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MUC1 EXPRESSION IN THE GASTRIC MUCOSA OF *HELICOBACTER PYLORI* POSITIVE GASTRITIS – A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract – *Objective:* MUC1, a membrane-bound mucin, is one of the important components of the gastric mucus unstirred layer, protecting the mucosa from the enzymatic attack of acid and pepsin, as well as from toxins and microorganisms. The aim of the study is to investigate the relationship between *H. pylori* and MUC1 expression in gastric mucosa.
Materials and Methods: English Medical literature searches were conducted for gastric MUC1 expression in *H. pylori*-infected patients *vs.* uninfected people. Searches were performed up to August 31, 2023, using PubMed, EMBASE, Scopus, and CENTRAL. Meta-analysis was performed by using Comprehensive meta-analysis software (Version 4, Biostat Inc., Englewood, NJ, USA). Pooled odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. Heterogeneity was evaluated using Cochran Q-test, and it was considered to be present if the Q-test *p*-value was less than 0.10. I^2 statistic was used to measure the proportion of inconsistency in individual studies, with $I^2 > 50\%$ representing heterogeneity. We also calculated a potential publication bias.
Results: 6 studies, which represent 9 sub-studies, were selected according to the inclusion criteria. The odds ratio of MUC1 expression in random effect analysis was 0.625, 95% CI 0.178-2.196, 37.5% lower in *H. pylori* gastritis than in normal mucosa. Heterogeneity and inconsistency were moderate, and no publication bias was demonstrated.
Conclusions: This meta-analysis showed that MUC1 expression is lower in *H. pylori* infected mucosa, which may have a significant effect on the survival of the bacterium and persistent chronic mucosal inflammation.

Keywords: *Helicobacter pylori,* MUC1, Mucin, Systematic review, Meta-analysis.

Abbreviations: MUC = mucin gene, OR = odds ratio, CI = confidence interval.

INTRODUCTION

Mucins, high molecular weight glycoproteins, are the main component of the mucus layer attached to the gastric mucosa. Mucins are heavily O-glycosylated with sugar side chains, and relatively stable to the active action of peptidases such as pepsin. They give the mucus unstirred layer the quality of viscosity and protect the mucosa from acid, pepsin, toxic material, and microorganisms.

Helicobacter pylori (*H. pylori*) needs the protection of the mucous layer against the hostile gastric environment of acid and pepsin and is adapted to live in the mucin environment, which enables moving in the viscous material by liquefying it using urease and higher pH. However, mucin has an antibiotic effect against the bug that controls its proliferation and colonization[1-3]. There are 3 main mucin types expressed in the gastric mucosa: MUC1, a membrane-bound mucin, and MUC5AC and MUC6, which are secreted mucins. MUC5AC is secreted by the superficial epithelium (Foveola) and MUC6 by the deep mucosal glands[4]. Mucin backbone has Variable Number Tandem Repeats (VNTR), composed of 25% amino acids serine, threonine, and proline, repeated 25-125 times. O-glycans are attached only to serine and threonine. The more amino acids in the VNTR the better the protection. MUC1 has 20 amino acids VNTR, MUC5AC (secreted by the surface epithelium) has 8 amino acids VNTR, and MUC6 (secreted by the deep mucosal glands) has 169 amino acids VNTR and gives the best protection. In this meta-analysis, we looked at studies that investigated the relationship between *H. pylori* and MUC1 expression in the gastric mucosa. Controversial results were found in several studies, and a dual effect was attributed to MUC1 in relation to *H. pylori* infection[5-10]. MUC1 has a dual effect on *H. pylori* colonization, releases the N-terminal subunits as a releasable decoy which binds the bacteria and prevents the attachment to the receptors in MUC5AC (such as Lewis b), but also inhibits toll-like receptors and decreases innate immune response. The net effect of MUC1 on *H. pylori* colonization is negative, as demonstrated by Sheng et al[11], comparing colonization in MUC1 knockout mice to wild type. We collected all the relevant studies that looked at MUC1 expression in the gastric mucosa of *H. pylori*-infected patients in comparison with healthy controls, trying to solve this controversy.

MATERIALS AND METHODS

Identification of Studies and Data Extraction

To identify studies and extract data in this meta-analysis, the PubMed, EMBASE, Scopus, and CENTRAL databases were searched until 31.8.2023 to identify human studies written in English using the following search text and/or Medical Topic Heading (MeSH) terms: *Helicobacter pylori OR H. pylori [All Fields] AND "MUC1" [MeSH Terms].* In addition, a manual search of all review articles published editorials and retrieved original studies, was made. Hand searches included articles bibliography. This meta-analysis was performed according to the PRISMA extension statement for interventions[12].

Selection Criteria – Primary Endpoints

We defined the inclusion and exclusion criteria before starting the study investigation. Thus, appropriate studies were included in the meta-analysis provided that the following criteria were met: a. published as complete articles with data that can be extracted; b. written in English, and c. were comparing *H. pylori* gastritis tissue with normal controls. Studies that did not meet these criteria were excluded. The resolution of OR and 95% CI was defined as the primary endpoint. Case-control studies comparing MUC1 expression in the gastric mucosa in patients positive and negative for *H. pylori* infection were included. *H. pylori* infection should be diagnosed with at least one of the following methods: histology, urease test, 13C-urea breath test, stool antigen test, or *H. pylori* DNA. We selected only studies that used standard immunohistochemistry with antibodies against mucin proteins and only those that expressed results by moderate and/or strong positive staining percentage. Thus, studies expressing the results with mean± SD of staining scores were excluded, since meta-analysis could not be performed.

Heterogeneity, Sensitivity, and Publication Bias

The heterogeneity of the studies was calculated using the Cochran Q test and I^2 inconsistency index and it was considered to be present if the *Q*-test *p*-value was less than 0.10. The higher the I^2, the greater the heterogeneity. The 25%, 50%, and 75% values indicate low, moderate, and high

heterogeneity, respectively. The sensitivity testing was conducted by removing individual studies from the overall result. The publication bias was analyzed using a funnel plot complemented by Begg-Mazumdar and Egger statistics. We constructed comparison-adjusted funnel plots and checked their symmetry to assess whether small-scale trials influence the efficacy results.

Statistical Analysis

Meta-analysis was performed by using Comprehensive meta-analysis software (Version 4, Biostat Inc., Englewood, NJ, USA). Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to compare mucin expression in individual studies by using the random effects model.

RESULTS

A Systematic Review of the Selected Studies[5-10]

Vinall et al[5] investigated the involvement of MUC1 in chronic gastritis using immunohistochemical analysis on endoscopic biopsy specimens from 95 patients, using antibodies against the tandem repeats of 20 amino acids. They found strong positive staining in 26 out of 41 normal specimens and *H. pylori* negative in 35 out of 36 specimens of gastritis positive for *H. pylori* (Table 2). The investigators assumed that this difference between normal and infected mucosa staining for MUC1 was due to the greater frequency of MUC1 short alleles in patients with *H. pylori* gastritis.

Cohen et al[6] looked at gastric biopsy specimens from 15 children with *H. pylori* chronic gastritis. Positive MUC1 staining was found in 4 out of 15 gastritis *H. pylori*-positive specimens (Table 2) and in none of the healthy mucosa. The investigators believe that the gastric mucosa of children may normally exhibit less MUC1 expression than that of adults.

Kocer et al[7] performed immunohistochemical staining for MUC1 in 30 *H. pylori*-positive and 15 *H. pylori*-negative antral gastric endoscopic biopsy specimens. *H. pylori* infection does not significantly affect the staining intensity and patterns of MUC1 expression but was higher in gastritis with *H. pylori*.

Wang et al[8] found positive immunohistochemical staining for MUC1 in 7 out of 21 gastric preneoplastic tissue specimens positive for *H. pylori*, and in 12 out of 16 negative for *H. pylori*. Similarly, 13 out of 26 gastric adenocarcinoma specimens were positive for *H. pylori*, and 16 out of 20 specimens were negative for *H. pylori.* The authors believe that *H. pylori* interacts with MUC1 and that functional allelic differences affect susceptibility to gastritis.

Rashid et al[9] used several different antibodies against MUC1, before and after deglycosylation. They believe that there is no substantial loss of the mucin domain of MUC1 from the apical surface in gastritis, but rather *H. pylori* influences the glycosylation of MUC1. This paper highlights the issue of epitope specificity of monoclonal antibodies directed against disease-associated markers, specifically when they are glycoproteins, as is the case for many cancer markers.

Niv et al[10] studied a cohort of randomly selected patients with *H. pylori*, NSAID, combined *H. pylori* and NSAID associated gastric ulcers, and patients with idiopathic gastric ulcers. Immunohistochemistry staining for MUC1 protein was strongly expressed on the apical membrane of the glands and mucosal surface foveola epithelial cells, higher for *H. pylori*-positive ulcer than NSAID-induced ulcer, but lower than in idiopathic ulcer.

Results of the Meta-Analysis

Our literature search revealed 87 studies that measured MUC1 expression in the gastric mucosa (Figure 1). We excluded 43 studies not involving human beings and not in full text and 38 studies not written in English, duplications, editorials, or review articles. Four studies (out of the excluded 38) were excluded for using the average score and standard deviation for comparison of *H. pylori* positive and negative states; thus, a meta-analysis could not be performed. From the results of these papers, we could not retrieve the relative strength of each study as we could for studies where results were expressed as a percentage of moderate or strong staining. We were left with 6 studies (9 data-sets) that fulfilled the inclusion criteria, in human beings that measured gastric MUC1 stain-

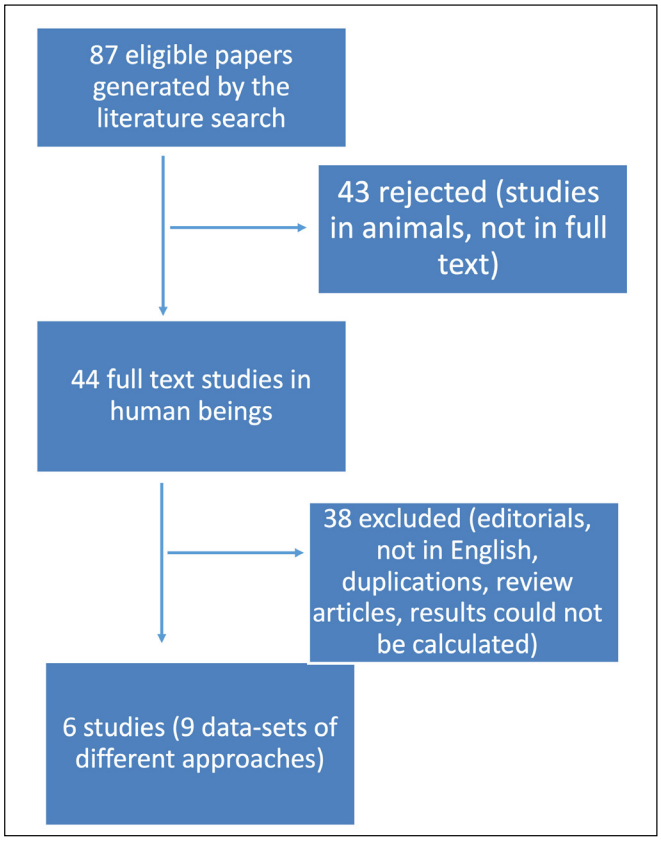

Figure 1. Flow chart of studies included in the meta-analysis.

ing intensity using immunohistochemistry, and compared the staining score between positive and negative *H. pylori* patients, published up to 31.8.2023 from 6 countries, the Netherlands, Argentina, Turkey, China, the UK, and Israel[5-10]. Altogether there were 230 *H. pylori* positive and 163 controls, of these 87 (37.82%) and 88 (53.98%), had positive staining for MUC1, respectively. Four of the 9 data sets have higher MUC1 expression in *H. pylori* gastritis (54 out of 209, 49.54% *vs.* 28 out of 77, 36.36%), and 5 data sets were lower than in controls (33 out of 121, 27.27% *vs.* 60 out of 86, 69.77%). The OR of MUC1 expression in random effect analysis (the mean effect size) was 0.625, 95% CI: 0.178-2.196, 37.5% lower in *H. pylori*-positive patients (Figure 2). The Z-value tests the null hypothesis that the mean effect size is 1.0. The Z-value is -0.733 with $p = 0.464$. Using a criterion

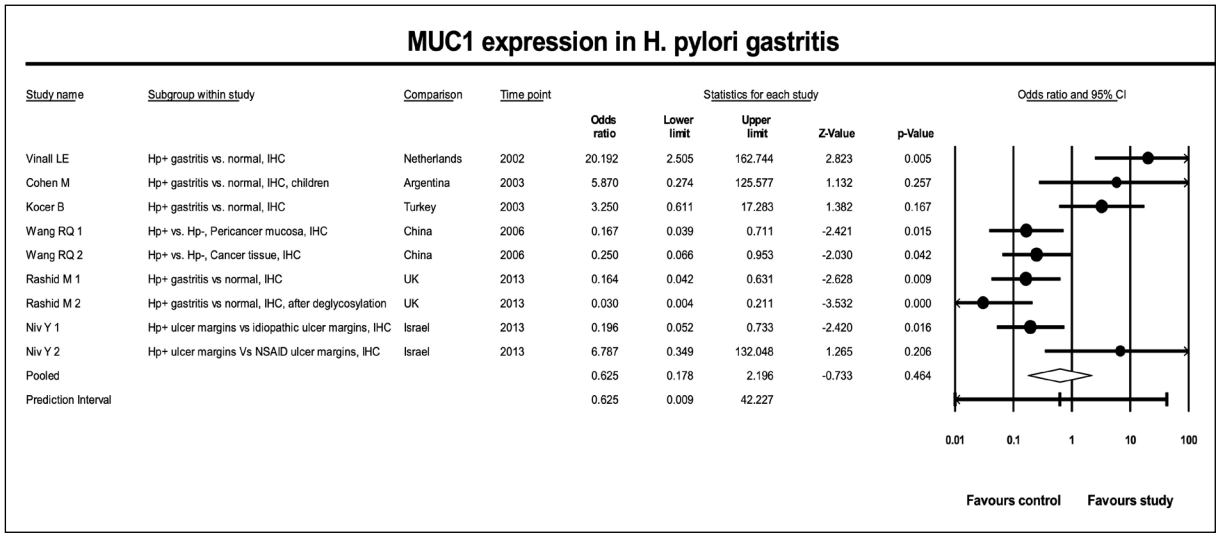

Figure 2. Forest plot illustrating OR and 95% CI for MUC1 expression in *H. pylori* gastritis *vs.* healthy controls.

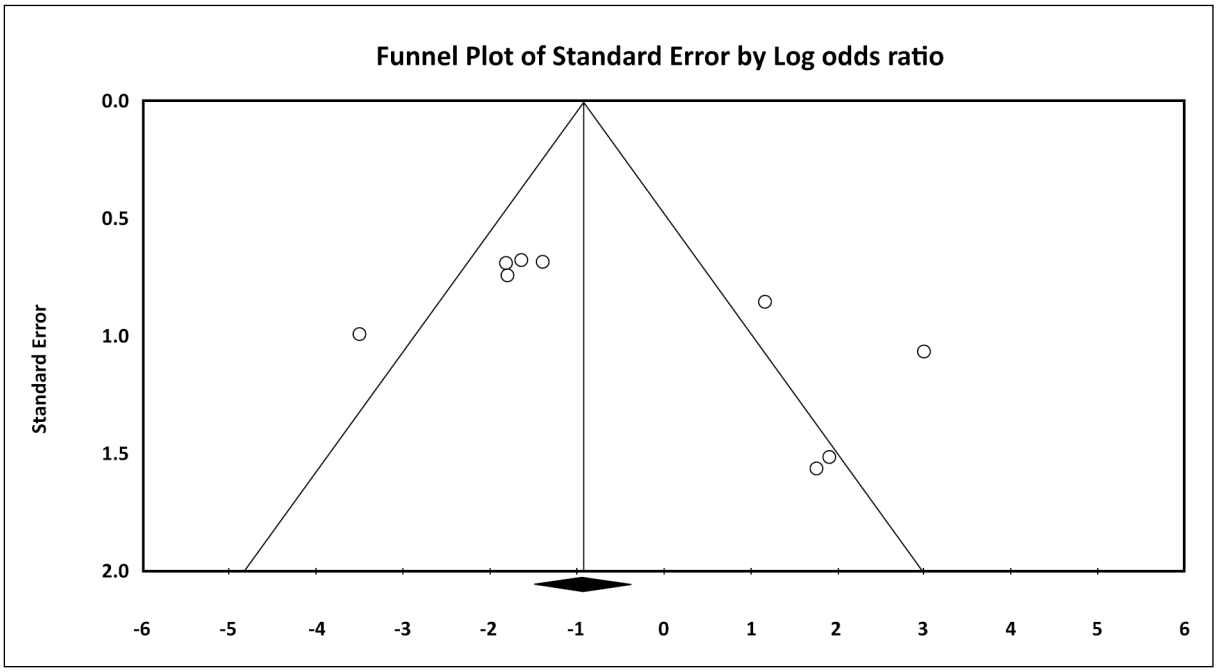

Figure 3. Funnel plot for publication bias.

alpha of 0.050, we cannot reject this null hypothesis. The relevant funnel plot (Figure 3) appeared symmetric, reflecting the lack of effects from small studies in the meta-analysis, and denies a significant publication bias. The Q-statistic provides a test of the null hypothesis that all studies in the analysis share a common effect size. If all studies shared the same true effect size, the expected value of Q would be equal to the degrees of freedom (the number of studies minus 1). The Q-value is 37.530 with 8 degrees of freedom and $p < 0.001$. Using a criterion alpha of 0.100, we can reject the null hypothesis that the true effect size is the same in all these studies. Thus, we have moderate heterogeneity. The I-squared statistic is 78.684%, $p < 0.0001$, which tells us that some 79% of the variance in observed effects reflects variance in true effects rather than sampling error. Inconsistency yielded insignificant overall results, meaning that the comparative effect sizes that were obtained by direct and indirect comparisons are consistent. The distribution of the true effect is shown in Figure 4. The mean effect size is 0.62 with 95% CI of 0.18-2.20, and falls in the interval

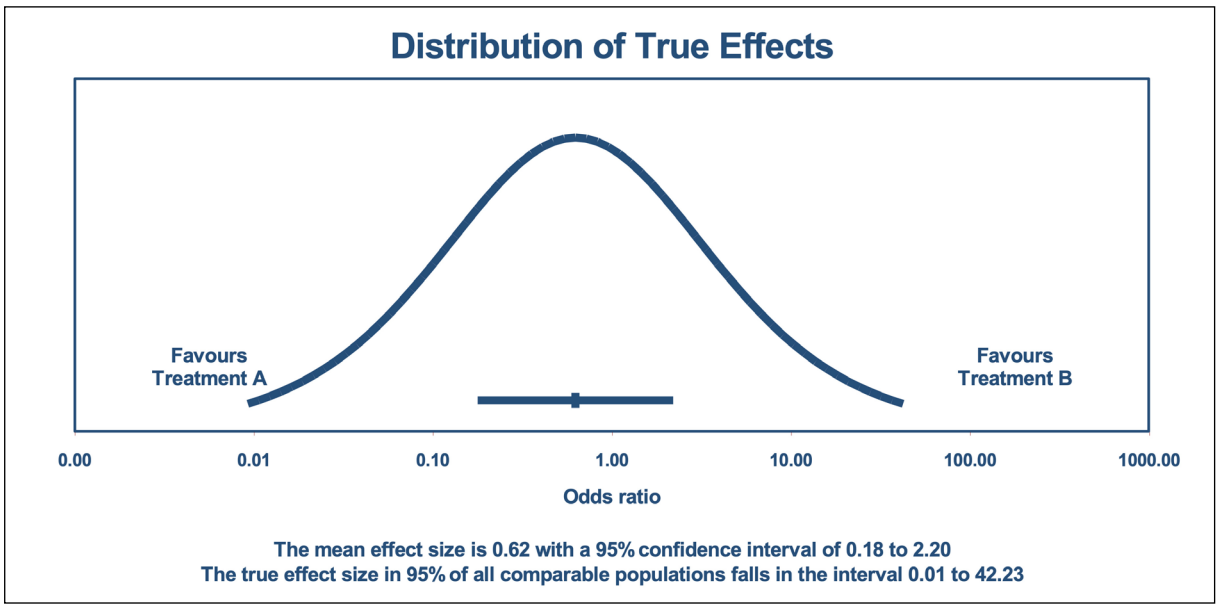

Figure 4. Distribution of true effect.

0.01-42.23, the true effect size in 95% of all comparable populations. We measured sensitivity by excluding individual studies and recalculating the overall meta-analysis outcome. This process was repeated for each of the studies. Deviations from the primary result were not significant, with ORs varying between 0.386 95% CI 0.129-1.157 and 0.886 95% CI 0.250-3.140 compared with the primary score of OR at 0.625 95% CI 0.178-2.196.

DISCUSSION

Lower MUC1 expression in the gastric epithelium of *H. pylori* positive patients than in healthy controls was demonstrated. This observation has limited importance since mucin synthesis in the gastric epithelium is a complex of many processes, and involves different kinds of secreted and membrane-bound mucins. MUC1 is the main membrane-bound mucin in the gastrointestinal epithelium and in addition to direct protection against bacteria and toxic material has C-terminal subunits that can be phosphorylated and function as a receptor, with cross-talk ability with intracellular, cytoplasmatic proteins, such as β-catenin, glycogen synthase kinase, APC, and E-cadherin, and also may activate the Wnt pathway and nuclear NF-kB. The effect of *H. pylori* infection on MUC1 expression should be further explored, since this may be a way for the bug to exert its carcinogenesis potential toward gastric adenocarcinoma or MALT lymphoma. Our paper has several limitations. First, our meta-analysis is based on studies that used different immunohistochemical methods, with different antibodies, which were manufactured by different companies. Second, we could only use studies comparing the proportion of positive expression, and exclude papers that compared average scores. Third, the study was performed in different populations of patients infected with different *H. pylori* species, about which we have no data. Thus, caution should be taken in interpreting the results.

CONCLUSIONS

In conclusion, *H. pylori* infection may decrease MUC1 expression in the human gastric epithelium. Since MUC1, a membrane-bound mucin, has a dual effect on *H. pylori* colonization, it remains to be found how this mucin expression is involved together with the secreted mucins MUC5AC and MUC6 in the complex relationship with *H. pylori* infection.

Conflicts of Interest

The authors have declared no conflicts of interest.

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Authors' Contribution

Both authors have the same contribution to the idea, searching the literature, discussing the results, and writing the paper.

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Ethics Statement and Informed Consent

Not applicable.

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