Gastric Precancerous Process in a High Risk Population: Cross-sectional Studies

Pelayo Correa, William Haenszel, Carlos Cuello, Diego Zavala, Elizabeth Fontham, Guillermo Zarama, Steven Tannenbaum, Tito Collazos, and Bernardo Ruiz

INTRODUCTION

Insight into the events and forces which characterize the human precancerous process might be gained by the close observation of biopsy material from subjects at high risk of a specific neoplasm. In the case of gastric carcinoma, the following precursor lesions have been described and postulated to be sequential in nature: superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, and dysplasia (1–3). The population of Nariño, Colombia, has been extensively studied in this regard and a series of cohorts have been followed for several years, the first of them recruited in 1973 (4–6). This report deals with the histopathological findings of the first stage of gastric biopsies of 1788 cohort participants and the correlation with demographic parameters and gastric juice chemistry.

MATERIALS AND METHODS

Because previous epidemiological studies demonstrated a high risk of gastric cancer in the Andean region of Nariño in the south of Colombia (4), a gastroscopy and gastric biopsy survey of subjects living in that area was conducted in 1973. Solicitation to participate in the study was made by a social worker who visited households in each town. Suspect precursors of gastric cancer were identified and correlated with demographic and environmental parameters. Later, individuals who participated in that cross-sectional study and were inhabitants of two towns (La Cruz and Guaitirilla) of special interest were followed up because of their high cancer rate and because acceptable logistic arrangements for rebiopsy could be made. These two towns yielded a first cohort of 412 individuals. Between 1981 and 1983 a second household-based cohort of 1376 individuals was recruited in and around the high risk town of Tuqueres where a hospital and health center were available. Gastroscopy and gastric biopsies were performed and an epidemiological questionnaire administered.

Participation in the study was voluntary. The study was explained to civic groups by the local health authorities, civic leaders, and local medical practitioners. The community has been highly motivated mainly because of general knowledge of and personal experiences with the high gastric cancer mortality rates, which have affected practically every family in the community. A social worker invited voluntary participants at the rate of 8/day, 2 or 3 times a week. They were transported every morning to the Hospital Departamental in Pasto, approximately 100 km away, where the procedures were performed. The participants were compensated for their meals and lost wages.

Gastric biopsies were taken as follows: a minimum of two from the antrum (midportion of antral lesser and greater curvatures), one from the transition zone, and at least one from the body, midanterior wall. Most of the time additional biopsies were taken and any grossly identifiable lesions were biopsied. Based on a random sample of 10% of the cases, the distribution of the number of adequate biopsies for histological interpretation (eliminating biopsies inadequate for evaluation) was as follows: 1 biopsy = 2.8%; 2 = 5.6%; 3 = 37.6%, 4 or more = 54%. Biopsies were fixed immediately in buffered formalin and sent to the central laboratory of pathology at Universidad del Valle in Cali for embedding, sectioning, and staining with hematoxylin-eosin and periodic acid Schiff-alcian blue.

In selected subsamples of the cohort ancillary studies were performed and additional samples of urine and blood taken. An attempt to obtain an adequate gastric juice sample was made in all cases; improvements were introduced in the chemical analytical techniques in the second cohort and therefore only data from this cohort are presented in the tables. The microscopic slides were studied by one of the authors (C. C.) and a global diagnosis was made based on the general evaluation of the case, following previously published diagnostic categories and criteria (2, 6). The global diagnosis involved one of the following categories: normal, superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, carcinoma. The global classification represented the most advanced lesion and did not imply the presence or absence of less advanced lesions. Additionally, a detailed protocol was applied independently to each fragment of tissue available, to assign grades for each of the histopathological findings: leukocytic infiltrate, hyperplasia of glandular necks, atrophy (gland loss), intestinal metaplasia and dysplasia. These criteria are illustrated in Figs. 1–6. A numerical score for each parameter was computed in terms of averages for the tissue fragments representing the antrum and the corpus. A final score for the case was computed with all the available material. The procedures and the criteria were different: the global diagnosis was the most advanced lesion in any tissue fragment; the protocol score was a semiquantitative estimation of the degree of abnormality for each lesion over all available fragments.

Global diagnosis and protocol scores together with identifiers, demographic data, questionnaire responses, and data on the ancillary studies performed for individual subjects, were recorded on computer diskettes using software that required verification of each record. The diskettes were sent to the Epidemiology Section, Louisiana State University Medical Center, for tabulation and analysis in a mainframe computer. Editing of the data was carried out in Cali and New Orleans. Discrepancies were reconciled by review of original data archived at the Tumor Registry in Cali. Standard t tests for statistical significance of

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1 Work supported by Grant P01-CA28842 from the National Cancer Institute.
2 To whom requests for reprints should be addressed, at Department of Pathology, Louisiana State University Medical Center, 1901 Perdido St., New Orleans, LA 70112-1393.

ABSTRACT

The gastric precancerous process is evaluated in 1788 participants in a gastroscopy survey in the population of Nariño, Colombia, which has one of the highest gastric cancer incidence rates on record. A detailed histological classification is used, and a hierarchical distribution of lesions is described with the main stages being gland neck hyperplasia, atrophy (gland loss), intestinal metaplasia and dysplasia. Acute inflammation was not found to be a specific stage in the sequence but rather a common finding in all stages of the precancerous spectrum. Indices of disease progression for the different steps are calculated and found to increase with gastric pH and nitrate and nitrite content of the gastric juice. The effects of high pH and nitrite content are intimately correlated. Relative risks of specific lesions, namely, hyperplasia, atrophy, metaplasia, and dysplasia, increase linearly with higher pH, nitrate, and nitrite values in the gastric juice. The severity of atrophy correlates with the prevalence of metaplasia and the severity of metaplasia correlates with the prevalence of dysplasia, suggesting a sequential relationship between the described stages, a finding supported by all parameters examined. The model of progression described may serve as a basis for comparisons with populations at different levels of gastric cancer risk but it fails to provide information concerning the time required for each change, which should be provided by follow-up (cohort) studies.
Fig. 1. Oxyntic mucosa with superficial gastritis. Inflammatory infiltrate limited to the upper lamina propria. Mild hyperplasia of gland necks.

percentage differences were used to evaluate the contrast of progression indices by sex, age, and gastric chemistry parameters. Odds ratios and confidence intervals were calculated by the Mantel-Haenszel method (7) and the Mantel extension was used to test for linear trend (8).

RESULTS

Although the total cohort involved 1788 subjects, individual values for some of the protocol parameters were missing or incomplete in 99 subjects. All tables are based on those cases with complete information for the parameter under study, excluding cases with missing values. Because the slides were interpreted over a period of approximately 10 years, the criteria set at the beginning of the study were strictly followed. Our initial definition of "normal" allowed for a certain degree ("mild") of chronic inflammatory cell infiltrate. A total absence of inflammatory cells, the ideal "normal" mucosa, is extremely rare (approximately 1%) in this population. This makes the distinction between superficial gastritis and normal somewhat subjective. Inflammatory WBC infiltrate was a constant finding at all stages of the process and by itself was not very useful in discriminating the different categories of lesions. Hyperplasia of glandular necks (Figs. 1 and 2) was a rather constant finding in specimens with clear inflammatory changes as well as in more advanced lesions of chronic atrophic gastritis, intestinal metaplasia, or dysplasia. Hyperplasia of glandular necks probably represents a reaction to cell injury or cell loss which appears to be a basic pathological change in the lesions under consideration. The degree of neck hyperplasia varies, which may reflect periods of more or less intense cell damaging forces and the adequacy of the reparative process. It is occasionally absent in advanced lesions, but in general it represents a good marker of the inflammatory and atrophic process, appears early in the chain of events, and persists afterward. Some specific types of chronic gastritis, such as the diffuse antral gastritis as well as the presence of Helicobacter pylori, were recognized after this study was begun and for that reason do not appear in the present material. They are the subject of separate evaluation and reports.

All individuals in the cohort with normal gastric mucosa as determined by the global classification were negative for hyperplasia, atrophy, metaplasia, and dysplasia when evaluated in accordance with the study protocol. All specimens assigned in the global classification to chronic atrophic gastritis were positive for atrophy but negative for metaplasia and dysplasia on
Fig. 3. Chronic gastritis with additional acute inflammation (activity). Polymorphonuclear leukocytes in the epithelium and the lumen of the loveola.

Protocol examination. There is a certain degree of interdependence between protocol findings based on a numerical score and global diagnosis based on the most advanced lesion after reviewing all the material available. This was addressed by defining the histological criteria beforehand (6) and assigning scores independently for each parameter. Some degree of uncertainty was experienced in cases of mild atrophy (loss of a few glands) and for that reason "mild" or "questionable" atrophy was not recorded as such. In the case of metaplasia, all cases were classified on a histochemical basis (alcian blue stain) evaluated in separate slides.

Fig. 4. Chronic atrophic gastritis. Most antral glands have disappeared. Hyperplasia of glandular necks is present.

Fig. 5. Chronic atrophic gastritis with intestinal metaplasia. Most antral glands have been replaced by intestinalized structures.

Fig. 7 is a schematic representation of the findings in the
gastric biopsies. All specimens positive for dysplasia (D+) were positive for metaplasia (M+), and glandular atrophy (A+). Only a few did not show hyperplasia of the glandular necks. Since this probably represents response to recent cell injury, it is reasonable to assume that hyperplasia was present at one point in the process. It appears then that the right side of the tree shown in Fig. 7 represents not only the great majority of cases but also the natural evolution of the process.

Acute inflammation represented by polymorphonuclear infiltrate was evaluated independently but in our analysis did not appear to follow the sequential pattern shown in Fig. 7 for the other parameters. Acute inflammation appears related but is not a necessary discriminant of glandular neck hyperplasia or other more advanced lesions. Recent investigations link acute inflammation (so-called active gastritis) with H. pylori infection, a very prevalent infection in our series.

Table 1 depicts the number of cases showing the specific histopathological lesions by age and sex, distributed by the highest grade of lesion observed. It also shows the proportion of subjects who have reached a more advanced lesion in terms of the original pool. Each step in the chain is presented as a function of the pool of subjects with the previous sequential event. The increase in the proportion of subjects positive for each lesion with increasing age is statistically significant; there are no differences by sex. Similarly, the last three columns represent the proportion of atrophy, metaplasia, and dysplasia as a function of the total number of subjects eligible. All 3 ratios show a statistically significant linear trend with age and no differences by sex.

Table 2 examines the correlation between pH and nitrite content of gastric juice below and above the median value of the second cohort, showing that the presence of nitrite is highly dependent on a high pH. Given the strong correlation between pH and nitrite status the similarity in findings is not surprising. The strong correlation also makes it difficult to assess the independent contribution of the two factors. Such strong correlation with pH is not observed for nitrate; at pH 3.7 or below, 36.4% of the subjects had positive nitrate values.

Table 3 shows numbers of cases and progression indices for histopathological parameters according to gastric juice pH and nitrate and nitrite content. The derivative ratios D+/M+, M+/A+, A+/H+, H+/N estimate the proportion of subjects positive for more advanced pathological phases and can be used as surrogate measures of rates of transition, or progression indices. Similarly, the derivative ratios in the last 3 columns estimate the proportions of the total pool which reached the stages of atrophy, metaplasia, or dysplasia. The methodology for gastric nitrite and nitrate evaluation was improved around 1980, after the first cohort had been recruited, and for that reason the table displays data for the individuals in the second cohort only. All indices of progression show a gradient with highest values for high pH and positive nitrite content and lowest values for low pH and negative nitrite. The gradient is more marked for the last 3 indices, based on all subjects eligible for change instead of the step by step indices. The progression indices for gastric juice nitrate followed the pattern depicted by the pH and nitrite contrasts.

The relationship of gastric chemistry and pathology can be elaborated by contrasting cases (pathology positive) and controls (pathology negative) with respect to gastric juice levels of pH, nitrite, and nitrate. Cast in this form Table 4 yields estimates of the risks of hyperplasia and succeeding pathology phases relative to unit risk for normal gastric mucosa. The nitrate contrasts produced small elevations of relative risks across all categories. For nitrite positive individuals, the magnitude of the relative risks was substantial. For pH levels of ≥3.7 (median) the relative risks increased monotonically with progression to the most advanced pathology (metaplasia, dysplasia). At pH 3.7 and above a gradient for nitrite concentration is still detectable. All 3 gastric chemistry measures showed a significant trend in relative risk by gastric pathology. How-
ever, for nitrates the major effect was the overall contrast of normal and abnormal and the contribution of differences among pathology stages was less marked.

The gastric lesions were classified by degree of abnormality in accordance with the criteria described. The question of interest is whether the severity of the lesions for hyperplasia, atrophy, and metaplasia is correlated with higher percentages of specimens positive for succeeding pathology phases. Table 5 shows that the degree of severity for atrophy influences the prevalence of metaplasia and the severity of metaplasia influences the prevalence of dysplasia. This is not the case for hyperplasia. These results lend support to the concept that metaplasia and dysplasia are linked in a sequential cause-effect relationship.

DISCUSSION

Our results support previous assumptions about the biology of the precancerous process made with smaller series and simpler protocols for the interpretation of the histopathological findings. The progression indices in Table 1 are consistent with other surveys in Colombia in describing a rise in the overall prevalence of precursor lesions with age, accompanied by greater representation of the more advanced stages at older ages. There has been little change in diet and other habits during recent years in Nariño, so that the current experience of persons aged 35-44 years may be used as an estimate of the status 10 years hence of persons now 25-34 years of age in the selected Colombian populations took essentially the same approach to the estimation of transitional probabilities. In the absence of direct information concerning the combination of rates of transition can be obtained from repeat gastric biopsies taken at intervals of 3-6 years, data on the joint presentation of lesions within individual specimens contain indirect information on the probability of progression to more advanced gastric pathologies. What is missing in this latter approach is information on the time span over which changes take place. The findings in Fig. 7 show that this assumption was well founded. A current model for the etiology of stomach cancer (1) hypothesizes a sequence for the etiology of stomach cancer (1) hypothesizes a sequence of events progressing from inflammation to atrophy, to metaplasia, to dysplasia, to carcinoma in situ, and finally to invasive gastric carcinoma. The model suggests that investigations should focus on the rates of transition among precursor states and on host characteristics and/or environmental exposures that might impede or accelerate them. While direct calculation of rates of transition can be obtained from repeat gastric biopsies taken at intervals of 3-6 years, data on the joint presentation of lesions within individual specimens contain indirect information on the probability of progression to more advanced gastric pathologies. What is missing in this latter approach is information on the time span over which changes take place. Dysplasia in the absence of metaplasia has been reported in other communities but was not observed in our series (9). Such a situation is seen mostly in the "nonepidemic" or "diffuse" type of carcinoma (10). The collective findings for the gastric
GASTRIC PRECANCEROUS PROCESS

Table 3 Protocol findings of gastric biopsies

<table>
<thead>
<tr>
<th>Gastric chemistry</th>
<th>Total subjects</th>
<th>No. positive</th>
<th>H+/N</th>
<th>A+/H+</th>
<th>M+/A+</th>
<th>D+/M+</th>
<th>A+/N</th>
<th>M+/N</th>
<th>D+/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH &gt; 6.6</td>
<td>430</td>
<td>389</td>
<td>287</td>
<td>186</td>
<td>46</td>
<td>0.90</td>
<td>0.74</td>
<td>0.65</td>
<td>0.25</td>
</tr>
<tr>
<td>2.5 - 6.5</td>
<td>445</td>
<td>323</td>
<td>167</td>
<td>81</td>
<td>7</td>
<td>0.73</td>
<td>0.52</td>
<td>0.49</td>
<td>0.09</td>
</tr>
<tr>
<td>&lt; 2.5</td>
<td>455</td>
<td>272</td>
<td>121</td>
<td>49</td>
<td>4</td>
<td>0.60</td>
<td>0.44</td>
<td>0.40</td>
<td>0.08</td>
</tr>
<tr>
<td>pH &gt; 6.6</td>
<td>NO2^-</td>
<td>303</td>
<td>277</td>
<td>214</td>
<td>138</td>
<td>33</td>
<td>0.91</td>
<td>0.77</td>
<td>0.64</td>
</tr>
<tr>
<td>NO3^-</td>
<td></td>
<td>127</td>
<td>112</td>
<td>73</td>
<td>48</td>
<td>13</td>
<td>0.88</td>
<td>0.65</td>
<td>0.66</td>
</tr>
<tr>
<td>pH 2.5 - 6.5</td>
<td>NO2^-</td>
<td>34</td>
<td>28</td>
<td>15</td>
<td>8</td>
<td>2</td>
<td>0.82</td>
<td>0.54</td>
<td>0.53</td>
</tr>
<tr>
<td>NO3^-</td>
<td></td>
<td>408</td>
<td>292</td>
<td>151</td>
<td>72</td>
<td>5</td>
<td>0.72</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td>pH &lt; 2.5</td>
<td>NO2^-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.60</td>
<td>0.44</td>
<td>0.41</td>
<td>0.08</td>
</tr>
<tr>
<td>NO3^-</td>
<td></td>
<td>453</td>
<td>270</td>
<td>120</td>
<td>49</td>
<td>4</td>
<td>0.60</td>
<td>0.44</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* H, hyperplasia; A, atrophy; M, metaplasia; D, dysplasia.

Table 4 Age-adjusted odds ratios of gastric pathology (normal mucosa = 1) for contrasts of gastric chemistry

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Hyperplasia</th>
<th>Atrophy</th>
<th>Metaplasia</th>
<th>Dysplasia</th>
<th>Test for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate &gt; 24.0 vs. ≤24.0 ppm</td>
<td>1.4</td>
<td>1.8</td>
<td>2.1</td>
<td>2.7</td>
<td>25.2 0.0001</td>
</tr>
<tr>
<td>Nitrite positive vs. negative</td>
<td>4.3</td>
<td>6.5</td>
<td>8.3</td>
<td>16.8</td>
<td>115.7 0.0001</td>
</tr>
<tr>
<td>pH &gt; 3.7 vs. ≤3.7</td>
<td>3.2</td>
<td>4.8</td>
<td>7.6</td>
<td>23.0</td>
<td>159.6 0.0001</td>
</tr>
<tr>
<td>pH &gt; 3.7: nitrite positive vs. negative</td>
<td>2.2</td>
<td>2.8</td>
<td>2.9</td>
<td>5.0</td>
<td>14.32 0.001</td>
</tr>
</tbody>
</table>

Table 5 Distribution of gastric lesions by severity of hyperplasia, atrophy, and metaplasia

<table>
<thead>
<tr>
<th>No. positive</th>
<th>H+*</th>
<th>A+</th>
<th>M+</th>
<th>D+</th>
<th>A+</th>
<th>M+</th>
<th>D+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia</td>
<td>611</td>
<td>339</td>
<td>204</td>
<td>45</td>
<td>55</td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td>Atrophy</td>
<td>387</td>
<td>244</td>
<td>118</td>
<td>13</td>
<td>63</td>
<td>48</td>
<td>11</td>
</tr>
<tr>
<td>Metaplasia</td>
<td>254</td>
<td>106</td>
<td>4</td>
<td>4</td>
<td>42</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* H, hyperplasia; A, atrophy; M, metaplasia; D, dysplasia.

juice chemistry parameters in Table 3 are consistent with earlier reports from Nariño on the strong correlation between gastric chemistry and gastric pathology. Nitrites may react with nitrogen-containing substances to produce carcinogenic N-nitroso compounds. Individual differences in the conversion of nitrates to biologically active nitrites and the fact that nitrites could be destroyed by ascorbic acid or combined with other nitrogen compounds could operate to dilute the associations of these compounds with gastric pathology.

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