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Cytokine-Mediated Modulation of SEN Virus Replication in Celiac Disease: Insights into Immune Signatures and Viral Persistence

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Abstract: Celiac disease (CD) is an autoimmune disorder triggered by gluten in genetically susceptible individuals, and viral infections have been proposed as potential cofactors in its pathogenesis. This study investigated the replication of SEN virus genotypes H and D in CD patients and their association with systemic cytokine levels. A total of 276 participants were enrolled, including 192 CD patients—115 on a gluten-containing diet (GCD) and 77 on a gluten-free diet (GFD)—alongside 84 healthy controls. SEN virus detection was performed using nested PCR, and serum cytokine levels (IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ) were quantified via ELISA. SENV-H was detected in 71.4% of CD patients on a GCD, 66.7% of those on a GFD, and only 19.0% of healthy controls. In contrast, SENV-D was found exclusively in healthy individuals (23.8%) and not in CD patients. CD patients on a GCD exhibited markedly elevated cytokine levels, particularly IL-6 (76.2 ± 12.3 pg/mL), IL-8 (112.0 ± 40.2 pg/mL), and IFN-γ (50.2 ± 15.2 pg/mL), compared to GFD patients and healthy controls. Multivariate logistic regression identified IL-1, IL-2, IL-4, IL-6, IL-8, and IL-10 as significantly associated with active disease (ORs < 1, $p < 0.05$). Strong to very strong positive correlations were observed between SENV-H positivity and cytokine levels, with IL-6, IL-8, and IL-1 each showing correlation coefficients around 0.99. These results suggest that SENV-H may play a role in promoting or amplifying mucosal immune responses in active CD, whereas SENV-D appears unrelated. The findings highlight a potential interaction between viral replication and immune activation in celiac disease, meriting further mechanistic investigation.

Keywords: Celiac disease; Cytokines; SEN virus; Gluten Free Diet (GFD); Gluten Containing Diet (GCD); Interleukin

1. Introduction

Celiac disease (CD) is an autoimmune disorder triggered by the ingestion of gluten in genetically predisposed individuals. Gluten, a protein found in wheat, rye, and barley, serves as the environmental trigger for this disease. Advances in screening tests have improved the diagnosis, understanding of the pathogenesis, and natural history of CD. These developments have led to an increase in diagnoses, including among older populations, contributing to the global rise in CD cases. While some theories suggest that globalization, increased gluten consumption, and changes in gluten quality may contribute to this rise, these hypotheses remain unproven. Moreover, the growing incidence of other autoimmune diseases points to the possibility of environmental factors beyond gluten playing a role (Caio et al., 2019).

CD affects approximately 0.5–1% of the general population, with a higher prevalence in Western countries. It is more common in first-degree relatives of affected individuals and in those with conditions such as Down syndrome and type 1 diabetes. (Catassi et al., 2010). The pathophysiology of CD involves both genetic factors, particularly HLA-DQ2 and HLA-DQ8, and an autoimmune response to tissue transglutaminase (tTG), an enzyme in the intestines (Hall & Batt, 1992). Gluten triggers this immune response, but the increasing incidence of CD also suggests other contributing factors, such as improved hygiene, reduced exposure to pathogens, and imbalances in the gut microbiome. These factors, combined with genetic predisposition and immune system dysfunction, drive

the autoimmune response seen in CD. (Hall & Batt, 1992; Verdu et al., 2015).

Early-onset CD is commonly observed in infants, particularly those with a first-degree relative diagnosed with the disease. (Olivares et al., 2015). Genetic studies have identified over 100 non-HLA genes associated with CD, although their impact on risk is limited. HLA-DQ2 and HLA-DQ8 remain the primary genetic contributors (Ditah et al., 2015). Gluten, which was introduced into the human diet around 10,000 years ago, is a major environmental factor triggering CD. Its complex proteins, especially gliadins, are difficult to digest and can elicit immune responses similar to infections, leading to increased intestinal permeability and inflammation (Fasano, 2003).

Research has shown that gliadin, a key component of gluten, can disrupt tight junctions between epithelial cells, increasing intestinal permeability (Jelínková et al., 2004). This allows gluten peptides to pass through the intestinal barrier and enter the lamina propria, where they trigger inflammation and exacerbate CD (Jelínková et al., 2004). These peptides can also enter the bloodstream, spreading the inflammatory response (Silano et al., 2009).

The innate immune system plays a central role in initiating CD. Cytokines such as IL-15 and interferon α activate immune cells, and specific gluten peptides can trigger innate immune responses (Kim et al., 2015). Combined with gliadin-induced disruption of the gut barrier, these processes contribute to the onset of CD enteropathy (Matysiak-Budnik et al., 2008). The adaptive immune response involves the activation of CD4+ T cells and the production of pro-inflammatory cytokines, leading to intestinal damage and crypt hyperplasia (Junker et al., 2012).

The gut microbiome also influences CD pathogenesis. Alterations in microbiota composition, particularly a reduction in beneficial bacteria such as *Lactobacillus*, have been linked to CD development, although causality remains unclear. In genetically predisposed infants, early-life imbalances in the microbiota may contribute to the development of gluten intolerance and CD. More research is needed to establish a definitive link between the microbiome and CD (Lionetti et al., 2014; Vriezinga et al., 2014).

The immune response in CD primarily involves gluten-specific CD4+ T cells that use the α/β T-cell receptor (TCR). These T cells are central to the disease's pathogenesis, particularly through the secretion of cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, which are toxic to intestinal epithelial cells. Studies have shown that T-cell clones derived from celiac lesions produce a mix of cytokines, predominantly IFN-γ, as well as TNF, transforming growth factor (TGF)-β, interleukin (IL)-6, and occasionally IL-4, IL-5, and IL-10 (Auricchio et al., 2023; Eidan & Mubark, 2024).

Using quantitative RT-PCR, researchers have demonstrated that gluten stimulation in treated celiac tissue samples leads to increased IFN-γ expression, peaking after about two hours. Low levels of other cytokines such as IL-2, IL-4, IL-5, IL-6, and TNF-α are also detected, while IL-10 and IL-12p40 are typically undetectable. Immunohistochemistry further confirmed a significant increase in the number of IFN-γ-positive cells in the mucosal lamina propria compared to healthy controls. These findings suggest that gluten activates Th1 or Th0 T cells in genetically predisposed individuals, leading to the production of high levels of IFN-γ, which, either directly or via macrophages, contributes to mucosal damage in untreated CD (Manavalan et al., 2010).

The SEN virus (SEN-V) is a newly identified group of DNA viruses, including SEN-V-D and SEN-V-H, linked to post-transfusion hepatitis (Umemura et al., 2001b). It is a small, single-stranded, circular, non-enveloped DNA virus with a genome of approximately 3600 to 3800 nucleotides and at least three open reading frames (ORFs) (Sagir et al., 2004). Eight strains of SEN-V have been identified and tentatively classified within the Circoviridae family, which includes small, single-stranded, non-enveloped circular DNA viruses like the TT virus (TTV), TUS01, SANBAN, PMV, and YONBAN. The prevalence of SEN-V varies widely across different populations, with notable differences between countries and groups (Umemura et al., 2002). While parenteral transmission is most likely, other modes of transmission, including mother-to-infant transmission, have also been observed. Research into the impact of SEN-V on chronic liver diseases has been conducted, and its effect on the response to HCV therapy has been explored in three studies, though results have been contradictory. Data on the virus's role in other liver conditions are limited, and more research is needed to fully understand its pathogenesis and clinical significance (Umemura et al., 2001a).

The antiviral and immune-modulatory properties of interferon have made them valuable therapeutic agents for various viral and non-viral diseases. Recombinant interferon are used to treat several conditions. For example, IFN-α2 is commonly used to treat chronic hepatitis C and hepatitis B infections, while IFN-β is frequently prescribed for relapsing-remitting multiple sclerosis (MS), although the precise mechanisms behind its effectiveness in MS remain unclear (Fensterl & Sen, 2009).

This study aims to investigate the immunological impact of SENV in celiac patients by analyzing the levels of key cytokines, including interferon and interleukins. Cytokines play a critical role in immune regulation and could provide insights into the mechanisms underlying SENV's association with celiac disease.

2. Material and Methodology

The study was conducted at the Molecular Virology Laboratory, COMSATS University Islamabad. A total of 276 participants were enrolled, comprising 192 patients diagnosed with celiac disease and 84 healthy controls. Celiac disease patients were further categorized into two groups based on diet status: 144 patients consuming a

gluten-containing diet (GCD) and 48 patients following a gluten-free diet (GFD). Healthy controls were selected to match the age distribution as closely as possible within the recruitment area. All participants, or in the case of minors, their parents or legal guardians, were informed about the study objectives and written informed consent was obtained. Ethical approval for the study was granted by the Ethical Review Committee of COMSATS University Islamabad, and all procedures were conducted in accordance with the Declaration of Helsinki.

Demographic information including age, gender, and diet history was collected using structured data forms and verified from patients' medical records. Patients were categorized by age into two groups: those younger than 10 years and those older than 10 years to help control for potential age-related differences in immune response.

Approximately 3 mL of peripheral blood was collected aseptically from each participant using sterile vacutainer tubes containing a clot activator gel. The blood samples were centrifuged at 5,000 rpm for 5 minutes to separate the serum. The resulting sera were aliquoted and stored at −20℃ until further analysis.

Serum cytokine levels for IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, and IFN-γ were quantified using the Evidence Investigator Human Cytokine and Growth Factor Biochip Array Kit (Randox Laboratories, UK). All assays were performed in triplicate according to the manufacturer's instructions. The assay used a multiplex sandwich immunoassay format, in which cytokines in the serum bind to specific antibodies immobilized at defined coordinates on a biochip. Detection was performed using a horseradish peroxidase–labeled conjugate that produces a chemiluminescent signal directly proportional to the concentration of cytokines present. Standard curves for each cytokine were generated during each run to ensure accuracy and reproducibility, and all detection limits complied with the kit specifications.

Viral DNA was extracted from the sera using the GF-1 Viral DNA/RNA Extraction Kit (Vivantis, Malaysia) according to the manufacturer's protocol. Briefly, 50 µL of Proteinase K was added to 200 µL of serum along with 215 µL of Buffer VL containing carrier RNA. After vortexing, samples were incubated at 65℃ for 10 minutes. Then, 280 µL of absolute ethanol was added and mixed immediately. The mixture was transferred to a spin column, washed sequentially with Wash Buffers 1 and 2, and DNA was eluted in 30–50 µL of Elution Buffer. The extracted DNA was stored at −20℃ until PCR amplification.

Nested PCR was employed to detect SEN virus genotypes H and D. In the first round, universal primers targeting the ORF1 region were used to amplify viral DNA from the extracted samples. This was followed by a second round of semi-nested PCR using genotype-specific primers to differentiate between the two strains. Two microliters of the first-round PCR product were used as the template for the second round. Table 1 presents the primers used for SEN virus detection.

Table 1. Primer used in SEN virus

Primer	Round	Sequence
Universal		
AI-IF	First	5-TWCYCMAACGACCAGCTAGACCT-3 W=A or T, Y=C or T, M=A or C
AI-IR	First	5-GTTTGTGGTGAGCAGAACGGA-3
SENV-D specific		
D-1148 F	Second	5-CTAAGCAGCCCTAACACTCATCCAG-3
D-1341 R	Second	5-GCAGTTGACCGCAAAGTTACAAGAG-3
SENV-H specific		
H-1020	Second	5-TTTGGCTGCACCTTCTGGTT-3
H-1138	Second	5-AGAAATGATGGGTGAGTGTTAGGG-3

PCR cycling conditions were as follows: an initial denaturation at 95℃ for 5 minutes; followed by 35 cycles of denaturation at 95℃ for 30 seconds, annealing at 55-60 °C (optimized for each primer pair) for 30 seconds, and extension at 72℃ for 1 minute; with a final extension at 72℃ for 7 minutes. The expected amplicon sizes were 124 base pairs (bp) for SEN H and 88 bp for SEN D, which were subsequently visualized using 2% agarose gel electrophoresis.

PCR products were separated by electrophoresis using 2%–3% agarose gels prepared in 1× TAE buffer and stained with ethidium bromide (10 µg/mL). Electrophoresis was run at 60 V for 5 minutes and then at 90 V for 35 minutes. Gels were visualized using AlphaQuant Imaging Software (Alpha Innotech, USA). A 50 bp DNA ladder was used as a molecular size marker, and band intensities were semi-quantitatively analyzed using ImageJ software to confirm product sizes and specificity.

All demographic differences (age, gender, and diet groups) were analyzed using Chi-square tests. The relationship between cytokine levels and diet status (GFD vs. GCD) was assessed using multivariable logistic regression, reporting odds ratios (ORs) with 95% confidence intervals (CI). The association between cytokine levels and SEN H virus detection was evaluated using Pearson's correlation coefficient. A two-sided p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 25. De-

identified data are available upon reasonable request under a standard data use agreement in compliance with open data policies.

3. Results

3.1 Demographic Study of Celiac Patients

The current study included a total of 276 samples, consisting of 192 patients diagnosed with celiac disease and 84 healthy individuals. The demographic distribution of participants is listed below.

Gender-wise, among the 192 celiac disease patients, 88 (45.83%) were male and 104 (54.17%) were female, while the gender distribution among healthy individuals included 24 (28.57%) males and 60 (71.43%) females. The p-value of 0.0074 indicates a statistically significant difference.

Age-wise, participants were divided into two groups: those under 10 years of age and those over 10 years. Among the 192 celiac patients, 144 (75.00%) were under 10 years old and 48 (25.00%) were over 10 years old. In the healthy group, 52 (61.90%) were under 10 years and 32 (38.10%) were over 10 years old. The p-value of 0.027 indicates a statistically significant difference.

Diet-wise, celiac disease patients were categorized based on their diet into two groups: those on a gluten-containing diet (GCD) and those on a gluten-free diet (GFD). Among the celiac patients, 144 (75.00%) were on a GCD, while 48 (25.00%) were on a GFD. The p-value of <0.001 indicates a highly significant difference. Table 2 represents the Demographic Study of Celiac Versus Healthy individuals.

Table 2. Demographic study of Celiac versus healthy

Category	Group	Subgroup	Count (n)	Percentage (%)	p-value
Gender	Healthy Controls	Male	24	28.57%	
		Female	60	71.43%	
	Celiac Patients	Male	88	45.83%	
		Female	104	54.17%	**0.0074**
Age	Healthy Controls	<10 years	52	61.90%	
		>10 years	32	38.10%	
	Celiac Patients	<10 years	144	75.00%	
		>10 years	48	25.00%	**0.027**
Diet	Celiac Patients	GCD	144	75.00%	
		GFD	48	25.00%	**<0.001**

Note: p<0.05

3.2 Cytokine Profiling

We fitted a multivariable logistic regression model with diet status as the binary outcome (0 = Active Disease, 1 = Gluten-Free Diet), and seven cytokine levels (IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ) as predictors. In logistic regression, each coefficient is on the log-odds scale and is typically reported as an exponentiated odds ratio (OR) with a 95% confidence interval. An OR < 1 indicates that a higher cytokine level is associated with lower odds of being on a GFD (i.e., more common in active disease), and vice versa. Table 3 summarizes the ORs, 95% CIs, and p-values for all cytokines. A two-sided $\alpha = 0.05$ was used to determine statistical significance.

Table 3. Cytokine profile level and multivariate regression logistic analysis

Variable	Participants (n=276)				
	Active (n= 115)	GFD (n=77)	Healthy Control (n=84)	OR (95% CI)	p-value
IL-1 (SD)	6.3 (5.2)	4.1 (3.9)	2.5 (1.7)	0.50 (0.26–0.95)	0.033
IL-2 (SD)	14.6 (4.9)	6.7 (1.3)	4.4 (2.6)	0.84 (0.73–0.95)	0.008
IL-4 (SD)	9.1 (2.2)	8.4 (2.1)	0.4 (0.2)	0.80 (0.68–0.95)	0.013
IL-6 (SD)	76.2 (12.3)	50.3 (10.3)	6.3 (1.1)	0.62 (0.41–0.94)	0.023
IL-8 (SD)	112.0 (40.2)	40.2 (18.2)	10.1 (5.1)	0.90 (0.83–0.97)	0.006
IL-10 (SD)	5.2 (2.3)	2.1 (1.4)	1.2 (.6)	0.74 (0.56–0.97)	0.030
INF-γ (SD)	50.2 (15.2)	30.2 (7.4)	10.2 (4.1)	0.98 (0.95–1.01)	0.205

Furthermore, the study investigated the effect of cytokine on the growth of SEN H virus in different groups, active CD patient, non-active CD patients and healthy control group.

Group 1: Among active celiac disease patients on a gluten-containing diet (GCD), elevated cytokine levels were observed, with SEN H virus detected in 71.4% of cases (82/115).

Group 2: This group include patient suffering with celiac disease on GFD with slightly elevated cytokines.

They showed 66.67% growth of SEN H virus.

Group 3: This group include healthy individuals, control group with normal level of cytokines. They showed the least SEN H viral replication, with only 19.4% of the samples. Figure 1 illustrates the detection of SEN H virus nucleic acid in the electropherogram.

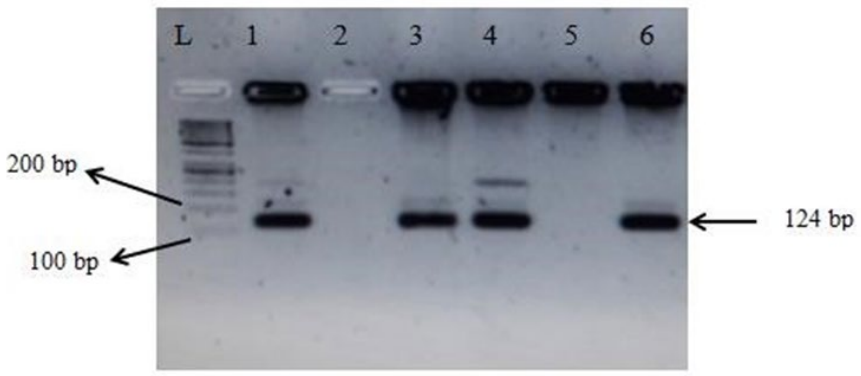

Figure 1. Electropherogram of SEN H virus in celiac patient and healthy control. L = ladder, lane 1&2 = Group 1, lane 3&4 = Group 2, lane 4&5 = healthy control

The detail of cytokines effects on viral replication is given in the Table 4.

Table 4. Effects of cytokine on the growth of SEN H and SEN D virus

Variables	Group 1	Group 2	Group 3
IL-1 (SD)	6.3(5.2)	4.1 (3.9)	2.5±1.7
IL-2 (SD)	14.6±4.9	6.7 (1.3)	4.4 (2.6)
IL-4 (SD)	9.1 (2.2)	8.4 (2.1)	0.4 (0.2)
IL-6 (SD)	76.2 (12.3)	50.3 (10.3)	6.3 (1.1)
IL-8 (SD)	112.0 (40.2)	40.2 (18.2)	10.1 (5.1)
IL-10 (SD)	5.2 (2.3)	2.1 (1.4)	1.2 (.6)
INF-γ (SD)	50.2 (15.2)	30.2 (7.4)	10.2 (4.1)
SEN H virus Growth	71.42% (82/115)	66.67% (51/77)	19% (16/84)
SEN D virus Growth	0.0% (0/115)	0.0% (0//77)	23.8% (20/84)

Note: Group 1 = Cytokines Elevated, Group 2 = Moderate Elevation, Group 3 = Healthy Control

Conversely, SEN D exhibited no growth in Group 1 and Group 2 while showed SEN D viral replication 23.8% in healthy control group (Group 3) with normal level of cytokines. Figure 2 illustrates the detection of SEN D virus nucleic acid in the electropherogram.

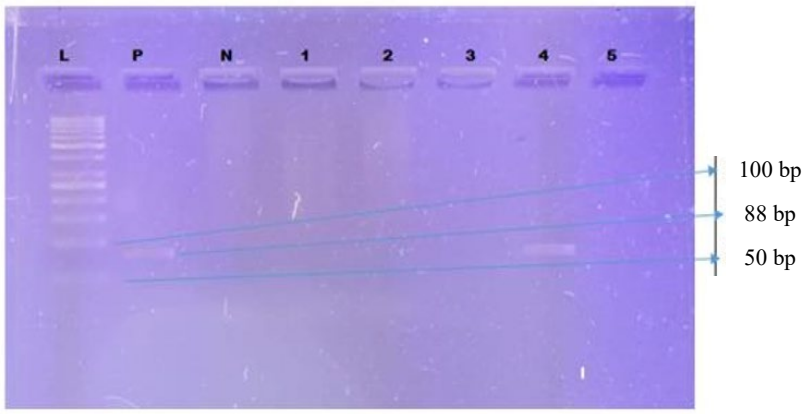

Figure 2. Electropherogram of SEN D virus in Celiac patient and healthy. L = ladder, lane N = negative control, lane p = positive, lane 1 = Group 1, lane 2 = Group 2, and lane 3,4&5 = Group 3

Table 5 represents the Correlation between Cytokine Levels and SEN H virus Positivity. Very strong positive

correlations ($r \geq 0.97$) were observed for most cytokines: **IL-1, IL-2, IL-6, IL-8, IL-10, and INF-γ**. This suggests that as levels of these cytokines increase, so does the prevalence of SEN H virus. **IL-6, IL-8, and IL-1** had the highest correlation values ($r \approx 0.99$), indicating a particularly tight association with SEN H virus positivity. These cytokines may play a key role in the immune response to the virus or reflect viral-induced inflammation. **IL-4** showed a weaker but still strong correlation ($r = 0.714$), potentially due to smaller variation in IL-4 levels between groups with high and moderate infection rates.

Table 5. Correlation between Cytokine levels and SEN H virus positivity

Cytokine	Pearson's r with SEN H Virus Positivity (%)	Strength of Correlation
IL-1	0.991	Very Strong
IL-2	0.977	Very Strong
IL-4	0.714	Strong
IL-6	0.991	Very Strong
IL-8	0.990	Very Strong
IL-10	0.985	Very Strong
INF-γ	0.985	Very Strong

4. Discussion

The aim of the current study was to explore cytokine profiling and its association with SEN virus replication in patients suffering from celiac disease. A total of 276 participants were examined, comprising 192 patients diagnosed with celiac disease and 84 healthy individuals**.** Participants were divided into three groups: Group 1 (celiac patients on a gluten-containing diet, GCD), Group 2 (celiac patients on a gluten-free diet, GFD), and Group 3 (healthy controls). The primary focus of the study was to investigate the effect of cytokine levels on SEN virus replication in celiac disease patients compared to healthy individuals.

Cytokine play important role in autoimmune diseases. It is a key regulatory protein involved in immune response and inflammations. In the current study, the cytokine level of different groups was compared with healthy control. Group 1, CD patients on GCD showed significant elevation particularly IL-6, IL-8 and slightly INF-γ. Group 2, CD patients on GFD exhibited moderate elevation and healthy control with no elevation. They showed normal level of cytokines. These reports show consistency with previous reports that IL-6, a pro-inflammatory cytokine, elevated. IL-6 plays a significant role in promoting intestinal inflammation and tissue damage in patients suffering with celiac disease (Garrote et al., 2008). IL-8 with a function of recruiting neutrophils, elevated in CD patients, indicating an increased inflammatory state (Nasserinejad et al., 2019).

Conversely, Group 2 (celiac disease patients on a GFD) showed a moderate elevation, with relatively lower levels of cytokines—particularly IL-6 and IL-8—compared to Group 1, suggesting that a GFD may help modulate inflammation but does not completely normalize cytokine levels. This conclusion supports the previous hypothesis that a GFD may reduce, but not entirely eliminate, immune activation in CD patients. Group 3 exhibited normal levels with the lowest cytokine elevation, as they do not experience the immune activation associated with CD (Manavalan et al., 2010).

These results emphasize the importance of cytokines in the pathophysiology of CD, particularly in the context of immune dysregulation and inflammation. Celiac disease can be aggravated by gluten ingestion. IL-6, IL-8, and IFN-γ may serve as markers of active celiac disease. The presence of these cytokines might reflect chronic inflammation in untreated celiac patients.

Additionally, the study investigates the relationship between elevated cytokines and viral replication, particularly SEN D and SEN H virus in patient suffering with celiac disease. SEN H virus shows positive correlation with cytokines elevation particularly IL-6, IL-8 and INF-γ. INF-γ showed slightly lower elevation as compared IL-6 and IL-8.

The current study clearly demonstrates a relationship between cytokine levels and viral replication. SENV-H showed significant replication (71.42%) in celiac patients on a gluten-containing diet (Group 1), who exhibited elevated levels of cytokines such as IL-6, IL-8, and moderately increased IFN-γ. These cytokines are known to contribute to an environment conducive to viral replication, possibly by modulating the antiviral response and promoting immune cell infiltration (Simón et al., 2023). Our findings align with previous reports suggesting that IL-6 and other inflammatory cytokines can facilitate viral persistence or reactivation by impairing antiviral control mechanisms.

Similarly, celiac disease patients on a gluten-free diet (Group 2) showed moderate SENV-H replication (66.67%), implying that reduced cytokine levels in response to dietary modification may partially suppress viral replication. Additionally, elevated IL-6 and TNF-α levels observed in SENV-H-positive CD patients suggest a possible synergistic role of viral replication in promoting or sustaining Th1-type inflammation.

In contrast, healthy controls (Group 3) showed the lowest level of SENV-H replication (19.4%), consistent with

the idea that a non-inflamed, low-cytokine environment is less favorable for viral proliferation. These data collectively indicate that SENV-H replication is closely linked to cytokine elevation, immune activation, and the inflammatory state of the host.

Interestingly, SENV-D replication showed a different pattern. It was completely absent in both Group 1 (CD on GCD) and Group 2 (CD on GFD), despite elevated cytokine levels. This suggests that pro-inflammatory immune activation does not universally support viral replication and may, in fact, suppress SENV-D replication through genotype-specific immune mechanisms. One possible explanation is that SENV-D is more susceptible to innate immune responses, particularly type I and II interferons, which are elevated during inflammation. Another possibility is that SENV-H and SENV-D utilize different cellular receptors or co-factors for replication, and these factors may be downregulated or altered in inflamed intestinal tissue. Alternatively, SENV-D may lack certain immune evasion strategies possessed by SENV-H, rendering it more vulnerable to cytokine-mediated restriction.

Surprisingly, healthy controls (Group 3) with normal cytokine levels showed detectable SENV-D replication in 23.8% of samples. This suggests that a low-inflammatory environment may be more permissive for SENV-D replication, possibly due to reduced immune surveillance. These findings point toward distinct host–virus interaction dynamics between the two SENV genotypes, underscoring the need for further studies to explore their receptor usage, immune sensitivity, and tissue tropism.

4.1 Clinical Implications and Future Directions

This study contributes significantly with regard to the impact of cytokines in celiac disease pathogenesis and viral infections. The results suggest that increased cytokines, particularly IL-6 and IL-8, seem to enhance SEN H virus replication, while the SEN D virus shows no evidence of such replication during the presence of high levels of the aforementioned cytokines. These particular impacts on viral replication may be due to the particular cytokine milieu and how it regulates immune response to various pathogens. Further studies are necessary to uncover the reasons that stem viral infections may aggravate celiac disease symptoms, dietary changes including gluten-free diet, and the infection's effects on the immune system.

The study emphasized the relevance of regularly monitoring the immune response in patients with celiac disease, especially in those with active disease, through systematic cytokine profiling in tracking disease progression and the possibility of complications. In addition to this, the observed patterns of viral replication emphasized a need for more investigations into how viral infections may coexist with autoimmune diseases and how such infections are associated with the severity of the illness as well as the outcome.

5. Conclusion

In conclusion, the present study provides evidence that active and severe celiac disease patients exhibit elevated concentrations of pro-inflammatory cytokines such as IL-6 and IL-8, which were associated with detectable replication of the SEN H virus. Notably, SEN D virus replication was observed only in healthy controls and not in patient groups, suggesting a potential interaction between inflammatory status and viral activity. However, the precise mechanisms underlying this pattern—whether driven by cytokine-mediated suppression, host receptor differences, or other immunological factors—remain unclear and require further mechanistic investigation. These findings highlight the complex interplay between systemic inflammation and viral persistence in autoimmune conditions like celiac disease. Future studies should explore these host–virus interactions in greater detail, including the role of immune modulation as a potential therapeutic avenue.

Data Availability

The datasets used and analyzed during this study are not publicly available due to restrictions from the corresponding author but can be provided upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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