Association of IgG Glycosylation and Esophageal Precancerosis Beyond Inflammation

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ABSTRACT

This study aimed to investigate the association of IgG glycosylation and esophageal precancerosis for squamous cell carcinoma and determine its role in inflammation. Primary glycans selected by the least absolute shrinkage and selection operator (LASSO) algorithm were validated using univariate and multivariate logistics models plus restricted cubic spline functions. In total, 24 direct glycans and 27 derived traits were detected, among which four glycans and three derived traits were primarily selected. Then, GP5 (adjusted OR: 0.805), GP17 (adjusted OR: 1.305), G12n (adjusted OR: 1.271), Gal_1 (adjusted OR: 0.776) and Fuc (adjusted OR: 0.737) were validated and significantly associated with esophageal precancerosis. In addition, there was a consistent positive association in GP17 and G12n and a negative association in GP5, Gal_1, and Fuc by restricted cubic spline function. Compared with esophageal inflammation, GP17, G12n, and Fuc were still independently associated with precancerosis. In brief, the IgG glycosylation profile was independently associated with esophageal precancerosis beyond inflammation, which could be an early biomarker for esophageal cancer.

Prevention Relevance: IgG glycosylation profile is associated with esophageal precancerosis and specific IgG glycans involves in the early stage of esophageal cancer, which is independent of inflammation.

Introduction

The incidence and mortality of esophageal cancer are relatively high, with 5.90 new cases and 5.48 deaths per 100,000 persons worldwide in 2017 (1), ranking 7th and 6th, respectively, among all cancers (2). The prognosis of esophageal cancer is poor without obvious symptoms in the early stage, leading to delayed detection in most cases (3). In addition, the 5-year overall survival rate was reported to be less than 20%, which emphasizes the importance of early diagnosis (4). Thus, exploring the potential biomarkers associated with the early pathologic stage of esophageal cancer and early prevention methods are of great significance to improve quality of life and reduce the disease burden.

The formation of cancer cells is commonly accompanied by changes in the cytomembrane glycoprotein structure (5). These glycoproteins or the attached glycans, such as sialyl Lewis X-i antigen, alpha-fetoprotein, and carbohydrate antigen 19-9, are secreted or degraded from the cell membrane and have been used as biomarkers for the diagnosis or prognostic evaluation of esophageal cancer (6, 7). In addition, IgG, as an abundant glycoprotein in serum that is modified by biantennary glycans bonded at the Fc region, mediates a variety of biological immune responses (8). Also, the variations in glycans in IgG have been investigated between healthy and diseased groups, and attached glycans are also associated with some gastrointestinal cancers, such as gastric cancer, colorectal cancer, and liver cancer (9). For instance, Frano and colleagues reported that colorectal cancer was associated with a decrease in IgG galactosylation, a decrease in IgG sialylation and changes in core fucosylation (10). However, the role of the IgG glycosylation profile in esophageal precancerosis, the early stage of esophageal cancer, remains unclear. In addition, there is little knowledge about whether the association between IgG glycosylation and esophageal precancerosis is mediated by inflammation status.

In this study, we aimed to investigate the linear and nonlinear relationships of IgG N-linked glycosylation and esophageal precancerosis for squamous cell carcinoma, which is the predominant type of esophageal cancer in China. We proposed that the observed association was independent of esophageal inflammation.

Materials and Methods

Study design and population

This population-based case–control study relied on a national screening project in two high-incidence regions of gastrointestinal cancers in China: Feicheng city (Shandong, P.R. China) and Wuwei city (Gansu, P.R. China). The incidence rates of esophageal cancer are approximately 120.50/100,000

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and 46.40/100,000 in Feicheng city and Wuwei city, respectively. Local residents with gastrointestinal symptoms voluntarily participated in the screening project and underwent an endoscopic examination (subjects with abnormalities underwent a subsequent biopsy examination). Two local hospitals were designated for this screening trial: Feicheng People's Hospital (Tai'an, Shandong, P.R. China) and Wuwei Cancer Hospital (Wuwei, Gansu, P.R. China). In addition, participants were asked to undergo a physical examination, complete a questionnaire, and provide a fasting blood sample. The questionnaire involved information about demographic characteristics, dietary habits, lifestyle, a history of gastrointestinal disease, and a family history of gastrointestinal cancer. The detailed questionnaire is shown in Supplementary Table S1. The data of endoscopic or biopsy examination, physical examination, and questionnaire information were acquired from the hospital electronic records. In addition, fasting blood samples were collected and stored at −80°C for subsequent experiments.

In total, 187 patients with esophageal precancerosis for squamous cell carcinoma and 195 age- and sex-matched controls (56 healthy subjects and 139 subjects with esophageal inflammation) were enrolled in 2018 for this case–control study. The following inclusion criteria were required: (i) informed consent was signed prior to enrollment; (ii) subject was at least 40 years old; (iii) patient had an initial confirmed diagnosis of esophageal precancerosis; and (iv) information from the questionnaire and physical examination and fasting blood sample data were available. The exclusion criteria were as follows: (i) a diagnosis of gastrointestinal cancer (esophageal cancer, gastric cancer, or intestinal cancer) in the screening; (ii) a history of mental illness, infectious disease, autoimmune disease, or other malignant cancers; and (iii) women who were pregnant or breastfeeding. This study was conducted following the Declaration of Helsinki and was approved by Ethics Committees of Capital Medical University (Beijing, China) and local hospitals (approval number Z2019SY012).

Definition criteria

The final diagnosis of esophageal precancerosis for squamous cell carcinoma was according to the endoscopic diagnosis and biopsy examination, while the judgment of the controls was based on the endoscopic diagnosis. The endoscopic examination involved similar procedures as described in a previous study (11): esophageal mucosa was pretreated with Lugol iodine solution; mucosal staining was observed, in which normal esophageal mucosa turns brown, and dysplastic lesions remain unstained; unstained tissues were processed with phosphate-buffered formalin and prepared on slides with paraffin for subsequent pathologic examinations. The diagnosis of esophageal precancerosis in biopsy examination required the presence of nuclear atypia, loss of normal cell polarity and abnormal tissue maturation without invasion of epithelial cells across the basement membrane (12, 13). Esophageal precancerosis in this study was defined as mild or moderate atypical dysplasia according to the severity of the esophageal epithelial lesion, while abnormalities confined to the lower third of the epithelium were defined as mild dysplasia, and those in the lower two-thirds of the epithelium were defined as moderate dysplasia. The healthy subjects or those with esophageal inflammation were referred to as controls. Esophageal inflammation was defined as congestion, edema, superficial erosion, and ulceration of the esophageal mucosa.

Covariates

Body mass index (BMI) was defined as weight (in kilograms)/height^2 (in meters squared) and was divided into ≤23.9 and >23.9. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were presented as the average of two measurements on the right arm using a sphygmomanometer after resting for at least 10 minutes. High blood pressure (HBP) was defined as SBP ≥ 140 or DBP ≥ 90. Education level was defined as illiteracy, primary school, middle or high school, bachelor's degree, or above. Marriage status was divided into married status or others. Family income was grouped into less than and more than 50,000 yuan per capita per year. Smoking was defined as smoking at least one cigarette per day on average in the past year, while drinking was defined as consumption of at least 100 mL of alcohol (content ≥50% alcohol) per day on average in the past year. The dietary frequency of vegetables/fruits, fermented food, fried food, hot food, and spoiled food was classified into never, seldom, and often. History of gastrointestinal diseases included gastroenteritis and peptic ulcer. Family history of gastrointestinal cancer included esophageal cancer, gastric cancer, and intestinal cancer.

Glycosylation experiment

The glycosylation experiment and analyses comprised four key processes: IgG protein isolation and purification from plasma, glycan enzyme digestion and release, glycan fluorescence labeling, and glycan quantitative detection, as described previously (14). In brief, the IgG protein was obtained and purified from 2 mL of plasma using a 96-well protein G monolithic plate and multiple elutions. The N-linked glycans were released at 37°C using 1.5 units of PNGase F after 18–20 hours. The glycans were fluorescently labeled using 2-aminobenzamide at 65°C for 3 hours and then eluted. Then, the glycans were quantitatively detected using an ultraperformance liquid chromatography platform (Waters).

Finally, 24 direct glycan peaks (GP) were presented and quantitatively expressed as the percentage of the total integrated peak area. In addition, 27 derived traits, were used to reflect the abundance of the specific structure, such as galactosylation, sialylation, bisecting *N*-acetylglucosamine (GlcNAc), core fucosylation, and mannose. The number of GPs and derived traits were scaled and centered after log transformation. In addition, batch size was considered and corrected for subsequent analyses. The detailed structure and information of each GP and derived trait are shown in Supplementary Fig. S1 and Supplementary Table S2.

Statistical analysis

Continuous variables were represented as the mean ± SD, and the differences between groups were tested by independent Student *t* tests; categorical variables were presented as *n* (%), and the differences were tested by χ^2 tests. Primary variable selection of IgG glycans and derived traits was performed using the least absolute shrinkage and selection operator (LASSO) regression model. The association effect of the selected glycans and derived traits was explored using univariate and multivariate binary logistics models, and age, sex, BMI, smoking, drinking, HBP, education, income, marriage status, and dietary habits were adjusted. We further investigated the nonlinear relationship by a restricted cubic spline function with three knots fitted. The associations of esophageal inflammation and precancerosis in healthy and esophageal inflammation subjects were explored and presented using forest plots. All reported *P* values were two tailed, and $P < 0.05$ was considered statistically significant. All the analyses presented above were performed using R software (version 3.6.3).

Results

Characteristics of participants

The mean age in the whole study population was 58 years (ranging from 42 to 72 years), and there were 205 men (53.7%) in the study. There were no significant differences in the basic demographic characteristics (age, sex, BMI, education level, marriage status, income, HBP, smoking, drinking, history of gastrointestinal diseases, and family history of gastrointestinal cancer) between the esophageal precancerosis group and the control group. The detailed information is shown in **Table 1**. In addition, the dietary habits, that is, eating frequency of vegetables/fruits, fermented food, fried food, hot food, and spoiled food, were similar between the esophageal precancerosis and control groups, as shown in Supplementary Table S3.

Table 1. The characteristics of the participants.

Variables[a]	Overall (*n* = 382)	Controls (*n* = 195)	Patients (*n* = 187)	*P*
Age	58.11 (6.18)	57.79 (5.84)	58.44 (6.52)	0.306
Sex				0.558
Male	205 (53.7)	108 (55.4)	97 (51.9)	
Female	177 (46.3)	87 (44.6)	90 (48.1)	
Education				0.98
Illiteracy	83 (21.7)	44 (22.6)	39 (20.9)	
Primary school	120 (31.4)	60 (30.8)	60 (32.1)	
Middle or high school	136 (35.6)	69 (35.4)	67 (35.8)	
Bachelor's degree or above	43 (11.3)	22 (11.3)	21 (11.2)	
BMI group				0.248
≤23.9	204 (53.4)	98 (50.3)	106 (56.7)	
>23.9	178 (46.6)	97 (49.7)	81 (43.3)	
Marriage status				0.074
Married	350 (91.6)	184 (94.4)	166 (88.8)	
Other	32 (8.4)	11 (5.6)	21 (11.2)	
Household income				0.775
<50,000 yuan	266 (69.6)	134 (68.7)	132 (70.6)	
≥50,000 yuan	116 (30.4)	61 (31.3)	55 (29.4)	
HBP				0.238
No	139 (36.4)	77 (39.5)	62 (33.2)	
Yes	243 (63.6)	118 (60.5)	125 (66.8)	
History[b]				1
No	324 (84.8)	165 (84.6)	159 (85.0)	
Yes	58 (15.2)	30 (15.4)	28 (15.0)	
Family history[b]				0.478
No	283 (74.1)	148 (75.9)	135 (72.2)	
Yes	99 (25.9)	47 (24.1)	52 (27.8)	
Smoking				0.084
No	246 (64.4)	117 (60.0)	129 (69.0)	
Yes	136 (35.6)	78 (40.0)	58 (31.0)	
Drinking				0.366
No	279 (73.0)	138 (70.8)	141 (75.4)	
Yes	103 (27.0)	57 (29.2)	46 (24.6)	

[a]Mean (SD), Student *t* test for continuous variables; numbers in each category (%), χ^2 test for categorical variables.
[b]History refers to a history of gastroenteritis and peptic ulcers; family history refers to a family history of esophageal cancer, gastric cancer, and intestinal cancer.

Association of IgG glycosylation and esophageal precancerosis

In the initial stage, four glycans and three derived traits were selected by the LASSO algorithm as the candidate profile. In the second stage, GP5, GP17, G12n, Gal_1, and Fuc showed significant associations with esophageal precancerosis in both univariate and multivariate analyses, and the adjusted ORs were 0.805, 1.305, 1.271, 0.776, and 0.737, respectively. The association effect of the selected glycans and derived traits is shown in **Table 2**, and the distribution plot is shown in **Fig. 1**.

Moreover, the nonlinear relationship of IgG glycosylation and esophageal precancerosis in the univariate model is shown in **Fig. 2**, and there was a consistent positive association for GP17 and G12n and a negative association for GP5, Gal_1, and Fuc. The nonlinear relationship pattern remained similar after other covariates were adjusted, as shown in Supplementary Fig. S2.

Distribution of IgG glycosylation in esophageal inflammation and precancerosis

Compared with those in the esophageal inflammation group, GP17 and G12n increased and Fuc decreased in the esophageal precancerous group, as shown in **Fig. 3A**. The adjusted OR values were 1.56, 1.29, and 0.72, respectively. There were no significant associations observed for GP5 and Gal_1. In addition, there was no difference in the distribution between the esophageal inflammation and healthy control groups, apart from the decrease in GP17 in the esophageal inflammation group, as shown in **Fig. 3B**.

Discussion

In this study, we investigated the association of IgG glycosylation profiles and esophageal precancerosis, which is the early stage of esophageal cancer. Glycans (GP5 and GP17) and derived traits (Gal_1, G12n, and Fuc) were selected and validated to be associated with esophageal precancerosis, but these glycans and derived traits were not associated with esophageal inflammation. In detail, a panel of GP17, G12n,

Table 2. Association of esophageal precancerosis with glycans and derived traits selected by LASSO.

Variable[a]	Controls ($n = 195$)	Patients ($n = 187$)	Univariate model			Multivariate model[b]		
			OR	95% CI	*P*	OR	95% CI	*P*
Glycan peak								
GP5	0.1 [0.02]	−0.11 [−0.23]	0.805	0.656–0.99	0.0395	0.805	0.652–0.988	0.04
GP8	0.08 [0.08]	−0.08 [−0.01]	0.606	0.344–1.067	0.0826	0.589	0.328–0.964	0.069
GP17	−0.11 [−0.13]	0.11 [−0.13]	1.259	1.014–1.562	0.037	1.305	1.052–1.648	0.019
GP24	−0.08 [0.03]	0.09 [0.28]	1.193	0.969–1.47	0.0969	1.174	0.953–1.46	0.138
Derived traits								
G12n	−0.09 [0]	0.09 [0.08]	1.203	1.081–1.475	0.0453	1.271	1.022–1.59	0.033
Gal_1	0.11 [0.28]	−0.11 [0.04]	0.797	0.645–0.986	0.0368	0.776	0.619–0.958	0.022
Fuc	0.12 [0.19]	−0.12 [0.14]	0.75	0.582–0.966	0.0261	0.737	0.558–0.932	0.019

[a]The mean and median values were given for selected glycans and derived traits.
[b]Age, sex, BMI, smoke, drink, HBP, education, income, marriage status, and dietary habits were adjusted in the multivariate model.

and Fuc was independently associated with esophageal precancerosis beyond esophageal inflammation. This IgG glycosylation profile could be a novel biomarker and potential drug target of esophageal precancerosis and could contribute to the early identification and prevention of esophageal precancerosis and cancer.

Some specific serum biomarkers of esophageal cancer, such as carcinoembryonic antigen, cytokeration fragment antigen 21-1, and squamous cell carcinoma antigen, have been discovered and applied in clinical practice (15, 16). Most of these FDA-approved biomarkers pertain to glycoproteins and are modified by attached glycans. However, these biomarkers reflect the variation in glycosylation in serum, while the glycan profile of a single protein has attracted increasing attention (17). IgG is one of the most common immunoglobulins, and biantennary glycans, which are the most widely described glycan, are covalently attached at the Fc region (18). Under precancerous or cancerous states, the amount of IgG is overexpressed (19, 20). Moreover, the bonded glycans tended to be abnormally modified, as the generated pathologic metabolites could change the effect of glycosyltransferase or glycosylhydrolase, especially in the tumor-infiltrating B lymphocytes (21, 22). These changed glycans could enhance or weaken the antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity effect of IgG (23, 24). In our study, we found that GP5 was positively associated with esophageal precancerosis, while GP17 was negatively associated with esophageal precancerosis. GP5 refers to high mannose glycans and GP17 refers to digalactosylated monosialylated biantennary glycans. In the derived traits, Gal_1 reflects the overall amount of monogalactosylated glycans, G12n reflects the proportion of digalactosylated biantennary glycans in the neutral glycans, and Fuc reflects the overall amount of glycans with core fucose (25, 26). These results revealed a glycosylation pattern of an increase in digalactosylated biantennary glycans (with and without monosialic acid) and a decrease in high mannose glycans, monogalactosylated glycans, and core-fucosylated glycans.

These findings were largely consistent with those from previous studies on the IgG glycosylation profile in gastrointestinal cancers. Vučković and colleagues (10) reported a concurrent decrease in core fucosylation of sialylated glycans

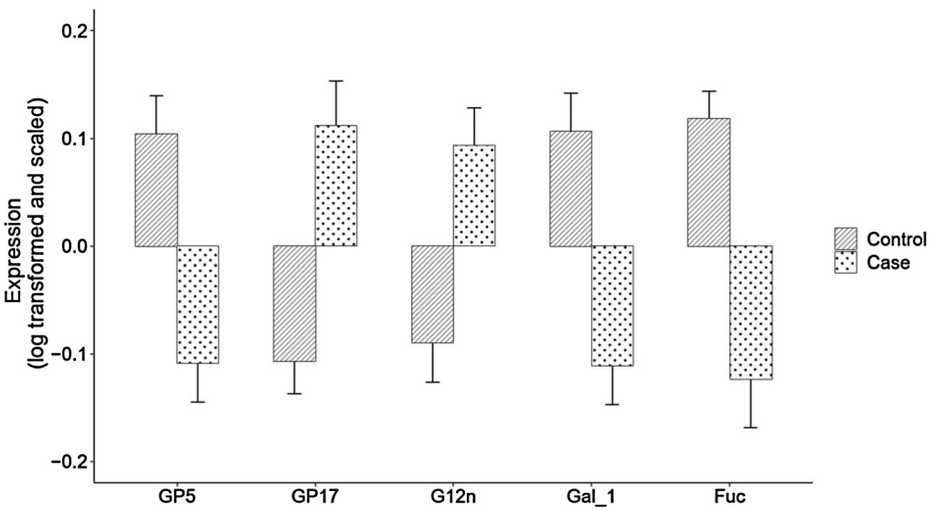

Figure 1.
The distribution plot of differential glycans and derived traits between the esophageal precancerous population and the control.

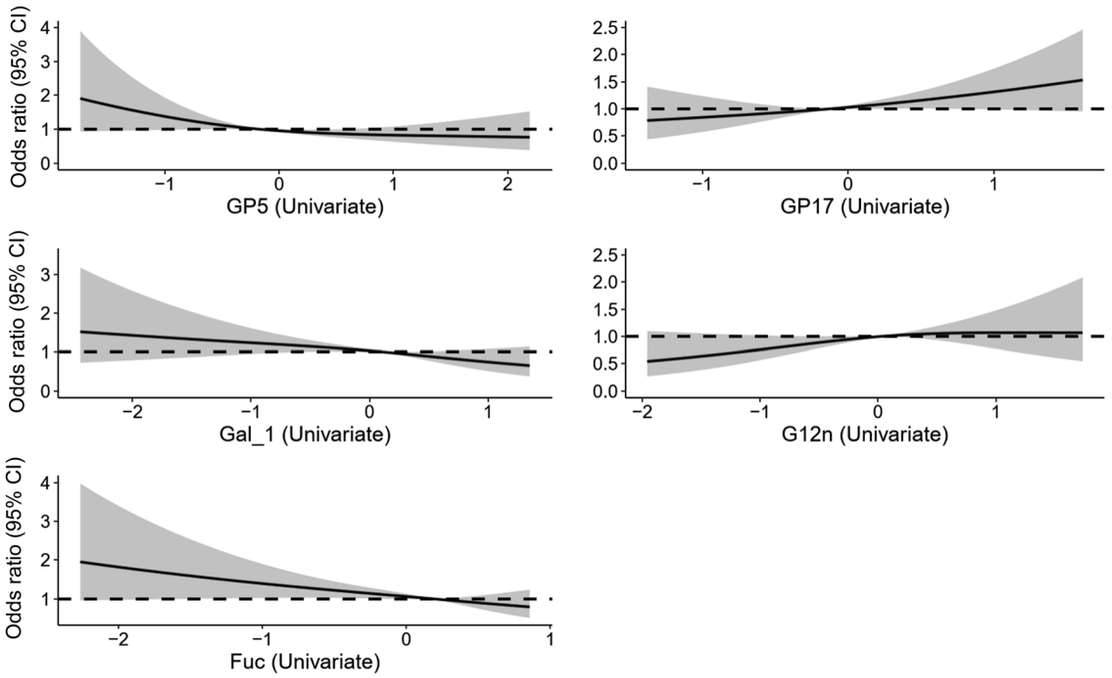

Figure 2.
The nonlinear relationship of esophageal precancerosis and IgG glycosylation by restricted cubic spline function.

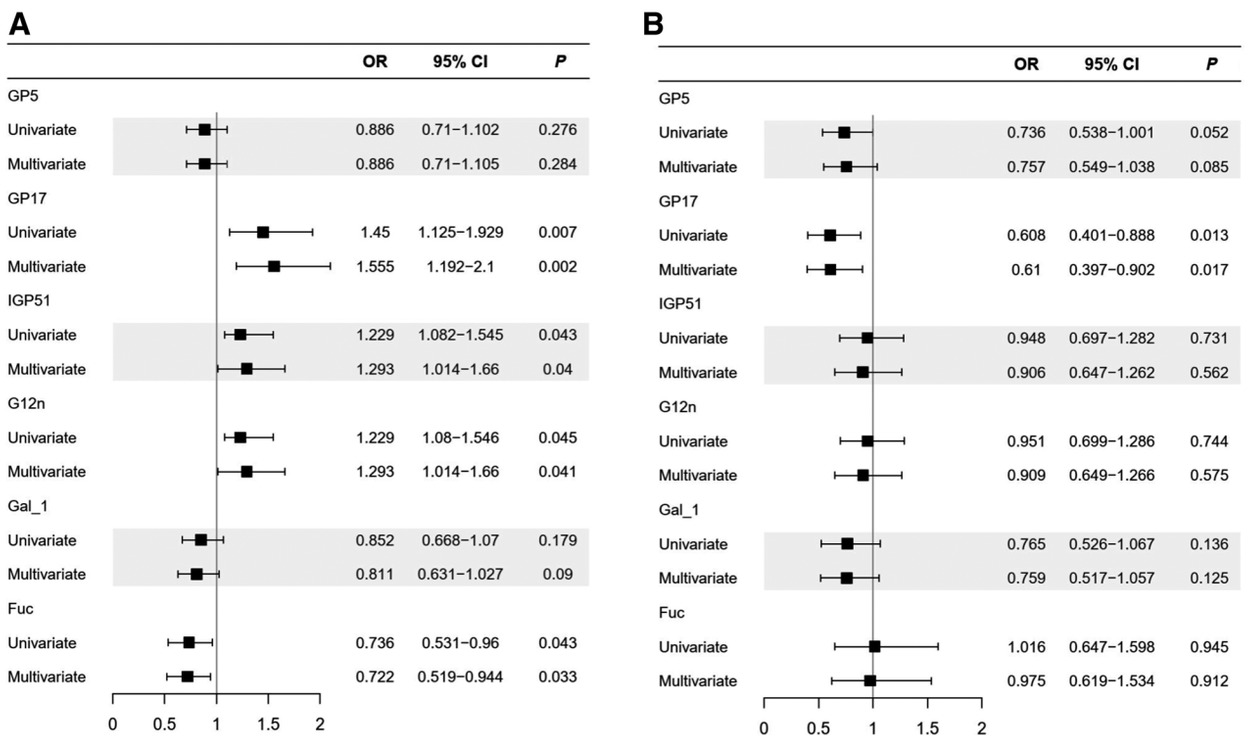

Figure 3.
Distribution of IgG glycosylation in esophageal inflammation and precancerosis patients (**A**) and in esophageal inflammation patients and healthy controls (**B**).

in colorectal cancer, and we found a decrease in glycans with core fucosylation in esophageal precancerosis. In addition, they found that IgG galactosylation decreased in colorectal cancer, while we found that monogalactosylation decreased and digalactosylation increased in esophageal precancerosis. Similarly, Ren and colleagues (9) suggested that IgG galactosylation could serve as a promising biomarker for cancer screening in multiple cancer types. We found a specific IgG galactosylation pattern in esophageal precancerosis. In addition, the high mannose glycan showed a decreasing trend, which needs to be investigated further.

The lack of core fucose in IgG glycans could influence the binding of IgG to FcγRIIIa and thus could greatly enhance its capacity to activate ADCC (27, 28), which has been proven in the clinical efficacy of mAbs. Apart from cancers, variations in IgG galactosylation have been reported in large studies of inflammatory diseases, such as inflammatory bowel diseases and systemic lupus erythematous (29–31). IgG glycosylation could also reflect the biological status between proinflammation and anti-inflammation (32, 33). In our study, we further added that IgG glycosylation was independently associated with esophageal precancerosis beyond esophageal inflammation, and this association has not been reported previously. Notably, compared with that in healthy control group, GP17 decreased in the esophageal inflammation group and increased in the esophageal precancerosis group, which implied that GP17 could have different patterns in benign and malignant lesions.

In this study, we illustrated significant differences in IgG glycome patterns between the esophageal precancerosis and the control groups. However, the results should be interpreted carefully due to the limitations of this study. First, the sample size was relatively small, causing an inadequate study power, and the proportion of subjects in the esophageal inflammation group and the healthy group was unbalanced. Second, we failed to claim a causal association due to the case–control study. We could not indicate whether the changes occurred before the disease processed. Third, our study was based on the Chinese population, and more collaborative studies are needed to validate the generalizability of the results.

In summary, considering the functional effect of IgG glycosylation on both immunosurveillance and ADCC, the variation in IgG glycans deserves more attention in the early prevention of esophageal precancerosis and cancer; thus, the underlying mechanism warrants more detailed investigations.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

Z. Wu: Methodology, writing–original draft, writing–review and editing. **H. Pan:** Methodology. **D. Liu:** Formal analysis, methodology. **D. Zhou:** Writing–original draft, writing–review and editing. **L. Tao:** Data curation. **J. Zhang:** Resources, data curation. **X. Wang:** Resources. **Y. Wang:** Conceptualization, supervision. **W. Wang:** Conceptualization, project administration. **X. Guo:** Conceptualization, resources, supervision, funding acquisition, project administration.

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