunology :2004 ,135:490-496.
32. Antonio A, Marilina T, Danilo V. A Clinical usefulness of the serological gastric biopsy for the diagnosis of chronic autoimmune gastritis; Journal ofImmunology Research, 2012: 5.

Conflict of Interest: Nil
Source of support:Nil