γ-Glutamyl Transpeptidase Localization in Esophageal Exfoliative Cytology of a Chinese Population at Risk for Carcinoma (42845)

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Abstract. **γ-Glutamyl transpeptidase (γ-GT) localization in exfoliative esophageal specimens was evaluated in a population of 911 individuals aged 35 or older who lived in an area of China with a high incidence of esophageal squamous carcinoma. Of the total population, 24.4% was positive for γ-GT. Graded histopathologic cellular classification revealed the following incidences: normal, 10.4% (43 of 413); hyperplasia, 22.0% (44 of 200); Grade I dysplasia, 42.4% (98 of 231); Grade II dysplasia, 46.0% (23 of 50); near-carcinoma, 100% (4 of 4); and squamous carcinoma, 77.0% (10 of 13). In these groups γ-GT-positive cells were usually from normal esophageal squamous epithelium. A lower incidence of γ-GT-positive cells was found for dysplastic cells (dysplasia grade I, 6.5% (15 of 231); grade II, 10.0% (5 of 50). The presence of γ-GT-positive cells in normal esophageal squamous epithelium may prove useful for identification of suspect populations; the presence in dysplastic cells may serve as a diagnostic marker for patients who will soon progress to the carcinoma stage and who should receive early treatment and careful follow-up.** [P.S.E.B.M. 1989, Vol 190]

The presence of γ-glutamyl transpeptidase (γ-GT) has been shown to be a common feature of both preneoplastic and neoplastic lesions in animal models of chemically induced carcinogenesis (1–3). In a previous study we identified γ-GT activity not only in esophageal neoplastic foci but also in regions of both dysplastic and basal cells near tumor foci (4). γ-GT-positive cells have been present in 95% of abrasive balloon exfoliative cytology collections from 113 patients with esophageal cancer (5). γ-GT-positive normal esophageal squamous epithelial (NESE) cells were frequently present with squamous cancer cells (5). Similar findings have been reported for oral, pharyngeal, and laryngeal carcinoma (6). These findings suggest that detection of γ-GT may be useful in the diagnosis of premalignant lesions and early cancer in the human. We report here our findings in a population of 911 individuals aged 35 or older who were studied in an area of China known to be a high risk area for esophageal squamous carcinoma.

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Materials and Methods

In 1975, it was found that age-adjusted mortality of esophageal cancer in the region studied was over 100/100,000/year according to the death rate mass survey of the inhabitants over 30 years of age (7). The population studied here consisted of 911 individuals who were residents of Lay-Ko Commune, An-Yang District, Henan Province, China. All of the male and female inhabitants aged 35 or older were informed of abrasive balloon collection. Thus, they were random samples.

Esophageal exfoliative cytology specimens were collected using the abrasive balloon collection technique. After intubation through the cardia, the balloon is first inflated with 30 ml of air and then slightly deflated so that it can be pulled back pass the cardia. When the balloon comes up into the lower segment of the esophagus, it is redistended to obtain complete contact with the mucous surface, then slightly deflated and retracted for 5 cm. This process is repeated until the 20-cm mark is reached, when the balloon should be deflated completely and withdrawn (8).

Direct smears were made onto eight slides for each subject, four slides were stained with Papanicolaou stain and the other four slides with γ-GT.

Cytologic classification was made according to the following five grades: normal, hyperplasia, dysplasia (Grade I and Grade II), near-carcinoma, and squamous carcinoma (8). The definition of near-carcinoma reads as follows: The nuclei in the near-carcinoma cells are five or more times greater in size than those in the normal cells of the same layer. The chromatin granules of the nuclei are not as course as those of typical cancer cells and are evenly distributed in the nuclei. The nuclear membrane is thickened but regular, and the cytoplasm is broader than that in the cancer cells (8).

γ-GT was detected according to the method of Rutenberg (4, 9). Nuclei were stained with hematoxylin. Two to four smears, depending on the density of cells, were required by cytologists for characterization of the cells with γ-GT activity. In this study, most of the cases considered to be γ-GT positive exhibited numerous cells with γ-GT activity. However, cases were judged to be γ-GT positive when even a single cell was observed to exhibit strong γ-GT activity. A similar rationale is employed in the evaluation of conventional cytological smears, wherein the presence of a single carcinoma cell is sufficient to warrant a diagnosis of malignancy.

Results

Cytological evaluation of the 911 individuals studied here revealed the following classification results: (i) normal, 45.3%; (ii) hyperplasia, 22.0%; (iiia) Grade I dysplasia, 25.4%; (iiib) Grade II dysplasia, 5.5%; (iv) near-carcinoma, 0.4%; and (v) squamous carcinoma, 1.4%.

γ-GT-positive findings increased with increasingly abnormal pathocytologic features. Positive staining was found in 10.4% of the normal cases (Figs. 1 and 2). Positive findings in the other groups were as follows: hyperplasia, 22%; Grade I dysplasia, 42.4% (Fig. 3); Grade II dysplasia, 46.0%; near-carcinoma, 100%; and squamous carcinoma, 77.0% (Fig. 4). It should be noted that the γ-GT-positive rate of the latter two groups may not be accurate enough, because of the small sample size (4 and 13 patients). The three cytologic diagnosis groups with greater sample size are presented in Table

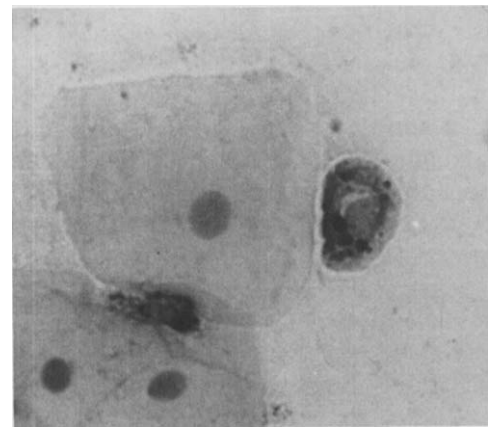

Figure 2. A parabasal cell (arrow) with γ-GT-positive diffuse and granular staining (original magnification ×400).

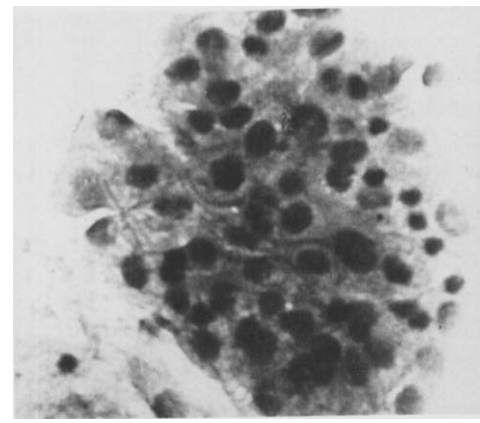

Figure 3. Groups of Grade I dysplastic cells with γ-GT-positive straining (original magnification ×400).

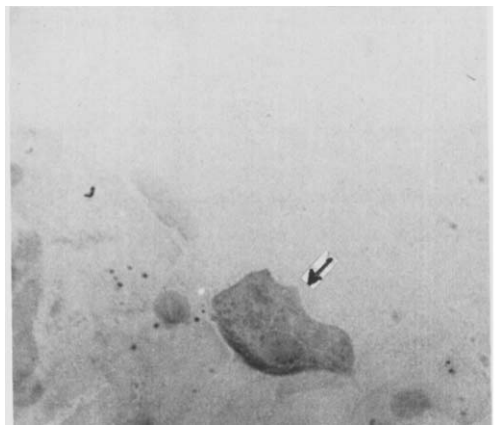

Figure 1. An intermediate cell (arrow) with γ-GT-positive diffuse and granular staining (original magnification ×400).

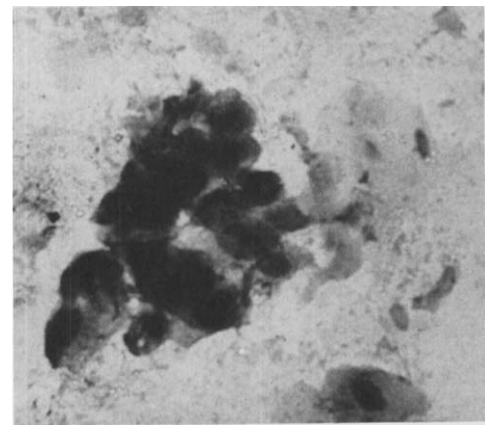

Figure 4. Groups of γ-GT-positive squamous cancer cells (original magnification ×400).

I. There seems to be no influence of age on γ-GT localization.

Although the incidence of γ-GT-positive staining varied within the five classification groups, γ-GT was found mainly in cells of the NESE present in the specimens. In all groups the frequency of γ-GT-positive NESE findings was as follows: normal, 100%; hyperplasia, 84.0%; Grade I dysplasia, 84.7%; Grade II dysplasia, 87.0%; near-carcinoma, 100%; and squamous carcinoma, 100%. Most of the dysplastic cells examined were γ-GT negative; the incidence was low in both Grade I (6.5%) and Grade II (10.0%) groups. Table II presents a summary of the γ-GT-positive findings according to our evaluation criteria.

Occasional γ-GT localization was observed in normal adenic epithelium, neutrophils, bacteria, and fungi present in the cytology specimens. Some bacteria adherent to the cell surfaces showed a granular γ-GT distribution similar to that seen in the cellular localization. Therefore, γ-GT detection must be combined with morphologic examination to exclude false positivity.

Discussion

The findings reported here are similar to those reported in another study of a Chinese population (12). The present data, however,show the applicability of γ-GT localization in a population with a high incidence of esophageal carcinoma.

The methodology for precise diagnosis of premalignant lesions is still under development. Several previous studies, however, have produced interesting results. Discrete populations of γ-GT-rich cells have been observed in liver and squamous epithelium of the rodent in early stages of chemically induced carcinogenesis (10, 11). γ-GT-positive NESE have been frequently found in human esophageal squamous carcinoma (5). γ-GT containing non-dysplastic epithelium present in sections of human carcinoma have been suggested to represent the very early premalignant lesions (6).

In the present study, 24.4% of the population studied were found to show γ-GT-positive cells on biopsy. Since it is unlikely that this high a fraction of the population would actually proceed to develop cancer, even in a high risk area, a role for the use of identification of γ-GT-positive dysplastic cells is suggested. γ-GT-positive NESE and hyperplastic cells may represent very early preneoplastic changes which may be reversible and nonlethal. γ-GT-positive dysplastic cells, however, may indicate irreversible premalignant lesion; this may point to later carcinoma development and may signal the presence of high risk.

In the present study γ-GT-positive NESE appeared before other histologic changes identifiable at the light microscopic level. Most hyperplastic and dysplastic cells observed were γ-GT negative. Thus, the presence of γ-GT-positive cells and abnormal histologic findings may be separate events. The γ-GT-positive rate increased with increasingly abnormal histopathologic findings; however, the true relationship between the two events remains unclear.

Our working hypothesis is as follows: (i) NESE and hyperplastic cells with γ-GT activity may be a sign of cells which have undergone very early steps in the transformation process. Screening for these γ-GT-pos-

Table I. γ-GT-Positive Rate in Different Age Groups

Cytologic diagnosis	Age groups			
	31–40	41–50	51–60	61–70
Normal	10.1% (15/149)	8.8% (10/114)	16.1% (18/112)	8.0% (3/38)
Hyperplasia	25.6% (20/78)	20.4% (10/49)	24.6% (14/57)	18.8% (3/16)
Grade I dysplasia	50.0% (27/54)	43.0% (23/54)	40.4% (40/99)	41.7% (10/24)
Total no. of cases	22.1% (62/281)	19.8% (43/217)	26.9% (72/268)	20.5% (16/78)

Table II. Frequency of γ-GT-Positive Cells in Different Cell Types[a]

Group	NESE	Hyperplastic cells	Dysplastic cells	Squamous cancer cells	Total no. of cases
Normal	10.4				413
Hyperplasia	18.5	4.0			200
Dysplasia					
Grade I	35.9	0.4	6.5		231
Grade II	40.0		10.0		50
Near-carcinoma	100.0				4
Squamous carcinoma	76.9			23.1[b]	13

[a] Some cases showed γ-GT-positive cells in different cell types simultaneously.
[b] γ-GT activity of invasive tumor cells may be lost, because the host recognition of neoplasia and natural inflammatory processes may only take place once stromal invasion has occurred. Frequency data are expressed as percentage.

itive cells is proposed as a method to identify individuals at risk in an area of high cancer incidence. (ii) Dysplastic cells with γ-GT activity may be a sign of cells which have undergone more early steps in the transformation process in which carcinoma may soon develop. This patient group should receive prompt treatment and regular follow-up.

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