Helicobacter pylori genotypes, salt intake, and sociodemographic factors associated with premalignant gastric lesions in a Colombian population. A case control study.

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Abstract

Introduction

Genetic variability of *Helicobacter pylori* is associated with various gastrointestinal diseases; however, little is known about its interaction with sociodemographic and dietary factors in the development of premalignant lesions.

Objective

To evaluate the association among *Helicobacter* genotypes, salt intake, and sociodemographic factors associated with precursor lesions of the stomach

Materials and Methods

An analytical study was conducted including cases (patients with gastric atrophy, intestinal metaplasia, and gastric dysplasia) and controls (patients with nonatrophic gastritis). Sociodemographic information and information about salt intake were obtained using a questionnaire. Histopathological diagnosis was performed according to the Sydney System. The cagA and vacA genotypes were established using polymerase chain reaction in paraffin blocks. ANOVA was used for analyzing quantitative variables. Categorical variables are presented as proportions and absolute frequencies. The effect of each variable on the study outcome (premalignant lesion) is presented as odds ratio (OR) and 95% CI. A *p*-value of <0.05 was considered as statistically significant.

Results

The vacA/s1m1 genotype increases the risk of developing precursor lesions of the stomach (OR: 3.82, 95% CI: 1.45–10.07, $p = 0.007$). Age and salt intake showed a positive interaction with the s1m1 genotype (adjusted OR: 5.19, 95% CI: 1.88–14.32, $p = 0.001$) and with bacterial coinfection (adjusted OR: 3.2, 95% CI: 1.06–9.59, $p = 0.038$). The cagA genotype was not correlated to the development of premalignant lesions of the stomach (OR: 1.21, 95% CI: 0.80–1.82, $p = 0.361$).

Conclusions

The vacA genotype, age, and salt intake are indicators of the risk of developing premalignant lesions of the stomach in the study population.

Introduction

Gastric cancer is a high-impact disease at the global level. According to GLOBOCAN, in 2012, 952,000 cases of gastric cancer were reported worldwide, making it the fifth most prevalent cancer and the third leading cause of death due to cancer (1). Colombia is one of the countries with the highest incidence of gastric cancer, along with Japan, Costa Rica, Singapore, Korea, and Chile (2). As reported by the National

Administrative Department of Statistics, the Cauca Department has one of the country's highest standardized mortality rate: 10.7/100,000 inhabitants/year for female and 18.7/100,000 inhabitants/year for male.

Besides its high incidence, late diagnosis of gastric cancer is one of the critical factors for mortality in Cauca. Adrada et al. showed that the proportion of cancer diagnosed at advanced stages in Cauca was >90% (3). Unfortunately, in most cases, its occurrence results in mortality due to the disease, with 5-year survival rates of <10% (4). Currently, prevention and early detection are the optimal strategies for mitigating the effects of the disease.

Considering these strategies, the carcinogenesis theory proposed by Dr. Pelayo Correa is particularly significant because it addresses the onset of histopathological lesions that precede the development of gastric cancer (5, 6). According to this theory, intestinal adenocarcinoma—the most common histotype in developing countries—is preceded by a series of progressive histopathological changes that begin with chronic atrophic gastritis, intestinal metaplasia, and gastric dysplasia (7). However, only a small proportion of patients with these lesions eventually develop cancer, with a higher risk associated with gastric dysplasia (6%) and lower risk associated with gastric atrophy (0.1%) (8).

It is challenging to predict the risk of progression, and this risk can be modulated by various genetic and environmental factors including salt intake habits and genetic variability of *Helicobacter pylori* (9, 10). For example, the vacA/s1m1 and cagA+ genotypes have been shown to be associated with an increased risk of presenting precursor lesions of gastric malignancy (11-16).

Few studies have been conducted in Colombia in which bacterial genotypes (17-19) or salt intake (20) has been associated with the onset of premalignant lesions of the stomach. In these studies, the approach is based on comparing genotype frequencies, and the results showed contradictory findings. However, to the best of the authors' knowledge, to date, no investigation has evaluated the interactive effect of the genetic variability of *H. pylori* on salt intake and sociodemographic factors. Therefore, the aim of the present study was to estimate the association among *H. pylori* genotypes, salt intake, and sociodemographic factors with regard to the precursor lesions of gastric malignancy (atrophy, metaplasia, and dysplasia) in the Cauca population.

Materials And Methods

An unpaired case–control analytical study was conducted with patients admitted to the Gastroenterology Units of the San José University Hospital and Endovideo in the city of Popayán (located in southwestern Colombia, in the mountainous area of the Andes, and is considered a high-risk area of gastric cancer) from January 2008 to December 2011. Samples were collected by convenience sampling, and included patients were aged >18 years with a histopathological diagnosis of nonatrophic gastritis (controls) and those with precursor lesions of gastric malignancy (cases). Inclusion criteria included the following: participants should be born in a municipality of Cauca; should be children of parents from Cauca; and should be diagnosed with *H. pylori* infection determined by histopathological tests and corroborated by

molecular diagnostics [polymerase chain reaction (PCR)]. Participants with a history of gastric surgery, who received treatment for *H. pylori* infection, who had an HIV infection, and who had gastric adenocarcinomas other than of the intestinal histotype were excluded.

Prior to sample collection, participants voluntarily signed an informed consent form and were interviewed via a survey to obtain information on sociodemographic and clinical variables. They were also queried regarding their salt intake habit using the question “Do you add additional salt to the food served on the table?” and three response categories were assigned—always, sometimes, or never.

Gastric samples were collected by experienced gastroenterologists via gastrointestinal endoscopy. Patients underwent gastrointestinal endoscopy following referral for dyspeptic symptoms and after fasting for at least 8 h. Although participants were not sedated, they received topical oropharyngeal anesthesia. Five samples corresponding to two antral biopsies (major and minor curvature), two corpus biopsies (major and minor curvature), and one incisura angularis biopsy were obtained. These samples were fixed in buffered formalin and stained with hematoxylin–eosin and Giemsa stains. Two pathologists conducted histopathological analyses of the samples. Patients were assigned to four diagnostic categories: nonatrophic gastritis, atrophic gastritis, intestinal metaplasia, and gastric dysplasia. The most severe lesion was selected as the final diagnosis in each patient. The visual analog scale was used according to the Sydney System. Genotyping studies of *H. pylori* were performed using DNA extraction of paraffin-embedded biopsies using the Chelex technique (No. C7901; Sigma, St. Louis, MO). PCR previously described by Sugimoto et al. (21) was performed for the amplification of vacA genes. PCR mixtures were prepared using 50 ng of genomic DNA, 100 µmol of dNTPs, 2.5 µL of 10× PCR buffer, 1.0 mM $MgCl_2$, 1 U of Taq DNA polymerase (No. M1665; Promega, Madison, WI), and 30 pmol of each primer shown in Table 1. The reactions commenced with a denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 94°C for 1 min, hybridization at 52°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The obtained products were analyzed using electrophoresis in 2% agarose gels at 80 V for 40 min, and the genotypes were identified according to the expected base pair size.

Table 1. Primer sequence for polymerase chain reaction amplification

Genes and regions	Sequence (5'→3')	Size (bp)
***vac*A s1/s2**	ATGGAAATACAACAAACACAC	259/286
	CTGCTTGAATGCGCCAAAC	
***vac*A m1/m2**	CAATCTGTCCAATCAAGCGAG	570/645
	GCGTCTAAATAATTCCAAGG	
***cag*A**	GATAACAGGCAAGCTTTTGAGG	349
	CGTCAAAAGATTGTTTGGCAGA	

The *H. pylori* strains NCTC-11637 and NCTC-11638 as well as the clinical isolate 3062 were provided by the Colombian National Cancer Institute and used as positive controls. The PCR tests included adequate internal amplification controls and molecular markers (ladders).

To control biases associated with sociodemographic information and salt intake, biologists and doctors who belonged to the GIGHA group were trained for standardizing questions in a closed questionnaire, which was completed before gastrointestinal endoscopy.

To reduce biases associated with histopathological information, diagnoses were validated by a different pathologist who was unaware of the previous diagnosis. In cases wherein diagnosis differed, the case was jointly re-evaluated to reach diagnostic consensus. To limit disagreement in cases of gastric dysplasia, they were grouped into a single category that included low- and high-grade gastric dysplasia. On the other hand, the histopathological diagnosis of *H. pylori* infection was corroborated using Giemsa staining and PCR.

Molecular diagnostics were conducted according to globally accepted protocols; the equipment was calibrated, and pilot tests were performed to verify the quality of reagents and extraction kits.

To determine the behavior of variables for each histopathological lesion, the frequencies of each precursor lesion of gastric malignancy were compared with the control group. Thereafter, patients with precursor lesions were regrouped as a single category for comparison with the control group.

Mean differences in age were evaluated using one-way ANOVA, along with post-hoc tests. Differences in proportions were evaluated using the chi-squared test of independence. Odds ratio (OR) and *p*-values were used to evaluate the effect of each variable of interest on the response variable (precursor lesion of gastric malignancy). Multivariate logistic regression analysis was performed including the variables that met the Hosmer–Lemeshow criteria and those reported in the scientific literature as significant variables. A *p*-value of <0.05 and 95% CI were considered statistically significant. Data were analyzed using SPSS version 23.

Participants provided their consent to participate in the study and signed informed consent. The ethical principles of the Declaration of Helsinki were followed, and the investigation was approved by the Scientific Research Ethics Committee of the Cauca University.

Results

Of a total of 821 patients, 389 met the inclusion criteria. Intestinal metaplasia was the most prevalent precursor lesion of malignancy. Patient distribution according to histopathological diagnosis were: Non atrophic gastritis 174(45%), Atrophy 49(12%), Metaplasia 135(35%) and dysplasia 31(8%).

The average patient age was 43 years for the nonatrophic gastritis group, 52 years for the atrophic gastritis and metaplasia groups, and 63 years for the dysplasia group. When average age was compared using ANOVA single-factor test, significant differences were observed among all groups (p = 0.000). Post-hoc analysis showed significant differences between each precursor lesion of gastric malignancy and gastric cancer with chronic nonatrophic gastritis (p = 0.000).

Regarding age, the most prevalent age group in the control category was 18–40 years; in the atrophic gastritis and metaplasia group, the prevalent age group was 41–60 years, whereas in the dysplasia group, the prevalent age group was >60 years. Regarding salt intake, 235 participants including 100 (43%) with nonatrophic gastritis denied the use of additional salt in their meals. On the other hand, 63 participants including only 19 (30%) with nonatrophic gastritis stated that they always added additional salt to their meals. Patient distribution according to age, sex, and salt intake is shown in Table 2.

Table 2. Distribution of participants in the study groups. CNAG: Chronic nonatrophic gastritis; CLMMW: Current Legal Minimum Monthly Legal Salary in Force Wage

CNAG*			Atrophy	Metaplasia	Dysplasia
Age	18–40 years	79 (45..4)	12 (24.5)	28 (20.7)	2 (6.5)
	41–60 years	65 (37.4)	23 (46.9)	70 (51.9)	9 (29)
	≥61 years	30 (17.2)	14 (28.6)	37 (27.4)	20 (64.5)
Sex	Female	114 (65.5)	29 (59.2)	96 (71.1)	16 (51.6)
	Male	60 (34.5)	20 (40.8)	39 (28.9)	15 (48.4)
Origin	Urban	104 (59.8)	28 (57.1)	76 (56.3)	16 (51.6)
	Rural	70 (40.2)	21 (42.9)	59 (43.7)	15 (48.4)
Income	>1 CLMMW	74 (42.5)	25 (51)	53 (39.3)	3 (9.7)
	<1 CLMMW	100 (57.5)	24 (49)	82 (60.7)	28 (90.3)
Ethnicity	Caucasian	8 (4.6)	3 (6.1)	2 (1.5)	1 (3.2)
	Black	7 (4)	2 (4.1)	0 (0)	1 (3.2)
	Mulatto	4 (2.3)	2 (4.1)	2 (1.5)	0 (0)
	Mestizo	152 (87.4)	41 (83.7)	128 (94.8)	25 (80.6)
	Indigenous	3 (1.7)	1 (2)	3 (2.2)	4 (12.9)
Salt intake	Never	102 (58.6)	27 (55.1)	66 (48.9)	15 (48.4)
	Sometimes	53 (30.5)	14 (28.6)	42 (31.1)	12 (38.7)
	Always	19 (10.9)	8 (16.3)	27 (20)	4 (12.9)

Each precursor lesion of gastric malignancy and cancer was compared with the chronic nonatrophic gastritis group to estimate the measures of association. Female sex, age of 18–40 years, and the “never add salt to meals” groups were selected as control categories. Analyses showed significant associations between the age groups of >40 and >60 years. Salt intake was associated with the development of intestinal metaplasia, whereas the income category lower than one minimum wage was associated with gastric dysplasia (Table 3).

Table 3. Odds ratio (OR) of sociodemographic factors and salt intake. *CNAG: chronic nonatrophic gastritis. ** CLMMW: Current Legal Minimum Monthly Wage. A *p*-value of <0.05 was considered statistically significant.

	Atrophy			Metaplasia			Dysplasia		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
Age									
18-40 years	1	1	1	1	1	1	1	1	1
41-60 years	2.33	(1.07-5.03)	0.032	3	(1.75-5.25)	0,000	5.47	(1.14-26.2)	0.034
>60 years	3.07	(1.27-7.4)	0.012	3.48	(1.82-6.64)	0,000	26.3	(5.8-119)	0,000
Sex									
Female	1	1	1	1	1	1	1	1	1
Male	1.31	(0.68-2.51)	0.415	0.77	(0.47-1.25)	0.296	1.78	(0.82-3.85)	0.142
Origin									
Urban	1	1	1	1	1	1	1	1	1
Rural	1.11	(0.58-2.11)	0.741	1.15	(0.73-1.82)	0.539	1.39	(0.64-2.99)	0.397
Income									
>1CLMMW	1	1	1	1	1	1	1	1	1
<1CLMMW	0.71	(0.37-1.34)	0.71	1.14	80.72-1.81)	0.562	6.9	(2.02-23.5)	0.002
Ethnicity									
Caucasian	1	1	1	1	1	1	1	1	1
Black	0.76	(0.09-5.95)	0.796	0	0	0.999	1.14	(0.06-21.8)	0.0929
Mulatto	1.33	(0.15-11.5)	0.794	2	(0.20-19.9)	0.55	0	0	0.999
Mestizo	0.72	(0.18-2.83)	0.638	3.36	(0.70-16.14)	0.13	1.31	(0.15-10.9)	0.8
Indigenous	0.88	(0.06-12.25)	0.93	4	(0.43-37.10)	0.22	10.6	(0.82-138.2)	0.07
Addition of salt									
Never	1	1	1	1	1	1	1	1	1
Sometimes	0.99	(0.48-2.06)	0.995	1.22	(0.73-2.04)	0.436	1.54	(0.67-3.52)	0.307
Always	1.59	(0.62-4.02)	0.327	2.19	(1.13-4.26)	0.02	1.43	(0.42-4.78)	0.56

To study the vacA genotypes, the s1, s2, m1, and m2 alleles were examined and grouped according to their virulence profile. Analysis showed the presence of the s2m2 genotype in 22 patients, which included 16 (72.7% isolates) with nonatrophic gastritis, 1(4.5% isolates) with gastric atrophy, 5 (22.7% isolates) with intestinal metaplasia, and none with gastric dysplasia. On the other hand, the s1m1 genotype was the most prevalent in the study population, being present in 314 patients, which included 113 with chronic nonatrophic gastritis, 36 with gastric atrophy, 101 with intestinal metaplasia, and 25 with gastric dysplasia. Cases of coinfection wherein the presence of a cagA+ bacterium was documented were considered the cagA+ category. cagA and vacA genotype distribution according to the type of diagnosis is shown in Table 4.

Table 4. Bacterial genotype distribution in the study groups. *CNAG. Chronic nonatrophic gastritis

		CNAG*	Atrophic gastritis	Metaplasia	Dysplasia
	s2m2	16(9.2)	1(2)	5(3.7)	0(0)
	s2m1	0(0)	0(0)	2(1.5)	0(0)
vacA genotypes	s1m2	10(5.7)	3(6.1)	8(5.9)	0(0)
	s1m1	113(64.9)	36(73.5)	101(74.8)	25(80.6)
	Coinfection	35(20.1)	9(18.4)	19(14.1)	6(19.4)
cagA genotypes	cagA-	71(40.8)	18(36.7)	53(39.3)	7(22.6)
	cagA+	103(59.2)	31(63.3)	82(60.7)	24(77.4)

To facilitate analyses and considering the absence of the s2m2 genotype in the dysplasia category, patients with gastric atrophy, intestinal metaplasia, and gastric dysplasia were grouped into a single category (precursor lesions of gastric malignancy) and compared with the control group. Chi-squared test showed significant differences among the vacA genotypes ($p = 0.025$) and showed no differences regarding the cagA genotype ($p > 0.05$). To analyze the measures of association, the vacA/s2m2 and cagA- genotypes were selected as control categories (Table 5).

Table 5. Measures of association of vacA and cagA bacterial genotypes. Patients with atrophy, intestinal metaplasia, dysplasia, and gastric cancer were added to one category. NA = Not applicable. A *p*-value of <0.05 was considered statistically significant.

		Non atrophic gastritis	Precursors lesions of gastric malignancy	OR	95%CI	p-value
	s2m2	16(9.2)	6(2.8)	1	1	1
	s2m1	0(0)	2(0.9)	NA	NA	NA
vacA	s1m2	10(5.7)	11(5.1)	2.99	0.82-10.44	0.097
	s1m1	113(64.9)	162(75.3)	3.82	1.45-10.07	0.007
	Coinfection	35(20.1)	34(15.8)	2.59	0.90-7.40	0.076
cagA	cagA-	71(40.8)	78(36.3)	1	1	1
	cagA+	103(59.2)	113(63.7)	1.21	0.80-1.82	0.361

Patients with gastric atrophy, intestinal metaplasia, and gastric dysplasia were grouped into a single category (precursor lesions of gastric malignancy) and compared with the control group to obtain the crude OR of the sociodemographic variables and salt intake. Only significant variables (categorized age, income category, and salt intake) were included in this analysis according to the findings of bivariate analysis by diagnostic categories (Table 3). In a previous analysis, male sex showed no significant associations either with the crude estimate (OR: 0.99, 95% CI: 0.66–1.51, $p = 0.98$) or in the model adjusted for genotypes and age (adjusted OR: 0.87, 95% CI: 0.54–1.37, $p = 0.557$). Moreover, the variable income category lower than one minimum wage showed no significant associations (crude OR: 1.22, 95% CI: 0.81–1.84, $p = 0.33$ and adjusted OR: 1.06, 95% CI: 0.68–1.66, $p = 0.779$). Considering these data, to evaluate the most parsimonious model, both sex and income category variables were excluded from the final logistic regression model. Table 6 illustrates the risk of developing precursor lesions of gastric malignancy according to age-adjusted vacA genotypes and the interactive effect enhancer of salt intake.

Table 6. Multivariate logistic regression model showing measures of association between the variables of interest and outcome (precursor lesions of gastric malignancy). aAdjusted for categorized age and bacterial genotype; bAdjusted for age, bacterial genotype, and salt intake categories. NA: Not applicable. A *p*-value of <0.05 was considered statistically significant.

Variable	Crude OR	95%CI	p-value	Adjusted OR	95%CI	p-value	Adjusted OR	95% CI	p-value
Age									
41-60 years	2.95	1.81-4.80	0,000	3.24	1.97-5.35	0,000	3.24	1.95-5.36	0,000
>60 years	4.45	2.52-7.85	0,000	4.65	2.60-8.30	0,000	4.78	2.67-8.58	0,000
Addition of salt									
Adds salt sometimes	1.21	0.77-1.99	0,403	NA	NA	NA	1.26	0.78-2.05	0.334
Always adds salt	1.93	1.05-3.57	0,034	NA	NA	NA	2.22	1.14-4.29	0.018
Genotypes									
VacA/s1m2	2.99	0.82-10.44	0,097	3.42	0.91-12.81	0.067	4.07	1.06-15.56	0.04
VacA/s1m1	3.82	1.45-10.07	0,007	4.62	1.70-12.53	0.003	5.19	1.88-14.32	0.001
Coinfection	2.59	0.90-7.40	0,076	2.74	0.93-8.07	0.066	3.2	1.06-9.59	0.038

Discussion

An association between age and onset of premalignant lesions of the stomach was determined in this study. The findings showed that the prevalence of gastric dysplasia is higher in patients aged >60 years, whereas injuries such as gastric atrophy and intestinal metaplasia are more prevalent in patients aged 40–60 years. Similar results have been reported by other authors (22, 23), showing a direct correlation between the severity of precursor lesions of gastric malignancy and age.

The greatest age-related risk is due to genomic instability acquired over the years owing to chronic inflammation, cumulative damage by free radicals, and inefficiency of DNA repair mechanisms (24-27). On the other hand, normal gastric mucosa reportedly lacks telomerase activity, and a progressive increase in the activity of this enzyme is directly related to premalignant lesions and cancer (28). Other studies and the present investigation suggest that preneoplastic lesions represent histological changes caused by tissue aging and dysfunctional adaptive responses, thereby increasing the risk of tumors.

Regarding bacterial genotypes, the s1m1 genotype was more prevalent in the case group, whereas the s2m2 genotype was more prevalent in the control group. Similar results have been reported by Colombian and foreign authors (29-31). The role of the s1m1 genotype can be explained via different mechanisms such as the synthesis of a vacuolizing protein, which induces greater epithelial damage, development of a more persistent inflammation, and blockage in the proliferation of T lymphocytes via its arrest in the G1 or S phase of the cell cycle (32-33).

In a recent meta-analysis, 33 studies were evaluated, which overall included 2697 controls and 1446 cases with gastric cancer and precursor lesions of gastric malignancy. In that study, the s1 allele showed an increased risk of gastric atrophy (RR: 1.11 95% CI: 1.019–1.222) and intestinal metaplasia (RR: 1.41, 95% CI: 1.03–1.94). Furthermore, the m1 vacA allele was associated with intestinal metaplasia (RR: 1.57, 95% CI: 1.24–1.98); however, there was no documented increase in the risk of gastric atrophy (34). The same study showed that adjusting the model for the incidence standardized by age decreased the association of bacterial genotypes with gastric cancer. Although *p*-values revealed significant associations in data analysis, the lower limit of 95% CI of the s1 allele was extremely close to the null hypothesis value. In contrast, the results of the present investigation showed significant associations of the s1m1 genotype with 95% CI far from the null hypothesis both in bivariate analysis (OR: 3.82, 95% CI: 1.45–10.07) and age-adjusted multivariate logistic regression model (adjusted OR: 4.62, 95% CI: 1.7–112.53).

Similarly, the analysis of genotype distribution by diagnostic category (Table 4) helps conclude that the prevalence of the s1m1 subtype increases with the increase in the severity of premalignant lesions, whereas the opposite seems to occur for the s2m2 genotype, suggesting a proportional relationship between the severity of the lesion and bacterial genotype. These findings highlight the conceptual value of the carcinogenesis model proposed by Dr. Correa and provide an important theoretical basis for its predictive capacity for cancer risk.

The carcinogenic effect of the cagA gene product is attributable to diverse mechanisms such as the reorganization of the cytoskeleton of epithelial cells, change in cell phenotype, and activation of signaling pathways that stimulate cell proliferation (35-37). These mechanisms would partly explain a higher incidence of gastric cancer in populations wherein approximately 90% isolates are cagA+ and a lower incidence of gastric cancer wherein the prevalence of cagA+ is lower (38, 39). In the present study, the prevalence of the cagA+ genotype between case and control groups did not significantly differ, and a relationship between the cagA genotype and development of precursor lesions of gastric malignancy was not documented. These results differ from those reported in the literature (16, 40, 41). A possible explanation for this finding is related to polymorphisms of the cagA gene and

phosphorylated EPIYA motifs. For example, it has been proposed that polymorphisms of the cagA gene and phosphorylated EPIYA motifs modulate the risk of diseases such as duodenal ulcer, degree of inflammation, and risk of gastric cancer (42, 43).

The role of salt intake in the genesis of precursor lesions of gastric malignancy has been evaluated in other investigations. Although methods to quantify salt intake differ among studies, a positive relationship with gastric pathology has consistently been demonstrated (44). For example, a systematic review published by Dias-Neto et al. showed a positive association between intestinal metaplasia and salt intake. However, this association was not significant (OR: 1.53, 95% CI: 0.72–3.24) (45). Furthermore, association studies between salt intake and gastric cancer have shown positive associations that were significant when high and low salt intake were compared (OR: 2.05, 95% CI: 1.60–2.62, $p < 0.005$) (46). The present study showed a significant association between regular salt intake and the development of preneoplastic lesions, and intestinal metaplasia was the diagnosis that appeared to best explain this association. These findings are consistent with those reported by Chen et al. in an advanced investigation at the Department of Nariño (20).

Dietary factors such as salt intake can modulate the risk of gastric carcinogenesis by modifying host mucosal factors, regulating inflammatory response, or inducing epigenetic changes (47) The effect of salt intake on *H. pylori* virulence has been evaluated in microbiological, transcriptional, and proteomic studies, showing changes in bacterial morphology and a higher transcription of the cagA gene under high-salt concentration conditions (48-50). A higher carcinogenic effect related to salt intake and cagA overexpression has been demonstrated in animal models (51). In the present study, no relationship was observed between premalignant lesions of gastric cancer and the cagA genotype, but it was shown that salt intake habit increases the risk of developing precursor lesions of gastric cancer in patients with vacA cytotoxic *Helicobacter* genotypes (adjusted OR for salt intake: 5.19, $p = 0.001$). To the best of the authors' knowledge, no studies that explore the relationship of vacA genotypes with *Helicobacter* and analyze the interactive effect in preneoplastic lesions have not yet been published.

Further, the present study evaluated bacterial coinfection, whose role in the development of gastric pathology is difficult to determine. For example, it has been suggested that coinfection generates a competitive growth disadvantage for the bacteria or favors growth at certain mucosal sites that would serve as niches (29). The present study shows that coinfection increases the risk of developing premalignant lesions and cancer, albeit at a much lower level than the s1m1 genotype. These results are difficult to compare with those in the literature because coinfection is associated with pathologies such as duodenal ulcer but not with the development of precursor lesions of gastric malignancy (52). This challenge is more evident in the inability to assign a particular pathological effect in cases wherein >1 bacteria are detected. However, the adjusted multivariate model showed that the regular salt intake habit increases the risk of developing preneoplastic lesions by >3 folds, suggesting that bacterial coinfection can have a primary injurious effect on gastric mucosa that could further be enhanced via dietary factors. This association of coinfection was evident in the logistic regression model and not in the bivariate model, which is consistent with the multifactorial nature of gastric cancer.

One of the limitations of the present study could be derived from the participants' perception of the harmful effect of salt intake on human health. This perception could eventually modify their responses, thereby generating the Hawthorne effect. In an attempt to limit this effect, the questionnaire was

completed before gastrointestinal endoscopy was performed, without the knowledge of the gastrointestinal endoscopic and histopathological diagnoses. Quantification of 24-h urine sodium excretion may be recommended in future studies for quantitative and precise assessment of salt intake.

The study results suggest that the s1m1 genotype is associated with the precursor lesions of gastric malignancy and that this association is strengthened with an increase in age and salt intake. On the other hand, it can be concluded that the severity of premalignant lesions of gastric malignancy is directly correlated with advanced age and cytotoxic *H. pylori* genotypes.

Declarations

Ethics approval and consent to participate:

Participants provided their consent to participate in the study and signed informed consent. The study was approved by the Scientific Research Ethics Committee of the Cauca University.

Availability of data and materials:

Data that support the findings of this study are available from Acosta-Astaiza CP (author) but restrictions apply to the availability of these data, which were used under license for the present study and so are not publicly available. However, data are available from the authors upon reasonable request and with permission of Acosta-Astaiza CP.

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Conflict of interests

The authors of this article declare no conflict of interest whatsoever.

Authors' contributions

All authors contributed to the conception and analysis of data. All authors were involved in drafting and revising the manuscript. All authors approved the final version of the manuscript.

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