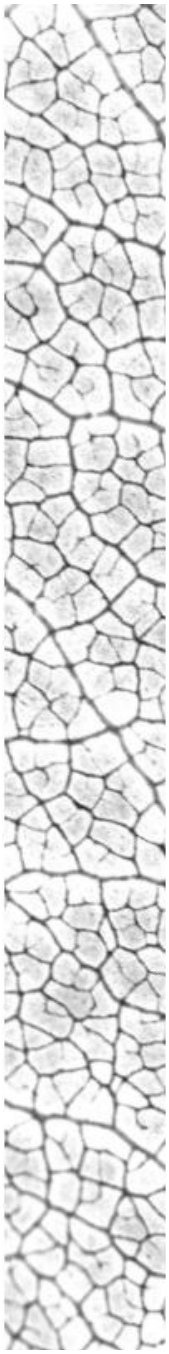

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Immunohistochemical evaluation of CD3 T-cell lymphocyte and CD20 B-cell markers in Iraqi patients with celiac disease

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Abstract

Celiac disease is an autoimmune disorder that affects the small intestine mucosa of a proportion of people with human leukocyte antigen (HLA)-DQ2 or -DQ8 haplotypes following gluten intake. The main pathologic lesion is composed of villous reduction in height with increase in crypt activity that is manifested by higher number of cell proliferation in addition to inflammatory infiltrate of epithelium and lamina propria with predominance of lymphocytes. Immunohistochemistry (IHC) is an important auxiliary method for pathologists as it specifically visualizes distribution and amount of a certain molecule in the tissue using specific antigen-antibody reaction. The aim of the current study was to correlate CD3 and CD20 immunohistochemical expression of lymphocytic population to histopathological changes of celiac disease. The current study was carried out at Al-Diwaniyah teaching hospital and a number of private histopathology laboratories in Al-Diwaniyah province, mid-Euphrates region of Iraq. The study was started on January 2019 and ended on March 2020. Sixty patients with clinical features suggestive of celiac disease were enrolled in the current study. Every patient was subjected to esaphagogastrodeuodenoscopy (OGD) at the gastrointestinal center. A number of duodenal biopsies were taken for each patient and were preserved in 10 % formalin solution. Later on, these biopsies were referred to private histopathology laboratory for purpose of histological handling and perpetration of histological sections.CD3 immunohistochemical expression was as following: Less than half staining pattern was seen in 10 (16.7 %) cases, approximately half staining pattern was seen in 10 (16.7 %) cases and most cases (56.7 %) showed more than half sating pattern. This fining correlated with histological finding that most cases are March 3 cases. CD20 immunohistochemical expression was as following: Mild crypt involvement was reported in 16.7 %, Moderate crypt involvement was reported in 43.3 % and intense crypt involvement was reported in 40.0 %.

Keywords: immunohistochemistry CD3, CD20, celiac disease, Iraq

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INTRODUCTION

Celiac disease is an autoimmune disorder that affects the small intestine mucosa of a proportion of people with human leukocyte antigen (HLA)-DQ2 or -DQ8 haplotypes following gluten intake (Lebwohl *et al*., 2018). Its prevalence, according to serologic tests, is 0.5% in North America and Africa, 0.4% in South America, 0.8% in Oceania and Europe, 0.6% in Asia, with predominantly female targeting (0.6% vs. 0.4%; $p < 0.001$) (Singh *et al*., 2018). The main pathologic lesion is composed of villous reduction in height with increase in crypt activity that is manifested by higher number of cell proliferation in addition to inflammatory infiltrate of epithelium and lamina propria with predominance of lymphocytes (Corazza *et al*., 2007). The clinical image is multifaceted, ranging from an overt malabsorption syndrome to seemingly asymptomatic manifestations of anaemia, isolated weakness, ambiguous hypertransaminasaemia, infertility, peripheral and core neurological disorders, osteopenia, short stature and dental enamel defects (Leffler *et al*., 2015).

In the vast majority of cases, a gluten-free diet results in an almost complete recovery of both mucosal lesions and clinical features (Lebwohl *et al*., 2018). Celiac disease is known to be associated with a number of autoimmune disorders and on top of the list is type 1 diabetes mellitus; the most probable explanation is the sharing of the same genetic and environmental factors in common between those autoimmune disorders

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(Lundin and Wijmenga, 2015). Nowadays, coeliac disease is widely considered an autoimmune disease that results from an aberrant immune response to gluten derivatives found in wheat, barley and seed in genetically susceptible people (Green and Jabri, 2006; Sollid and Jabri, 2005).

From histological point of view the alterations can be assessed according to "The modified Marsh-Oberhuber classification". Lesions can be described in terms of a range of architectural, cytological and ultrastructural characteristics that combine to create a blurry range of histopathological permutations. These characteristics alone are not unique and should be assumed to be shared among a range of diseases affecting the small intestine. Several characteristics of coeliac disease, such as increased intraepithelial lymphocytes (IELs), crypt hyperplasia and villous atrophy, may be indicative when screening small bowel biopsy specimens (Oberhuber *et al*., 1999).

Immunohistochemistry (IHC) is an important auxiliary method for pathologists as it specifically visualizes distribution and amount of a certain molecule in the tissue using specific antigen-antibody reaction. The applications of IHC have recently been expanded explosively as more and more molecules involved in pathogenesis, diagnosis, and treatment of diseases are discovered. The unique feature that makes IHC stand out among many other laboratory tests is that it is performed without destruction of histologic architecture, and thus the assessment of an expression pattern of the molecule is possible in the context of microenvironment (Kim *et al*., 2016). The aim of the current study was to correlate CD3 and CD20 immunohistochemical expression of lymphocytic population to histopathological changes of celiac disease.

MATERIALS AND METHODS

Study Design

The current study was carried out at Al-Diwaniyah teaching hospital and a number of private histopathology laboratories in Al-Diwaniyah province, mid-Euphrates region of Iraq. The study was started on January 2019 and ended on March 2020.

Participants

Sixty patients with clinical features suggestive of celiac disease were enrolled in the current study. Every patient was subjected to esaphagogastrodeuodenoscopy (OGD) at the gastrointestinal center.

A number of duodenal biopsies were taken for each patient and were preserved in 10 % formalin solution. Later on, these biopsies were referred to private histopathology laboratory for purpose of histological handling and perpetration of histological sections.

Five Paraffin sections of duodenal biopsy were retrieved from these laboratories. One section was made from each paraffin block and stained by the conventional hematoxylin and eosin (H & E) stain to be reviewed by two pathologists who confirmed the diagnosis and other related histological features. Four further sections were made for immunohistochemical staining by CD3, CD20, lambda and kappa antibodies.

Table 1. Demographic characteristics of celiac disease patients

Characteristic		Result
Number of cases		60
Age (years)		
	Mean ±SD	22.1 ±11.60
	Range	3 -40
	< 10, *n* (%)	12 (20 %)
	10-19, *n* (%)	12 (20 %)
	20-29, *n* (%)	16 (26.7 %)
	30-40, *n* (%)	20 (33.3 %)
Gender		
	Male, *n* (%)	26 (43.3 %)
	Female, *n* (%)	34 (56.7 %)

The sections made were thin (5 µm) and were put on positive charged slides. Deparaffinization step was then done using xylene bath, three times 5 minutes for each. Rehydration step was done using descending ethanol concentrations baths 95%, 90% and 75 %, 5 minutes for each then followed by distil water bath for 5 minutes. The antigen retrieval step was carried out at microwave with EDTA buffer (pH of 8) for 20 minutes. Endogenous peroxidase was inhibited by running tissue through 6 % oxygenated water for 5 minutes. Incubation with primary antibody was then made after washing with PBS solution for 5 minutes and the incubation step lasted for one hour at 37 ºC. "The primary antibodies used were H. pylori (monoclonal human anti- CD3, CD20, kappa and lambda; DAKO Cytomation, Denmark) in 1:50 dilution". The tissues were then washed with PBS/ Tween and then incubated with En Vision HPR detection system for 30 minutes at room temperature. Washing with water was then carried out and then followed by signal visualization using 3-3' diaminobenzidine DAB. Hematoxylin was then used for nuclei counterstaining. Dehydration step was then performed with ascending ethanol concentrations baths followed by clearing step and mounting using Canada balsam and covered by cover slip.

The degree of histopathological changes including chronic inflammation, activity and atrophy where done according to the modified Marsh-Oberhuber classification (Oberhuber *et al*., 1999; Yerkinbayeva, et al, 2015).

RESULTS

The demographic characteristics of celiac disease patients enrolled in the current study are outlined in **Table 1**. The mean age was 22.1 ±11.60 years and there was almost even distribution of cases according to age intervals. There was 26 (43.3 %) males and 34 (56.7 %) females.

Table 2. Frequency distribution of cases according to March classification

March class	Number	%
1-2	10	16.7
3A	16	26.7
3B	22	36.7
3C	12	20

Table 3. CD3 immunohistochemical expression

CD3 expression	Frequency	%
< half	10	16.7
Approximately half	16	26.7
More than half	34	56.7

Table 4. CD20 immunohistochemical expression

CD20 expression	Number	%
Mild crypt involvement	10	16.7
moderate crypt involvement	26	43.3
Intense crypt involvement	24	40

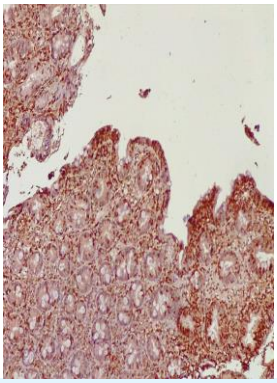

Fig. 1. CD3 immunohistochemical expression. Diffuse involvement of more than half of villi in patient with sever villous atrophy corresponding to March 3C. 10X

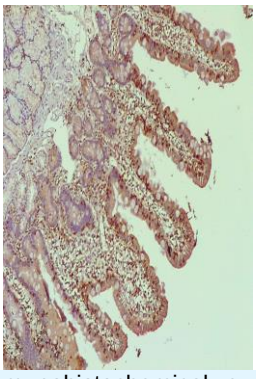

Fig. 2. CD3 immunohistochemical expression. Diffuse involvement of more than half of villi in patient with mild villous atrophy corresponding to March 3A. intraepithelial lymphocytes are very clearly demonstrated. 10X

The frequency distribution of cases according to March classification is demonstrated in **Table 2**. CD3 immunohistochemical expression was shown in **Table 3**. Less than half staining pattern was seen in 10 (16.7 %) cases, approximately half staining pattern was seen in 10 (16.7 %) cases and most cases (56.7 %) showed more than half sating pattern. This fining correlated with histological finding that most cases are March 3 cases. CD20 immunohistochemical expression was shown in

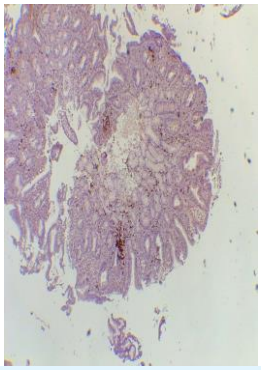

Fig. 3. CD20 immunohistochemical expression. Scattered involvement of some crypts in patient with mild villous atrophy corresponding to March 3A. 4X

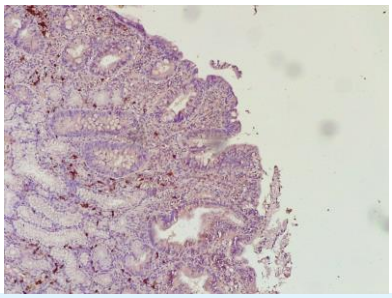

Fig. 4. CD20 immunohistochemical expression. Diffuse involvement of most crypts in patient with moderate to severe villous atrophy corresponding to March 3B to C. 10X

Table 4. Mild crypt involvement was reported in 16.7 %, Moderate crypt involvement was reported in 43.3 % and intense crypt involvement was reported in 40.0 %. **Fig. 1** showed CD3 immunohistochemical expression with diffuse involvement of more than half of villi in patient with sever villous atrophy corresponding to March 3C. **Fig. 2** showed CD3 immunohistochemical expression with diffuse involvement of more than half of villi in patient with mild villous atrophy corresponding to March 3A and intraepithelial lymphocytes were very clearly demonstrated. **Fig. 3** showed CD20 immunohistochemical expression with scattered mild involvement of some crypts in patient with mild villous atrophy corresponding to March 3A. **Fig. 4** showed CD20 immunohistochemical expression with diffuse moderate involvement of most crypts in patient with moderate to severe villous atrophy corresponding to March 3B to C.

DISCUSSION

In early stages of celiac disease, the histological evaluation and final diagnosis relies up on the existence of lymphocytes within the lining mucosa of small intestine, specifically speaking, inside the epithelium just above the basement membrane. This procedure is relatively difficult and sometimes the contrast between the blue color of lymphocyte nucleus and that of epithelial cell nucleus is inconclusive; therefore, the use of more sophisticated method to distinguish

lymphocytes from epithelial cells is essential in early stages of celiac disease. The immunohistochemical expression of CD 3 by T-lymphocytes represents a sensitive and a specific tool to distinguish intra-epithelia lymphocytes from epithelial cells. In addition, the extent and the number of lymphocytic infiltration can be estimated more precisely than using conventional histological stains (Mubarak *et al*., 2015).

In the current study, histological diagnosis of celiac disease was almost easy with conventional histological staining method with the exception of some cases of early stages of celiac disease in which histological changes and inflammatory infiltrate was mild and very difficult to be interpreted; nevertheless, the use of CD3 immunohistochemistry permitted the distinguishing of such mild early celiac disease cases.

Immunohistochemistry can provide highly sensitive technique for the characterization of Marsh I cases of celiac disease. The occurrence of intraepithelial lymphocytes by itself is not specific for celiac disease and can be seen in forms of intestinal inflammation such as Giardiasis (Husby *et al*., 2012). Therefore, the existence of intra-epithelial lymphocytes should be seriously considered only when there are highly suggestive clinical features and positive serological results. In our study, cases with mild early stages have in addition positive serological findings and highly suggestive clinical features, thus, it was easy to combined histological, immunohistochemical and serological investigation and clinical features to clearly diagnose celiac disease cases. Marsh I histological changes are early stage of celiac disease and can therefore develop into overt celiac disease with passage of time (Kurppa *et al*., 2010).

Moreover, a gluten challenge can cause mucosal changes that permit a diagnosis of celiac disease in number of patients with Marsh I celiac disease (Wahab *et al*., 2001). Indeed, a number of studies have demonstrated that patients with Marsh I lesions can benefit, at least in the short term, from a gluten-free diet (Mubarak *et al*., 2015). Immunohistochemical study of CD3, CD8, CD4, and CD56 lymphocytes have been studies in celiac disease patients in comparison with normal healthy mucosa and the results indicate that CD3 was the best diagnostic tool of celiac disease cases (Arévalo Suárez *et al*., 2016; Tosco *et al*., 2015).

In the current study, we were able to show that CD20 B lymphocytes were mainly associated with cryptic location and lymphoid follicle formation, a finding that was previously documented by other authors (Iftikhar *et al*., 2016). Indeed, the use of CD20 immunohistochemistry in the current study has shown a significant role for B-lymphocytes in the pathogenesis of this autoimmune disease.

CONCLUSION

In conclusion, the use of CD 3 immunohistochemistry is essential in the diagnosis of early stages (March I) of celiac disease in suspicious cases and that CD20 immunohistochemistry documented the role of B-lymphocytes in the pathogenesis of celiac disease.

REFERENCES

Abu-Zeid YA, Jasem WS, Lebwohl B, Green PH, ElGhazali G. (2014). Seroprevalence of celiac disease among United Arab Emirates healthy adult nationals: a gender disparity. World J Gastroenterol. 20(42):15830-15836.

Al-Bayatti SM. (2002). Etiology of chronic diarrhea. Saudi Med J. 23:675-679.

Aljebreen AM, Almadi MA, Alhammad A, Al Faleh FZ. (2013). Seroprevalence of celiac disease among healthy adolescents in Saudi Arabia. World J Gastroenterol. 19(15):2374-2378.

Arévalo Suárez F, Portugal S, Barreda C, Montes P, Perez-Narrea MT, Rodríguez O, Vergara G, Monge E. (2016). Celiac disease and negative serology villous atrophy: histological comparison and immunohistochemical study of CD3, CD4, CD8 and CD56 lymphocytes. Rev Gastroenterol Peru. 36(2):123-8.

Biagi F, Bianchi PI, Campanella J. (2008). The prevalence and the causes of minimal intestinal lesions in patients complaining of symptoms suggestive of enteropathy. A follow-up study. J Clin Pathol. 61:1116-1118.

Caio G, Riegler G, Patturelli M (2017). Pathophysiology of non-celiac gluten sensitivity: where are we now? Minerva Gastroenterol Dietol. 63:16-21.

Caio G, Volta U, Sapone A. (2019). Celiac disease: a comprehensive current review. BMC Med. 2019;17(1):142.

Caraceni P, Benazzi B, Caio G. (2011). Hyposplenism as a cause of pneumococcal meningoencephalitis in an adult patient with celiac disease. Ital J Med. 5:124-127.

Catassi C, Fasano A. (2010). Celiac disease diagnosis: simple rules are better than complicated algorithms. Am J Med. 123:691-693.

Corazza GR, Villanacci V. (2005). Coeliac disease: some considerations on the histological diagnosis. J Clin Pathol. 58:573-574.

Fasano A, Catassi C. (2012). Celiac disease. N Engl J Med. 367:2419-2426.

Greco L, Timpone L, Abkari A. (2011). Burden of celiac disease in the Mediterranean area. World J Gastroenterol. 17:4971-4978.

Greco L, Timpone L, Abkari A. (2011). Burden of celiac disease in the Mediterranean area. World J Gastroenterol. 17(45):4971-4978.

Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C. (2012). European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 54:136-160.

Iftikhar R, Jamal S, Zafar A, Saadia A. (2016). Histopathological and Immunohistochemical Analysis of Small Intestinal Biopsies in Adults Suspected of Celiac Disease. J Coll Physicians Surg Pak. 26(10):827-830.

Ilus T, Kaukinen K, Virta LJ (2014). Incidence of malignancies in diagnosed celiac patients: a population-based estimate. Am J Gastroenterol. 109:1471-1477.

Junker Y, Zeissig S, Kim SJ. (2012). Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J Exp Med. 209:2395-2408.

Kim SM, Mayassi T, Jabri B. (2015). Innate immunity: actuating the gears of celiac disease pathogenesis. Best Pract Res Clin Gastroenterol. 29:425-435.

Kim SW, Roh J, Park CS. (2016). Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. J Pathol Transl Med. 50(6):411-418.

Kurppa K, Ashorn M, Iltanen S, Koskinen LL, Saavalainen P, Koskinen O, Mäki M, Kaukinen K. (2010). Celiac disease without villous atrophy in children: a prospective study. J Pediatr. 157:373-80, 380.e1.

Leonard MM, Sapone A, Catassi C, Fasano A. (2017). Celiac disease and nonceliac gluten sensitivity: a review. JAMA. 318:647-656.

Lionetti E, Castellaneta S, Francavilla R. (2014). Introduction of gluten, HLA status, and the risk of celiac disease in children. N Engl J Med. 371:1295-1303.

Liu E, Lee HS, Aronsson CA. (2014). Risk of pediatric celiac disease according to HLA haplotype and country. N Engl J Med. 371:42-49.

Ludvigsson JF, Leffler DA, Bai JC. (2013). The Oslo definitions for coeliac disease and related terms. Gut. 2013;6:43-52.

Ludvigsson JF, Leffler DA, Bai JC. (2013). The Oslo definitions for coeliac disease and related terms. Gut. 6:43-52.

Lundin KE, Wijmenga C. (2015). Coeliac disease and autoimmune disease-genetic overlap and screening. Nat Rev Gastroenterol Hepatol. 12:507-515

McAllister BP, Williams E, Clarke K. (2018). A Comprehensive Review of Celiac Disease/Gluten-Sensitive Enteropathies. Clin Rev Allergy Immunol. Jun 02;

Mohammadibakhsh R, Sohrabi R, Salemi M, Mirghaed MT, Behzadifar M. (2017). Celiac disease in Iran: a systematic review and meta-analysis. *Electron Physician*. 9(3):3883-3895.

Mubarak A, Wolters VM, Houwen RH, ten Kate FJ. (2015). Immunohistochemical CD3 staining detects additional patients with celiac disease. *World J Gastroenterol*. 21(24):7553-7557.

Oxentenko AS, Murray JA. (2015). Celiac disease: ten things that every gastroenterologist should know. Clin Gastroenterol Hepatol. 13:1396-1404.

Pagliari D, Urgesi R, Frosali S. (2015). The interaction among microbiota, immunity, and genetic and dietary factors is the condicio sine qua non celiac disease can develop. J Immunol Res. 123653.

Parzanese, I., Qehajaj, D., Patrinicola, F., Aralica, M., Chiriva-Internati, M., Stifter, S., … Grizzi, F. (2017). Celiac disease: From pathophysiology to treatment. World journal of gastrointestinal pathophysiology, 8(2), 27-38.

Roshan B, Leffler DA, Jamma S. (2011). The incidence and clinical spectrum of refractory celiac disease in a north American referral center. Am J Gastroenterol. 106:923-928.

Rostami K, Marsh MN, Johnson MW. (2017). ROC-king onwards: intraepithelial lymphocyte counts, distribution & role in coeliac disease mucosal interpretation. Gut. 66:2080-2086.

Rostami-Nejad M, Villanacci V, Hogg-Kollars S. (2013). Endoscopic and histological pitfalls in the diagnosis of celiac disease: a multicentre study assessing the current practice. Rev Esp Enferm Dig. 105:326-333.

Rubio-Tapia A, Ludvigsson JF, Brantner TL. (2012). The prevalence of celiac disease in the United States. Am J Gastroenterol. 107:1538-1544.

Savvateeva LV, Erdes SI, Antishin AS, Zamyatnin AA. (2018). Current Paediatric Coeliac Disease Screening Strategies and Relevance of Questionnaire Survey. Int. Arch. Allergy Immunol. 177(4):370-380.

Silano M, Volta U, Mecchia AM, (2007). Delayed diagnosis of coeliac disease increases cancer risk. BMC Gastroenterol. 7:8.

Singh P, Arora A, Strand TA. (2018). Global prevalence of celiac disease: Systematic review and meta-analysis. Clin Gastroenterol Hepatol. 16(6):823-836.

Singh P, Arora S, Singh A, et al. Prevalence of celiac disease in Asia: a systematic review and meta-analysis. J Gastroenterol Hepatol. 2016;3:1095-1101.

Stamnaes J, Sollid LM. Celiac disease: autoimmunity in response to food antigen. Semin Immunol. 2015;27:343-352.

Tjon JM, van Bergen J, Koning F. Celiac disease: how complicated can it get? Immunogenetics. 2010;62:641-651.

Tosco A, Maglio M, Paparo F, Greco L, Troncone R, Auricchio R. Discriminant score for celiac disease based on immunohistochemical analysis of duodenal biopsies. J Pediatr Gastroenterol Nutr 2015; 60 (5): 621-5.

Verdu EF, Galipeau HJ, Jabri B. Novel players in celiac disease pathogenesis: the role of gut microbiota. Nat Rev Gastroenterol Hepatol. 2015;185:2969-2982.

Villalta D, Tonutti E, Prause C, et al. IgG antibodies against deamidated gliadin peptides for diagnosis of celiac disease in patients with IgA deficiency. Clin Chem. 2010;56:464-468.

Volta U, Caio G, Boschetti E, et al. Seronegative celiac disease: shedding light on an obscure clinical entity. Dig Liver Dis. 2016;48:1018-1022.

Volta U, Caio G, Stanghellini V, De Giorgio R. The changing clinical profile of celiac disease: a 15-year experience (1998-2012) in an Italian referral center. BMC Gastroenterol. 2014;14:194.

Volta U, Tovoli F, Piscaglia M, et al. Old and new serological test for celiac disease screening. Exp Rev Gatroenterol Hepatol. 2010;4:31-35.

Vriezinga SL, Auricchio R, Bravi E, et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med. 2014;371:1304-1315.

Wahab PJ, Crusius JB, Meijer JW, Mulder CJ. Gluten challenge in borderline gluten-sensitive enteropathy. Am J Gastroenterol. 2001;96:1464-1469.

West J, Fleming KM, Tata LJ, Card TR, Crooks CJ. Incidence and prevalence of celiac disease and dermatitis herpetiformis in the UK over two decades: population-based study. *Am J Gastroenterol*. 2014;109(5):757-768.

Yap TW, Chan WK, Leow AH, Azmi AN, Loke MF, et al. (2015) Prevalence of serum celiac antibodies in a multiracial Asian population--a first study in the young Asian adult population of Malaysia. *PloS one*, *10*(3), e0121908.

Yerkinbayeva L, Sherimova N, Borodina A (2015). The Problems of Legal Regulations, Protection and Use of Groundwater (International Experience and Practice in Kazakhstan). International Journal of Sustainable Energy and Environmental Research, 4(4): 73-85.