Mucin Expression in the Small Bowel of Celiac Disease: A Systematic Review

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ABSTRACT **Background:** Mucins, heavily glycosylated glycoproteins, are synthesized by mucosal surfaces and play an important role in healthy and malignant states. Changes in mucin synthesis, expression, and secretion may be a primary event or may be secondary to inflammation and carcinogenesis.

Objectives: To assess current knowledge of mucin expression in the small bowel of celiac disease (CD) patients and to determine possible associations between mucin profile and gluten-free diet.

Method: Medical literature searches of articles in English were conducted using the terms *mucin* and *celiac*. Observational studies were included. Pooled odds ratios and 95% confidence intervals were calculated.

Results: Of 31 articles initially generated by a literature search, 4 observational studies that fulfilled the inclusion criteria remained eligible for meta-analysis. These studies included 182 patients and 148 controls from four countries (Finland, Japan, Sweden, United States). Mucin expression was significantly increased in small bowel mucosa of CD patients than in normal small bowel mucosa (odds ratio [OR] 7.974, 95% confidence interval [95%CI] (1.599–39.763), P = 0.011] (random-effect model). Heterogeneity was significant: Q = 35.743, df (Q) = 7, P < 0.0001, I^2 = 80.416%. ORs for MUC2 and MUC5AC expression in the small bowel mucosa of untreated CD patients were 8.837, 95%CI 0.222–352.283, P = 0.247 and 21.429, 95%CI 3.883–118.255, P < 0.0001, respectively.

Conclusions: Expression of certain mucin genes in the small bowel mucosa of CD patients is increased and may serve as a diagnostic tool and assist in surveillance programs.

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KEY WORDS: celiac disease (CD), gene expression, meta-analysis, mucin, small bowel

Mucins are heavily glycosylated, high-molecular-weight glycoproteins, synthesized by mucosal surfaces. They play an important role in healthy state and malignant diseases [1,2]. Change in mucin synthesis, expression, and secretion may be a primary event or secondary to inflammation and carcinogenesis.

There are more than 20 known mucin genes in the human genome, encoding for two types of mucins: secreted and membrane-bound [3]. In addition to their protective role, membrane-bound mucins are involved in cell signaling and processes such as growth, motility, adhesion, and immune modulation.

Celiac disease (CD) is an immune-mediated disease that is induced by consumption of gluten in genetically predisposed individuals, results in inflammation and damage to the intestinal mucosa, and is associated with an increased risk of lymphoproliferative malignancies, especially when there is persistent villous atrophy [4].

The role of mucins in CD has never been thoroughly studied. Laparra and colleagues [5] demonstrated in vitro that dietary glycosaminoglycans (components of mucin) interfered with bacterial adhesion and gliadin-induced pro-inflammatory response in intestinal epithelial cells. Capuano and co-authors [6] found a significant decrease in MUC2 expression in small bowel mucosa of active celiac disease patients as well as inactive/treated celiac patients in comparison with healthy controls. Their research was not included in our meta-analysis since only small number of patients and biopsies were studied, without quantification. Cinova et al. [7], using the rat model, found that gliadin fragments and/or interferon-gamma reduced the number of PAS-positive goblet cells but increased mucin secretion; changes typical for early stages of any enteropathy. These changes were more pronounced when these agents were combined with pathogenic enterobacteria.

The aim of our study was to assess the current knowledge about mucin expression in the small bowel of CD patients, and to determine possible associations between mucin profile and gluten-free diet.

METHODS

SEARCH STRATEGY

Medical literature searches were conducted in English for the terms mucin and celiac. Searches were performed until 31 May 2022 using MEDLINE/PubMed, Scopus, EMBASE, and CENTRAL. No beginning date limit was used. Hand searches

of medical articles were also performed. Only fully published human studies in English were included [Figure 1].

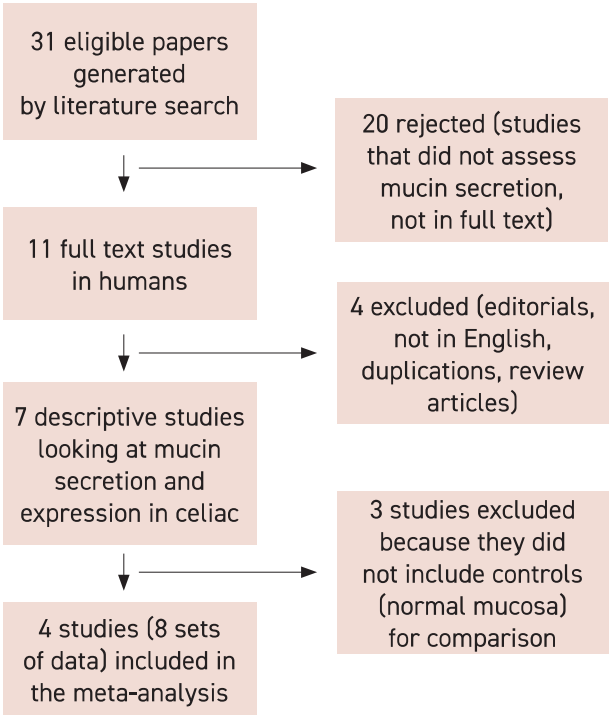
STUDY SELECTION

The meta-analysis was performed according to the PRISMA checklist [8]. Inclusion and exclusion criteria were delineated before the commencement of the literature search. Observational studies describing mucin expression in small bowel normal mucosa, celiac in remission, and celiac in relapse were included. We selected only studies that clearly included data on small bowel of celiac patients compared with normal small bowel mucosa of healthy controls.

DATA EXTRACTION

The name of the first author, country of origin, year of study publication, number of patients with celiac disease and controls that were included in the study and the number of positive staining for a specific mucin was extracted. Next, data were stratified according to the lesion (celiac in relapse or remission) and according to the mucin expressed (MUC2 or MUC5AC). The different sub-studies (data-sets) were considered separately. The advantages and disadvantages of each included study were briefly abstracted.

Figure 1. Flow chart of the articles identified for the meta-analysis



STATISTICAL ANALYSIS

We calculated the pooled odds ratios (ORs) and 95% confidence intervals (95%CIs) and compared the results of individual studies by using the random-effect model [9,10]. Forest plots were constructed for visual display of ORs of individual studies. Heterogeneity among studies was evaluated using the Cochran Q-test [11] and was considered to be present if the Q-test P value ≤ 0.10. I^2 statistic was used to measure the proportion of inconsistency in individual studies. We also calculated a potential publication bias by constructing a funnel plot, which was obtained by plotting the log ORs versus standard error (SE) of individual studies [12]. Symmetry was estimated by the Begg and Mazumdar adjusted rank correlation test [13]. All analyses were performed by using Comprehensive Meta-Analysis Software (Version 4, Biostat Inc., Englewood, NJ, United States).

RESULTS

DESCRIPTIVE ASSESSMENT AND STUDY CHARACTERISTICS

A flow chart describing the process of study selection is shown in Figure 1. Of 31 titles initially generated by the literature searches, 4 observational studies that fulfilled the inclusion criteria remained eligible for meta-analysis [14-17]. They included 182 patients and 148 controls from four countries (Finland, Japan, USA, Sweden). The main characteristics of the papers eligible for meta-analysis are shown in Table 1. There was no evidence of publication bias as judged by the construction of funnel plot and estimation of its symmetry [Figure 2].

MUCIN EXPRESSION

In the four studies eligible for meta-analysis (8 sets of data), mucin expression was significantly increased in small bowel mucosa of celiac patients compared to normal small bowel mucosa (OR 7.974, 95%CI 1.599–39.763, P = 0.011; random-effect model) [Figure 3]. Heterogeneity was significant: Q = 35.743, df (Q) = 7, $P < 0.0001$, $I^2 = 80.416\%$ [Figure 3, Table 1]. ORs for MUC2 and MUC5AC expression in the small bowel mucosa of untreated CD patients were 8.837, 95%CI 0.222–352.283, $P = 0.247$ and 21.429, 95%CI 3.883–118.255, $P < 0.0001$, respectively. There was no significant publication bias (Begg and Mazumdar adjusted rank correlation test).

DESCRIPTION OF STUDIES

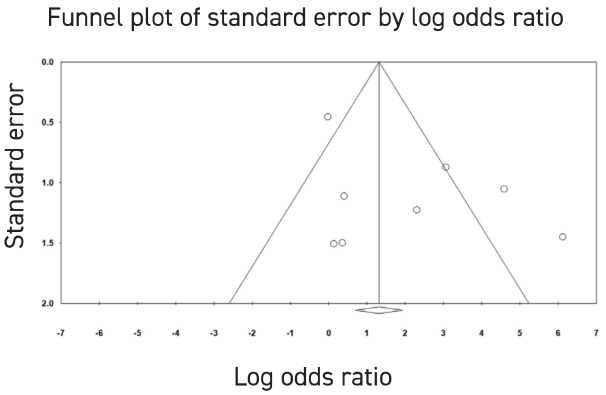
Teppo and Maury [14] found an increase in serum antibodies level against epithelial glycoprotein in celiac patients. Shaoul et al. [15] looked at mucin expression in duodenal biopsies of 22 untreated children compared with controls. Marked villous atrophy was associated with a significant increase in MUC5AC expression. They interpreted their findings by the development of gastric metaplasia, characterized by MUC5AC and gastric trefoil factor expression, as well as by positive staining for

Table 1. The main characteristics of studies included in the meta-analysis

Author name [Reference number]	Publication year	Country	Mucin studied (method)	Pathology	Sets of data	Total number of cases	Total number of controls
Teppo [14]	1984	Finland	Serum antibodies to glycoprotein (EIA)	Proved celiac disease	Celiac patients with a positive serology	16	30
Shaoul [15]	2000	Japan	MUC5AC (IHC)	Villous atrophy	Untreated children	22	22
Ciacci [16]	2002	USA	MUC2 (IHC and RT-PCR)	Villous atrophy	Untreated patients	9	8*
					Treated patients	11	8
Forsberg [17]	2004	Sweden	MUC2 (RT-PCR)	Proved celiac disease	Untreated patients	55	78*
					Treated patients	53	78
	2004	Sweden	MUC2 (UEA1 lectin)	Proved celiac disease	Untreated patients	11	10*
					Treated patients	5	10
Total						182	148

EIA = enzyme immunoassay, IHC = immunohistochemistry, RT-PCR = reverse transcription polymerase chain reaction
*Used twice

Figure 2. Funnel plot for publication bias



neutral mucin by periodic-acid-Schiff. Gastric metaplasia may protect the atrophic duodenal mucosa from acid and pepsin, being hyper secreted in untreated CD. Interestingly, Ciacci and colleagues [16] found a significant decrease of intestinal trefoil factor in distal duodenum goblet cells of untreated patients with CD, with no parallel significant decrease of MUC2. Unfortunately, MUC5AC was not assessed in this study. Forsberg et al. [17] found that rod-shaped bacteria were frequently associated with the mucosa of CD patients. The lectin Ulex europaeus agglutinin I (UEAI) stained goblet cells in the mucosa of all CD patients but not of controls (attached to alfa-L-fucose on MUC2). mRNA levels of MUC2 were significantly increased in active CD, correlated to the interferon-γ mRNA levels in intraepithelial lymphocytes.

DISCUSSION

Mucin is an important component of the mucus layers protecting epithelial surfaces of the respiratory, digestive, urinary, and reproductive organs and as such has been studied intensively. Mucin has an important protective role in the small bowel that is exposed to gastric acid, bile acids, and toxic materials in the food.

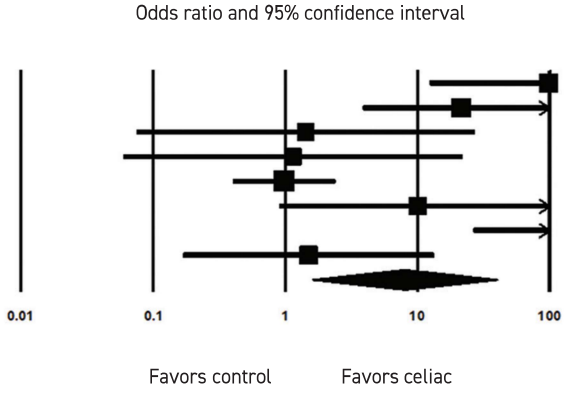
Gluten is a complex protein that increases intestinal permeability [18] and when consumed cannot be fully hydrolyzed by human gastrointestinal proteases [19,20]. Both hydrolyzed and non-hydrolyzed fractions are involved in eliciting toxicity to the gut mucosa that involves various mechanisms including cytotoxic and immunomodulator pathways [21]. All of these conditions resolve on a gluten-free diet.

While data are available on mucin expression in diseases of the gastrointestinal tract, interestingly, there is a lack of knowledge about mucin expression in the small bowel of CD patients on gluten-containing or gluten-free diets.

In our meta-analysis, we found a significant increase in mucin expression in the small bowel of CD patients compared with healthy controls. The specific mucins that increased were MUC2 and MUC5AC. MUC2 is the mucin usually secreted from the small bowel mucosa and the main component of the goblet cell. MUC5AC is usually secreted by the gastric epithelium and is a marker for gastric metaplasia. We hypothesize that mucosal challenge with gluten stimulates secretion of protective mucins. If this protective response is adequate, the small bowel mucosa is unlikely to become inflamed or undergo malignant transformation. However, if aggressive factors overwhelm this protective response, chronic mucosal inflammation can occur, which results in the development of a new cellular phenotype from stem cell proliferation, columnar

Figure 3. Forest plot concerning mucin expression in the small bowel of celiac disease patients (4 studies, 8 sub-studies)

Study author	Subgroup within study	Comparison	Time point	Statistics for each study				
				Odds ratio	Lower limit	Upper limit	z-value	*P*-value
Teppo	Celiac patients, antibodies to glycoprotein, EIA	Finland	1984	98.000	12.462	770.669	4.357	0.000
Shaoul	Untreated children, MUC5AC, IHC	Japan	2000	21.429	3.883	118.255	3.517	0.000
Ciacci	Treated patients, MUC2, IHC and RT-PCR	USA	2002	1.429	0.076	26.895	0.238	0.812
Ciacci	Untreated patients, MUC2, IHC and RT-PCR	USA	2002	1.143	0.060	21.870	0.089	0.929
Forsberg	Treated patients, MUC2, RT-PCR (positive > 20 mRNA copies/18SrRNA unit)	Sweden	2004	0.977	0.401	2.376	-0.052	0.959
Forsberg	Treated patients, MUC2, RT-PCR (positive > 0.4, no=0, weak=1, strong=2)	Sweden	2004	10.000	0.907	110.282	1.880	0.060
Forsberg	Untreated patients MUC2, RT-PCR (positive > 20 mRNA copies/18SrRNA unit)	Sweden	2004	454.742	26.593	7776.221	4.225	0.000
Forsberg	Untreated patients, MUC2, RT-PCR (positive > 0.4, no=0, weak=1, strong=2)	Sweden	2004	1.500	0.170	13.225	0.365	0.715
				7.974	1.599	39.763	2.533	0.011



C = control, EIA = Enzyme immunoassay, IHC = immunohistochemistry, L = lesion, RT-PCR = reverse transcription polymerase chain reaction

epithelium (morphologically better equipped to cope with acid), pepsins (gastric type), or bile acids (intestinal type of epithelium). Our hypothesis is supported by the findings of recent studies, which demonstrate changes in the microbiome in the small bowel epithelium of celiac disease patients [22-25]. Several genes of the mucosal cells that have a role in mucosal innate immunity such as toll-like receptors, trefoil peptides, interferon-gamma, and mucin are activated in active celiac disease by these bacteria, and de-activated in remission following gluten-free diet.

Our research has limitations, including the small number of included studies, the variation among ORs, the different classification of the sub-studies, and the high heterogeneity between them, which may limit the interpretation of the results. In addition, the different methods used in the studies to evaluate mucin expression, different antibodies, and staining protocols or scoring techniques may have affected the validity of our results.

CONCLUSIONS

Expression of certain mucin genes in the small bowel mucosa of celiac disease patients may serve as a diagnostic tool and may add important information to the surveillance program of celiac disease. Changes in mucin synthesis, expression, and secretion may be a primary event or secondary to inflammation and carcinogenesis. Mucin expression was significantly increased in the small bowel mucosa of celiac disease patients than in normal small bowel mucosa. Future prospective, controlled studies are needed to confirm our observations.

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He is a hard man who is only just, and a sad one who is only wise.
François-Marie Arouet (M. de Voltaire) (1694–1778), French Enlightenment writer, historian, and philosopher

Capsule

Taking the STING out of host defenses

The papain-like proteases (PLPs) from coronaviruses remove the proteins ubiquitin and ISG15 from host proteins to suppress antiviral signaling. **Xiong** et al. found that in contrast to those from the highly pathogenic coronaviruses, PLPs from mildly pathogenic coronaviruses preferred ubiquitin over ISG15. Coronavirus pathogenicity also correlated with the PLP's ability to inhibit antiviral signaling. In a related paper, **Cao** et al. showed that the SARS-CoV-2 PLP deubiquitinated the host defense protein STING. A STING agonist and a PLP inhibitor synergistically reduced SARS-CoV-2 replication in human lung cells.

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Eitan Israeli