Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 09, pp.39403-39408, September, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

A STUDY OF CLINICAL, SEROLOGICAL AND HISTOPATHOLOGICAL PROFILE IN CELIAC DISEASE

***Dr. Pansi Gupta, Dr. Sonia Chhabra, Dr. Sunita Singh, Dr Parveen Malhotra and Dr. Rajeev Sen**

Department of Pathology, Pt.B.D.S. Post Grauate Institute of Medical Sciences, Rohtak – 124001 (Haryana) India

ARTICLE INFO

Article History:
Received 15th June, 2016
Received in revised form
20th July, 2016
Accepted 08th August, 2016
Published online 30th September, 2016

Key words:
Celiac Disease,
Modified Marsh Grading,
Anti-TTG.

ABSTRACT

Background: Celiac disease (CD), is a chronic immune-mediated disorder of small intestine that occurs in genetically predisposed populations. It is characterized by permanent intolerance to wheat gliadins and other cereal prolamins. The epidemiology of CD has iceberg characteristics¬ with more undiagnosed cases. The pathogenesis entails a T cell- mediated immune response with production of autoantibodies directed against tissue transglutaminase or endomysium. The diagnosis of CD is currently based on both typical small bowel biopsy findings with clinical and serological parameters.

Aims: This study conducted with aims to classify the endoscopic duodenal biopsies using modified Marsh grading in cases of suspected celiac disease and compare these grades with various clinical and serological parameters including serum tTG levels.

Setting and Design: Biopsies from second part of duodenum in total of 100 consecutive cases of suspected CD (on the basis of clinical and serological profile) formed the study group. Marsh grades were compared with anti-tTG levels, hemoglobin, endoscopy, and clinical presentations.

Materials and Methods: Histopathological diagnosis was established on routine haematoxylin and eosin stained sections. The histopathological grading was performed as per modified Marsh grading. Representative section was also subjected for immunohistochemical staining with antihuman CD3 antibody for evaluating intraepithelial lymphocytes. Comparison of these grades with the serological (anti tTg levels) and other clinical parameters (symptoms, weight, endoscopy and hemoglobin levels) were done.

Statistical Analysis: These data were subsequently analysed using SPSS 20.0 software. Chi square test and other relevant statistics were used to assess the relationship between two variables. P-value less than 0.05 was accepted as statistically significant.

Results and

Conclusions: Majority of patients presented with typical gastrointestinal symptoms and significantly correlated with higher Marsh grades (p= 0.0326) but atypical symptoms can be the primary presentation of the disease. Patients with higher serum anti-tTG levels, have a high-degree probability of duodenal damage. Anti-tTG levels have conclusively been proven to correlate with increasing histological grades (p=0.005). So, in selected conditions with strong clinical suspicion and high titres of anti tTG, a duodenal biopsy may be avoided especially in children and it could be the basis to prescribe a GFD.

Citation: Dr. Pansi Gupta, Dr. Sonia Chhabra, Dr. Sunita Singh, Dr Parveen Malhotra and Dr. Rajeev Sen, 2016. "A study of clinical, serological and histopathological profile in celiac disease", *International Journal of Current Research*, 8, (09), 39403-39408.

INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated disorder of small intestine that occurs in genetically predisposed populations (Harris *et al*., 2012). The pathogenesis entails a T cell- mediated immune response with production of autoantibodies directed against tissue transglutaminase or endomysium. Wheat, rye and barley prolamines are toxic for celiac patients due to their high glutamine and proline content (Volta and Villanacci, 2011). Initially the suspicion of CD was based on clinical gastrointestinal (GI) symptoms. Subsequently, the disease has been found with variety of atypical symptoms and even in asymptomatic subjects (Lohi *et al*., 2007).

****Corresponding author: Dr. Pansi Gupta,***
Department of Pathology, Pt.B.D.S. Post Grauate Institute of Medical Sciences, Rohtak – 124001 (Haryana) India.

The histologic changes in CD vary from severe villous atrophy to more subtle changes (with or without increased density of intraepithelial lymphocytes and crypt hyperplasia). Although villous atrophy is not specific to CD. Serology has become increasingly relevant to CD diagnosis. Anti-tissue transglutaminase antibodies) are the most sensitive test for CD (Volta and Villanacci, 2011). Cases with high celiac autoantibody might not need histological confirmation. Consensus is needed on the diagnostic criteria for cases with mild mucosal changes or high antibody levels.

MATERIALS AND METHODS

Biopsies from a total of 100 consecutive cases of suspected CD formed the study group. Various symptoms considered for clinical suspicious of CD including GI symptoms (diarrhea, vomiting and pain abdomen), and other atypical symptoms.

Complete history and clinical examination was done to rule out other causes of malabsorption. The biopsy was taken from second part of duodenum through eosophagoduodenoscopy with the assessment of duodenal endoscopic markers including scalloping of folds, grooving, and nodularity of mucosa. Histopathological diagnosis was established on routine haematoxylin and eosin stained sections (Bancroft and Layton, 2012). Immunohistochemical (IHC) staining (Jackson, 2012) with antihuman CD3 antibody was done for evaluating intraepithelial lymphocytes (IELs) with positive (tonsillar tissue) and negative (substituting the primary antibody with an antibody of irrelevant specificity) controls. The histopathological grading was performed as per modified Marsh grading (Oberhuber, 1999). (Table-I) Anti-tTG levels were performed by enzyme-linked immunosorbent assay (ELISA). Patients with anti-tTG>15 U/ml were considered to be suspicious even the absence of clinical symptoms. These patients were again divided into two groups, including 15-99U/ml and ≥100U/ml. Complete hematologic work-up was performed to classify anaemia. Reference range for anaemia for age was taken from WHO guidelines (Lerner, 2011). Serum iron studies could not be performed due to financial limitations. Reference range for weight for age was as per Advanced Pediatric Life Support guidelines (Seddon *et al.*, 2012). All patients were started on gluten-free diet (GFD) along with hematinics. Celiac status of a patient was confirmed by clinical response to GFD, defined as improved symptoms such as weight gain, and increase in hemoglobin concentration at 4 and 24 weeks.

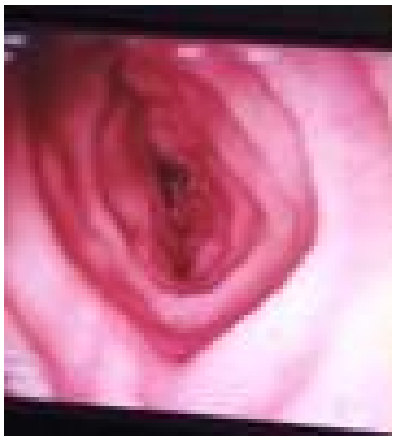

Fig 1a. Endoscopic View of Duodenum Indicating Nodularity

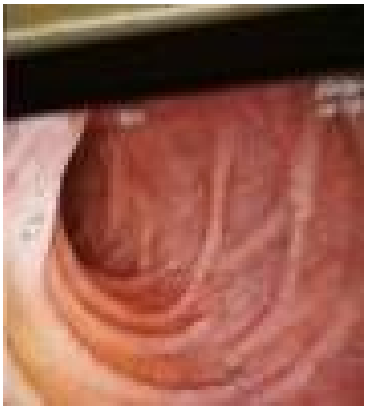

Fig 1b: Endoscopic View of Duodenum Indicating Granularity

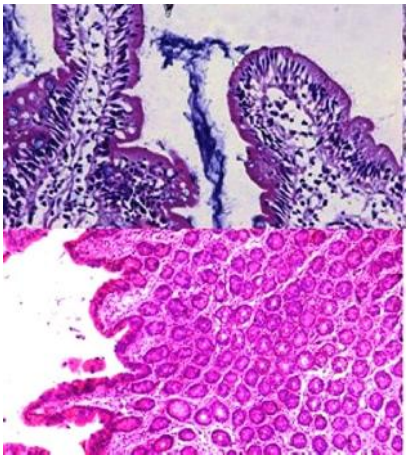

Fig 2a: H&E And Ihc View Showing Modified Marsh Grade I (400x)

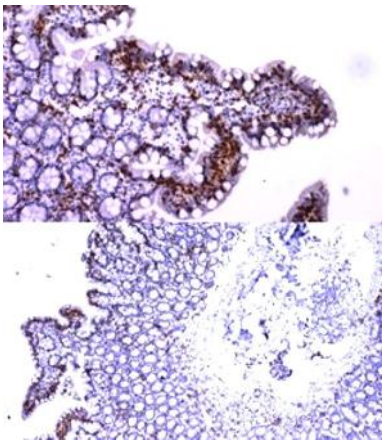

Fig 2b
H&E And Ihc View Showing Modified Marsh Grade Ii (200x)

Comparison of these histological grades with the anti-tTg levels and other clinical parameters (symptoms, weight, endoscopy and hemoglobin levels) were done using SPSS 20.0 software. Chi square test and other relevant statistics were used to assess the relationship between two variables. P-value less than 0.05 was accepted as statistically significant.

RESULTS

Biopsies from second part of duodenum of 100 consecutive cases of suspected CD formed the study group. Maximum number of the cases (71%) lied in age group 11-30 years. Mean age of study group was 21.16 year. Children and adolescents (<18 years) constituted 40% while adults (≥18 years) constituted 60% of study group.

Table 1. Modified Marsh Classification of histological findings in celiac disease (Seddon *et al*., 2012)

Marsh Type	IEL/ 100 enterocytes	Crypt hyperplasia	Villi
0	<40	Normal	Normal
1	>40	Normal	Normal
2	>40	Increased	Normal
3a	>40	Increased	Mild atrophy
3b	>40	Increased	Marked atrophy
3c	>40	Increased	Complete atrophy
4	<40	Normal	Complete atrophy

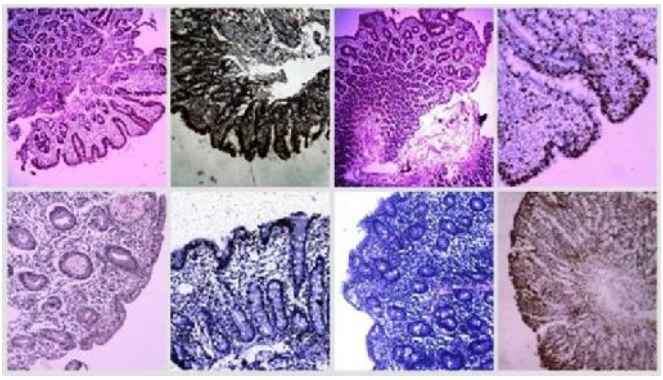

Fig 3a. H and E And Ihc View Showing Modified Marsh Grade IIIA (100x)
Fig 3b. H and E And Ihc View Showing Modified Marsh Grade IIIB (100x)
Fig 3c. H and E And Ihc View Showing Modified Marsh Grade IIIC (200x)
Fig 3d. H and E And Ihc View Showing Modified Marsh Grade Iv (40x)

Maximum number of the cases among children and adults were belong to age group 11-17 (26) and 21-30 (32) years respectively. Female patients constituted 54% of total study group. Out of 40 children, 23 were females and 17 were males. Out of 60 adults, 31 were females and 29 were males. Histologically, 87 cases were positive for CD. Most of them belonged to grade IIIa (36 cases). Remaining 51 cases were distributed as follows: Grade I (13), grade II (10), grade IIIb (16), grade IIIc (10) and grade IV (02). Maximum number of patients (82%) presented with typical/gastrointestinal symptoms (including diarrhoea, abdominal pain and vomiting). Among these, 20 cases presented with all the three GI symptoms. Diarrhoea (85.36%) was the most common complaint. Other 18 cases had atypical symptoms, in which short stature (07), hypothyroidism (03), and diabetes (03) were predominant in study group.

Difference in presentation of GI symptoms between children and adult groups was statistically insignificant (p=0.339). Preponderance of GI over atypical symptoms was statistically significant (p= 0.0001). Gastrointestinal and atypical symptoms predominated in higher modified Marsh grades (III and IV), but this was significant statistically (p=0.0326) for GI symptoms only. Anaemia was diagnosed in majority of our cases (90%). Anaemia was observed more in adults (93.33%) than children (85%) which was statistically insignificant (p=0.161). There was a fall in hemoglobin levels with higher grades in both children and adults, but this was statistically insignificant (p=0.729 and 0.726 respectively). Sixty three cases were underweight. Underweight were seen more in children (70%) than adults (58.33%) and difference was statistically insignificant (p=0.117). There was a decrease in weight with higher Marsh grades in both children and adults but not significant statistically (p=0.189 and 0.40 respectively). Endoscopic findings (nodularity, granularity and scalloping) were present in 45% of the cases. Maximum endoscopically positive cases had scalloping (88.89%). Distribution of endoscopic findings in children and adults was not significant (p=0.682). Increasing severity of endoscopic damage had been found with increasing Marsh grades, but was statistically insignificant (p=0.431). The sensitivity, specificity, positive and negative predictive value for endoscopic finding in relation to CD were 47.13%, 69.23%, 91.11% and 16.36% respectively.

On the basis of serum tTG levels, cases were categorised into two groups with cut off value of 15U/ml. Sixteen cases had tTG level <15 U/ml. Eighty four cases had serum tTG levels (≥ 15U/ml); among these 53 cases had serum tTG ≥100 U/ml. (Table II) We found a statistically significant increase in anti-tTG values from histologically milder forms of the disease to more severe forms (p= 0.005). Though 16 cases had tTG <15 U/ml, but histologically different grades of CD was seen in 14 cases, of which grade IIIa was predominated. Out of rest 84 cases with tTG ≥ 15 U/ml, 11 cases were histologically negative. The sensitivity, specificity, positive predictive value and negative predictive value for serum tTG levels in relation to CD were 83.91%, 15.38%, 86.90% and 12.50% respectively. Fifty three cases with higher tTG level (≥100 U/ml) predominated in higher grades, which was statistically significant (p= 0.009). (Table II)

Table 2. Association between tissue transglutaminase (ttg) and histopathological grades

GRADES	TTG <15	TTG=15 -99.99	TTG <100	TTG ≥100	TOTAL
I	2	8	10	3	13
II	4	3	7	3	10
IIIa	5	8	13	23	36
IIIb	1	2	3	13	16
IIIc	2	4	6	4	10
IV	0	0	0	2	02
NOT SEEN	2	6	8	5	13
TOTAL	16	31	47	53	100

DISCUSSION

The disease was first described in 1st century AD. Since then, insight into CD has undergone a revolutionary development regarding epidemiology, diagnostics and treatment. CD also known as intestinal infantilism, idiopathic steatorrhoea, non-tropical sprue, and gluten-sensitive enteropathy (Kennedy and Feighery, 2000). Various etiological factors are considered for CD including genetic (HLA class II antigen) and environmental risk factors (Kennedy and Feighery, 2000) including GI infections (Plot and Amital, 2009). Gliadin is a glycoprotein extract from gluten, directly toxic to the enterocytes of individuals with CD. Transglutaminase enzyme crosslinks gliadin and causes specific deamidation of glutamine into glutamic acid. With such deamidation, the gliadin peptides are able to be more efficiently presented to gliadin-reactive CD-4 T cells. Without Transglutaminase, it is believed that gliadin is less immunogenic. Thus Transglutaminase autoantibodies play a role in disease pathogenesis, but lacks sufficient supportive evidence (Barker and Liu, 2008).The epidemiology of CD has iceberg characteristics as there are more undiagnosed cases. The female-to-male ratio is 2:1. The prevalence of CD is globally 1%. The prevalence of CD in India is nearly the same as that in Western Caucasian populations (Gujral *et al.*, 2012).The clinical presentation of CD depends on the severity of small intestine damage. Depending on the clinical, histopathological and immunological features, CD can be classified into the following forms: classical (typical), subclinical (atypical or mono-symptomatic), silent (asymptomatic) and potential/latent. A number of idiopathic neurological disorders associated with atypical CD such as epilepsy, cerebellar ataxia, intellectual deterioration, peripheral neuropathy and multiple sclerosis. Reproductive system dysfunction may be observed in the form of menstrual irregularities, infertility and obstretic complication in females

and impotence, infertility and abnormalities of sperm in males (Volta and Villanacci, 2011). Several disorders are associated with CD. The most well-known association is with dermatitis herpetiformis and other pathological conditions include autoimmune disorders (diabetes mellitus type 1, thyroid disorders, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, alopecia, vitiligo, Addison's disease, Sjogren's syndrome, IgA nephropathy and IgA deficiency), idiopathic disorders (primitive dilated cardiomyopathy, atopy and inflammatory bowel disease) and chromosome disorders (Down, Turner and Williams syndromes) (Volta and Villanacci, 2011; Collin *et al*., 1994; Lauret, 2013). These associations may be due to common pathogenic basis. Various malignancies have been linked to CD such as enteropathy-associated T-cell lymphoma, multiple myeloma, Hodgkin's disease, gastric cancer, melanoma of the skin, renal cell carcinoma, breast cancer, carcinoid tumour of the pancreas, meningioma, lung cancer, liposarcoma of the abdomen and prostate cancer (Collin *et al*., 1994).In current practice, the diagnostic protocol of CD is based on (1) History and clinical presentation; (2) Serological screening; (3) Histological findings; (4) Obvious clinical and serological response to a gluten free diet; (5) Age >2 years and (6) Exclusion of other clinical conditions mimicking CD (Lohi *et al*., 2007). It has been suggested that cases with high celiac autoantibody values might not need histological confirmation. Consensus is needed on the diagnostic criteria for cases with mild mucosal changes or high antibody levels (Bai *et al*., 2013).

Auto-antibodies against endomysial (EMA), tissue transglutaminase (tTG), gliadin and deamidated gliadin peptides (DGPs) are measure in serologic tests. All of these antibodies are immunoglobulin A (IgA) or G (IgG) type. Anti-tTG antibodies are highly sensitive and specific for CD. ELISA tests for IgA anti-tTG antibodies are now widely available. But there are still substantial differences between the cut-off points suggested by the manufacturers.[15] The characteristic findings of CD on endoscopy include: a) Scalloped folds, fissures and a mosaic pattern, b) Flattened folds, and c) Smaller size and/or disappearance of folds with maximum insufflations (Kalhan *et al*., 2011). An intestinal biopsy and serology represents the gold standard in diagnosing CD. It is also essential that biopsy samples are correctly oriented to avoid tangential artifacts and under or over estimation of villi atrophy and counting of IELs. Modified Marsh classification allows a short and precise classification of the intestinal lesion at the time of first diagnosis and follow up Giardiasis, Tropical/Collagenous/ Hypogammaglobulinaemic sprue, Autoimmune enteropathy, Infectious gastroenteritis, Intestinal T-cell lymphoma and Food protein hypersensitivity are differential diagnosis of CD (Oberhuber *et al*., 1999). Adult celiac were more than children in our study. In both groups, females were predominant. Maximm number of patients presented with GI symptoms than atypical symptoms in which diarrhoea was the most common complaint as seen in other studies in literature (Kalhan *et al*., 2011; Makhajda *et al*., 2011; Osman *et al*., 2012; Thakur *et al*., 2006; Donaldson *et al*., 2007; Barker *et al*., 2005). Presentation of gastrointestinal symptoms was statistically significant over atypical symptoms (p=0.0001).Typical GI symptoms predominated in higher histological grades, which is statistically significant (p=0.0326). Study by Donaldson *et al* and other authers suggested that the severity of symptoms followed a linear trend toward more severe histopathological grades (Kalhan *et al*., 2011; Makhajda *et al*., 2011; Donaldson *et al*., 2007; Donaldson *et al*., 2008; Vivas *et al*., 2008). Anaemia was diagnosed in majority of our patients (90%). Prevalence of anaemia among celiac patients was also observed in various studies (Thakur *et al*., 2006; Donaldson *et al*., 2008). Anaemia was observed more in adults as compared to children. There was a fall in hemoglobin levels with increase in tTG levels and higher grades in both children and adults, which was statistically insignificant. However Dipper *et al* found significant association between anaemia and anti-tTG antibody (Dipper *et al*., 2009). This may be due to more specific test was used for diagnosis of anaemia like serum ferritin. We observed that 63% celiac patients were underweight and more in children. There was a decrease in weight with increase in tTG levels and higher Marsh grades in both children and adults. This decrease in weight was statistically significant in adults only (p=0.05). Similar association was observed by Dipper *et al*. (2009). Endoscopic findings (nodularity, granularity and scalloping) were absent in 55% cases. Sensivity, specificity, positive and negative predictive value were 47.13%, 69.23%, 91.11% and 16.36% respectively for the endoscopic findings. Association between endoscopic findings with various Marsh grades was not significant (p=0.431). Similar insignificant observations were found by Oxentenko *et al*. (2002) However in few studies, these endoscopic changes were significantly associated (Tursi *et al*., 2002; Reyes *et al*., 2008). This may be due to various factors including selection of the patients, sensitivity and specificity of endoscopic method used or histological criteria used for evaluation of duodenal biopsy.

We classified the study population in two groups on the basis of cut off value of tTG (15U/ml). Eighty four cases had serum tTG levels ≥15U/ml, among these 53 cases had ≥100 U/ml. Concordant findings were also observed by Donaldson *et al* and Kotze *et al*. (2007) (Kotze *et al*., 2003) Sensitivity, specificity, positive and negative predictive value were 83.91%,15.38%, 86.90% 12.50% for serum tTG levels. We found a statistically significant increase in anti-tTG values from histologically milder to severe forms (p=0.005). Similar observations were found in various studies (Kalhan *et al*., 2011; Osman *et al*., 2012; Donaldson *et al*., 2007; Donaldson *et al*., 2008; Vivas *et al*., 2008; Kotze *et al*., 2003; Mubarak *et al*., 2012; Alessio *et al*., 2012). Fourteen case had different grades with tTG <15 U/ml. Similar observation was seen by Kalhan *et al*. (2011). Higher tTG level (≥100 U/ml) was predominant in higher grades, which was statistically significant (p=0.009). It has been proposed that higher IgA tTG level can be considered diagnostic tool for CD even without biopsy (Barker *et al*., 2005; Vivas *et al*., 2008; Mubarak *et al*., 2012). Allesio and colleagues concluded high probability of duodenal damage if anti-tTG serology ≥ 7 times the cut-off (Alessio *et al*., 2012).

Conclusion

To conclude, majority of patients with CD present with typical gastrointestinal signs and symptoms but atypical symptoms can be the primary presentation. Endoscopic markers have been predictive of the disease and are guides for directing small bowel biopsies in patients suspected of having CD. Patients with serum anti-tTG levels ≥100U/ml, have a high-degree probability of duodenal damage. So, in selected conditions with strong clinical suspicion and high titres of anti tTG, a duodenal biopsy may be avoided and it could be the

basis to prescribe GFD. Increased awareness among clinicians and pathologists especially in its atypical forms, will aid in diagnosing more cases from the "celiac iceberg".

Acknowledgement

I am deeply indebted to my esteemed teacher and my supervisor Dr. Sonia Chhabra, Professor, Department of Pathology, Pt. B.D. Sharma University of Health Sciences, Rohtak (Haryana). With high esteem and profound regards, I take the privilege to acknowledge my sincere gratitude to my worthy teacher and co-supervisor, Dr. Parveen Malhotra, Associate Professor and Head, Department of Gastroenterology, Pt. B.D. Sharma University of Health Sciences, Rohtak, for his expert guidance, constant encouragement, sublime suggestions, outstanding help and painstaking support throughout the entire span of present work. My heart fills with gratitude while expressing my sincere thanks and indebtedness to my esteemed teacher Dr. Rajeev Sen, Senior Professor and Head and Dr. Sunita Singh, Professor Department of Pathology, Pt. B.D. Sharma University of Health Sciences, Rohtak for his easy approachability, excellent foresight, constructive criticism, keen interest, inexhaustable encouragement and unhesitant and generous help. It's my fortune to gratefully acknowledge the support of some special individuals.

REFERENCES

Alessio, M.G., Tonutti, E., Brusca, I., Radice, A., Licini, L., Sonzogni, A. *et al*. 2012. Correlation between IgA tissue transglutaminase antibody ratio and histological finding in celiac disease. *J Pediatr Gastroenterol Nutr*., 55:44-9.

Bai, J.C., Fried, M., Corazza, G.R., Schuppan, D., Farthing, M., Catassi, C. *et al*. 2013. World Gastroenterology Organisation global guidelines on celiac disease. *J Clin Gastroenterol*, 47:121-6.

Bancroft, J.D., Layton, C. 2012. The hematoxylins and Eosin. In: Suvarna SK, Layton C, Bancroft JD, editors. Theory and Practice of Histological techniques. 7th ed. New York: Churchill Livingstone; p. 173-86.

Barker, C.C., Mitton, C., Jevon, G., Mock, T. 2005. Tissue transglutaminase antibody, celiac disease, biopsy. Pediatrics, 115:1341-6.

Barker, J.M., Liu, E. 2008. Celiac disease: Pathophysiology, clinical manifestations and associated autoimmune conditions. Adv Pediatr, 55:349-65.

Collin, P., Reunala, T., Pukkala, E., Laippala, P., Keyrilainen, O., Pasternack, A. *et al*. 1994. Coeliac disease-associated disorders and survival. Gut 35:1215-8.

Dipper, C.R., Maitra, S., Thomas, R., Lamb, C.A., McLean-Tooke, A.P.C. Ward, R. *et al*. 2009. Anti-tissue transglutaminase antibodies in the follow-up of adult coeliac disease. *Aliment Pharmacol Ther.*, 30:236-44.

Donaldson, M.R., Book, L.S., Leiferman, K.M., Zone, J.J., Neuhausen, S.L. 2008. Strongly positive tissue transglutaminase antibodies are associated with Marsh 3 histopathology in adult and pediatric celiac disease. *J Clin Gastroenterol*, 42:256-60.

Donaldson, M.R., Firth, S.D., Wimpee, H., Leiferman, K.M., Zone, J.J., Horsley, W. *et al*. 2007. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. *Clin Gastroenterol Hepatol5*:567-73.

Gujral, N., Freeman, H.J., Thomson, A.B. 2012. Celiac disease: Prevalence, diagnosis, pathogenesis and treatment. *World J Gastroenterol*, 18:6036-59.

Harris, L.A., Park, J.Y., Voltaggio, L., Himlin, D.L. 2012. Celiac disease: clinical, endoscopic and histopathologic review. Gastrointest endosc76:625-40.

Jackson, P., Blythe, D. 2012. Immunohistochemical techniques. In: Suvarna SK, Layton C, Bancroft JD, editors. Theory and Practice of Histological techniques. 7th ed. New York: Churchill Livingstone; p. 381-426.

Kalhan, S., Joseph, P., Sharma, S., Dubey, S., Dudani, S., Dixit, M.2011. Comparative study of histopathological Marsh grading with clinical and serological parameters in celiac iceberg of north India. *Indian J Pathol Microbiol*, 54:279-83.

Kennedy, N.P., Feighery, C. 2000. Clinical features of coeliac disease today. *Biomed & Pharmacother*, 54:373-80.

Kotze, L.M., Utiyama, S.R., Nisihara, R.M., De Camargo, V.F., Ioshii, S.O. 2003. IgA class anti-endomysial and anti-tissue transglutaminase antibodies in relation to duodenal mucosa changes in celiac disease. Pathology 2003;35:56-60.

Lauret, E., Rodrigo, L. 2013. Celiac disease and autoimmune-associated conditions. Biomed Res Int;2013:127589. doi:10.1155/2013/127589.

Lerner, N.B., Sills, R. 2011. Iron deficiency anaemia. In: Kliegman R, Nelson WE, editors. Nelson Textbook of Pediatrics. 19th ed. Philadelphia: Elsevier Suanders; p. 1655-7.

Lohi, S., Mustalahti, K., Kaukinen, K., Laurila, K., Collin, P., Rissanen, H. *et al*. 2007. Increasing prevalence of coeliac disease over time. Aliment Pharmacol Ther, 26:1217-25.

Makhajda, E., Tiszai, A., Lenart, Z., Balint, L., Tiszlavicz, L., Wittmann, T. 2011. Comparison the modified Marsh classification, The clinical symptoms and laboratory parameters in coeliac disease (CD). Z *Gastroenterol*;8:49-55.

Mubarak, A., Wolters, V.M., Gmelig-Meyling, F.H., Ten Kate, F.J., Houwen, R.H. 2012. Tissue transglutaminase levels above 100 U/mL and celiac disease: a prospective study. *World J Gastroenterol* 18:4399-403.

Oberhuber, G., Granditsch, G., Vogelsang, H. 1999. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol*, 11:1185-94.

Osman, M.T., Nasiry, S.A., Fayadh, M.H., Taha, B.I. 2012. Innovative clinicopathological study of an iraqi patients group suspected to have coeliac diasease. IJMHS2:98-103.

Oxentenko, A.S., Grisolano, S.W., Murray, J.A., Burgart, L.J., Dierkhising, R.A., Alexander, J.A. 2002. The insensitivity of endoscopic markers in celiac disease. *Am J Gastroenterol*, 97:933-8.

Plot, L., Amital, H. 2009. Infectious associations of celiac disease. Autoimmun Rev., 8:316-9.

Reyes, H., Niveloni, S., Moreno, M.L., Vazquez, H., Jer, H.H., Argonz, J. *et al*. 2008. A prospective evaluation of endoscopic markers for identifying celiac disease in patients with high and low probability of having the disease. *Acta Gastroenterol Latinoam.*, 38:178-86.

Seddon, C., Lockitt, L., Dhanjal, S., Eisenhut, M. 2012. Validation of Advanced Paediatric Life Support Formulas for Weight Calculation in a Multiethnic Population. ISRN Pediatrics, 2012:869634. doi:10.5402/2012/869634.

Thakur, B., Mishra, P., Desai, N., Thakur, S., Alexander, J., Sawant, P. 2006. Profile of chronic small-bowel diarrhea in

adults in Western India: A hospital-based study. *Trop Gastroenterol*, 27:84-6.

Tursi, A., Brandimarte, G., Giorgetti, G.M., Gigliobianco, A.2002. Endoscopic features of celiac disease in adults and their correlation with age, histological damage, and clinical form of the disease. *Endoscopy*, 34:787-92.

Vivas, S., Ruiz de Morales, J.M., Fernandez, M., Hernando, M., Herrero, B., Casqueiro, J. *et al*. 2008. Age-related clinical, serological, and histopathological features of celiac disease. Am J Gastroenterol, 103:2360-5.

Volta, U., Villanacci, V. 2011. Celiac disease: diagnostic criteria in progress. *Cell Mol Immunol*., 8:96-102.

****