***Short Research Article***

Folate Deficiency and Depression Risk in Celiac Disease: A Retrospective Cohort Analysis

**Abstract**

 The goal of this study was to determine the significance of folate deficiency in individuals diagnosed with celiac disease and their subsequent risk of developing depressive episodes. Antibody production against deamidated gluten and autoantibodies against the enzyme transglutaminase 2 (TG2) results. T cell epitopes are created in the gut, and thus both T cells and B cell antigens allow for the characterization of disease-specific immune cells to be identified in samples of CD patients. Data for this retrospective study was acquired through TriNetX, a global, health online platform that uses real world patient electronic health records (EHR) with over 100 million patients from 95 healthcare organizations (HCOs). Data analysis was conducted via TriNet’s built-in analysis software. Descriptive statistics were used to compare patient demographics with continuous variables measured with standard deviation and categorical variables measured with frequencies. The established role of folate in monoamine neurotransmitter biosynthesis and the reduction of homocysteine levels may explain the results of our study. Our odds ratio reflects the decreased incidence of depressive episodes in celiac patients who are folate replete. Our hazard ratio of 0.22 was not statistically significant, as the calculated p-value was 0.7705. This could be attributed to a variety of factors, including the presence of other vitamin deficiencies, other possible causes of depressive episodes unrelated to folate deficiency, and limited follow-up duration. Randomized control studies should be conducted to determine if folate supplementation mitigates depressive symptoms in Celiac patients. These future directions could provide valuable insight into folate’s role in psychiatric comorbidities in Celiac disease and support more informed tailored nutritional therapies.

Keywords: Celiac disease, neurotransmitter, nutritional therapies, disease-specific immune cells

**INTRODUCTION:**

Celiac disease (CD) is a common chronic autoimmune disorder triggered by the ingestion of gluten in genetically predisposed individuals. More specifically, the gluten proteins present in wheat, barley, rye, and cross-contaminated oats. (Rewers M 2005). While traditionally regarded as a gastrointestinal condition, CD is increasingly recognized as a systemic disorder with a wide range of clinical manifestations beyond the small intestine. (Holtmeier W & Caspary WF 2006). Notably, emerging evidence suggests a significant association between CD and mood disorders, including depression and anxiety.

The immunopathogenesis of CD is particularly well understood among other human leukocyte antigen (HLA) associated disorders. (Brown NK et al. 2019). It has been established that individuals carrying the DQ2 or DQ8 human leukocyte antigen haplotypes will develop the disease when ingesting gluten. (De Re V, Magris R, & Cannizzaro R 2017). That is, T cell reactivity against gluten peptides undergoes a modification, deamidation, whereby certain glutamine residues are converted into glutamic acid. (du Pré MF & Sollid LM 2015). Antibody production against deamidated gluten and autoantibodies against the enzyme transglutaminase 2 (TG2) results. T cell epitopes are created in the gut, and thus both T cells and B cell antigens allow for the characterization of disease-specific immune cells to be identified in samples of CD patients. (Iversen R & Sollid LM 2023). This process leads to intestinal inflammation and villous atrophy, which in turn impairs nutrient absorption. The classical presentation of the disease ranges from an asymptomatic patient to one with severe malnutrition. (Schuppan D & Zimmer KP 2013).

Until recently, it was thought that the disease was limited to individuals of European descent. However, there has been a substantial increase in the prevalence and incidence of CD over the last two decades, which has a global extent not limited to those of European origin. (Ludvigsson JF & Murray JA 2019). With the surge in cases and individuals diagnosed with CD, it is important to be aware of the significant risks that may come as a result related to depression, anxiety, and panic disorder. (King JA et al. 2020). With the certainty of the disorder being linked to a decreased quality of life (QoL) and certain mood disorders, this is not to be overlooked. Although the disease primarily affects the small intestine, previous studies have confirmed higher rates of neuropsychiatric diseases among CD patients compared to non-CD controls. (Sharma N, Singh K, & Senapati S 2021).

Common complications of CD related to malabsorption may indicate this link. Folate (vitamin B-9) is a group of water-soluble compounds that play various roles in central nervous system functions such as the synthesis of neurotransmitters such as serotonin, dopamine, and norepinephrine, all of which are involved in mood regulation. (Liwinski T & Lang UE 2023). Folate is of particular interest as low serum levels are a common hematological manifestation of the disease. (Halfdanarson TR, Litzow MR, & Murray JA 2007). The link between inadequate diet and the risk of depression is well-established, (Berk M & Jacka FN 2019). However, malabsorption due to CD can also lead to the same low levels of serum and red blood cell folate often seen in depressed individuals. Thus, it is crucial to take into consideration the role of folate deficiency in such patients. Additionally, folate is associated with an increased risk of depression, severity of symptoms, prolonged duration of depressive episodes, likelihood of relapse, and poor response to antidepressant treatment. (Liwinski T, & Lang UE 2023).

**METHODS:**

Data for this retrospective study was acquired through TriNetX, a global, health online platform that uses real world patient electronic health records (EHR) with over 100 million patients from 95 healthcare organizations (HCOs). All patient information is de-identified and follows the Health Insurance Portability Act (HIPAA) Privacy Rule. TrinetX utilizes standardized coding systems such as the International Classification of Diseases (ICD-10), Anatomical Therapeutic Chemical (ATC), Current Procedural Terminology (CPT) to identify specific diagnoses, procedures, medications and demographics. In addition, Institutional Review Board (IRB) approval was not required to gain access to TriNetX network.

 **COHORTS:**

Two cohorts were utilized using the TriNetX platform. All patients were female and between the ages of 18-80 years old due to celiac disease is more prevalent in women and is common in older adults. Any individuals with a history of depressive episodes prior to celiac diagnoses were excluded. To reduce potential confounding variables, Trinet’s 1:1 propensity matching was used to balance comorbid conditions of intestinal malabsorption including ulcerative colitis, Crohn’s disease (ICD K50-K52), and pernicious anemia (ICD D51.0) as well as demographics. We did not analyze pediatric associations between folate deficiency and depression.

Cohort 1 consisted of Celiac patients (ICD K90.0) without a diagnosis of folate deficiency (ICD D52.9) and could not have a depressive episode (ICD 10 F31.0). Cohort 2 consisted of individuals with celiac disease (ICD K90.0) and folate deficiency (ICD D52.9) and could not have a depressive episode (ICD F31.0) before the diagnosis of celiac disease. Primary outcomes were the prevalence of a depressive episode (F32.2) within a five-year follow-up period.

Data analysis was conducted via TriNet’s built-in analysis software. Descriptive statistics were used to compare patient demographics with continuous variables measured with standard deviation and categorical variables measured with frequencies. Cohort 1 (Celiac patients without folate deficiency) and Cohort 2 (Celiac patients with folate deficiency) were compared using chi-squared tests and paired t-tests.

Primary outcomes of interest were the prevalence of a depressive episode (ICD F32.2) within a five-year follow-up period. To measure the correlation between folate deficiency and depressive episodes, odd ratios with 95% confidence intervals were calculated by TriNet’s logistic regression models. Time-to-event analysis was performed using Cox proportional hazard models to estimate hazard ratios (HR) for the development of a depressive episode in patients with folate deficiency. Statistically significance was defined as a p value <0.05.

**RESULTS:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   |  Demographics | Mean ± SD | Patients | % of Cohort | P-Value  | Std diff.  |
| 12 | Current Age | 58.4 ± 17.658.8 ± 17.7 | 206206 | 100%100% | 0.8147  | 0.0231  |
|
|
| 12 | Age of Index  | 53.8 ± 1754.1 ± 17 | 206206 | 100.00%100.00% | -  | -  |
|
| 12 | Female |    | 206206 | 100.00%100.00% | - |  - |
|
| 12 | White |    | 158159 | 76.699%77.184% | 0.0.9069  | 0.0115  |
|
| 12 | Not Hispanic or Latino |    | 127130 | 61.65%63.107% | 0.7603 | 0.0301 |
|
| 12 | Unknown Ethnicity |    | 6057 | 29.126%27.67% | 0.7431  | 0.0323  |
|
| 12 | Hispanic or Latino |    | 1919 | 9.223%9.233% | 1.000 | <0.0001 |
|
| 12 | Other Race |    | 1713 | 8.252%6.311% | 0.4482 | 0.0748 |
|
|
| 12 | Unknown Race |    | 1818 | 8.738%8.738% | 1.000 | <0.0001 |
|
| 12 | Asian |    | 1010 | 4.854%4.854% | 1.000  | <0.0001  |
|
| 12 | Black or African American |    | 1113 | 5.34%6.311% | 0.6740  | 0.0415  |
|
| 12 | Native Hawaiian or Other Pacific Islander |    | 1010 | 4.854%4.854% | 1.000  | <0.0001  |
|
| 12 | American Indian or Alaska Native |   | 00 | 0.00%0.00% | -- | -- |
|

TABLE 1: Demographic comparison between Celiac patients and folate deficiency

Cohort 1: Celiac disease without folate deficiency

Cohort 2: Celiac disease with folate deficiency

Demographic analysis showed no statistical significance between cohorts.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Outcome | Cohort  | Patients in cohort | Patients with outcome | Odds ratio (95% CI) |
| Depression | 1 | 206 | 14 | 0.464(0.236,0.909) |
|   | 2 | 206 | 28 |   |

Table 2: Cross-sectional analysis of folate deficiency on depressive episodes in Celiac patients

Cohort 1 (Celiac patients with no folate deficiency) was compared to Cohort 2 (Celiac patients with folate deficiency) to estimate the incidence of depressive episodes at a single point in time. The odds ratio (OR) with 95% confidence intervals (CI) was calculated showing patients without folate deficiency were 46.4% less likely to have depression compared to those with folate deficiency (Table 2).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Hazard Ratio | 95 % Confidence Interval  | χ2 | df | p |
| 0.465 | (0.245,0.885) | 2.221 | 1 | 0.1362 |

Table 3: Cox-proportional hazard ratio on development of depressive episode in Celiac patients with folate deficiency.

This table shows the Cox proportional hazards model used to compare the time to event outcome of depression in between Celiac patients with and without folate deficiency. The hazard ratio of 0.465 suggests a reduced risk of developing depression in celiac patients with folate deficiency but these findings were not statistically significant p>0.05 (Table3).

**DISCUSSION:**

The goal of this study was to determine the significance of folate deficiency in individuals diagnosed with celiac disease and their subsequent risk of developing depressive episodes. Overall, the data shows statistically significant higher odds of developing depressive episodes in patients with celiac disease who are deficient in folate. Folate’s role in the synthesis of neurotransmitters responsible for mood regulation, the maintenance of hemostasis, and reducing levels of homocysteine may be clinically relevant in preventing the occurrence of depressive episodes in those with celiac disease (Morris M 2003).

Folate has been proven to be a necessary component in the biosynthesis of monoamine neurotransmitters (dopamine, norepinephrine, serotonin) (Alpert M 1997). 5-Methyltetrahydrofolate (5-MTHF), the biologically active metabolite of folate, serves as a methyl group donor to homocysteine, creating methionine and S-adenosylmethionine (SAMe) (Bender A 2017). Folate and SAMe have been shown to increase the biosynthesis of tetrahydrobiopterin (BH4), a vital cofactor in the synthesis of monoamine neurotransmitters (Coppen A, Fanet H 1989). The lack of the these neurotransmitters (dopamine, serotonin, norepinephrine) has been hypothesized to be the pathophysiological basis for the development of depression and other mood disorders. (Delgado PL 2000). Thus, the supplementation of folate may decrease the risk of depressive episodes in individuals who have folate deficiency due to celiac disease.

In addition to its function in monoamine synthesis, 5-MTHF serves as a methyl group donor to reduce homocysteine levels in the body via conversion of homocysteine to methionine. Increased homocysteine levels have been documented to be positively associated with an increased incidence of depression (Tolmunen T 2004). Of interest is the enzyme methylenetetrahydrofolate reductase (MTHFR), which functions in folate and homocysteine metabolism (Leclerc D 2013). One meta-analysis of 3478 women found that those with decreased MTHFR activity due to being homozygous for the MTHFR C677T polymorphism possessed higher homocysteine levels and were 1.37 times more likely to have been diagnosed with depression when compared to those without (Lewis SL 2006). Homocysteine is a cytotoxic compound that can cause increased oxidative stress in the central nervous system (CNS) (Mattson M 2003). The increased oxidative stress on the CNS leads to the generation of reactive oxygen species (ROS), which cause inflammation, apoptosis, and neurodegeneration (Correia A 2023). These factors combined provide a compelling explanation for the increased incidence of depression among patients with increased homocysteine levels. Consequently, folate supplementation provides the cofactors to prevent elevated homocysteine levels in patients with celiac disease.

The established role of folate in monoamine neurotransmitter biosynthesis and the reduction of homocysteine levels may explain the results of our study. Our odds ratio reflects the decreased incidence of depressive episodes in celiac patients who are folate replete. Our hazard ratio of 0.22 was not statistically significant, as the calculated p-value was 0.7705. This could be attributed to a variety of factors, including the presence of other vitamin deficiencies, other possible causes of depressive episodes unrelated to folate deficiency, and limited follow-up duration. Overall, this study has provided evidence of the association between folate deficiency and increased risk of the development of depressive episodes in celiac patients, which isconcurrent with prior research. However, the use of folate deficiency as a predictive element in the development of depressive episodes in celiac patients warrants further study.

The use of TriNetX inherently comes with certain limitations. First, the platform mainly utilizes electronic health records data. These records may be incomplete and may be missing relevant clinical information. Additionally, the ICD-10 code for folate deficiency used to create this study’s dataset does not include specific folate level measurements, thus limiting detailed comparisons of deficiency severity. Due to the observational study design, there is an inability to infer causality from the results. Furthermore, though the results of our study were statistically significant, the relatively small sample size may limit external validity.

**CONCLUSION**:

Our findings support an association with folate deficiency and depression in patients with Celiac disease. Given folate’s immunological role in monoamine neurotransmitter synthesis, folate deficiency may contribute to neuroinflammation due to interruption of serotonin, dopamine, and norepinephrine synthesis. Future studies should incorporate laboratory measured folate levels to quantify deficiency levels in Celiac patients. Longitudinal studies can clarify the temporal relationship between folate deficiency and onset of depressive symptoms. Randomized control studies should be conducted to determine if folate supplementation mitigates depressive symptoms in Celiac patients. These future directions could provide valuable insight into folate’s role in psychiatric comorbidities in Celiac disease and support more informed tailored nutritional therapies.

**REFERENCES:**

1. Alpert, Jonathan E., and Maurizio Fava. “Nutrition and Depression: The Role of Folate.” *Nutrition Reviews* 55, no. 5 (May 1, 1997): 145–49.<https://doi.org/10.1111/j.1753-4887.1997.tb06468.x>.
2. Bender, Ansley, Kelsey E. Hagan, and Neal Kingston. “The Association of Folate and Depression: A Meta-Analysis.” *Journal of Psychiatric Research* 95 (December 1, 2017): 9–18.<https://doi.org/10.1016/j.jpsychires.2017.07.019>.
3. Berk M, Jacka FN. Diet and Depression-From Confirmation to Implementation. JAMA. 2019;321(9):842-843. doi:10.1001/jama.2019.0273
4. Brown NK, Guandalini S, Semrad C, Kupfer SS. A Clinician's Guide to Celiac Disease HLA Genetics. Am J Gastroenterol. 2019;114(10):1587-1592. doi:10.14309/ajg.0000000000000310
5. Coppen, A., C. Swade, S. A. Jones, R. A. Armstrong, J. A. Blair, and R. J. Leeming. “Depression and Tetrahydrobiopterin: The Folate Connection.” *Journal of Affective Disorders* 16, no. 2 (March 1, 1989): 103–7. [https://doi.org/10.1016/0165-0327(89)90062-1](https://doi.org/10.1016/0165-0327%2889%2990062-1).
6. Correia, Ana Salomé, Armando Cardoso, and Nuno Vale. “Oxidative Stress in Depression: The Link with the Stress Response, Neuroinflammation, Serotonin, Neurogenesis and Synaptic Plasticity.” *Antioxidants* 12, no. 2 (February 13, 2023): 470.<https://doi.org/10.3390/antiox12020470>.
7. Delgado, P. L. “Depression: The Case for a Monoamine Deficiency.” *The Journal of Clinical Psychiatry* 61 Suppl 6 (2000): 7–11.
8. De Re V, Magris R, Cannizzaro R. New Insights into the Pathogenesis of Celiac Disease. Front Med (Lausanne). 2017;4:137. Published 2017 Aug 31. doi:10.3389/fmed.2017.00137
9. du Pré MF, Sollid LM. T-cell and B-cell immunity in celiac disease. Best Pract Res Clin Gastroenterol. 2015;29(3):413-423. doi:10.1016/j.bpg.2015.04.001
10. Fanet, H., L. Capuron, N. Castanon, F. Calon, and S. Vancassel. “Tetrahydrobioterin (BH4) Pathway: From Metabolism to Neuropsychiatry.” *Current Neuropharmacology* 19, no. 5 (April 29, 2021): 591–609.<https://doi.org/10.2174/1570159X18666200729103529>.
11. Halfdanarson TR, Litzow MR, Murray JA. Hematologic manifestations of celiac disease. Blood. 2007;109(2):412-421. doi:10.1182/blood-2006-07-031104
12. Holtmeier W, Caspary WF. Celiac disease. Orphanet J Rare Dis. 2006;1:3. Published 2006 Mar 1. doi:10.1186/1750-1172-1-3
13. Iversen R, Sollid LM. The Immunobiology and Pathogenesis of Celiac Disease. Annu Rev Pathol. 2023;18:47-70. doi:10.1146/annurev-pathmechdis-031521-032634
14. King JA, Jeong J, Underwood FE, et al. Incidence of Celiac Disease Is Increasing Over Time: A Systematic Review and Meta-analysis. Am J Gastroenterol. 2020;115(4):507-525. doi:10.14309/ajg.0000000000000523
15. Leclerc, Daniel, Sahar Sibani, and Rima Rozen. “Molecular Biology of Methylenetetrahydrofolate Reductase (MTHFR) and Overview of Mutations/Polymorphisms.” In *Madame Curie Bioscience Database [Internet]*. Landes Bioscience, 2013.<https://www.ncbi.nlm.nih.gov/books/NBK6561/>.
16. Lewis, S. J., D. A. Lawlor, G. Davey Smith, R. Araya, N. Timpson, I. N. M. Day, and S. Ebrahim. “The Thermolabile Variant of MTHFR Is Associated with Depression in the British Women’s Heart and Health Study and a Meta-Analysis.” *Molecular Psychiatry* 11, no. 4 (April 2006): 352–60.<https://doi.org/10.1038/sj.mp.4001790>.
17. Liwinski T, Lang UE. Folate and Its Significance in Depressive Disorders and Suicidality: A Comprehensive Narrative Review. Nutrients. 2023;15(17):3859. Published 2023 Sep 4. doi:10.3390/nu15173859
18. Ludvigsson JF, Murray JA. Epidemiology of Celiac Disease. Gastroenterol Clin North Am. 2019;48(1):1-18. doi:10.1016/j.gtc.2018.09.004
19. Mattson, Mark P., and Thomas B. Shea. “Folate and Homocysteine Metabolism in Neural Plasticity and Neurodegenerative Disorders.” *Trends in Neurosciences* 26, no. 3 (March 1, 2003): 137–46. [https://doi.org/10.1016/S0166-2236(03)00032-8](https://doi.org/10.1016/S0166-2236%2803%2900032-8).
20. Morris, M.S., M. Fava, P.F. Jacques, J. Selhub, and I.H. Rosenberg. “Depression and Folate Status in the US Population.” *Psychotherapy and Psychosomatics* 72, no. 2 (2003): 80–87.<https://doi.org/10.1159/000068692>.
21. Rewers, M. (2005). Epidemiology of celiac disease: what are the prevalence, incidence, and progression of celiac disease?. Gastroenterology, 128(4), S47-S51
22. Schuppan D, Zimmer KP. The diagnosis and treatment of celiac disease. Dtsch Arztebl Int. 2013;110(49):835-846. doi:10.3238/arztebl.2013.0835
23. Sharma N, Singh K, Senapati S. Celiac disease poses significant risk in developing depression, anxiety, headache, epilepsy, panic disorder, dysthymia: A meta-analysis. Indian J Gastroenterol. 2021;40(5):453-462. doi:10.1007/s12664-021-01215-2 [Original source: https://studycrumb.com/alphabetizer]
24. Tolmunen, T., J. Hintikka, S. Voutilainen, A. Ruusunen, G. Alfthan, K. Nyyssönen, H. Viinamäki, G.A. Kaplan, and J.T. Salonen. “Association between Depressive Symptoms and Serum Concentrations of Homocysteine in Men: A Population Study.” *American Journal of Clinical Nutrition* 80, no. 6 (2004): 1574–78.<https://doi.org/10.1093/ajcn/80.6.1574>.