Elevation of Serum Steroid Sulfatase Level in Gynecologic cancers

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Abstract : *Steroid sulfatase (STS) desulfates sulfated 3 β-hydroxysteroid family, such as, estrone sulfate, androstenediol sulfate, dehydroepiandrosterone sulfate and cholesterol sulfate. This enzyme possesses an important role in the converting the inactive steroid hormone to the active form. We have established an enzyme-linked immunosorbent assay (ELISA) of STS to measure the amount of the enzyme protein in sera. ELISA was performed by "Sandwich" method using a peroxidase conjugated anti STS IgG Fab' fragment. A range of STS was 10 to 1,500 ng per ml serum in this method. When gynecologic carcinomas were assayed by this ELISA, the serum STS level was significantly ($P<0.01$) elevated in endometrial carcinoma and ovarian carcinoma patients, as compared to that of normal women. Measurement of serum STS protein may be useful for clinical applications as a tumor marker. (Thai J Obstet Gynaecol 1995;7:33-40.)*

Key words : steroid sulfatase; gynecologic cancers; enzyme-linked immunoassay; ovarian cancer; endometrial carcinoma

Steroid sulfatase (E.C. 3.1.6.2.) is widely distributed in the mammalian tissues such as liver, ovary, testis, and uterine endometrium and is especially abundant in placenta[(1)]. This enzyme catalyzes desulfation all the 3 β-hydroxysteroid sulfates, including estrone sulfate, and pregnenolone sulfate. Sulfated form of the steroid hormones is inactive because of failure to bind to their receptors. The hydroxysteroid sulfates become active after desulfation by STS[(2)]. Sulfated steroid hormones which represent in blood as the major form of the steroid hormones acts as a reservoir or precursors of active hormones[(3)]. The development and growth of endometrial carcinoma[(4)], ovarian carcinoma[(5)] depends on estrogen. These observations suggest that STS is involved in the genesis and the

maintenance of steroid hormone depended on tumors such as endometrial carcinoma an ovarian carcinoma[6]. Some recent reports have demonstrated that STS activity was significantly higher in endometrial carcinoma tissue than normal endometrium[7]. It will be of value to know STS level in blood for elucidation of the tumor growth and diagnostic and prognostic meaning in steroid dependent carcinomas. However, STS activity level in blood is under detectable level by the methods so far developed. In this paper, we have developed an enzyme-linked immunosorbent assay (ELISA) of serum STS to measure an amount of the enzyme protein in sera of patients with gynecological carcinomas.

Materials and Methods

Sera from normal subjects and patients. The normal control sera (69 women and 8 men) were obtained from healthy volunteers. The patients' sera prior to any treatment were from 30 patients with cervical carcinoma, 17 with endometrial carcinoma, 13 with ovarian carcinoma. All samples were kept frozen at - 20°C before analysis.

Material [7-^{3}H] dehydroepiandrosterone sulfate was purchased from New England Nuclear (Boston, USA); non labeled dehydroepiandrosterone from Sigma (St. Louis, USA); concanavaline A (Con-A) Sepharose, Blue-Sepharose, octyl Sepharose, Mono P, and PD-10 from Pharmacia (Uppsala, Sweden); pepsin, 2 mercaptoethylamine and Triton X-100 from Sigma (St Louis, USA); N-succinimidy l-6-maleimidohexanoate (EMCS) and peroxidase from Dojinkagaku (Kumamoto, Japan) and from Toyoby (Tokyo, Japan), respectively; microtiter-plate for ELISA from Nunk (Roskilde, Denmark); Centricon from Amicon (Danvers, USA); Tween 20 from Wako Jyunyaku (Tokyo, Japan). All other reagents are of reagent grade.

Purification of human steroid sulfatase. STS was purified from term human placenta, as previously described by Yen et al[8] with a slight modification, the human placenta was homogenized with three volumes of 10 mM Tris-HCl, pH 7.5 containing 0.05% Triton X-100. The homogenate was centrifuged at 10,000 x g for 30 min. The supernatant was discarded. The precipitate was dissolved in three volumes of 10 mM Tris-HCl, pH 7.5 containing 1% Triton X-100 and stirred. Following centrifugation at 10,000 x g for 30 min, the supernatant containing STS activity was subjected to sequential chromatographies on Con A Sepharose, Blue Sepharose, octyl Sepharose, and chromatofocusing on Mono P. Purification was monitored by assaying STS activity using [7^{3}H] dehydroepiandrosterone sulfate as described previously[9]. One unit (U) of the enzyme activity was defined as one nmol of the product per hour.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) and

2-mercaptoethanol was performed on 10% polyacrylamide gel as described previously by Laemmli[10]. The gel was stained using silver staining kit (Kanto Chemical, Tokyo, Japan).

Preparation of antibody against steroid sulfatase. Rabbit antisera against STS was produced by immunizing 3 time with the purified preparation (100 μ g each) emulsifed three times in Freund's complete adjuvant. The IgG was purified by ammonium sulfate precipitation and DEAE-cellulose chromatography. Immunoblot was carried out as described previously[11].

Conjugation of anti STS-IgG Fab' with peroxidase. This was carried out as described previously by Yoshitake[12]. Briefly, the IgG (32mg) was digested with pepsin (0.6 mg) followed by isolation of F(ab')2 by chromatography on a Sephacryl S-300 column. After reduction of F(ab')2 to Fab' by 2-mercaptoethylamine, the Fab' was labeled with peroxidase using N-succinimidyl 6-maleimidohexanoate.

ELISA procedures. ELISA was performed by a "Sandwich" method microtiterplater for ELISA was coated with 100 ul of 0.1 mg/ml anti-STS IgG in 50 mM NaHCO3 buffer (pH 9.6) overnight at 4°C. The plate was washed 3 times with phosphate buffered-saline (PBS) blocked with 2% (W/V) skim milk for 1 hour at room temperature. After washing 3 times with PBS containing 0.05% Tween20 (washing buffer), 100 μl of serum or a solution containing STS in phosphate-buffered saline was added to the wells and incubated at room temperature for 3 hour. After the plate was washed 4 times with the washing buffer, 100 μl of the peroxidase-conjugated anti-STS Fab' diluted at 1:100 was added and left for 3 hour at room temperature, followed by washing 5 times with the washing buffer. Two wells were added 100 μl of peroxidase substrate solution (3 mg of ophenylenediamine in 5 ml of 10 mM citrate phosphate, pH 5.0, containing 1.7 μl of hydrogen peroxide), and the reaction was allowed to develop at room temperature for 10 min. The reaction was stopped by adding 100 μl of 2 N H_2SO_4 and the absorbance was measured at 490 nm.

Results

Establishment of ELISA Method of Serum Steroid Sulfatase. STS was purified 120-fold with a yield of 6% from placenta. A single protein band was given indicating that STS has been purified to an apparent homogeneity from human placenta. Molecular weight was estimated approximately 63,000 on reducing SDS-PAGE (Fig.1). The extent of specific activity of the purified enzyme, 1,900 U/mg protein, was equal to that obtained previously[8].

Specificity of antibody against STS. To evaluate the specificity of the raised antibody, the STS preparation was subjected to SDS-PAGE and then transferred to a nitrocellulose sheet followed by immunoblot with the antibody. As shown in Fig. 2, the

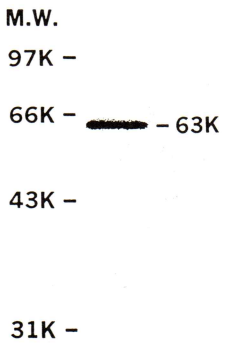

Fig. 1 SDS-PAGE of the purified STS from human placenta. The enzyme protein, 2ug was electrophresed on 10% acrylamide gel containing SDS. The band was visualized by silver-staining.

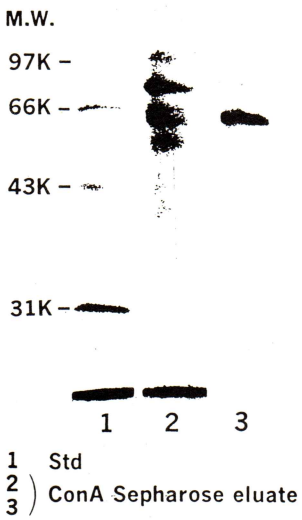

Fig. 2 Western blotting of a STS-containing fraction. The eluant on Con-A Sepharose was electrophoresed and immunobloted using anti-STS IgG. Lane 1, molecular standard markers; Lane 2, 3, eluant (about 40 μg proteins) from ConA Sepharose; Lane 1,2, with stained amido black; Lane 3, stained with peroxidase technique.

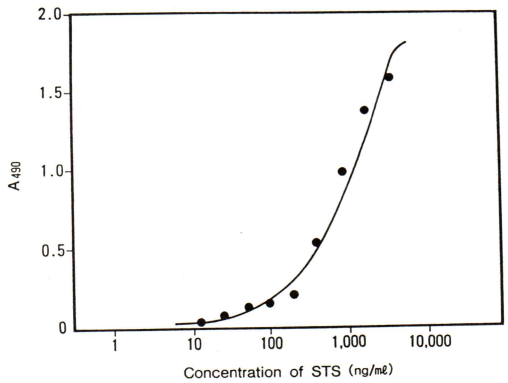

Fig. 3 Standard curve of the ELISA assay for STS enzyme protein.

antibody detected a single band at a position corresponding to molecular weight of the purified STS.

Sensitivity of ELISA. The standard curve using by this assay method as shown in Fig. 3, a range of 10 to 1500 ng/ml, STS protein was possible to assay.

Serum STS level in normal women and cancer patients. Normal level : The serum level of STS protein in healthy women (37.0 ± 13.6 year at the year of age, n = 69) was 74.5 ± 27.7 ng/ml (mean ± S.D.). On the other hand, the serum level of STS (43.0 ± 19.3 ng/ml) in normal men (33.7 ± 6.7 year at the year of age, n = 8) was lower than that in women.

Serum steroid sulfatase Gynecologic patients. Although serum STS concentrations of gynecologic patients were distributed somewhat in a wider range, the level in gynecologic cancer patients were elevated compared to that in healthy women (Fig. 4) The serum STS protein in

cervical carcinoma patients (117.8 ± 14.5 ng/ml) was significantly ($p < 0.05$) elevated than that in normal women. The serum STS in endometrial carcinoma patients (190.8 ± 31.3 ng/ml) was significantly ($p > 0.01$) higher than normal. The STS level (176.1 ± 22.6 ng/ml) was also significantly ($p < 0.01$) elevated in ovarian carcinoma. Cut-off value was determined at 130 ng/ml, which is Mean ± 2 SD of normal control.

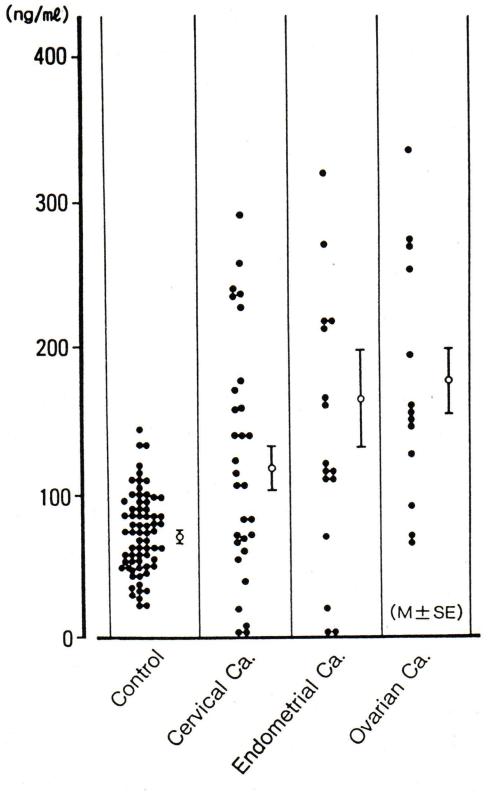

Fig. 4 The serum levels of STS in patients with cervical carcinoma, endometrial carcinoma, ovarian carcinoma. The cut-off value was set at the mean +2S.D.(130 ng/ml) of healthy female controls.

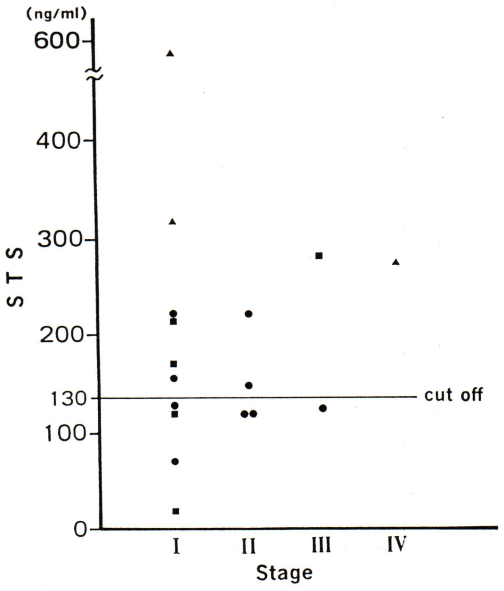

Fig. 5 Relationship between serum STS and staging in endometrial carcinoma. The histologic type was diagnosed as G1 (●), G2 (■) and G3 (▲). G1 ; highly differentiated adenomatous carcinoma. G2; moderated differentiated adenomatous carcinomas with partly solid areas. G3; predominantly solid or entirely undifferentiated carcinoma.

Relationship between serum steroid sulfatase and stage of cancer. The relationship between the serum steroid sulfatase, clinical stage and histology in patients with endometrial carcinoma and ovarian cancer are shown in Fig. 6. On the whole, stage dependent the serum steroid sulfatase levels could be observed. The serum STS of patients with histological grade 3 was higher than those of grade 1 and 2, independently clinical staging. Even though at stage, the elevation of that in patients with mucinous adenocarcinoma was noticeable. The serum levels of steroid

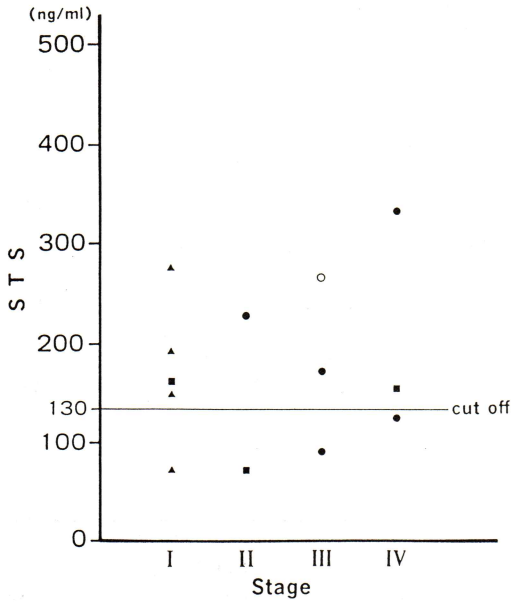

Fig. 6 Relationship between serum STS and staging in ovarian carcinoma. Each histological type was diagnosed as serous cystadenocarcinoma (●), mucinous cystadenocarcinoma (▲), clear cell carcinoma (O) and undifferentiated carcinoma (■).

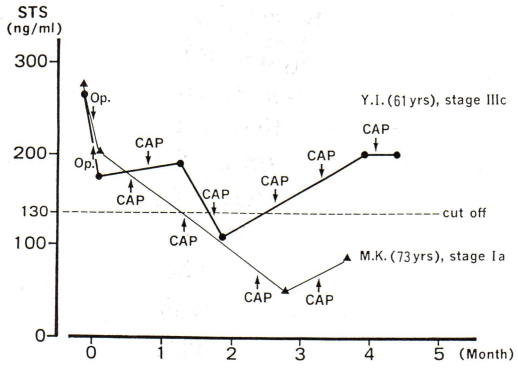

Fig. 7 Changes in serum STS levels in patients with ovarian carcinoma.

sulfatase in the patients of cervical squamous carcinoma did not have any relationship between the clinical stage (data not shown).

Effect of therapy on the appearance of steroid sulfatase. We examined for correlation between serum STS and clinical course in two patients with ovarian carcinoma. After surgery, the serum STS level decreased in patient with stage IIIc clear cell carcinoma, however, the lowest value was higher than the cut-off value. In these patients, the carcinoma did not respond to CAP (cyclophohosphamide, adriamycin and cisplatin) therapy, and that serum STS became elevated. On the other hand, the serum STS level of stage Ia with mucinous adenocarcinoma decreased as the tumor responded to the chemotherapy.

Discussion

Commonly, the enzymatic activity of STS is assayed using titrated steroid sulfate as a substrate. Such methodology precludes measuring the enzyme activity in serum where concentration of endogenous steroid sulfates is approximately 100 times higher than that of the exogenously added substrate[13]. To overcome this limitation we developed an ELISA method to measure the level of STS protein in serum. The present assay method enabled to assay STS protein as low as 10 ng/ml. In this method endogenous sulfated steroids which interfered the activity assay did not inhibit the assay.

The STS gene is localized on the X chromosome, and escapes from

Lyon's inactivation[14]. It may be for this process that the serum level of STS in normal men was lower than that in women.

Estrogen plays a role in promoting the development of endometrial cancers[4]. The endometrial carcinoma is associated with local production of estrone through aromatization of androstenedione[15,16] and desulfating estrone sulfate[7]. In post menopausal women, estrone sulfate is the most important estrogen precursor[17], and is concentrated higher than in pre menopausal women[18]. The steroid sulfatase activity in endometrial cancer tissue is significantly higher than in corresponding normal tissue[7]. It is probable that elevated STS enzyme activity in endometrial cancer tissue is reflected the increased level of STS protein serum. Normal ovary tissue has also STS activity[1] together with estrogen receptor. The estrogen receptor is present in some ovarian cancers, and the tumor tissues are responsive to estrogen by STS[5]. It is possible that the desulfatation of estrone sulfate may serve a role in the maintenance and promoting development of such ovarian tumors[6]. The elevation of serum STS level of cervical carcinoma was lower compared to that in endometrial carcinoma and ovarian carcinoma. These results suggest that STS may play a role in development and proliferation of gynecologic adenocarcinoma.

Although the releasing mechanism of STS, which is an integral endoplasmic reticulum protein, into blood circulation is unclear, it is postulated that STS leaks from destroyed cancer cells or is secreted from cancer cells.

Determination of serum STS protein by this newly developed ELISA will be useful for clinical application as a possible tumor marker in gynecologic carcinomas, especially of adenocarcinoma type.

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