Original Article

Improving Serum Sialic Acid Determination with Cetyltrimethylammonium Bromide and Clinical Significance of Sialic Acid Levels in Colorectal Cancer

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Abstract: Background and Objectives: Sialic acid levels may be useful for evaluating important biological reactions, such as inflammation. Thus, the precise determination of its levels in clinical specimens should provide significant clinical information. We herein improved the sensitivity of determining sialic acid levels by preprocessing samples with cetavlon.

Materials and methods: Serum samples from healthy volunteers (n=20), rectal cancer patients (n=5), and colon cancer patients (n=13) were preprocessed with cetavlon. Samples were then hydrolyzed and derivatized for HPLC fluorescent analysis.

Results: The linearity of our determination method was confirmed using a standard curve. The detection and quantification limits of our method were 3.5 and 10.5 to 10.7 pmol/ml, respectively. The mean recovery rate was 81.5 to 97.2%. Significant differences were observed in sialic acid levels between cetavlon-treated samples obtained from healthy subjects and colon cancer patients.

Discussion: Our analytical method provided reliable data regarding sialic acid levels in biological specimens. Significant differences were observed in sialic acid levels between cetavlon-treated samples obtained from healthy subjects and cancer patients. The odds ratios for this method suggested that pre-processing serum samples with cetavlon provided a stronger relationship between sialic acid levels and the development of colorectal cancer.

Key Words: HPLC, Sialic acid, Fluorescence labeling, DMB, Cetavlon.

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Introduction

Sialic acids, neuraminic acid derivatives, are widely distributed in animal tissues as the polysaccharide chain component of glycoproteins and glycolipids. These acids are mostly associated with acute phase reactants in serum, and their concentrations are increased in inflammatory diseases, such as infections and rheumatoid arthritis. Therefore, serum sialic acid levels are a useful marker of inflammation.

Matsuzaki et al. measured serum sialic acid levels in cirrhosis patients and liver cancer patients, and found a positive correlation between serum sialic acid levels and protein synthesis in the liver. Serum sialic acid levels were found to be increased in liver cancer patients with normal levels of alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) tumor markers[1]. Reshad suggested that sialic acid may be a marker for secondary inflammation to cancer, as well as a cancer marker[2].

Sialic acids are present in serum in both free and bound forms with sugar chains in glycoproteins. Previous studies reported that free sialic acid and protein-bound sialic acid were associated with different diseases. Protein-bound sialic acid and α 1-acid glycoprotein serum levels were reported to be higher in laryngeal cancer patients than in healthy subjects. Furthermore, protein-bound sialic acid serum levels positively correlated with cancer stages[3]. Protein-bound sialic acid serum levels were also found to be higher in colorectal cancer patients than in healthy subjects[4], suggesting that the sialic acid-to-total serum protein ratio could be a useful marker for colorectal cancer[5]. Previous studies demonstrated that the ingestion of alcohol increased free sialic acid serum levels, whereas others argued that free sialic acid serum levels were not as useful as total sialic acid levels as a marker for colorectal cancer[6].

Sialic acid levels are a useful parameter for inflammation, and can be used to evaluate other important biological reactions. Thus, the precise determination of sialic acid levels in clinical specimens may provide important clinical information. Four main methods are currently used to determine serum sialic acid levels: a colorimetric method[7], enzymatic method[8], fluorescence method[9], and high performance liquid chromatography (HPLC)[10][11]. Colorimetric and enzymatic methods are insensitive and less accurate due to interference with substances in specimens. The fluorescence method is sensitive ($\approx 10^{-8}$ M) and can measure glycoprotein-bound sialic acid without hydrolyzation[9]. Hara et al. determined serum sialic acid levels in humans and experimental animals using HPLC with a fluorescence detector. The detection limit was a few tens of fmol and they only reported N-acetylneuraminic acid in human serum[11].

In the present study, we measured N-acetylneuraminic acid levels in human serum samples by a fluorescence method. The fluorescence labeling method was based on that described by Hara et al. Okazaki et al. obtained reliable data using an XBridge C18 column in their HPLC determination of allose, a rare monosaccharide[12]; therefore, we employed the XBridge C18 column to determine DMB-labeled N-acetylneuraminic acid levels. Various determination conditions were examined, and the analytical method was evaluated according to the International Conference on Harmonization (ICH) guidelines. Many N-linked sugar chains exist in serum, and when measurements are performed in serum samples without any pre-processing, N-acetylneuraminic acid unrelated to any disease condition may influence the level measured. Pre-processing samples to selectively concentrate disease-associated N-acetylneuraminic acid may improve the utility of N-acetylneuraminic acid in diagnosing

colorectal cancer.

Quarternary ammonium salts, such as cetylpyridinium chloride and cetyltrimethylammonium bromide, form complexes and precipitates with acidic complex carbohydrates, such as proteoglycans, glycosaminoglaycans, and mucin-type glycoproteins[13]. Cetyltrimethylammonium bromide (cetavlon) has been used to screen mucopolysaccharidosis[14] and the extraction of DNA[15].

Therefore, we herein investigated the extraction of N-acetylneuraminic acid-containing glycoproteins and quantitation of N-acetylneuraminic acid using cetavlon.

Materials and Methods

Reagents

1, 2-Diamino-4, 5-methylenedioxybenzene dihydrochloride (DMB) was purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). N-acetylneuraminic acid (biochemistry grade), 2-mercaptoethanol (biochemistry grade), sodium hydrosulfite (biochemistry grade), cetavlon (cetyltrimethylammonium bromide), methanol (HPLC grade), acetonitrile (HPLC grade), SDS-polyacrylamide gel electrophoresis reagents (for electrophoresis), and other reagents (special grade) were all obtained from Wako Pure Chemical Industries, Inc (Osaka, Japan). Sambucus sieboldiana-biotin was purchased from J-OIL MILLS, Inc. (Tokyo, Japan). Streptavidin-HRP was purchased from Thermo Fisher Scientific K.K. (Yokohama, Japan).

Instruments and HPLC conditions

HPLC instruments consisted of a Hitachi HPLC pump (L-6200), Autosampler (L-7200), Column oven (L-7300), Fluorescence Detector (L-7480), and Interface (D-7000) (Hitachi High-Technologies; Tokyo Japan). Excitation and fluorescence wavelengths were 370 and 450 nm, respectively. XBridge C18 (5 μm 3.0 × 250 mm; Waters; Milford, MA) was used as a column with a column temperature of 35 ℃ and flow rate of 0.4 ml/min. The mobile phase for the analysis was a mixture of methanol and RO water (3: 7; v/v), and the mobile phase for rinsing was a mixture of acetonitrile and RO water (1: 1; v/v). Since a fraction of the analyte could not be eluted into the mobile phase for analysis, the column was washed with the mobile phase for rinsing until no peak was detected.

Derivatization

A total of 10.0 mg of DMB was added into a brown test tube with a round glass stopper, followed by 3.2 ml of RO water to dissolve DMB and 0.6 ml of 2-mercaptoethanol with mixing. Thirty milligrams of sodium hydrosulfite was added into another test tube, followed by 2.8 ml of RO water. These solutions were mixed to make the DMB reagent for derivatization. The reagent was stored in a refrigerator and used within 48 hours of being prepared. Sample and standard solutions (100 μl) were poured into a light-shielded screw-capped microtube (2.0 ml), mixed with 100 μl of DMB reagents, and allowed to react for 60 min at 70 ℃. The tubes were cooled with water, then centrifuged (8000～10000 g; 4 ℃) for approximately 10 seconds. One hundred microliters of 0.2 M potassium borate buffer at pH 9.0 was added to the solution, and a 10- μl aliquot of the solution was subsequently used for HPLC.

Serum samples

The protocol of the study was approved by the Ethical Committee of Kyoto Biseibutu Kenkyusho. After providing informed consent, serum samples were obtained from rectal cancer (5) and colon cancer (13) outpatients at Torii Clinic, as well as 20 healthy volunteers. These samples were kept frozen at −70 ℃ until use.

Hydrolyzing condition

Hara et al. (1987) 11 previously described the conditions used to hydrolyze human serum with 25 mM sulfuric acid at 80℃ for 1 hour. Since hydrolyzing conditions are critical for determining sialic acid levels, we explored optimal hydrolyzing conditions with 0.05 N sulfuric acid at 80℃ or 95℃.

Serum pre-processing

Pre-processing for total sialic acid

The pre-processing procedure used to determine total N-acetylneuraminic acid (TSA) was as follows: 5 μl of a serum sample was mixed with 995 μl of 0.05N sulfuric acid to make a 200-fold diluted solution. Ten microliters of the diluted solution was then placed into a light-shielded screw-capped microtube, and 90 μl of 0.05 N sulfuric acid was added to make a final 2000-fold dilution. After hydrolyzing the serum, the tubes were cooled with water and centrifuged (8000～10000g; 4℃) for approximately 10 seconds.

Pre-processing procedure with cetavlon

The pre-processing procedure of N-acetylneuraminic acid treated with cetavlon (CTSA) was as follows: 50 μl of a serum sample was mixed with 50 μl of 2% cetavlon-0.2 M sodium acetate solution. The sample was kept for 10 min. at room temperature and then centrifuged (13000 g for 10 min.; 20℃). After the supernatant was completely removed, 500 μl of 2 % cetavlon-0.2 M sodium acetate solution was added, the sample was mixed well, and then centrifuged again (13000 g for 10 min.; 20℃). After completely removing the supernatant, 500 μl of 2% acetic acid solution was added to the sample to completely dissolve the residual (10-fold dilution). The 10-fold diluted sample (5 μl) and 0.05 N sulfuric acid (95 μl) were placed into a light-shielded screw-capped microtube (200-fold dilution) to hydrolyze the serum. The tubes were then cooled with cooled water and centrifuged (8000～10000g; 4℃) for approximately 10 seconds.

Evaluation of the analytical method

A total of 4 nmol/ml of N-acetylneuraminic acid standard solution (0.05 N sulfuric acid) was diluted with 0.05 N sulfuric acid to make 800, 400, 160, 32, and 6.4 pmol/ml solutions. Standard curves with/without hydrolyzation were plotted (n=3). The regression coefficients of the non-hydrolyzed and hydrolyzed curves were calculated (N=8). The limits of detection and quantitation were determined from the standard deviation of the response and slope of the standard curve (n=3). In the recovery tests, 800 or 160 pmol/ml N-acetylneuraminic acid solution (50 μl) was added to the 2-fold diluted samples (50 μl) to make 400 or 80 pmol/ml solutions. The recovery tests were repeated in triplicate (n=3) for each concentration. Tests were repeated for 5 days to determine intra-day (n=15) and inter-day (n=2) fluctuations.

SDS-PAGE Western Blot Lectin Analysis

The sample treatment solution was prepared from 0.1 M Tris HCl (pH 6.8), 1% SDS, and 20% glycerin (without 2-mercaptoethanol). Serum samples were diluted 20 times. The cetavlon pre-processed residual (obtained from 50 μl of a serum sample) was dissolved with 20 μl of 2% acetic acid, and 30 μl of the sample treatment solution was then added to make 50 μl of SDS-PAGE sample solution. All samples were processed under non-reducing conditions.

Rapidas AE-6530 and Horizblot 2M AE-6687 (·S) (ATTO CORPORATION, Tokyo, Japan) were used as the SDS-PAGE chamber and blotting apparatus, respectively. A constant power supply was provided by the PowerPhoreStar 3810 (Anatech Corporation, Tokyo, Japan). A 9% Hand-Cast Gel was

prepared for the gel.

After blotting, proteins were transferred onto a PVDF membrane (Clear Blot; ATTO CORPORATION)[16], stained with 0.1% Coomassie Brilliant Blue G250, and then with Sambucus sieboldiana-biotin/Streptavidin-HRP.

Statistical Analysis

All statistical analyses were performed with R software, and differences were considered significant when $P<0.05$.

Results

Hydrolyzing condition

Fig. 1 shows the results of the hydrolyzing experiments. When hydrolyzed at 95℃, the recovery rate of sialic acid was slightly low. Thus, we established the optimum hydrolyzing conditions to be 80℃ for 40 min. with 0.05 N sulfuric acid.

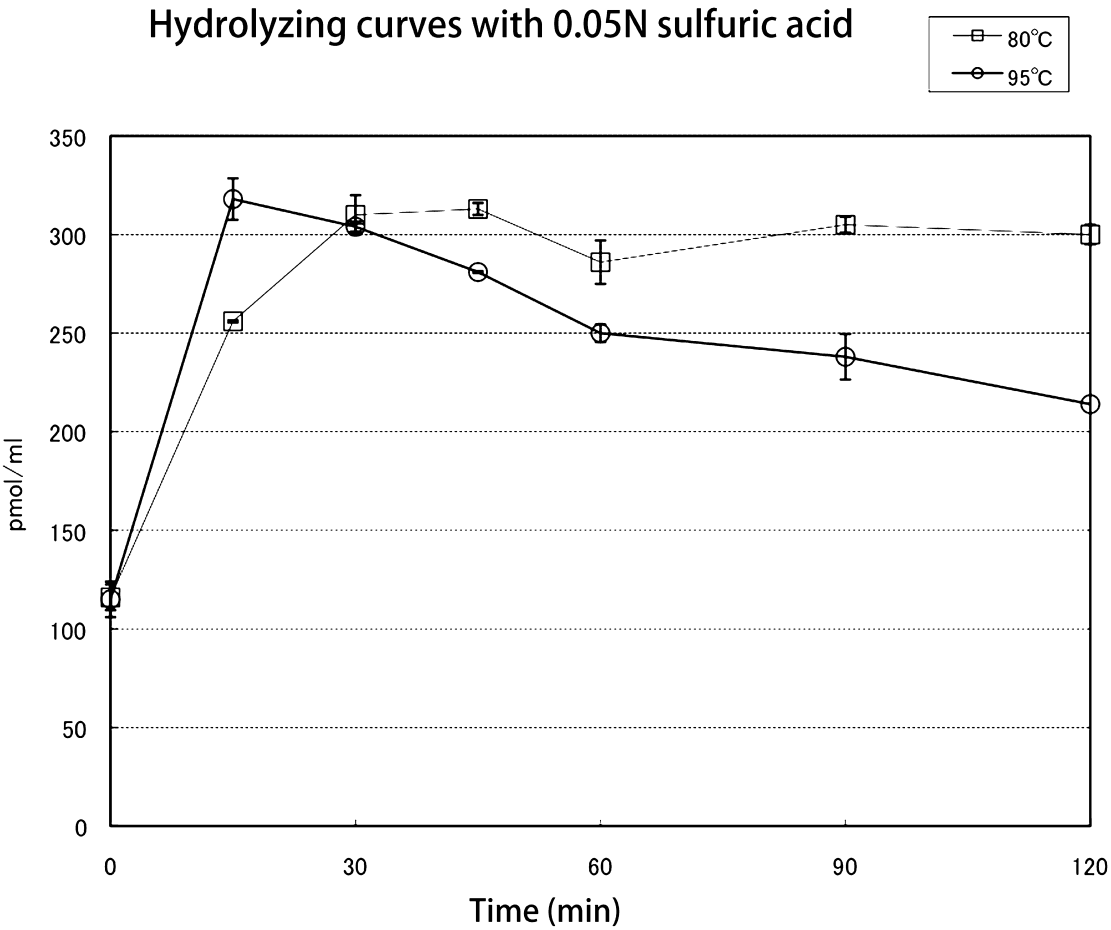

Fig. 1. Hydrolyzing curves with 0.05 N sulfuric acid.
The temperature was set at either 80℃ or 95℃. Measuring points: before heating and after 15, 30, 60, 90, and 120 min. Results are shown as the mean±SEM.

Evaluation of analytical methods

Fig. 2 shows the standard curves for N-acetylneuraminic acid. Contribution rates (r^2) were 1 and 0.999 for non-hydrolyzing conditions and hydrolyzing conditions, respectively. The regression coefficients were 5758.2 and 4895 for non-hydrolyzing conditions and hydrolyzing conditions, respectively. The regression coefficient for the non-hydrolyzing standard curve was significantly higher than that for the hydrolyzing standard curve (mean difference 137.6; the paired Student's t-test; $p = 0.000757$; 95% confidence interval (CI), 80 to 195).

Fig. 3 shows detection and quantification limits. The detection limits for the non-hydrolyzing and hydrolyzing conditions were 3.5 pmol/ml, while the quantification limits were 10.5 and 10.7 pmol/ml, respectively.

The results of the recovery tests showed that the recovery rates for TSA and CTSA (mean± SEM) at 80 pmol/ml were 81.5±3.1% (95% CI: 68 to 95%) and 93.0±6.9% (95% CI: 88 to 101%), respectively, and at 400 pmol/ml, 94.3±1.4% (95% CI: 88 to 101%) and 97.2±1.0% (95% CI: 94 to 101%), respectively.

The chromatograms of CTSA for blank and sample solutions with standard concentrations are shown in Fig. 4.

Intra-day and inter-day fluctuations are shown in Table 1. Intra-day and inter-day fluctuations for TSA were 5.1% and 7.1%, respectively, and were 6.6% and 10.9%, respectively, for CTSA.

SDS-PAGE Western Blot Lectin Analysis

The results of the SDS-PAGE Western blotting analysis are shown in Fig. 5.

Determination of TSA and CTSA in serum samples

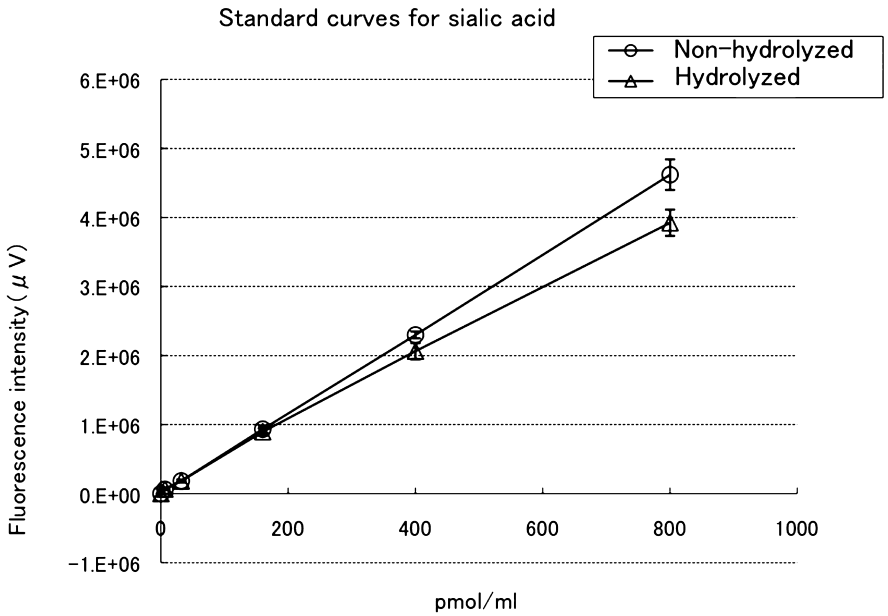

Fig. 2. Standard curves for N-acetylneuraminic acid.
Fluorescence was determined at 6.4, 32, 160, 400, and 800 pmol/ml. Results are shown as the mean ± SEM (n=3).

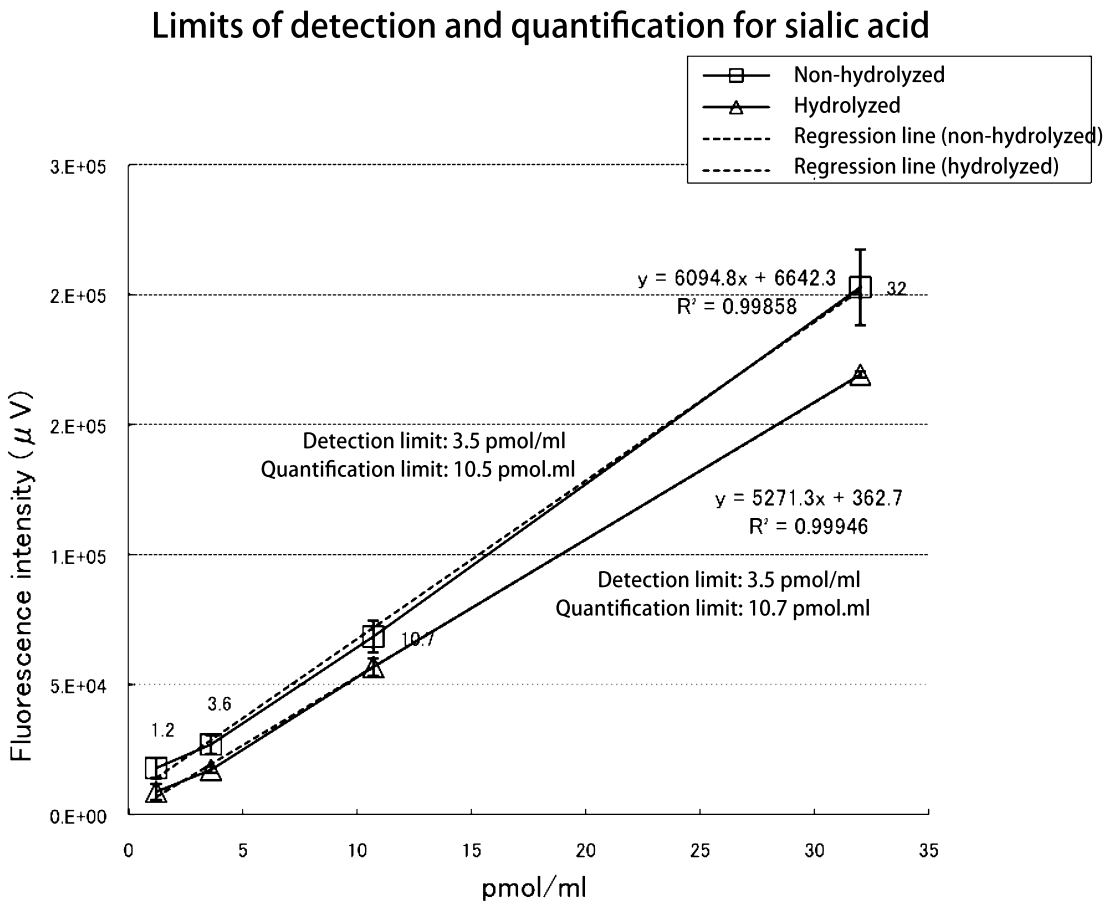

Fig. 3. Detection and quantification limits for N-acetylneuraminic acid.
Results are shown as the mean ± SEM (n=3). The detection limit (DL) was calculated from the equation: DL=3.3 σ/S ; and the quantification limit (QL) was calculated from the equation: QL=10 σ/S (S: regression coefficient).

The results of serum TSA and CTSA determinations are shown in Table 2. Fig.s 6 and 7 show the dot charts and box plots for TSA and CTSA. Fig. 8 shows the Receiver Operating Characteristic (ROC) curves of TSA and CTSA.

Discussion

The hydrolyzing experiments (Fig. 1) performed in the present study revealed that the recovery of N-acetylneuraminic acid at 95℃ was lower than expected, whereas no decrease was observed in the recovery rate of N-acetylneuraminic acid at 80℃ with a heating time from 30 to 120 min. Thus, we concluded that hydrolyzing with 0.05 N sulfuric acid at 80℃ for 40 min. was sufficient.

As shown in Fig. 2, the regression coefficient for the hydrolyzing standard curve was significantly lower than that for the non-hydrolyzing standard curve. This may have been due to the degradation of some N-acetylneuraminic acid during the hydrolyzing process. Thus, a calibration should be performed for the hydrolyzing standard curve. Since the detection limit was 3.5 pmol/ml and quantification limit

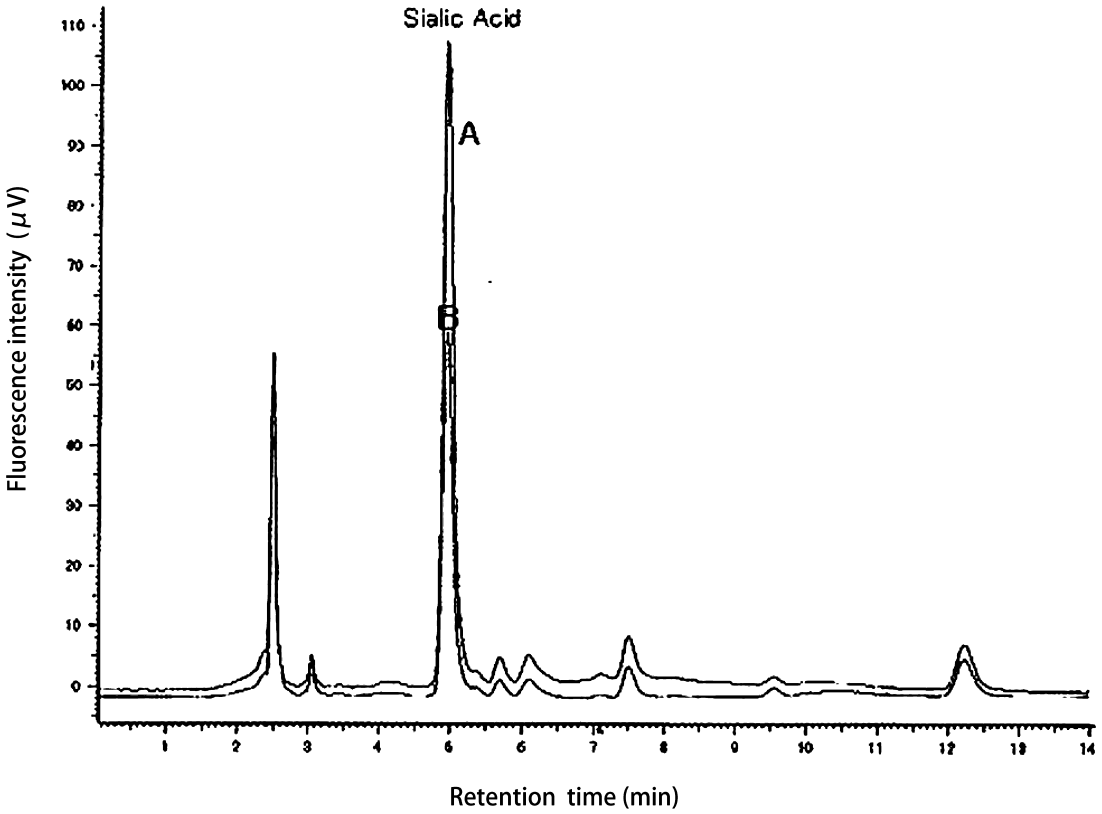

Fig. 4. HPLC chromatograms for CTSA blank and standard solution samples.
A: Standard CTSA solution chromatogram; B: Blank chromatogram.
The retention time of N-acetylneuraminic acid = 4.95 min.

Table 1. Intra-day and inter-day fluctuations in the determination of TSA and CTSA levels

	Intra-day fluctuation			Inter-day fluctuation		
	mean (nmol/ml)	SD	CV (%)	mean (nmol/ml)	SD	CV (%)
TSA	1491.6	76.1	5.1	1191.8	84.7	7.1
CTSA	84.6	5.6	6.6	81.4	8.9	10.9

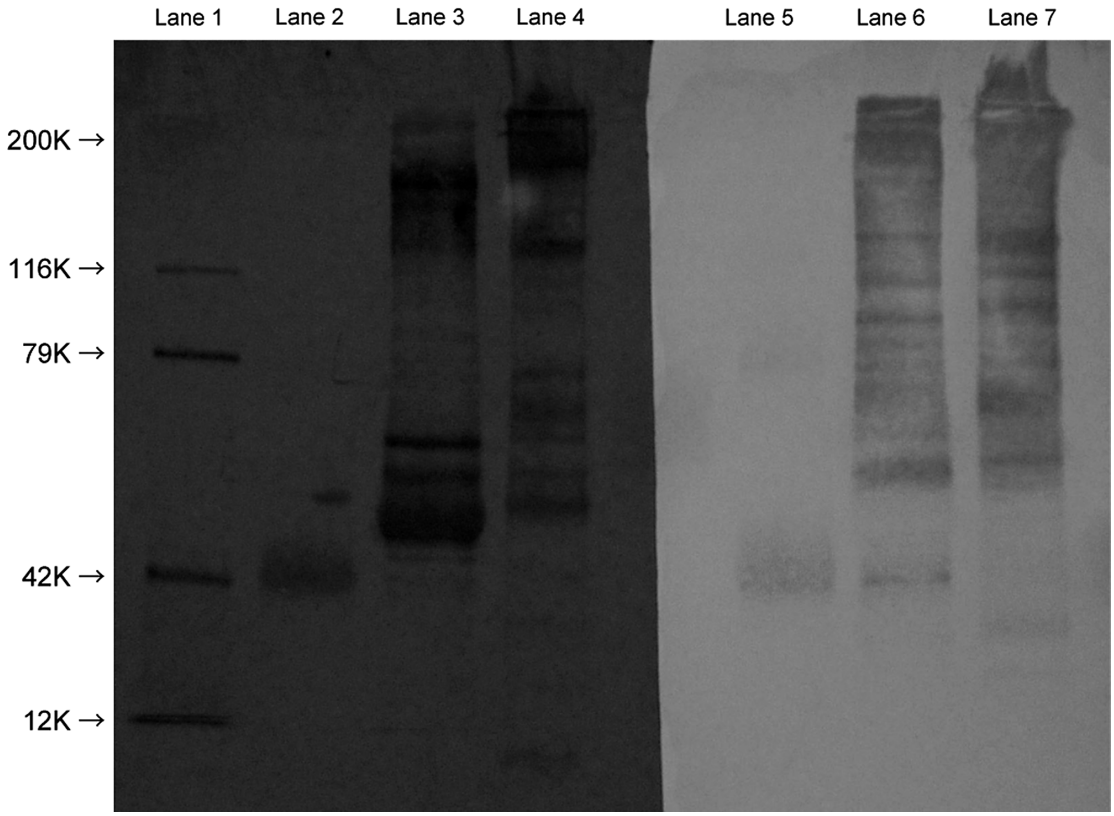

Fig. 5. SDS-PAGE Western blot Lectin Analysis of serum and cetavlon-treated samples under non-reduced/no heat conditions.
Lanes 1-4 were stained with Coomassie Brilliant Blue G250, and Lanes 5-7 were stained with Sambucus sieboldiana-biotin/Streptavidin-HRP.
Lane 1: M.W. marker
Lane 2, Lane 5: α1-acid glycoprotein
Lane 3, Lane 6: human serum
Lane 4, Lane 7: cetavlon-treated serum

was approximately 11 pmol/ml (Fig. 3), determination showing level 20 pmol/ml or less, should be repeated at a different dilution.

When the results of the recovery tests ($\mu 0 = 100\%$) were analyzed using one-sample Student's t-tests, the 95% CI for the 80 pmol/ml spike did not cross the 100% level ($p = 0.029$); thus, an alternative hypothesis was adopted. However, based on the results of other recovery tests, we concluded that the recovery results were sufficient.

The chromatogram shown in Fig. 4 revealed that there was acceptable specificity.

Although the results of intra-day and inter-day fluctuations were favorable, with approximately 10% fluctuations, the inter-day fluctuation of CTSA was considered to be high (CV = 10.9%). This may have been due to the complexity of pre-processing samples, which contributed to large fluctuations in day-to-day processing. Furthermore, the complex-forming rate of samples treated with cetavlon may have been influenced by the reaction environment, which could also have contributed to the large inter-

Table 2. TSA and CTSA levels in human serum samples levels

	TSA			CTSA		
	Healthy control	Rectal cancer	Colon cancer	Healthy control	Rectal cancer	Colon cancer
mean	1114	1125	1280	61	116	98
SD	36.7	109.4	75.7	3.7	15.6	9.4
SE	164.3	244.6	273.1	16.8	34.8	34.0
median	1080	1130	1287	61	115	85
number of samples	20	5	13	20	5	13

Values are nmol/ml, except for the number of samples

Abbreviations: SD = Standard Deviation; SE= Standard error

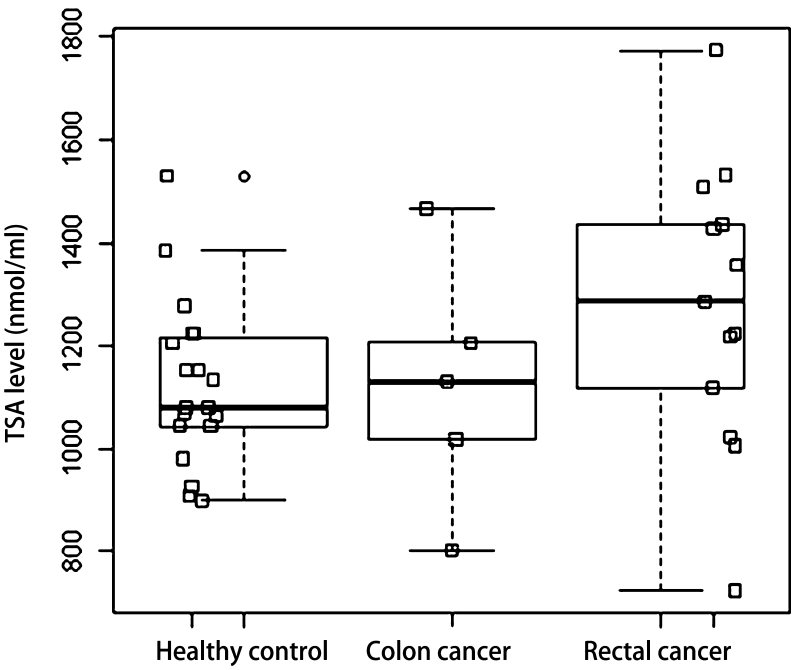

Fig. 6. Dot chart-box plot of TSA levels for healthy controls and rectal cancer and colon cancer patients.

Healthy controls, 20; rectal cancer patients, 5; colon cancer patients, 13.

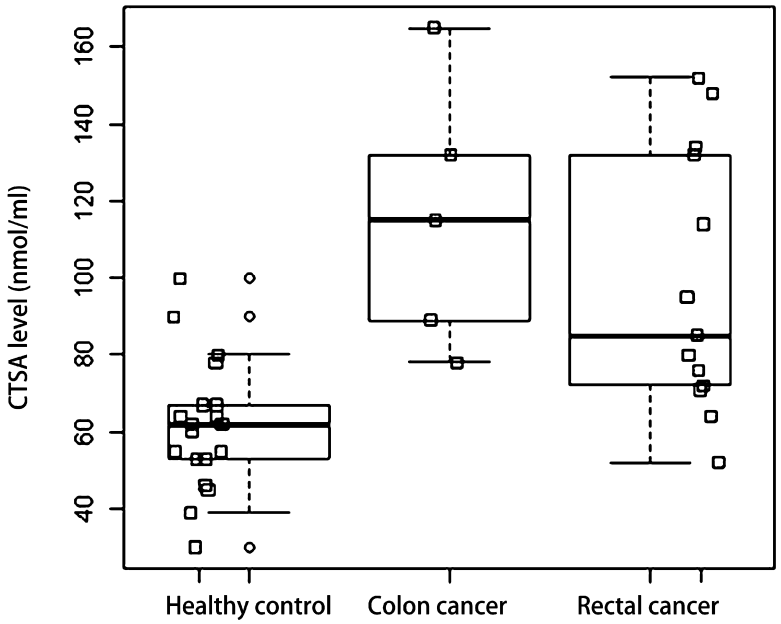

Fig. 7. Dot chart-box plot of CTSA levels for healthy controls and rectal cancer and colon cancer patients.
Healthy controls, 20; rectal cancer patients, 5; colon cancer patients, 13.

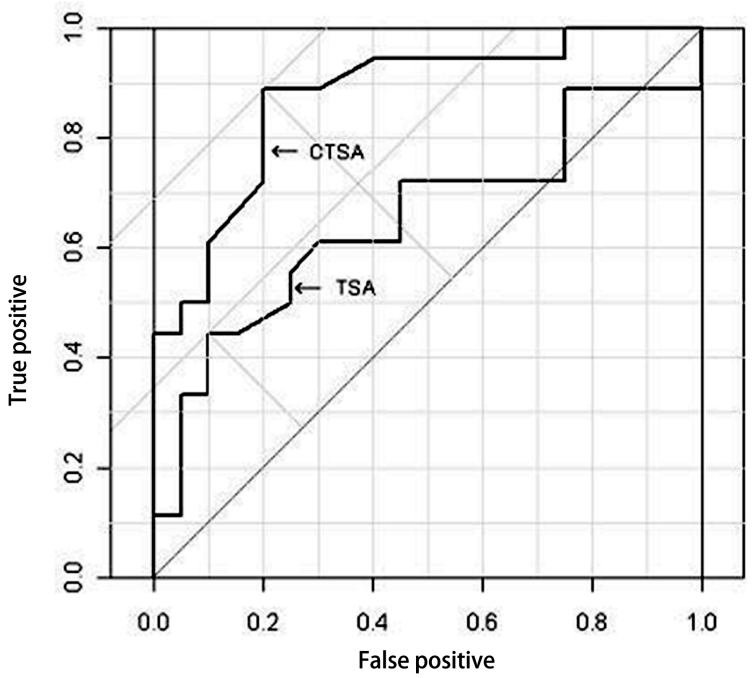

Fig. 8. ROC curves for TSA and CTSA.
"True" was assigned to rectal cancer and colon cancer, and "False" was assigned to healthy controls. AUC: TSA, 0.65, CTSA, 0.88; Threshold: TSA, 1287, CTSA 71; Odds ratio: TSA, 7.2; CTSA, 32.

day fluctuation.

The distribution of proteins in TSA and CTSA samples are shown in Fig. 5 as the SDS-PAGE Western blot Lectin analysis. When we compared unprocessed human serum (lane 3) with cetavlon-processed human serum (lane 4), albumin was almost completely removed from cetavlon-processed serum, and a larger amount of proteins with molecular weights (m.w.) higher than that of albumin could be recovered. In the Western blot Lectin analysis, the α1-acid glycoprotein was detected in human serum (Lane 6), but not in cetavlon-treated serum (Lane 7). In cetavlon-treated serum (Lane 7), several intense bands were observed in the area corresponding to molecules with molecular weights larger than that of albumin over the globulin area.

The cetavlon treatment may have precipitated anionic macromolecular mixtures of acidic glycoproteins including sialic acid, acidic polysaccharides, and nucleic acids; however, that of α1- acid glycoprotein was not confirmed.

Since the proteins that may play a role in the relationship between N-acetylneuraminic acid and diseases are α1-acid glycoprotein (m.w.=ca. 44,000), haptoglobin (ca. 100,000 to 400,000), ceruloplasmin (ca. 130,000), and transferrin (ca. 78,000),[17)18)] further analyses are required in order to determine the characteristics of these proteins.

Inagaki et al. performed an immunohistochemical lectin analysis on cancer tissue using sialic acid-specific lectin[19)]. We herein investigated the quantitation of serum N-acetylneuraminic acid.

In the present study, the measured TSA level was 1114 ± 164.3 nmol/ml (mean ± SD) (Table 2), whereas it was previously reported to be 502.6 ± 89.5 mg/L by Celil et al.3 The mean value of 1114 nmol/ml is equivalent to approximately 344 mg/L (molecular weight of sialic acid=309), which was smaller than that reported by Celil and may have been due to differences in the methods used. When the results in Table 2 were analyzed by a one-way ANOVA, no significant differences were observed in TSA ($p=0.1031$). However, we cannot draw any concrete conclusions because of the small sample size. A significant difference was noted in CSTA ($p=0.0039$).

Tukey's multiple comparison test revealed a significant difference between healthy controls and rectal cancer patients, and also between healthy controls and colon cancer patients, but not between rectal cancer and colon cancer patients (Fig. 6, 7). The results of the ROC curves are shown in Figure 8. Based on the results of a one-way ANOVA, "True" was assigned to rectal cancer and colon cancer patients, and "False" was assigned to healthy controls. The odds ratios obtained were 7.2 and 32, with 95% CI 1.3 to 40.7 and 5.1 to 200.1, for TSA and CTSA, respectively. The AUCs were 0.65 and 0.88, for TSA and CTSA, respectively.

The cetavlon treatment significantly increased the sensitivity of colorectal cancer screening. It currently remains unknown how sialic acid was selected through the pre-treatment with cetavlon; however, the separation of glycoprotein-bound and free sialic acid may have contributed to its selection. The results of the Western blot analysis suggested that this selection could also be attributed to differences in the molecular weight of sialic acid-bound glycoprotein.

The Western blot analysis showed that the molecular weight of glycoprotein precipitated by the cetavlon treatment was larger than that of albumin. However, the sensitivity of detecting all cancer types was not necessarily increased by the cetavlon treatment. Although sensitivity was increased in patients with colon and rectal cancers in our study, an increase in α 1-acid glycoprotein levels was previously reported in laryngeal cancer patients[3)], suggesting that detection sensitivity may be

enhanced by investigating changes in the supernatant of samples obtained from patients with this cancer type. In any case, a pre-treatment that alters the properties of sialic acid-bound glycoprotein may specifically increase the detection sensitivity of various cancer types. The results of the present study demonstrated that determining sialic acid levels following a cetavlon treatment, which mainly bound to glycoprotein with a higher molecular weight than that of albumin, increased the screening sensitivity of rectal and colon cancer patients.

These results indicated that CTSA values have a stronger relationship with certain diseases than TSA values; however, this could not be confirmed with other diseases.

Further studies are required to improve the efficiency of pre-processing and determining relationships with other diseases.

The authors indicated no potential conflict of interest.

References

1）Matsuzaki S, Itakura M, Iwamura K, and Kamiguchi H. Serum sialic acid levels in liver cirrhosis and liver cancer. Nippon Shokakibyo Gakkai Zasshi 1981; 78: 2395-2401.

2）Reshad K, Suzuki K, Shimada T. A Clinical Study on Serum Sialic Acid and Immunosuppressive Acidic Protein (IAP) as Inflammatory Parameter in Non-Malignant Pulmonary Diseases. Bulletin of the Chest Disease Research Institute, Kyoto University 1986; 19: 56-70.

3）Uslu C, Taysi S, Akcay S, Yavuz Sutbeyaz M, and Bakan N. Serum Free and Bound Sialic Acid and Alpha-1-Acid Glycoprotein in Patients with Laryngeal Cancer. Ann. Clin. Lab. Sci 2003; 33: 156-159.

4）Feijoo C, Paez de la Cadena M, Rodriguez-Berrocal FJ, Matrinez-Zorzano VS. Sialic acid levels in serum and tissue from colorectal cancer patients. Cancer Lett 1997; 112: 115-60.

5）Feijoo-Carnero C, Rodriguez-Berrocal FJ, Paez de la Cadena M, Ayude D, de Carlos A, Martinez-Zorzano VS. Clinical significance of preoperative serum sialic acid levels in colorectal cancer: Utility in the detection of patients at high risk of tumor recurrence. Int. J. Biol. Markers 2004; 19: 38-45.

6）Chrostek L, Cylwik B, Korcz B, et al. Serum Free Sialic Acid as a Marker of Alcohol Abuse. Alcohol Clin. Exp. Res 2007; 31: 996-1001.

7）Warren L. The thiobarbituric acid assay of sialic acid. J. Biol. Chem 1959; 234: 1971-1975.

8）Sugahara K, Sugimoto K, Nomura O, and Usui T. Enzymatic Assay of Serum Sialic acid, Clin. Chim. Acta 1980; 108: 493-498.

9）Matsuno K, and Suzuki S. Simple fluorimetric method for quantification of sialic acids in glycoproteins. Anal. Biochem 2008; 375: 53-59.

10）Honda S, and Suzuki S. Common conditions for high-performance liquid Chromatographic microdetermination of aldoses, hexosamines, and sialic acids in glycoproteins. Anal.Biochem 1984; 142: 167-174.

11）Hara S, Takemori Y, Yamaguchi M, Nakamura M, and Ohkura Y. Fluorometric High-Performance Liquid Chromatography of N-Acetyl- and N-Glycolylneuraminic Acids and Its Application to Their Microdetermination in Human and Animal Sera, Glycoproteins, and Glycolipids. Anal. Biochem 1987; 164: 138-145.

12）Okazaki K, and Izumori K. High-performance Liquid Chromatography for Analysis of p-Aminobenzoic Ethyl Ester-converted D-Allose and D-Altrose on XBridge Column Containing Polyethoxysilane. Tech. Bull. Fac. Agr. Kagawa Univ 2008; 60: 47-52.

13）Iozzo, R. V. ed., Methods Molecular Biol., 171, Humana Press 2001, p.117.

14）Isshiki G. Conditions for cetavlon reaction and molecular weights of acidic mucopolysaccharides in urine. [in Japanese] Reports on Monitoring of Congenital Anomalies, Studies on Physical and Mental Disorders, Ministry of Health, Labor and Welfare 1981: 251-252.

15）Doyle JJ, and Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue.

Phytochem. Bull. 1987; 19: 11-15.
16) Kyhse-Andersen J. Electroblotting from multiple gels: A simple apparatus without buffer tank for rapid transfer of proteins to nitrocellulose membrans. J. Biochem. Biophys. Methods 1984; 10: 203-209.
17) Lopez-Saez JJB, Senra-Varela A. Evaluation of lipid-bound sialic acid as a tumor marker. Int. J. Biol. Markers 1995; 10: 174-179.
18) Taysi S, Akcay F, Uslu C, Dogru Y, and Gulcin I. Trace elements and some extracellular antioxidant protein levels in serum of patients with laryngeal cancer. Biol. Trace Elem. Res 2003; 91: 11-18.
19) Inagaki Y, Tang W, Guo Q, Kokudo N, Sugawara Y, Karako H, Konishi T, Nakata M, Nagawa H, Makuuchi M. Sialoglycoconjugate expression in primary colorectal cancer and metastatic lymph node tissues. Hepatogastroenterology 2007; 54: 53-57.

〈和文抄録〉

セチルトリメチルアンモニウムブロミド処理によるシアル酸定量および血清中シアル酸の大腸癌における臨床的有用性について

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背景：シアル酸は炎症のパラメーターとして有用であるが，さらに重要な生体反応の指標となる可能性もあり，精度良く測定する意義は大きい．シアル酸の高感度定量について検討し，セタブロンによる血清前処理を行うことにより，大腸癌とシアル酸の関係について検討した．

材料および方法：健常人 20 名，直腸癌 5 名，結腸癌 13 名についてセタブロンによる前処理を行い，加水分解，蛍光標識後シアル酸定量を行った．

結果：検出限界は 3.5 pmol/ml，定量限界は 10.5〜10.7 pmol/ml だった．添加回収試験では，81.5〜97.2％だった．血清中シアル酸は CTSA では差は有意となった（$\alpha = 0.05$）．

考察：血清中のｼｱﾙ酸分析法評価については良好な結果であると考える．
CTSA について Tukey の多重比較では，健常人と直腸癌，健常人と結腸癌で差は有意となった．オッズ比を求めたところ，セタブロン処理を加えた血清中シアル酸定量値のほうが大腸癌と関連が強いことが確認できた．

キーワード：HPLC，シアル酸，蛍光標識，DMB，セタブロン．