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SERUM LACTIC DEHYDROGENASE ISOZYME IN MALIGNANT DISEASES FOR EVALUATION OF TREATMENT

BY

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ABSTRACT

Recently the relationship between malignant diseases and serum lactic dehydrogenase (SLDH) is being studied with increasing intensity.

The purpose of the present study was made to determine the clinical usefulness of SLDH-III activity in the diagnosis of malignant diseases and was made not only to detect the clinical usefulness of SLDH-III activity in the evaluation of the chemotherapeutic effect on cancer but also to detect the clinical value of SLDH-III activity during the follow-up period after the removal of the tumor.

The present report is based on studies on one hundred and twenty-four patients with carcinoma of various origins and twenty-six patients with benign diseases in whom the SLDH activity and isozymes were measured.

The following findings were obtained.

SLDH activity increased above the normal range in about seventy per cent of ninety-eight malignant diseases.

SLDH-III activity had an intimate correlation with the clinical therapeutic response.

During the follow-up period, the SLDH-III activity apparently decreased in three weeks after the removal of the tumor and increased steadily above the normal when the tumor recurred. These changes were named as the U pattern of SLDH-III.

The measurement of the SLDH-III activity is considered to be useful in the diagnosis of malignant diseases and has a considerable value in the evaluation of the chemotherapeutic effect on cancer and in the early detection on the recurrence of the tumor.

INTRODUCTION

Since the original observation by Hill and Levi[1] in 1954 that the SLDH activity increases above the normal range in ninety-five per cent of the patients with malignant diseases, many similar reports[2–16] have been reported.

Vessel and Bearn[17] first reported in 1954 that SLDH was separated

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into five fractions of isozymes by electrophoresis.

The clinical significance of the SLDH isozymes in the diagnosis of various diseases including heart diseases, liver diseases and leukemia were recognized by Wroblewski[18] et al. and other investigators.

Regarding the clinical application of the SLDH isozymes in the diagnosis of malignant diseases, several reports were made by Wieme et al.[19], Wroblewski et al.[20] and others[21–26].

Utsunomiya et al.[27], Hamaguchi et al.[28] and Yoshida[13] reported that SLDH-III has a more diagnostic significance than the total SLDH activity in patients with stomach cancer.

The present study was made to extend this result to a more routine use in the diagnosis of malignant diseases and in the evaluation of the therapeutic effect as well as in the early detection of the recurrence of the diseases.

Methods

The total SLDH activity was measured by the spectrophotmetric method described by Hill[29].

For the isozyme separation, the agar-gel electrophoresis method originally described by Wieme[30] and Shibata[31] was used.

I) Preparation of agar gel matrix

1) Agar solution: Agar (Special Agar Noble, Difco) was dissolved at the rate of 0.8% in veronal buffer (pH 8.6).

2) Agar gel slide: 5.5 ml of the solution was placed on a slide glass to make a gel film of a thickness of 3 mm. After being left overnight, two small transverse linear grooves of 5 mm in length were made on the agar plate at the position 5 mm from the center line towards the cathode.

II) Electrophoresis

1) Sample: 0.006 ml to 0.008 ml of serum was placed into the grooves using a special micropipette.

2) Condition: Electrophoresis was carried out at 50 mA for four slides for 90 minutes.

III) Staining

1) Preparation of stock solution

Stock sol. I: 50% sodium lactate sol. 1.4 ml
Sodium cyanide 50.0 mg
Phosphate buffer (pH 7.2, 0.1 M) 90.0 ml
Nitroblue tetrazolium 25.0 mg

Stock sol. II: Phenazine methosulphate 10.0 mg
Phosphate buffer 10.0 ml

2) Staining sol. (for four slides)
Stock sol. I: 21.6 ml
Stock sol. II: 0.6 ml
DPN: 24 mg
NBT: 6.0 mg
Staining: About 5 ml or more of the staining solution were poured on each slide in the staining box and incubated at 37°C for three hours.

IV) Fixation

Each slide was placed in the acetic-alcohol solution for forty minutes.
Acetic-alcohol sol.: Ethanol 70 ml
Acetic acid 5 ml
Dist water 25 ml

V) Decolorization

Rinsed in tap water for over six hours.

VI) Drying

The gel slide was covered with filter paper and kept overnight.

The isozyme fractions thus separated were designated as SLDH-I, SLDH-II, SLDH-III, SLDH-IV and SLDH-V from the anode toward the cathode.

The percentage of each isozyme was calculated by the densitometer.

The activity of each isozyme was calculated from the total SLDH activity.

In the present study, the clinical usefulness of the activity of SLDH isozymes was evaluated because of the observation by Yoshida[13] and Utsunomiya et al.[27] that the SLDH isozyme expressed as the activity is considered to have a more intimate correlation with the progression of the disease than that expressed by the percentage.

VII) Determination of normal range

The activity over 300 Hill unit was determined as the abnormal increase.

The normal range of SLDH isozymes which were determined by Yoshida[13] was adapted for this study as shown in Table 5.

Materials

One hundred and twenty-four malignant diseases, including twenty-six recurrent cases, and twenty-six benign diseases as shown in Table 1~3 were

Table 1. Material

	Cases
Malignant diseases	124
Benign diseases	26
Total	150

Table 2. Recurrent malignant diseases

	Cases
Carcinoma of the stomach	14
Carcinoma of the large intestine	4
Carcinoma of the bile duct	1
Carcinoma of the breast	3
Lympho-sarcoma	2
Fibro-sarcoma	1
Carcinoma of the ovary	1
Total	26

Table 3. Benign diseases

	Cases
Gastric ulcer	13
Polyp of the stomach	2
Duodenal ulcer	1
Cholelithiasis	4
Polyp of the intestine	1
Parotis tumor	1
Haemangioma	1
Subdural haematoma	1
Others	2
Total	26

Table 4. Malignant diseases

	Operable cases	Inoperable cases
Carcinoma of the esophagus	1	2
Carcinoma of the stomach	36	14
Carcinoma of the large intestine	6	5
Carcinoma of the bile duct	1	10
Carcinoma of the lung	0	3
Carcinoma of the breast	6	5
Others	4	5
Total	54	44

Table 5. Normal range of activities of SLDH and isozymes

SLDH activity

Mean	231
Range	120–306

Proportion of SLDH isozymes

	I	II	III	IV	V
Mean	44.8	47.6	6.4	0.9	0.4
Range	31–59	34–61	2–10	0–3	0–2

Activity of SLDH isozymes

Mean	121.5	113	20.5	4.5	3.9
Range	47–150	50–145	0–30	0–9	0–5

submitted for the study.

Fifty-four cases out of ninety-eight malignant diseases were resectable, the rest being terminal cases as shown in Table 4.

Venous blood was obtained periodically from these patients. Sera were separated carefully to avoid hemolysis.

Results

(I) Comparison of the activities of SLDH and isozymes between malignant and benign diseases. (Table 6 and Fig. 1).

While the SLDH activity increased above the normal range in three out of twenty-six benign diseases (11%), in malignant diseases it increased above the normal in forty-nine out of ninety-eight (50%).

Among the malignant diseases, the SLDH activity increased abnormally in twenty-three out of fifty-four operable cases (43%) and above the normal in twenty-six out of forty-four advanced cases (59%).

Table 6. Comparison of activities of SLDH and SLDH-III which increased above the normal range between malignant and benign diseases

	Benign disease	Malignant diseases	
		Operable	Inoperable
SLDH	3/26 (12%)	23/54 (43%)	26/44 (59%)
SLDH-III	4/26 (15%)	30/54 (56%)	35/44 (80%)

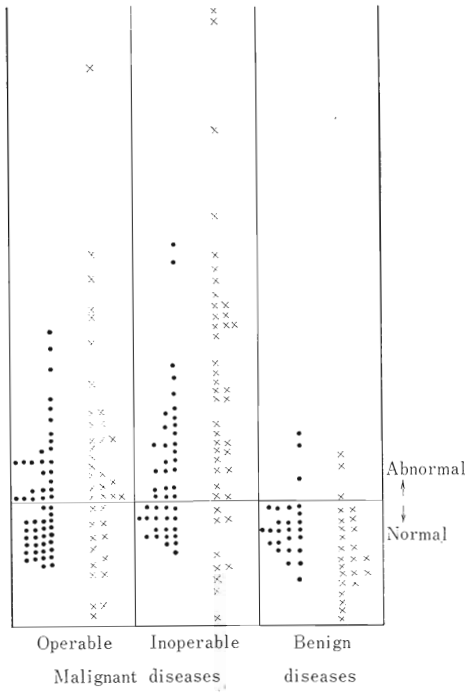


Fig. 1. Distribution of activities of SLDH and SLDH-III

While the SLDH-III activity increased above the normal in four out of twenty-six benign diseases (15%), in the malignant diseases it increased in sixty-five out of ninety-eight (67%).

The SLDH-III activity increased abnormally in thirty out of fifty-four operable cases (56%) and above the normal in thirty-five out of forty-four advanced cases (80%).

In the benign diseases, the activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V which increased to abnormal levels were less than seventeen per cent.

In the malignant diseases, the activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V which increased to abnormal levels were also less than twenty-four per cent.

(II) Comparison of the activities of SLDH and isozymes in malignant diseases of various organs.

a) In the carcinoma of the stomach, the SLDH activity increased above the normal in thirty-four out of sixty-four cases (53%) and above

Table 7. Comparison of activities of SLDH and SLDH–III which increased above normal range in carcinoma of stomach

	Operable	Inoperable
SLDH	16/36 (44%)	9/14 (64%)
SLDH–III	20/36 (56%)	11/14 (79%)

the normal in sixteen out of thirty-six operable cases (44%) and in nine out of fourteen advanced cases (64%).

On the other hand, the SLDH-III activity increased above the normal in forty-three out of sixty-four cases (67%) and above the normal in twenty out of thirty-six operable cases (56%) and in eleven out of fourteen advanced cases (79%) as shown in Table 7 and Fig. 2.

The activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V which increased to abnormal levels were less than seventeen per cent.

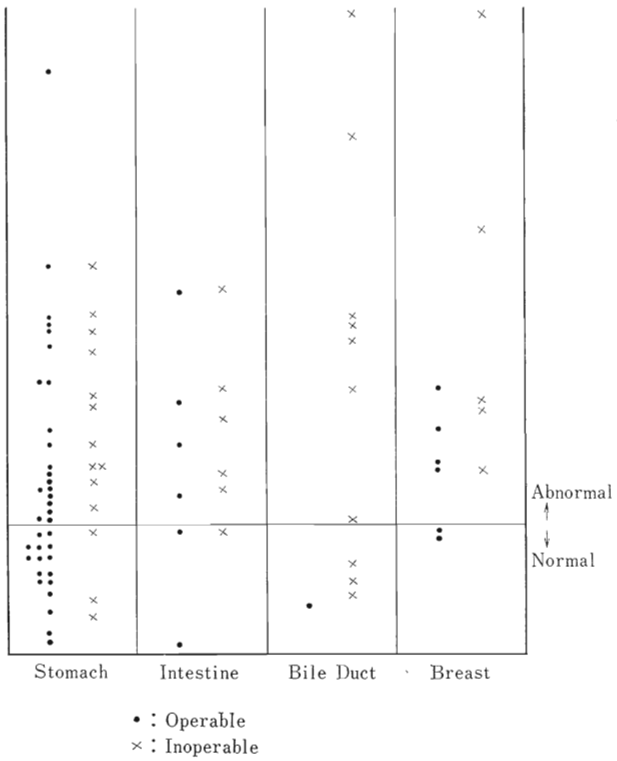


Fig. 2. Distribution of SLDH-III activity in carcinoma of various organs.

Table 8. Comparison of activities of SLDH and SLDH–III which increased above normal range in carcinoma of large intestine

	Operable	Inoperable
SLDH	3/6 (50%)	3/6 (50%)
SLDH–III	4/6 (67%)	5/6 (83%)

Table 9. Comparison of activities of SLDH and SLDH–III which increased above normal range in carcinoma of bile duct

	Operable	Inoperable
SLDH	0/1	6/10 (60%)
SLDH–III	0/1	7/10 (70%)

b) In the carcinoma of the large intestine, the SLDH activity increased abnormally in eight out of sixteen cases (50%) and abnormally in fifty per cent of both operable and advanced cases.

On the other hand, the SLDH-III activity increased abnormally in twelve out of sixteen cases (75%) and abnormally in four out of six operable cases (67%) and in five out of six advanced cases (83%) as shown in Table 8 and Fig. 2.

The activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V which increased to abnormal levels were less than seventeen per cent.

c) In the carcinoma of the bile duct, ten out of twelve cases were inoperable.

The SLDH activity increased abnormally in six out of these ten advanced cases (60%).

The SLDH-III activity increased abnormally in seven out of ten cases (70%) as shown in Table 9 and Fig. 2.

The activities of SLDH-I, SLDH-II, and SLDH-IV which increased to abnormal levels were less than twenty-seven per cent. The SLDH-V activity increased abnormally in forty-five per cent.

d) In the carcinoma of the breast, the SLDH activity increased abnormally in eight out of fourteen cases (57%) and abnormally in two out of six operable cases (33%) and in four out of five advanced cases (80%).

On the other hand, the SLDH-III activity increased abnormally in twelve out of fourteen cases (86%) and abnormally in four out of six operable cases (67%) and in five out of five advanced cases (100%) as shown in Table 10 and Fig. 2.

Table 10. Comparison of activities of SLDH and SLDH–III which increased above normal range in carcinoma of breast

	Operable	Inoperable
SLDH	2/6 (33%)	4/5 (80%)
SLDH–III	4/6 (67%)	5/5 (100%)

The activities of SLDH-I, SLDH-II and SLDH-V which increased to abnormal levels were less than twenty per cent.

The SLDH-IV activity increased to abnormal levels in forty per cent.

e) In the carcinoma of the lung, which were all in the advanced stage, the SLDH activity increased abnormally in one out of three cases (33%) while the SLDH-III activity increased abnormally in two out of three cases (67%) as shown in Fig. 3.

The activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V were all within the normal range.

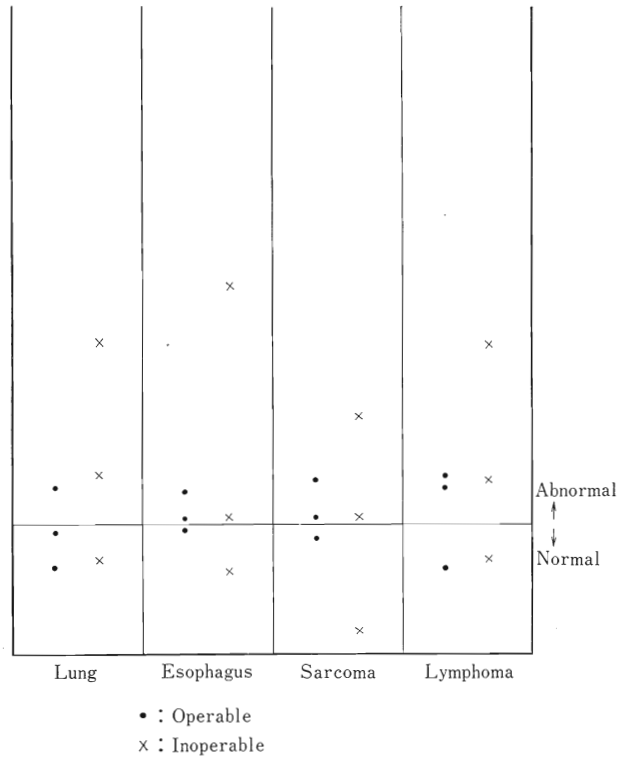


Fig. 3. Distribution of SLDH-III activity in carcinoma of various organs.

f) In the carcinoma of the esophagus, one was operable and two were advanced.

The activities of SLDH and SLDH-III were of normal level in an operable case, but in the advanced cases the SLDH and SLDH-III activity increased to abnormal levels as shown in Fig. 3.

The activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V were all within the normal range.

g) The activities of SLDH and SLDH-III increased abnormally in two out of three sarcoma cases (one was fibrosarcoma of the breast and two were lymphosarcoma) as shown in Fig. 3.

The activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V were all within the normal range.

h) In the operable case of malignant lymphoma, the activities of SLDH and SLDH-III were within the normal range while the activities of SLDH and SLDH-III increased abnormally in two advanced cases as shown in Fig. 3.

The activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V were all within the normal range.

(III) Changes in activities of SLDH and isozymes beyond the difference of ±20% in advanced cases treated by cancer chemotherapy.

For the determination of significant discrepancy of the enzyme activity in the following study, three kinds of variation ranges such as ±10%, ±15% and ±20% were compared with each other, respectively.

±20% was found to provide the most significant correlation between the enzyme activity and the symptoms.

Thirty-seven advanced malignant diseases of various origins as shown in Table 11 were treated by different agents including Tespamin, Endoxan, Toyomycin, Mitomycin C and 5-fluorouracil.

Generally these agents were administrated intravenously but ocassion-

Table 11. Inoperable malignant diseases treated by cancer chemotherapy

	Cases
Carcinoma of the stomach	21
Carcinoma of the large intestine	5
Carcinoma of the bile duct	3
Carcinoma of the lung	2
Carcinoma of the breast	3
Lympho-sarcoma	1
Fibro-sarcoma of the breast	1
Mediastinal tumor	1
Total	37

Table 12. Changes of activities of SLDH and isozymes beyond the difference of ± 20 per cent by cancer chemotherapy (12 cases responding objectively)

	Increased cases	Decreased cases	Unchanged cases
Total SLDH	2/12 (17%)	3/12 (25%)	7/12 (58%)
SLDH–I	3/12 (25%)	2/12 (17%)	7/12 (58%)
SLDH–II	5/12 (41%)	3/12 (25%)	4/12 (33%)
SLDH–III	0/12	10/12 (83%)	2/12 (17%)
SLDH–IV	3/12 (25%)	5/12 (41%)	4/12 (33%)
SLDH–V	3/12 (25%)	3/12 (25%)	6/12 (50%)

ally directly into the artery or intraperitoneally or intrapleurally.

These cases were divided into the following three groups based on the responsiveness to the chemotherapy; 12 cases responded objectively, 13 cases became aggravated objectively and 12 cases were stationary.

In the group responding objectively, among the total SLDH and the isozymes the SLDH-III showed a significant decrease in the activity in the largest number of cases or 83%, while it showed a significant increase in none of the cases as shown in Table 12 and Fig. 4 and 5.

In the group aggravated objectively, among the total SLDH and the isozymes the SLDH-III showed a significant increase in the activity in the largest number of cases or 85%, while it showed a significant decrease in none of the cases as shown in Table 13 and Fig. 4 and 6.

The symptoms were stationary in twelve cases.

The SLDH-III activity decreased in five cases (41%), unchanged in

Table 13. Changes of activities of SLDH and isozymes beyond the difference of ± 20 per cent by cancer chemotherapy (13 cases with symptoms aggravated)

	Increased cases	Decreased cases	Unchanged cases
Total SLDH	8/13 (62%)	1/13 (7%)	4/13 (31%)
SLDH–I	5/13 (37%)	2/13 (15%)	6/13 (48%)
SLDH–II	5/13 (37%)	3/13 (23%)	5/13 (37%)
SLDH–III	11/13 (85%)	0/13	2/13 (15%)
SLDH–IV	8/13 (62%)	4/13 (31%)	1/13 (7%)
SLDH–IV	7/13 (53%)	3/13 (23%)	3/13 (23%)

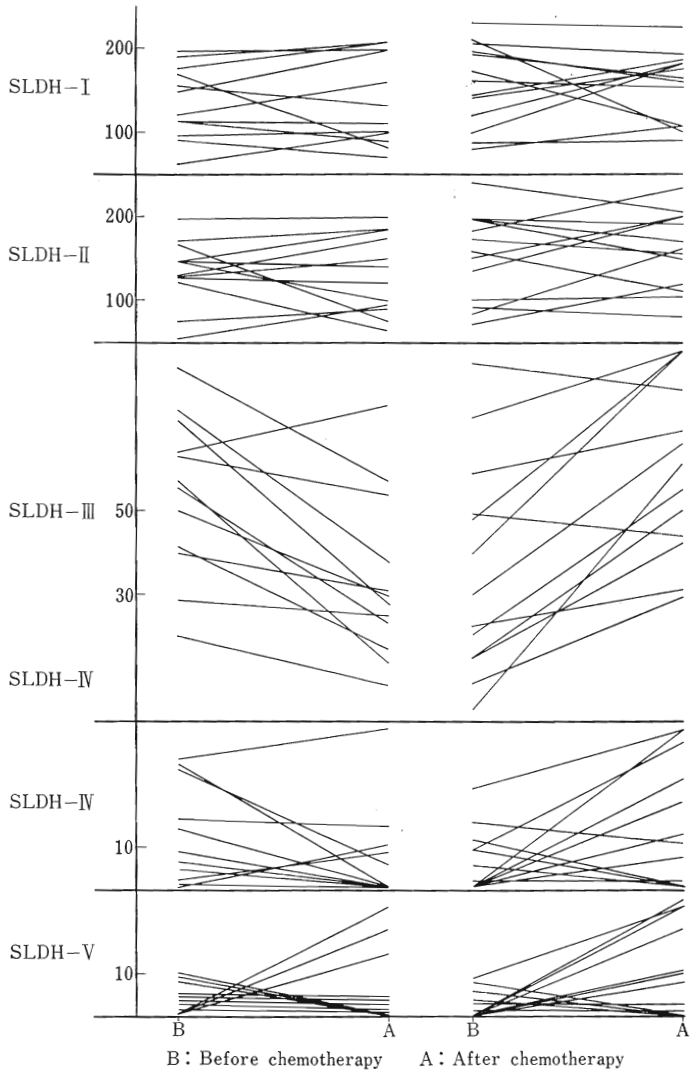


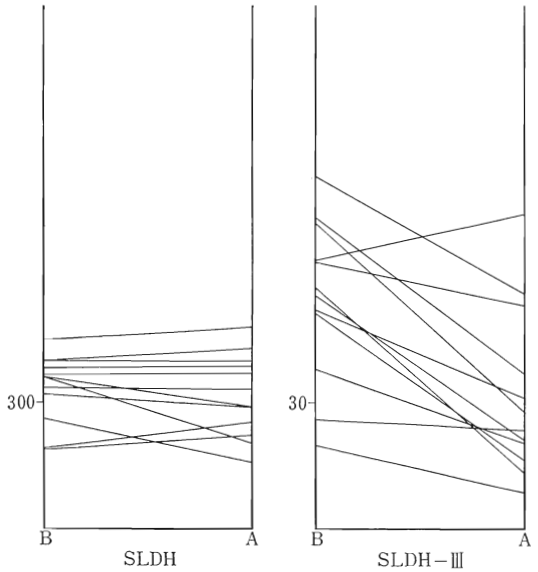
Fig. 4. Changes of activities of SLDH isozymes before and after cancer chemotherapy.

three (25%) and increased in one (9%).

The activity of SLDH, SLDH-I, SLDH-II, SLDH-IV and SLDH-V did not show any significant changes.

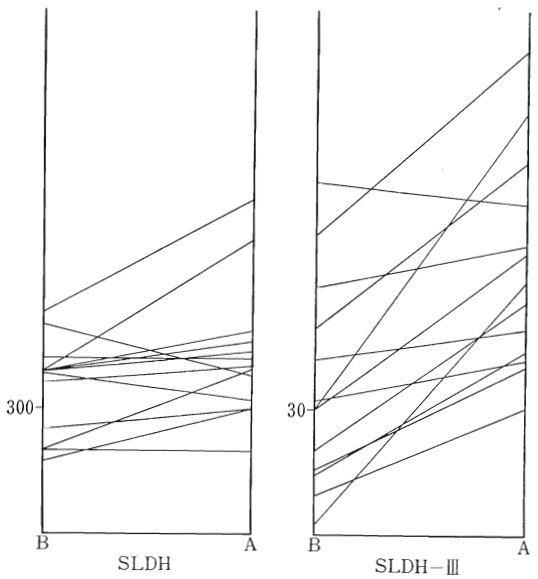
The above findings indicated that the SLDH-III activity has a more intimate correlation with the clinical response than with the activity of SLDH and other isozymes.

(IV) Changes in the activities of SLDH and SLDH-III in sixteen



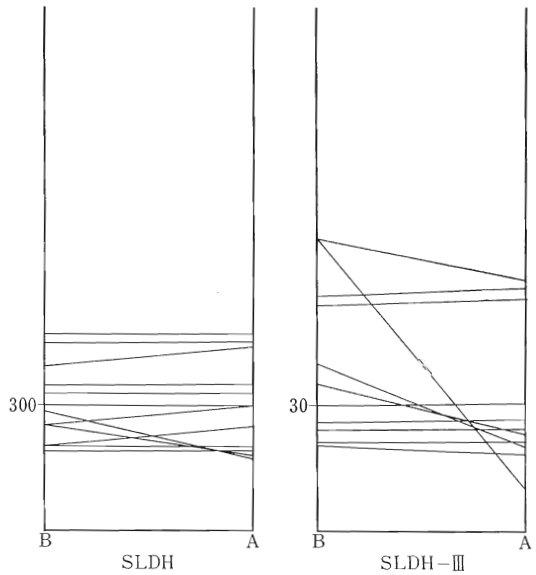
B : Before treatment
A : After treatment

Fig. 5. Changes of activities of SLDH and SLDH-III by cancer chemotherapy. (12 cases responding objectively)



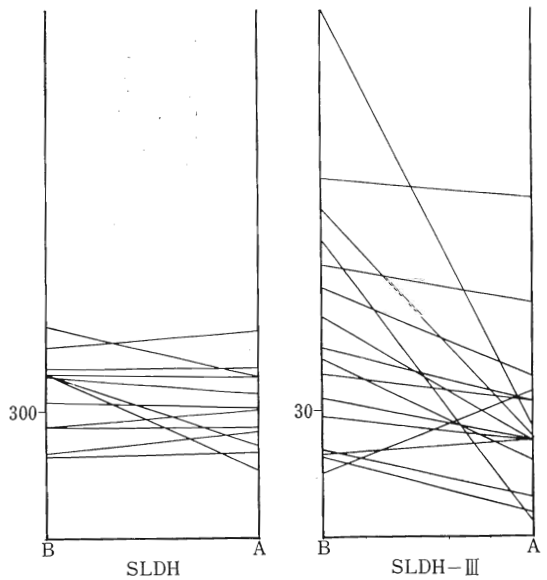
B : Before treatment
A : After treatment

Fig. 6. Changes of activities of SLDH and SLDH-III by cancer chemotherapy. (13 cases with symptoms aggravated)



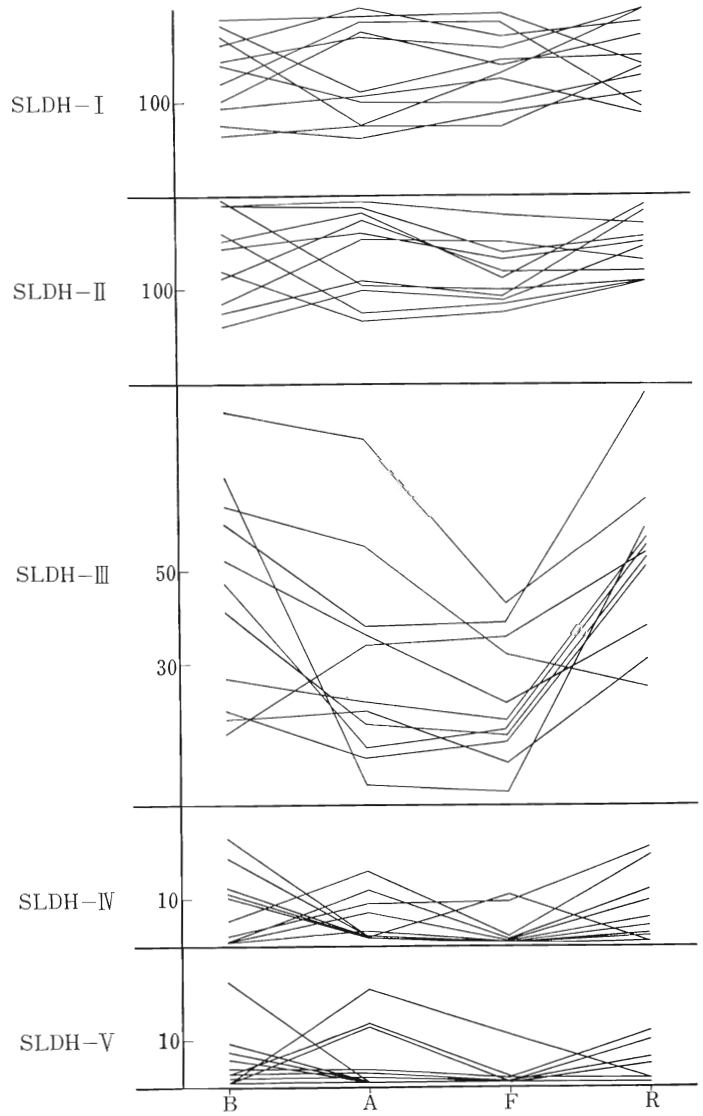
B : Before treatment
A : After treatment

Fig. 7. Changes of activities of SLDH and SLDH-III by cancer chemotherapy. (12 cases being stationary)



B : Before operation
A : After operation

Fig. 8. Changes of activities of SLDH and SLDH-III in malignant diseases before and after operation.



B : Before operation F : Follow-up period
A : After operation R : Recurrence

Fig. 9. Changes of activities of SLDH isozymes during follow-up period.

malignant diseases (mostly gastric cancer) before and three weeks after the resectional operation.

The SLDH-III activity apparently decreased in eighty-seven per cent of these sixteen cases as shown in Fig. 8.

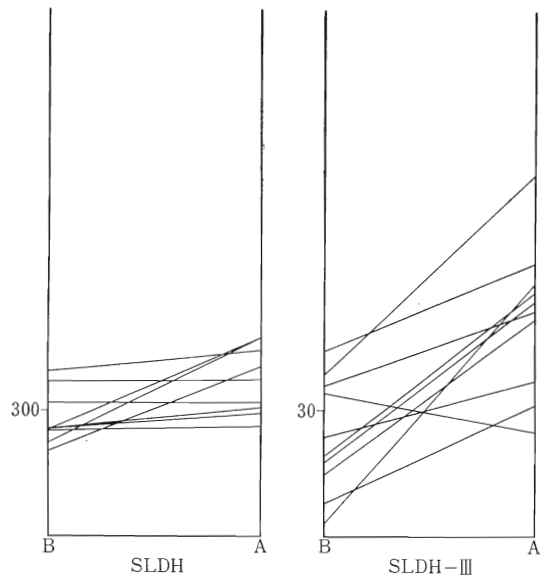
Table 14. Changes of activities of SLDH and isozymes beyond the difference of ± 20 per cent after operation
(Follow-up of 11 cases)

	Increased cases	Decreased cases	Unchanged cases
Total SLDH	2/11 (18%)	5/11 (45%)	4/11 (36%)
SLDH–I	3/11 (27%)	3/11 (27%)	5/11 (45%)
SLDH–II	3/11 (27%)	3/11 (27%)	5/11 (45%)
SLDH–III	1/11 (9%)	9/11 (82%)	1/11 (9%)
SLDH–IV	4/11 (36%)	6/11 (55%)	1/11 (9%)
SLDH–V	3/11 (27%)	4/11 (36%)	4/11 (36%)

Eleven cases of these sixteen cases were followed until the tumor recurred.

(V) During the follow-up period from six months up to two years after the resectional operation the SLDH activity and isozymes and other routine blood chemistry were periodically examined in these eleven cases.

Tables 14 and 15 and Fig. 9 and 10 show changes in the activities of



B : Before recurrence
A : After recurrence

Fig. 10. Changes of activities of SLDH and SLDH-III in malignant diseases during follow-up period.

Table 15. Changes of activities of SLDH and isozymes beyond the difference of ± 20 per cent when tumor recurrud (Follow-up of 11 cases)

	Increased cases	Decreasedc ases	Unchanged cases
Total SLDH	4/11 (36%)	0/11	7/11 (64%)
SLDH–I	2/11 (18%)	3/11 (27%)	6/11 (55%)
SLDH–II	3/11 (27%)	1/11 (9%)	7/11 (64%)
SLDH–III	10/11 (91%)	1/11 (9%)	0/11
SLDH–IV	6/11 (55%)	1/11 (9%)	4/11 (36%)
SLDH–V	4/11 (36%)	0/11	7/11 (64%)

SLDH and isozymes during the follow-up period.

After the operation, the following findings were obtained as shown in Table 14 and Fig. 9.

Among the total SLDH and the isozymes the SLDH-III showed a significant decrease in the activity in the largest number of cases or 82%, while it showed a significant increase in the activity in only one case (9%).

When the tumor recurred, the following findings were obtained as shown in Table 15 and Fig. 10.

Among the total SLDH and the isozymes the SLDH-III showed a significant increase in the activity in the largest number of cases or 91%, while it showed a significant decrease in the activity in only one case (9%).

The changes in the SLDH-III activity during the follow-up period such as the decrease after the operation and the increase when the tumor recurred were named as the U pattern.

The U pattern of the SLDH-III activity was observed in eight out of eleven cases (73%).

On the other hand, the U pattern of the other isozymes were observed only in less than twenty-seven per cent of eleven cases.

It is concluded that the U pattern of the SLDH-III during the follow-up period was considered as typically representing the progression of the disease and the measurement of the SLDH-III activity is considered to be useful in the early detection of the recurrence after the operative treatment.

Discussion

The original observation of Hill and Levi[1] in 1954 that the sera from many individuals with malignant diseases exhibited an abnormal elevation

of LDH in ninety-five per cent has been confirmed by many authors[2–16].

Most of the other reports, however, indicated that the abnormal elevation of total SLDH in the malignant diseases was found in about fifty per cent, agreeing with the fifty-three per cent obtained by the author.

Since the introduction of the biochemical method of diagnosis of SLDH isozyme by Vessel and Bearn[17], the study of SLDH turns out to be a qualitative analysis.

Vessel and Bearn indicated that the proportion of SLDH-III significantly increased in the patients with leukemia.

The clinical significance of SLDH isozyme in the heart disease, liver disease and leukemia was also reported by Wroblewski et al.[18,20] and Wieme et al.[19]

Plageman[21] reported on the evidence that the sera of tumor-bearing animal and tumor tissue contain increased the proportion of LDH-III and IV and suggested that the LDH isozyme pattern of the tumor tissue is reflected in the sera.

Denis et al.[32], Zondag[33], Abe et al.[34], Miyamoto[35] and Oohata[36] reported on a similar observation in the patients of neoplastic diseases.

Asano et al.[37–39] also reported that the elevation of the proportion of SLDH-III was a significant change in the neoplastic disease.

On the other hand, there is an opinion by Yamamura et al.[40] that the SLDH isozyme did not show any significant changes in the patients with lung cancer.

Utsunomiya et al.[27] and Yoshida[13] confirmed that the SLDH-III activity had a significant relationship with the presence of cancer in the gastric cancer patients and concluded that the SLDH-III activity was a more useful diagnostic tool than the total SLDH activity, and the above opinion agrees with the results obtained by the authors study.

West et al.[6], Brindley et al.[41], Sameshima et al.[42] and Yoshida[13] reported on the observation that the SLDH activity had an intimate correlation with the cancer chemotherapeutic response.

But with regard to the SLDH-III, no evidence was reported on these aspects.

According to the authors results, the SLDH-III activity was demonstrated to have an intimate correlation with the clinical therapeutic response.

With regard to the SLDH isozymes, especially the SLDH-III activity during the follow-up period, no reports can be found except the opinion by Yoshida that the SLDH and SLDH-III activity showed a significant change at the time of the recurrence in two gastric cancer patients.

The author confirmed sufficiently the above findings in eleven neoplastic diseases which was followed from six months up to two years after the operation and concluded that the SLDH-III activity showed apparent changes called the U pattern during the follow-up period, and the measurement of the SLDH-III activity is considered to be useful in the early detection of the recurrence.

Conclusion

The SLDH isozymes in the neoplastic diseases were studied and the findings described below were obtained.

(1) The SLDH activity increased above the normal in fifty per cent of the neoplastic diseases. It increased above the normal in fifty-nine per cent of the advanced cases, while it increased above the normal in only forty-three per cent of the resectable cases.

(2) While the activities of SLDH-I, SLDH-II, SLDH-IV and SLDH-V increased to abnormal levels in less than twenty-four per cent of the neoplastic diseases, the SLDH-III activity increased abnormally in sixty-seven per cent of the neoplastic diseases and in eighty per cent of the advanced cases.

(3) The SLDH-III activity had an intimate correlation with the clinical therapeutic response.

(4) The SLDH-III activity decreased apparently after the operation in eighty-seven per cent of sixteen patients with resectable neoplastic diseases.

(5) The SLDH-III activity increases steadily above the normal when the tumor recurred.

(6) The changes in SLDH-III during the follow-up period called the U pattern was considered as typically representing progression of the disease.

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