

## A Modification of YAMANAKA Test for the Diagnosis of Gastric Cancer

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### INTRODUCTION

Simple and accurate tests for the diagnosis of gastric cancer are long-awaited by the clinicians. In 1962, YAMANAKA and KOBAYASHI<sup>11)</sup> developed a new test, which is based on dithizone reaction for zinc contained in gastric juice. They reported that the accuracy of this test was as high as 95.3 per cent and positive results were obtained even in the early stage of gastric cancer.

On the re-investigation of this test, we have noticed that its accuracy of positive diagnosis of gastric cancer is not so high in the patients with anacidity or hypoacidity and is greatly elevated by the previous addition of HCl prior to the test. These observations indicate an important role of gastric acidity in the test.

The object of the present paper is to record the results obtained by our modification and establish the validity of the addition of HCl in this procedure.

### PROCEDURE OF THE TEST

Gastric juice was aspirated through a nasogastric tube after about 12-hour overnight fasting period. The stimulants of gastric secretion were not used. In principle, the gastric aspirate obtained was divided into two samples. One sample was tested by the original method described by YAMANAKA and KOBAYASHI (henceforth referred to as YAMANAKA test). Another sample was adjusted with 2N HCl to pH 1.5. After 3-hour standing, it was tested by YAMANAKA and KOBAYASHI method (henceforth referred to as modified YAMANAKA test). In all process, care was taken to minimize the contamination of zinc.

The procedure of YAMANAKA test is as follows:<sup>11)</sup>

1. Test sample is filtrated.
2. Ten ml of the filtrate is added to 80 ml of 20 % citrate-ammonia buffer of pH 8.3 in a test tube.
3. The mixture is shaken vigorously and afresh adjusted with ammonia to pH 8.3.

4. Ten ml of 25  $\gamma$ /ml dithizone chloroform solution is added to the mixture. The test tube is corked up and shaken vigorously.

5. It is stored in a cold dark place for 2-3 hours. The mixture is divided into two layers; upper clean layer and lower chloroform layer.

In the course of the above procedure, zinc contained in the filtrate of gastric juice is extracted into chloroform layer. Pink color in this layer indicates positive reaction. Appearance of pink stained zone at the highest portion of chloroform layer indicates doubtful reaction. In case of negative reaction, it is stained blue.

### CLINICAL OBSERVATIONS

In this study, YAMANAKA test and the modified YAMANAKA test were performed in 100 patients with gastric cancer and 98 patients with gastric diseases other than gastric cancer (87 with peptic ulcer and 11 with gastritis). In each patient, diagnosis was confirmed by laparotomy and histologic examination. The patients with marked gastric stasis were not included.

*YAMANAKA test* The result obtained by YAMANAKA test is shown in Table 1.

Table 1. Accuracy of YAMANAKA test

	No. of Cases	Positive		Doubtful		Negative	
		no.	%	no.	%	no.	%
Cancer	100	73	73.0	3	3.0	24	24.0
No Cancer	98	14	14.3	8	8.2	76	77.6

Diagnosis was correct in 73 cases (73.0 per cent) of the cancerous group and 76 cases (77.6 per cent) of the non-cancerous group. The error included 24 false negative results (24.0 per cent) and 14 false positive results (14.3 per cent). As shown in Table 2, the accuracy of diagnosis in the cancerous group was greatly affected by gastric acidity; 23 of 25 cases (92.0 per cent) gave positive results at pH less than 4.0, while its accuracy was considerably lower at higher pH levels.

Table 2. Relationship of gastric acidity to accuracy of YAMANAKA test in cancerous group

pH of Gastric Juice	No. of Cases	Positive		Doubtful and Negative	
		no.	%	no.	%
Less than 4.0	25	23	92.0	2	8.0
4.0 to 6.0	40	28	70.0	12	30.0
More than 6.0	35	22	62.9	13	37.1

*Modified YAMANAKA test* As shown in Table 3, the accuracy of positive diagnosis of gastric cancer in the modified YAMANAKA test was markedly higher than that in YAMANAKA test; positive results were obtained in 92.0 per cent of the cancerous group. Only 8.0 per cent exhibited negative findings. In contrast, the accuracy of negative diagnosis in the non-cancerous group was 77.6 per cent, being the same as that in YAMANAKA test. There were 16 false positive cases (16.3 per cent), indicating that 2 doubtful cases in YAMANAKA test gave false positive results by the addition of HCl.

Table 3. Accuracy of modified YAMANAKA test

	No. of Cases	Positive		Doubtful		Negative	
		no.	%	no.	%	no.	%
Cancer	100	92	92.0	0		8	8.0
No Cancer	98	16	16.3	6	6.1	76	77.6

### STUDIES ON THE NATURE OF THE TEST

The clinical observations that in the cancer patients with anacidity or hypoacidity the accuracy of YAMANAKA test was lower and greatly elevated by the addition of HCl indicate an intimate relationship of gastric acidity to the nature of the test. Thus, the following studies were performed to elucidate the nature of YAMANAKA test and establish the validity of the addition of HCl.

#### *Modified YAMANAKA test in the supernatant and sediment of gastric juice in gastric cancer*

After centrifugation at 1500g for 20 minutes, the supernatant solution and the sediment suspended in distilled water were tested by the modified YAMANAKA test. The result is shown in Table 4. In the gastric juice with a pH of about 3.0, the supernatant solution gave positive results in all of 3 cases, while the sediment did in only one case. This was reverse in the gastric juice with a pH of 7.0 or more; the supernatant solution gave positive results in only one of 5 cases, while the sediment did in all cases. These findings suggest that zinc originally contained in the sediment is dissolved out

Table 4. Modified YAMANAKA test in supernatant and sediment of gastric juice in cancerous group

Case	pH of Gastric Juice	Reaction	
		Supernatant	Sediment
1	3.5	+	-
2*	3.2	++	--
3	3.0	++	-
4	5.5	++	+
5	7.0	--	--
6	7.5	-	+
7*	7.2	-	+
8	7.2	-	++
9	7.5	-	-

\* A considerable amount of food debris was contained.

into the supernatant solution at lower pH levels.

*Effects of acid on gastric cancer cells in vitro*

In preparing of the suspension of gastric cancer cells, a cancerous tissue of the stomach obtained by gastrectomy was ground up in a glass mortar and percolated through gauzes. After centrifugation at 500g for 5 minutes, the sediment obtained was suspended in 50 vol. portion of 0.9 % NaCl solution (pH 5.5). Microscopic examination revealed that many cancer cells and some non-malignant cells were suspended in this solution.

*YAMANAKA test and modified YAMANAKA test* The suspension of gastric cancer cells was serially diluted and tested by YAMANAKA test without or with the addition of HCl. The former gave positive results in a dilution of 1 : 2<sup>1</sup>, while the latter did in a dilution of 1 : 2<sup>3</sup>. This indicates that the previous addition of HCl greatly enhances the sensitivity of YAMANAKA test in the suspension of gastric cancer cells.

*Morphological findings* Phase contrast microscopic examination revealed that HCl had striking effect on cancer cells as early as 1½ hours after its addition. At this time, non-malignant cells did not undergo obvious changes. As shown in Fig. 1, the cancer cells became inflated and the cell border was indistinct.

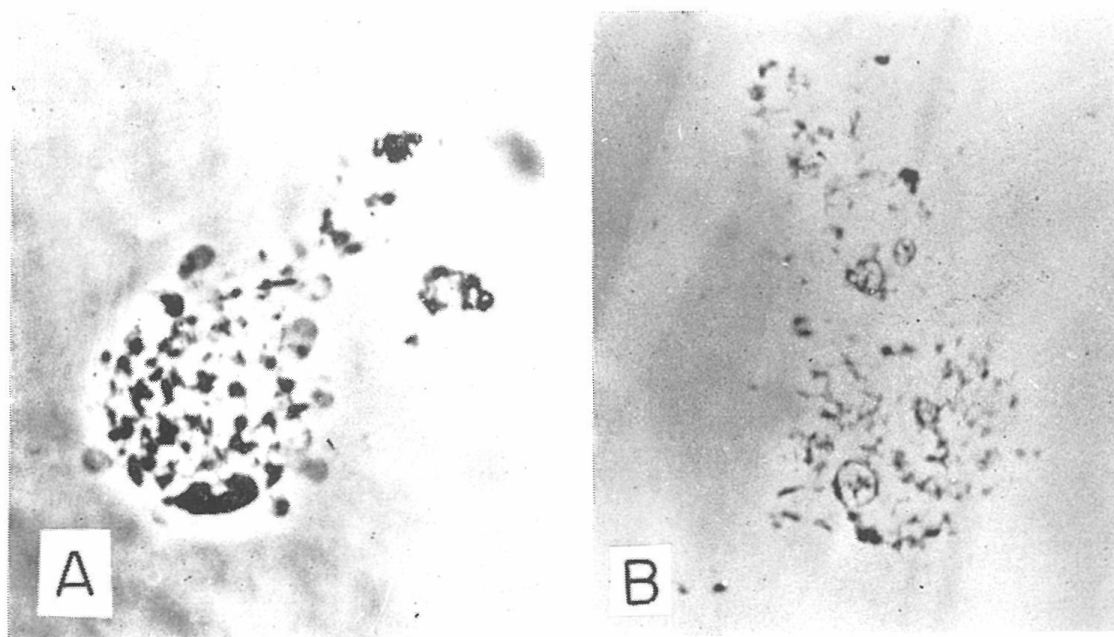


Fig. 1. Effect of HCl on gastric cancer cells. The suspension of gastric cancer cells was adjusted to pH 1.5 by adding HCl.

(A) 1½ hours after the addition of HCl. Swollen cytoplasm with an indistinct cell border.

(B) 3 hours after the addition of HCl. Disintegrated cell.

(phase contrast photomicrograph x 600)

The cytoplasm was abnormally vacuolated. Pyknosis and karyolysis were also

common. After 3 hours, the cells were on the verge of disintegration. It should be emphasized that such degenerative changes occurred more intensely and rapidly in cancer cells. In the control without the addition of HCl, the above changes were markedly delayed and cancer cells did not show distinct alterations until the 6 hour interval.

*Dialyzability* The suspension of gastric cancer cells was adjusted with HCl to pH 1.5 and dialyzed against 0.9 % NaCl solution with the same pH at 4°C for 48 hours. The solution inside the dialysis bag showed negative YAMANAKA test. In contrast, the suspension without the addition of HCl showed positive modified YAMANAKA test after dialysis. These findings indicate that zinc derived from gastric cancer cells is existent in a dialyzable form after the addition of HCl.

## DISCUSSION

YAMANAKA test is based on dithizone reaction for zinc contained in gastric juice and its procedure is very simple.<sup>11)</sup> If its reliability is satisfactorily high, the test will be an excellent method for the diagnosis of gastric cancer. In our present study, however, its accuracy of correct positive diagnosis was less than 80 per cent, contrary to our expectation. This was due to the lower accuracy in the patients with anacidity or hypoacidity; better results were obtained at the higher acidities. In view of greater accuracy of this test at lower pH levels, we have developed a modification which is characterized by the previous addition of HCl prior to the test. In this modification, the accuracy of correct positive diagnosis was increased to 92 per cent. These observations indicate the intimate relationship of gastric acidity to the accuracy of YAMANAKA test. Since the patients with gastric cancer show a tendency of hypoacidity or anacidity, the procedure of the addition of HCl seems to be essential.

The nature of YAMANAKA test is not established. There are two possibilities which can explain the origin of zinc in the test. The first possibility is that the parietal cells, which have been shown to exhibit highly positive reactions with dithizone,<sup>4)</sup> are its main origin. However, there are no reports that non-malignant cells including parietal cells are abundantly desquamated in the stomach with gastric cancer.<sup>2)</sup> On the other hand, isotopic<sup>6)</sup> and histochemical studies<sup>4)</sup> suggest that little or no zinc is excreted or secreted from the stomach. Therefore, the first possibility seems to be excluded.

Another possible origin is gastric cancer cells. It is well known that cancerous tissues are abundant in zinc.<sup>1)7)8)9)</sup> Especially, TOKUOKA (1957)<sup>8)</sup> has shown that the cancerous tissue of the stomach contains zinc about twice as great as in the parent tissue. Since the gastric juice of the patients with gastric cancer immerse the cancerous tissue and contains exfoliated cancer cells,<sup>2)</sup> it is very

likely that zinc participated in YAMANAKA test is derived from gastric cancer cells. This view is also supported by the present observation that the suspension of gastric cancer cells showed positive YAMANAKA test, especially when HCl was added prior to the test.

The process that the sensitivity of YAMANAKA test for zinc derived from gastric cancer cells is enhanced by the addition of HCl is considered to consist of two steps. The first step consists in the process that gastric cancer cells undergo disintegration by HCl and zinc-containing substances are released from the cancer cells. KUROKAWA (1956)<sup>3)</sup> has shown in his phase contrast microscopic study that the degenerative changes of gastric cancer cells are argued in highly acidic gastric juice or 1N HCl solution. This finding was ascertained by our present observation in the suspension of gastric cancer cells; the degenerative changes of gastric cancer cells produced by HCl occurred more intensely and rapidly than those of non-malignant cells. The next step is that the zinc-containing substances originating in gastric cancer cells are altered to the form, in which zinc reacts with dithizone easily. It has been shown that zinc in the biological system usually exists in the form of zinc-protein complex or zinc metalloprotein.<sup>9)10)</sup> Since the latter does not easily react with dithizone,<sup>5)</sup> it is unlikely that zinc metalloprotein *per se* in gastric cancer cells are participated in YAMANAKA test. However, if zinc metalloprotein is decomposed to the isolation of zinc by HCl, dithizone reaction will become positive. The present finding that, after the addition of HCl, zinc originating in gastric cancer cells was finally dialyzable supports the above presumption. In addition, the observation that YAMANAKA test gave positive results in the supernatant solution of gastric juice at lower pH levels, in contrast with negative results at a pH near neutrality, strongly suggests that such process can also occur *in vivo*.

From the above discussion, it may be concluded that YAMANAKA test is principally based on zinc originating in gastric cancer cells and the addition of HCl is a reasonable procedure in the standpoint that HCl accelerates the degenerative changes of gastric cancer cells and transforms the released zinc-containing substances to react with dithizone easily.

In exfoliative cytologic diagnosis, efforts should be made to obtain a satisfactory specimen of gastric content.<sup>2)</sup> In contrast, the procedure of YAMANAKA test is very simple and has no need of special consideration, since the damage of cancer cells are beneficial to the test. In addition, its accuracy of positive diagnosis of gastric cancer is greatly elevated by the addition of HCl, as shown in the present study. Thus, it may be said that our modification of YAMANAKA test can be recommended as a valuable method for the diagnosis of gastric cancer.

The evaluation of the test in early gastric cancer and the improvement to lower the incidence of false positive results will be the subjects for future

study.

### SUMMARY

1. YAMANAKA test, a test for the diagnosis of gastric cancer using dithizone reaction for zinc in gastric juice, was applied to 100 patients with gastric cancer and 98 patients with gastric diseases other than cancer. There were 73 correct positive results (73.0 per cent) and 76 correct negative results (77.6 per cent). The accuracy of positive diagnosis was lower in the cancer patients with anacidity or hypoacidity.

2. The accuracy of YAMANAKA test was greatly elevated by the previous addition of HCl prior to the test. In this modification, correct positive results were obtained in 92 of 100 (92.0 per cent) patients with gastric cancer. However, the accuracy of correct negative diagnosis was not elevated.

3. The present model experiments strongly suggest that YAMANAKA test is principally based on zinc originating in gastric cancer cells and that HCl accelerates the degenerative changes of cancer cells and transforms the released zinc-containing substances to react with dithizone easily. Thus the addition of HCl is a reasonable procedure.

4. In view of its simplicity and reliability, our modification of YAMANAKA test can be recommended as a valuable method of diagnosis of gastric cancer.

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### REFERENCES

- 1) Cristol, P.: *Bull. Soc. Chim. Biol. Paris*, **5**: 23, 1923.
- 2) Graham, R. M.: *Advances in Internal Medicine*, **6**: 59, 1954.
- 3) Kurokawa, T. and Saito, T.: *Saishin-Igaku*, **11**: 1683, 1956.
- 4) Mager, M., McNary, W. F., Jr. and Lionetti, F.: *J. Histochem. Cytochem.*, **1**: 493, 1953.
- 5) Okamoto, K., Ueda, M., Maeda, R. and Mizutani, A.: *Microscopic Histochemistry*, **3. ed.**, Igaku Shoin, Tokyo-Osaka, 1965.
- 6) Spencer, H., Rosoff, B., Feldstein, A., Cohn, S. H. and Gusmano, E.: *Radiation Res.*, **24**: 432, 1965.
- 7) Sugai, M.: *J. Kyoto Pref. Med. Univ.*, **21**: 1197, 1939.
- 8) Tokuoka, S.: *Saishin-Igaku*, **12**: 771, 1957.
- 9) Vallee, B. L.: *Physiol. Rev.*, **39**: 443, 1959.
- 10) Vallee, B. L.: *Advances in protein chemistry*, **10**: 317, 1955.
- 11) Yamanaka, S. and Kobayashi, H.: *Internal Medicine and Pediatrics* **17**: 777, 1962.