Original Article

Immunological Study of Cytomegalovirus in the Serum of Iraqi Patients with Celiac Disease

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Abstract

Background: Human cytomegalovirus (HCMV) is one of the important viruses involved in the progression of autoimmune diseases affecting the gastrointestinal system. **Objectives:** This study was conducted to evaluate the immune status of celiac disease (CD) with cytomegalovirus (CMV). **Materials and Methods:** This study included 68 subjects with suspected CD, which was collected during the period extent from January 2024 to March 2024. The immune status was evaluated using the enzyme-linked immunosorbent assay (ELISA) test to determine anti-Ttg A, anti-Ttg G, anti-gliadin IgA, and anti-gliadin IgG as well as anti-CMV IgM/IgG. **Results:** The results of the present study showed that 22 cases of CD were confirmed, with significantly higher percentages of positive results (18.2%, 68.2%) for patients compared to the control groups (2.2%, 13%) according to anti-gliadin A/G, respectively. Moreover, the level of anti-HCMV IgG antibodies in the serum samples of patients suffering from CD revealed significant differences of 3.39 ± 1.12 and 2.51 ± 0.835 between the patient and the control groups, respectively. **Conclusion:** It could be concluded that CMV infection may be involved in CD in terms of factors that stimulate or enhance autoimmunity or in the immunosuppressive state of the disease. Thus, CMV is a major contributor to the increased incidence of CD and likely worsens the prognosis of the disease.

Keywords: Anti-CMV IgM/IgG, Anti-gliadin A/G, Anti-Ttg A/G, Celiac disease

Introduction

Autoimmunity and infection have a complex interaction in genetically high-risk individuals, with viral infections or other infections as environmental triggers that initiate or escalate autoimmune and inflammatory processes, leading to the clinical presentation of the disease.[1] Therefore, viral infections such as enterovirus, rotavirus, HCV, HBV, Epstein–Barr virus (EBV), and cytomegalovirus (CMV) as well as other infections such as Bacteroides species, Campylobacter jejuni, Pneumococcus, Mycobacterium tuberculosis, and Helicobacter pylori are associated to celiac disease (CD).[2]

CD, also known as gluten-sensitive enteropathy, is an autoimmune intestinal disease triggered by gluten in individuals with genetic predispositions.[3] Its characteristic features include flattened small intestine mucosa with lymphocytic infiltration, villous atrophy, and crypt hyperplasia.[4] A strong correlation was observed between the patients' grain intake and this mean.[5] Global data displayed in Iraq indicate that one in every 2800 people has sensitivity caused by wheat components.[6] Therefore, serological tests measuring anti-gliadin (AGA), anti-endomysial (EMA), and anti-transglutaminase (anti-tTG) antibodies are used to diagnose CD. Anti-tTG and IgA AGA concentrations from early serological testing may

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accurately predict the chance of later biopsy-proven CD.[7] Thus, when CD patients consume gluten, the primary class of proteins that are retained in grains to promote seed germination and growth is called casein.[8] Here, it is clear that the gluten proteins found in dietary gluten include two main types (soluble gliadin and insoluble glutenin).[9] Gluten proteins, among other environmental, immunological, and genetic variables, combine to create CD.[10]

CMV, a member of the Herpesviridae family, is endemic, widespread, and human-specific. This virus is the leading infectious cause of congenital anomalies worldwide.[11] In those who are otherwise healthy, CMV infection does not result in significant medical issues. Serious infections, however, are typically only a problem for newborns who contract the disease before birth and for those who have compromised immune systems, such as those with acquired immune deficiency syndrome (AIDS) or organ transplant recipients.[12] A person is protected for the rest of their life by CMV-IgG.[13] Seroprevalence of human cytomegalovirus (HCMV) varies by area. Seroprevalence is more common in elderly individuals, women, and persons from lower socioeconomic groups.[14] Initial CMV infection causes the formation of CMV-specific IgM antibodies and then CMV-specific IgG antibodies. These persons can transfer CMV by coming into contact with bodily fluids (such as blood, urine, vaginal secretions, saliva, and so on) from an infected person.[15,16]

Some case studies have linked CD with CMV infection,[17] and potential connections between the two illnesses have been investigated through anti-HCMV antibody levels, which were found to be much higher in controls than in CD patients with malabsorption. Thus, it became clear that there was a role in this case.[18] On the other hand, the noticeably decreased prevalence of anti-HCMV antibodies in CD patients would imply that CMV protects against CD in individuals who are afflicted.[19] Further support for this correlation came from a study by Jansen *et al.*[20] which discovered a strong inverse relationship between the amount of anti-tTG antibody positivity and CMV infection. Serological tests are required to determine whether CMV is the source of CD-related or celiac crisis-related symptoms. These assays measure the presence of IgM and IgG antibodies, which are protective against CMV. High HCMV IgG titers signify prior HCMV infection; however, they may not always indicate the exact time when the infection was active until there is a four-fold increase in titer.[21] It is possible to ascertain whether an individual has been infected lately, though, if IgG is tested twice and there is a gap of one to three months between the samples. This will occur if the first sample yields a negative result, and the second sample yields a positive result. IgM assays for CMV with initial infection.[22] This work aimed to evaluate the immune status of CD with CMV.

Materials and Materials

Study of design and participants

In this cross-sectional study, 68 serum specimens were obtained from subjects suspected of CD at Al-Imam Al-Sadiq Teaching Hospital from January 2024 to March 2024. These subjects were distributed between 32 males and 36 females, ranging in age from 1 to 55 years old.

Measurement of a serological assay

A solid phase (quantitative and qualitative) enzyme-linked immunosorbent assay (ELISA) kit was used to measure anti-Ttg A, anti-Ttg G, anti-gliadin IgA, and anti-gliadin IgG (ESKU.DIAGNOSTICS GmbH & Co. KG, Wendelsheim, Germany), as well as Anti-CMV IgM/IgG (DRG International Inc., USA.) in all samples. Parameters were measured according to the manufacturer's instructions.

Statistical analysis

For the statistical study, IBM Co., Chicago, and IL's SPSS software version 26.0 were utilized. The data were presented as number (n), percentage, mean, and standard deviation, and the means of the various groups were compared using the Fisher's exact test, Mann-Whitney test, independent samples t test, and chi-square (χ^2) test. A P value of 0.05 indicated that the difference was statistically significant.

Ethical approval

The study was conducted in accordance with the moral guidelines found in the Helsinki Declaration. Before taking a sample, the patient's verbal and analytical consent were obtained. According to Document No. 2145 dated December 28, 2023, an ethical committee at the University of Babylon, Hammurabi College of Medicine, Babylon, Iraq, evaluated and approved the study protocol as well as the subject information and permission form.

Results

The current study of 68 subjects suspected of CD found 22 confirmed cases of this disease according to anti-Ttg A and anti-Ttg G assay [Figure 1].

Participants were distributed based on sex (males: 45.5% and females: 54.5%) to patients and controls (males: 47.8% and females: 52.2%) as shown in Table 1, which did not show any significant differences, while significant differences with high statistical evidence were observed in this study through higher percentages of positive results (18.2% and 68.2%) to patients compared to controls (2.2% and 13%) according to anti-gliadin A/G, respectively.

Table 2 showed a difference in the percentages for positive and negative results when examining CMV in patients and control groups, and no significant

differences appeared. They were the results for serum anti-HCMV IgM with CD patients (positive 27.3% and negative 72.7%) and the control group (positive 30.4% and negative 69.6%), while the results for serum anti-HCMV IgG with CD patients (positive 95.5% and negative 4.5%) and the control group (positive 78.3% and negative 21.7%).

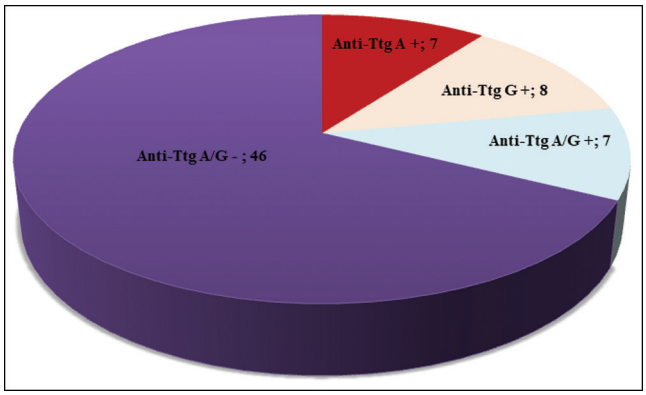

Figure 1: The suspected and confirmed cases of CD according to human tissue anti-transglutaminase

Table 3 revealed the relationship among age groups, BMI, and anti-gliadin A/ G, indicating a very high significant relationship between anti-gliadin A/G with CD, while no significant relationship was found between age groups and BMI with CD.

The level of anti-HCMV IgG antibodies in the serum samples of patients suffering from CD showed significant differences, represented by 3.39 ± 1.12 and 2.51 ± 0.835, compared to the levels of anti-HCMV IgM antibodies, which did not show any significant difference, represented by 0.922 ± 0.68 and 0.988 ± 0.753, between the patient group and the control group, respectively, as shown in [Table 4].

Discussion

Previous studies have shown an association between some viral infections (particularly enteroviruses, such as

Table 1: Percentages of sex and specific antibody assay according to this study

Parameters		Patients group (*N* = 22)	Controls group (*N* = 46)	*P* value
		N (%)	*N* (%)	
Sex	Male	10 (45.5%)	22 (47.8%)	*0.855
	Female	12 (54.5%)	24 (52.2%)	
Anti-gliadin A U/mL	Positive	4 (18.2%)	1 (2.2%)	**0.035
	Negative	18 (81.8%)	45 (97.8%)	
Anti-gliadin G U/mL	Positive	15 (68.2%)	6 (13%)	*<0.001
	Negative	7 (31.8%)	40 (87%)	

*Chi-square (χ^2) test,
**Fisher's exact test, level of significance is $P < 0.05$

Table 2: Percentages of CMV infection among sera from participant groups

Parameters		Patients group (*N* = 22)	Controls group (*N* = 46)	*P* value	Odds ratio
		N (%)	*N* (%)		
Anti-HCMV IgM U/mL	Positive	6 (27.3%)	14 (30.4%)	*0.789	0.857
	Negative	16 (72.7%)	32 (69.6%)		
Anti-HCMV IgG U/mL	Positive	21 (95.5%)	36 (78.3%)	**0.089	5.833
	Negative	1 (4.5%)	10 (21.7%)		

*Chi-square (χ^2) test,
**Fisher's exact test, Level of significance is $P < 0.05$

Table 3: Concentration of database characteristics and specific antibodies assays among sera from participant groups

Parameters	Patients group (*N* = 22)	Controls group (*N* = 46)	*P* value
	Mean ± SD	Mean ± SD	
Age/years	15.86 ± 15	11.98 ± 11.22	*0.237
BMI/kg/m²	15.82 ± 5.12	15 ± 5.18	*0.578
Anti-gliadin A U/mL	20.94 ± 63.12	2.28 ± 6.38	**<0.001
Anti-gliadin G U/mL	42.51 ± 44.3	8.67 ± 15.56	*0.002

*Independent samples *t* test,
**Mann-Whitney test, Level of significance is $P < 0.05$

Table 4: Level of HCMV antibodies among sera from participant groups

Parameters	Patients group ($N = 22$)	Controls group ($N = 46$)	*P* value
	Mean ± SD	Mean ± SD	
Anti-HCMV IgM U/mL	0.922 ± 0.68	0.988 ± 0.753	*0.727
Anti-HCMV IgG U/mL	3.39 ± 1.12	2.51 ± 0.835	*0.03

*Independent samples *t* test, Level of significance is $P < 0.05$

Reovirus, EBV, and CMV) with autoimmune diseases (CD, idiopathic arthritis, type 1 diabetes, and others) because they stimulate the immune system to overreact to gluten and lead to the development of CD.[23-26] Sharquie *et al.*[27] demonstrated the existence of a highly statistically significant relationship between serum levels of antibodies specific for CD and infection with the human cytomegalovirus, and this is consistent with our current study.

Also, in this study, the use of anti-transglutaminase biomarkers is one of the best diagnostic signs, which is consistent with the study.[28,29]

However, CD is still underdiagnosed, which indicates the need to study other new biomarkers due to the estimated 90% or more cases of people with CD who did not show any symptoms, as in our study, and which has been confirmed by many previous studies. Therefore, it has been suggested that genotype patients using HLA-DQ2 and/or HLA-DQ8 markers be genotyped, but it is very expensive.[30,31]

The prevailing belief in the pathophysiology of this disease has several important steps, one of which is tTG, due to its effective action on glutamine-rich gliadin peptides. The resulting deamidated, negatively charged peptides have a much higher affinity for the HLA-DQ2 and HLA-DQ8 molecules.[32]

In any case, CD is considered an immune disorder that results from foods that contain gluten (such as wheat, barley, or rye). In addition, patients may suffer from non-specific symptoms, including diarrhea, bloating, abdominal pain, and other symptoms such as failure to thrive, vitamin deficiencies, anemia, dermatitis herpetiformis, aphthous stomatitis, and psychological conditions (anxiety and depression).[33]

Therefore, when food is ingested, it is broken down by the digestive system and absorbed by microvilli in the small intestine. Dendritic cells (DC) take up peptides, which lead to the stimulation of regulatory T cells (Treg). Thus, an inhibitory response to the inflammatory cascade occurs to the specific ingested food, and this process is abnormal in patients with CD as it stimulates persistent streams of gluten-specific CD4+ cells rather than stimulating Treg cells.[34]

Thus, viral infection stimulates natural killer (NK) pathways, and this leads to the activation of type I interferons (IFNs), which results in the breakdown of oral tolerance (which means a lack of immune response to ingested foods), and thus, the immune response to food begins with the production of cellular toxins and food-specific antibodies.[34-37]

HCMV infection is common in the intestines, especially in people with weakened immune systems. This affects epithelial cells, fibroblasts, histiocytes, and smooth muscle cells and may end in tissue necrosis and erosion, in addition to ulceration of the mucous membrane with bleeding.[34]

Thus, the characteristics of CMV include many autoimmune disorders due to its replication in multiple tissues in the lytic phase, its persistence throughout life with intermittent states of latency and acute reactivation, in addition to its possession of a large complex genome and molecular mimicry. In addition, comprehensive modification of adaptive and innate immunity triggered by this virus occurs in the case of autoimmunity.[36]

Therefore, the current study is consistent with many previous results that the presence of this virus is highly likely to be one of the causes of the disease.[17,18]

This study showed that there are very high and noticeable significant differences in anti-gliadin IgG and IgA between the patient group and the control group, supporting what was stated by Husby *et al.*[37]

Thus, anti-gliadin IgG and IgA have a strong similarity in terms of sensitivity and specificity. The anti-gliadin IgA test produced an accurate test for CD with a specificity of 97% and sensitivity of 71%, while anti-gliadin IgG had a specificity of 91% and sensitivity of 87%. Thus, the combined IgA and IgG antibody test for anti-gliadin has a sensitivity of 95% and specificity of 90%.[38,39]

Conclusion

It could be concluded that the distribution of CD was equal among males and females, while its prevalence in children was higher than in adults. In addition, CMV infection may be involved in CD in terms of factors that stimulate or enhance autoimmunity or in the immunosuppressive state of the disease. Thus, CMV is a major contributor to the increased incidence of CD and thus likely worsens the prognosis of the disease. Thus, early detection of CD allows doctors to have a lower threshold for screening in this group of patients and allows the implementation of a

gluten-free diet, which reduces the risk of the disease and the severity of the condition's complications.

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Conflicts of interest
There are no conflicts of interest.

References
1. Arleevskaya MI, Manukyan G, Inoue R, Aminov R. Editorial: Microbial and environmental factors in autoimmune and inflammatory diseases. Front Immunol 2017;8:243.
2. Lerner A, Arleevskaya M, Schmiedl A, Matthias T. Microbes and viruses are bugging the gut in celiac disease. Are they friends or foes? Front Microbiol 2017;8:1392.
3. Khan A, Li S, Han H, Jin WL, Ling Z, Ji J, *et al*. A gluten degrading probiotic Bacillus subtilis LZU-GM relieve adverse effect of gluten additive food and balances gut microbiota in mice. Food Res Int (Ottawa, Ont.) 2023;170:112960.
4. Dewar DH, Ciclitira PJ. Clinical features and diagnosis of celiac disease. Gastroenterology 2005;128:S19-24.
5. Sudha M, Viveka S. A comprehensive review of architecture, classification, challenges, and future of the internet of medical things (IoMTs). Med J Babylon 2022;19:311-17.
6. Ahmed SA, Ali GM, Sultan SM, Hammed AE. Evaluation of serum level of trace elements (iron, zinc) in patients with celiac disease (review study). NTU J Pure Sci 2021;1:27-37.
7. Toftedal P, Nielsen C, Madsen JT, Titlestad K, Husby S, Lillevang ST. Positive predictive value of serological diagnostic measures in celiac disease. Clin Chem Lab Med 2010;48:685-91.
8. Shewry P. What is gluten—Why is it special? Front Nutr 2019;6:101.
9. Wieser H. Chemistry of gluten proteins. Food Microbiol 2007;24:115-9.
10. Lebwohl B, Ludvigsson JF, Green PH. Celiac disease and non-celiac gluten sensitivity. BMJ 2015;351:h4347.
11. Fernandez C, Chasqueira MJ, Marques A, Rodrigues L, Marçal M, Tuna M, *et al*. Lower prevalence of congenital cytomegalovirus infection in Portugal: Possible impact of COVID-19 lockdown? Eur J Pediatr 2022;181:1259-62.
12. Almishaal AA. Knowledge of cytomegalovirus infection among women in Saudi Arabia: A cross-sectional study. PLoS One 2022;17:e0274863.
13. Ali KS. The sero-prevalence of cytomegalovirus infection among women with abortion and intrauterine death in Erbil City Kurdistan Region, Iraq. Diyala J Med 2020;18:77-90.
14. Bate SL, Dollar SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: The national health and nutrition examination surveys, 1988–2004. Clin Infect Dis 2010;50:1439-47.
15. Auriti C, De Rose DU, Santisi A, Martini L, Piersigilli F, Bersani I, *et al*. Pregnancy and viral infections: Mechanisms of fetal damage, diagnosis, and prevention of neonatal adverse outcomes from cytomegalovirus to SARS-CoV-2 and Zika virus. Biochim Biophys Acta Mol Basis Dis 2021;1867:166198.
16. Shimada K, Toriyabe K, Kitamura A, Morikawa F, Minematsu T, Ikejiri M, *et al*. Primary cytomegalovirus infection during pregnancy and congenital infection: A population-based, mother–child, prospective cohort study. J Perinatol 2021;41:2474-81.
17. Kelly E, Cullen G, Aftab AR, Courtney G. Coeliac crisis presenting with cytomegalovirus hepatitis. Eur J Gastroenterol Hepatol 2006;18:793-5.
18. Nemes E. Pathologic Antibody Responses in Celiac Disease: Specificity and Immunological Correlations. PhD Thesis. Debrecen: University of Debrecen; 2008.
19. Plot L, Amital H, Barzilai O, Ram M, Nicola B, Shoenfeld Y. Infections may have a protective role in the etiopathogenesis of celiac disease. Ann N Y Acad Sci 2009;1173:670-4.
20. Jansen MA, van den Heuvel D, van der Zwet KV, Jaddoe VW, Hofman A, Escher JC, *et al*. Herpesvirus infections and transglutaminase type 2 antibody positivity in childhood: The generation R study. J Pediatr Gastroenterol Nutr 2016;63:423-30.
21. CDC. Cytomegalovirus (CMV) and congenital CMV infection. CDC 2020.
22. Ross SA, Novak Z, Pati S, Boppana SB. Overview of the diagnosis of cytomegalovirus infection. Infect Disord Drug Targets 2011;11:466-74.
23. Janahi EM, Das S, Bhattacharya SN, Haque S, Akhter N, Jawed A, *et al*. Cytomegalovirus aggravates the autoimmune phenomenon in systemic autoimmune diseases. Microb Pathog 2018;120:132-9.
24. Harley JB, Chen X, Pujato M, Miller D, Maddox A, Forney C, *et al*. Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity. Nat Genet 2018;50:699-707.
25. Houen G, Trier NH. Epstein-Barr virus and systemic autoimmune diseases. Front Immunol 2021;11:587380.
26. Talipova D, Smagulova A, Poddighe D. Toll-like receptors and celiac disease. Int J Mol Sci 2022;24:265.
27. Sharquie IK, Abdullah SF, Bayati AM. The role of human cytomegalovirus (HCMV) in patients with active celiac disease (CD). East J Med 2021;26:204-9.
28. Vermeersch P, Geboes K, Marien G, Hoffman I, Hiele M, Bossuyt X. Diagnostic performance of IgG anti-deamidated gliadin peptide antibody assays is comparable to IgA anti-tTG in celiac disease. Clin Chim Acta 2010;411:931-5.
29. Bragde H, Jansson U, Fredrikson M, Grodzinsky E, Soderman J. Potential blood-based markers of celiac disease. BMC Gastroenterol 2014;14:176.
30. Vives-Pi M, Takasawa S, Pujol-Autonell I, Planas R, Cabre E, Ojanguren I, *et al*. Biomarkers for diagnosis and monitoring of celiac disease. J Clin Gastroenterol 2013;47:308-13.
31. Clouzeau-Girard H, Rebouissoux L, Taupin JL, Le Bail B, Kalach N, Michaud L, *et al*. HLA-DQ genotyping combined with serological markers for the diagnosis of celiac disease: Is intestinal biopsy still mandatory? J Pediatr Gastroenterol Nutr 2011;52:729-33.
32. Di Sabatino A, Vanoli A, Giuffrida P, Luinetti O, Solcia E, Corazza GR. The function of tissue transglutaminase in celiac disease. Autoimmun Rev 2012;11:746-53.
33. Welch NL, Welch T, Songtanin B. Viral triggered celiac disease: A case report. Cureus 2023;15:e40429.
34. Brown JJ, Jabri B, Dermody TS. A viral trigger for celiac disease. PLoS Pathog 2018;14:e1007181.
35. Al-Kaif LAIK, Al-Ameri H, Alfatlawi WRO, Mahdi AE, Al-Khafaji YA, Al-Saadi MAK, *et al*. Detection of CTLA-4 level and humeral immune response after the second dose of COVID-19 vaccine in certain Iraqi provinces participants. PLoS One 2024;19:e0296521.
36. Abadie V, Sollid LM, Barreiro LB, Jabri B. Integration of genetic and immunological insights into a model of celiac disease pathogenesis. Annu Rev Immunol 2011;29:493-525.
37. Halenius A, Hengel H. Human cytomegalovirus and autoimmune disease. Biomed Res Int 2014;2014:472978.
38. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, *et al*. European society pediatric gastroenterology, hepatology, and nutrition guidelines for diagnosing coeliac disease 2020. J Pediatr Gastroenterol Nutr 2020;70:141-56.
39. Laftah RF, Roudhan BG, Hassan AM. Celiac disease screening in short stature children using anti-gliadin IgA and IgG. Med J Babylon 2013;10:336-42.