Helicobacter pylori genotypes, salt intake, and sociodemographic factors associated with premalignant stomach lesions in a Colombian population

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Abstract

INTRODUCTION Genetic variability of Helicobacter pylori is associated with various gastrointestinal diseases; however, little is known regarding its interaction with sociodemographic and dietary factors in the development of premalignant lesions.OBJECTIVE To evaluate the association between Helicobacter genotypes, salt intake, and sociodemographic factors with precursor lesions of gastric malignancyMATERIALS AND METHODS An analytical study was conducted including cases (patients with gastric atrophy, intestinal metaplasia, and dysplasia) and controls (patients with non-atrophic gastritis). Sociodemographic information and salt intake habits were obtained using a questionnaire. Histopathological diagnosis was made according to the Sydney System. Genotypes cagA and vacA for H. pylori 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 outcome (premalignant lesion) is presented as OR and 95%CI. A p-value of <0.05 was considered as significant.RESULTS The genotype vacA/s1m1 increases the risk of developing precursor lesions of malignancy (OR: 3.82; 95%CI: 1.45–10.07; p = 0.007). Age and salt intake showed a positive interaction effect for the genotype s1m1 (adjusted OR: 5.19; 95%CI: 1.88–14.32; p = 0.001) and bacterial coinfection (adjusted OR: 3.2; 95%CI: 1.06–9.59; p = 0.038). The cagA genotype was not related with the development of premalignant lesions (OR: 1.21; 95%CI: 0.80–1.82; p = 0.361).CONCLUSIONS The vacA genotypes, age, and salt intake are indicators of the risk of premalignant lesions in the study population.

Background

Gastric cancer is a disease with a high impact disease at the global level. According to GLOBOCAN, in 2012, there were 952,000 cases reported worldwide, making it the fifth most prevalent cancer and the third cause of death attributable to cancer (1). Colombia is one of the countries with the highest incidence, 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—10.7/100,000 inhabitants/year for women and 18.7/100,000 inhabitants/year for men.

Besides the high incidence, late diagnosis is one of the most important factors affecting the mortality of patients with cancer in Cauca. Adrada et al. showed that the proportion of cancers diagnosed in advanced stages in Cauca is >90% (3). Unfortunately, in most cases, this occurrence results in fatality due to the disease, with the 5-year survival rates of <10% (4). Currently, prevention and early detection are the best 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 dysplasia (6%) and a lower risk associated with 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, vacA-s1m1 and cagA+ genotypes have been shown to be associated with an increased risk of presenting precursor lesions of malignancy (11-16).

Few studies have been conducted in Colombia in which bacterial genotypes (17-19) or salt intake (20) is associated with the onset of premalignant stomach lesions. In these studies, the approach is based on comparing genotype frequencies, and the results show contradictory findings. However, to the best of the authors' knowledge, to date, none of the investigations have 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 between *H. pylori* genotypes, salt intake, and sociodemographic factors with regard to the precursor lesions of malignancy (atrophy, metaplasia, and dysplasia) in the Cauca population.

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 for gastric cancer) from January 2008 to December 2011. The samples were obtained by convenience sampling method and included patients aged >18 years with a histopathological diagnosis of non-atrophic gastritis (controls) and patients with precursor lesions of malignancy (cases). The inclusion criteria included the following: participants should be born in a municipality of Cauca; should be children of parents from Cauca; and should have a diagnosis of *H. pylori* infection determined by histopathological tests and corroborated by molecular biology tests [polymerase chain reaction (PCR)]. Participants who underwent previous gastric surgery, who received treatment for *H. pylori*, who had an HIV infection, and who had gastric adenocarcinomas other than intestinal histotype were excluded.

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

Gastric samples were obtained by experienced gastroenterologists via digestive endoscopy. Patients underwent the 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. Biopsies were fixed in buffered formalin and

stained with hematoxylin–eosin and Giemsa stains. Two pathologists conducted the histopathological analyses of the samples. Patients were assigned to four diagnostic categories: non-atrophic gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia; the most severe lesion was selected as the final diagnosis in each patient. Visual analog scales were used according to the Sydney System. Genotyping studies of *H. pylori* were performed from the DNA extraction of paraffin embedded biopsies using the Chelex technique (No. C7901, Sigma, St. Louis, MO). PCR technique previously described by Sugimoto et al. (21) was used for the amplification of vacA genes. PCR mixtures were prepared using 50 ng of genomic DNA, 100 µmol 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 of the primers shown in Table 1. The reactions commenced with a denaturation step 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 a final extension step at 72°C for 10 min. The 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.

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 the questions in a closed questionnaire, which was completed before the endoscopy.

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

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

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

Mean differences in the age variable were evaluated using the one-way ANOVA, supplemented by post-hoc tests. Differences in proportions were evaluated using the Chi-Square Test of Independence. The OR and its p-value were used to evaluate the effect of each variable of interest on the response variable (precursor lesion of malignancy). A multivariate logistic regression analysis was performed, including the variables that met the Hosmer–Lemeshow criteria and those reported in the scientific literature as

significant. A p-value of <0.05 and 5%CI were considered statistically significant. Data was analyzed using version 23 of the SPSS program.

Participants provided their consent to participate in the study and signed the informed consent. The ethical principles of the Declaration of Helsinki were respected, 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%).

Mean patient age was 43 years for the non-atrophic gastritis group, 52 years for the atrophic gastritis and metaplasia group, and 63 years for the dysplasia group. When comparing the mean ages between groups using ANOVA single-factor test, significant differences were observed between groups (p = 0.000). Post-hoc analysis showed significant differences between each precursor lesion of malignancy and cancer with non-atrophic chronic gastritis (p = 0.000).

Regarding age, the most prevalent age group in the reference 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. With regard to salt intake, 235 participants, who included 100 (43%) patients with non-atrophic gastritis, reported never adding additional salt to their meals. On the other hand, 63 participants, who included only 19 (30%) patients with non-atrophic 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.

Each precursor lesion of malignancy and cancer was compared with the non-atrophic chronic gastritis group to estimate the measures of association. Female gender, age of 18–40 years, and the “never added salt to meals” groups were selected as reference categories. Analyses showed significant associations in the age groups >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 dysplasia (Table 3).

To study the vacA genotypes, alleles s1, s2, m1, and m2 were examined and grouped according to virulence profile. The analysis showed the presence of the s2m2 genotype in 22 patients, which included 16 (72.7% of the isolates) patients with non-atrophic gastritis, 1(4,5% of the isolates) with atrophy, 5 (22,7% of the isolates) with metaplasia, and none with 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 non-atrophic chronic gastritis, 36 with atrophy, 101 with metaplasia, and 25 with dysplasia. Cases of coinfection where the presence of a cagA+ bacterium was documented were considered within the cagA+ category. The cagA and vacA genotype distribution according to type of diagnosis is shown in Table 4.

To facilitate the analyses and considering the absence of the s2m2 genotype in the dysplasia category, patients with atrophy, metaplasia and dysplasia were grouped into a single category (precursor lesions of malignancy) and compared with the reference 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, vacA/s2m2 and cagA genotypes were selected as reference categories (Table 5).

Patients with atrophy, metaplasia, and dysplasia were grouped into a single category (precursor lesions of malignancy) and compared with the reference group to obtain the crude OR of the sociodemographic variables and salt intake. Only the statistically significant variables (categorized age, income category, and salt intake) were included in this analysis according to the findings of the bivariate analysis by diagnostic categories (Table 3). In a previous analysis, male gender showed no statistically significant associations either in 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 malignancy according to age-adjusted vacA genotypes as well as the interactive effect enhancer of salt intake.

Discussion

An association between age and onset of premalignant gastric lesions was determined in this study. The findings show that the prevalence of dysplasia is higher in patients aged >60 years whereas injuries, such as atrophy and 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 malignancy and age.

The greatest age-related risk is due to genomic instability acquired over the years owing to chronic inflammation, cumulative damage of free radicals, and the 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). Results from other studies and those of this present investigation suggest that preneoplastic lesions represent histological changes caused by tissue aging and dysfunctional adaptive responses, thereby increasing the risk for tumors.

Regarding bacterial genotypes, the s1m1 genotype was more prevalent in the case group whereas 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 division cycle (32-34)

In a recent meta-analysis, 33 studies were evaluated, which included overall 2696 controls and 1446 cases with gastric cancer and precursor lesions of malignancy. In that study, the s1 allele showed an increased risk for 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. The same study showed that adjusting the model for the incidence standardized by age decreased the association of bacterial genotypes with gastric cancer. Although the p values revealed significant associations in the data analysis, notably, the lower limits of the confidence intervals for the s1 alleles were extremely close to the null hypothesis value. In contrast, the results of the present investigation showed significant associations for the s1m1 genotype with confidence intervals far from the null hypothesis, both in the 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) also allows us to conclude that the prevalence of s1m1 subtypes increases with the increase in the severity of premalignant lesions, whereas the opposite seems to occur with s2m2 genotypes, 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 regarding 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 epithelial cell cytoskeleton, change of cellular phenotype, and activation of signaling pathways that stimulate cell proliferation (35-37). These mechanisms would partly explain the higher incidence of gastric cancer in populations where approximately 90% of isolates are cagA-positive and a lower incidence where the prevalence of positive cagA is lower (38, 39). In our study, the prevalence of the cagA-positive genotype in the cases and the reference group did not significantly differ, and a relationship between the cagA genotype and development of precursor lesions of malignancy was not documented. These results differ from those reported in the literature (16, 40, 41). A possible explanation for this finding could be related to the polymorphisms of the cagA gene and the phosphorylation state of the EPIYA motifs. For example, it has been proposed that the polymorphisms and phosphorylation status of EPIYA motifs can 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 are significant when comparing high and low salt intake (OR = 2.05; 95%CI 1.60–2.62; p < 0.005) (46). Our 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 the information published by Chen et al. in an advanced investigation in the department of Nariño (20).

Dietary factors, such as salt intake, can modulate the risk of gastric carcinogenesis by modifying host mucosal factors, regulating the 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 as well as a greater transcription of the cagA gene when salt concentrations are high (48-50). A greater carcinogenic effect related to salt intake and cagA overexpression has been demonstrated in animal models (51). In our study, no relationship was observed between premalignant lesions and cagA genotype, but it was shown that the salt intake habit increases the risk of developing precursor lesions 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 yet been published.

Further, the present investigation 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 in certain mucosal sites that would serve as niches (29). Our research shows that coinfection increases the risk of having premalignant lesions and cancer, albeit at a much lower level than the s1m1 genotypes. 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 malignancy (52). This challenge is more evident in the inability to assign a particular pathological effect in cases wherein more than one bacterium is detected. However, the adjusted multivariate model showed that the regular salt intake habit increases the risk of developing preneoplastic lesions by more than three-fold, suggesting that bacterial coinfection could have a primary injurious effect on the gastric mucosa that could further be enhanced via dietary factors. The association of coinfection was evident in the logistic regression model and not in the bivariate model, which is consistent with the gastric cancer's multifactorial nature.

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 the responses of the participants, thereby generating a Hawthorne effect. In an attempt to limit this effect, the questionnaire was completed before gastroscopy was performed, without the knowledge of the endoscopic and histopathological diagnoses. A quantification of 24-h urine sodium excretion may be recommended in future studies for a quantitative and precise assessment of salt intake.

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

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Declarations

Ethics approval and consent to participate:

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

Availibility of data and materials:

The 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 current study,

and so are not publicly available. Data are however 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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Tables

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	

Table 1. Primer sequence for PCR amplification

		NACG*	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	More than 1 CLMMW	74 (42.5)	25 (51)	53 (39.3)	3 (9.7)
	Less than 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)

Table 2. Distribution of participants in the study groups. NACG: Non-atrophic chronic gastritis; CLMMW: Current Legal Minimum Monthly Legal Salary in Force Wage

	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	3.04	(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
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									
More than 1 CLMMW	1	1	1	1	1	1	1	1	1
Less than 1 CLMMW	0.71	(0.37–1.34)	0.71	1.14	(0.72–1.81)	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	0.99	1.14	(0.06–21.8)	0.929
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

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

		NACG*	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)

Table. 4. Bacterial genotype distribution in the study groups. *NACG. Non-atrophic chronic gastritis

		Non-atrophic gastritis	Precursor lesions of 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)	137 (63.7)	1.21	0.80–1.82	0.361

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

Variable	Crude OR	95%CI	p-value	Adjusted OR[a]	95%CI	p-value	Adjusted OR[b]	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	0.040
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

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