

# Age-dependent decrease in serum transforming growth factor (TGF)-beta 1 in healthy Japanese individuals; population study of serum TGF-beta 1 level in Japanese

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**Abstract.** Transforming growth factor-beta1 (TGF- $\beta$ 1), a multi-functional cytokine, is involved in regulating a variety of cellular activities and the serum/plasma TGF- $\beta$ 1 level is altered with various diseases. However, most published reports have described adult patients, and so we investigated the clinical significance of serum TGF- $\beta$ 1 level in pediatric patients. The diagnostic application of the measurement of serum TGF- $\beta$ 1 level depends critically on the control value, however, there is no information on the control value of serum TGF- $\beta$ 1 for children.

In the present study, we determined the serum TGF- $\beta$ 1 level of healthy Japanese children as a control value with enzyme-linked immunosorbent assay (ELISA). The serum TGF- $\beta$ 1 level of children (0–14 years old) was significantly higher than that of adults (over 15 years old) ( $p < 0.01$ ). Thus, it is recommended that when the serum TGF- $\beta$ 1 levels of patients are evaluated, they should be compared with those of age-matched controls.

**Keywords:** Transforming growth factor (TGF)- $\beta$ 1, human, serum

## 1. Introduction

Transforming growth factor-beta1 (TGF- $\beta$ 1), a multi-functional cytokine, plays an important role in fibrosis and regulation of repair and regeneration after tissue injury [1]. TGF- $\beta$ 1 also has various functions in the immune system, mostly suppressive [2]. It can suppress the proliferation of T cells, the expression of

interleukin (IL) –2 mRNA [3], H<sub>2</sub>O<sub>2</sub> production by macrophages [4], and the adhesion between endothelial cells and lymphocytes and neutrophils [5].

Serum/plasma TGF- $\beta$ 1 level has been measured in patients with several diseases. Elevated levels of TGF- $\beta$ 1 in the serum/plasma are detected in patients with such tumors as bladder cell carcinoma [6], renal cell carcinoma [7,8], and hepatocellular carcinoma [9–11], as well as systemic sclerosis [12], Alzheimer's disease [13], and renal allograft transplantation [14]. Conversely, decrease in the serum level of TGF- $\beta$ 1 is detected in patients with Kawasaki Disease [15]. However, there is little information on pediatric patients.

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The diagnostic application of the measurement of serum TGF- $\beta$ 1 level depends critically on the control value. The levels of various cytokines in body fluids of healthy individuals change with age [16–18], thus, the serum TGF- $\beta$ 1 level of a pediatric patient should not be compared with that of a normal adult as a control value. At present, there is little information regarding the level of plasma/serum TGF- $\beta$ 1 in healthy children [18,19].

In the present study, to investigate the correlation between the level of serum TGF- $\beta$ 1 and the disease state of children, the serum TGF- $\beta$ 1 level in healthy Japanese children was determined by enzyme-linked immunosorbent assay (ELISA).

## 2. Materials and methods

### 2.1. Samples

Serum samples were obtained from Japanese children (1–14 years old) undergoing corrective surgery for inguinal hernia or circumcision repairs, which were considered to be different from diseases with TGF- $\beta$ 1 shift. All children had been free of infectious diseases and were not undergoing any medical treatments. The age distribution of the serum samples from children was 1–14 years old (mean  $5.2 \pm 3.8$  years old). Specimens from healthy Japanese adults were collected at a mass screening examination. The age range of adults was 21–67 years old (mean  $41.4 \pm 13.2$  years old). Serum samples were immediately frozen and stored at  $-70^\circ\text{C}$  until use. The purpose, procedures, and benefits of our project were explained to the participants or their parents, and we obtained their consent for our study.

### 2.2. Measurement of human TGF- $\beta$ 1 with ELISA

The concentration of TGF- $\beta$ 1 was measured by ELISA as follows. Microtitre plates (Immunoplate Maxisorp, Nunc, Roskilde, Denmark) were coated with  $100 \mu\text{l}$  of  $2 \mu\text{g/ml}$  of monoclonal anti-human TGF- $\beta$ 1 antibody (R&D systems Inc., MN, USA) in carbonate buffer (0.02 mol/l, pH9.5) overnight at  $4^\circ\text{C}$ . Thereafter, the wells were blocked with  $300 \mu\text{l}$  of 1% BSA, 5% sucrose and 0.05% Tween 20 for 90 min at room temperature. The plates were then washed three times with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBS-T). Recombinant (r) human TGF- $\beta$ 1 (R&D systems Inc. MN, USA), which was used to construct the standard curve (0–20 ng/ml), was serially diluted with 0.5% BSA/PBS-T. One hundred  $\mu\text{l}$  of

each activated serum sample, which was acidified with 2.5 mol/l acetic acid/10 M urea for 15 min at  $25^\circ\text{C}$  followed by neutralization with 2.7 mol/l sodium hydroxide, was incubated in the wells at  $4^\circ\text{C}$  overnight. After the incubation, the plate was washed and biotinylated goat anti-human TGF- $\beta$ 1 ( $100 \mu\text{l}$ , 100 ng/ml, R&D systems Inc., MN, USA) was added and incubated for 2 hours at  $25^\circ\text{C}$ . The plates were washed and  $100 \mu\text{l}$  of horseradish peroxidase (HRP)-conjugated streptavidin diluted 1/2000 in 0.5% BSA/PBS-T was added. Incubation was carried out for 30 min at room temperature. The plates were washed and  $100 \mu\text{l}$  of 0.05 mol/l citrate phosphate buffer, pH 5.8 containing  $0.4 \mu\text{g/ml}$  o-phenyldiamine (DAKO Japan, Kyoto, Japan) and 0.0013% hydrogen peroxide, was added to each well. After 15 min the enzyme-substrate reaction was terminated by the addition of  $100 \mu\text{l}$  0.5 mol/l sulfuric acid. The absorbance at 490 nm was measured in Microplate reader Model 3550UV (Biorad Laboratories, CA, USA). Data reduction and calculation of sample TGF- $\beta$ 1 value were carried out with analysis software package (Microplate Manager ver.2, BioRad). The detection limit of the assay was 0.08 ng/ml. The intra-assay and inter-assay coefficients of variation were 3.9% and 3.3% respectively. Mean recovery rate from serum samples ranged from 89 to 97%.

### 2.3. Statistical analysis

Mann-Whitney U rank sum test was used to investigate significant differences. Probability values of  $p < 0.01$  were considered significant. The Pearson correlation test was applied to evaluate possible correlations between the serum TGF- $\beta$ 1 level and age.

## 3. Results and discussion

The diagnostic application of the measurement of serum TGF- $\beta$ 1 level depends critically on the control value. However, reliable information on the serum TGF- $\beta$ 1 level of healthy children has not been available. Thus, we measured the serum TGF- $\beta$ 1 level in a total of 55 healthy children aged from 1 to 14 years old and 44 healthy adults aged from 21 to 67 years old with a TGF- $\beta$ 1 specific ELISA.

The entire age (1–67 years old)-related profile of serum TGF- $\beta$ 1 is shown in Fig. 1. The result showed a significant negative correlation between age and serum TGF- $\beta$ 1 level ( $r = -0.517$ ,  $p < 0.0001$ ,  $n = 99$ ). Mean serum TGF- $\beta$ 1 value of children was  $61.7 \pm 18.5$

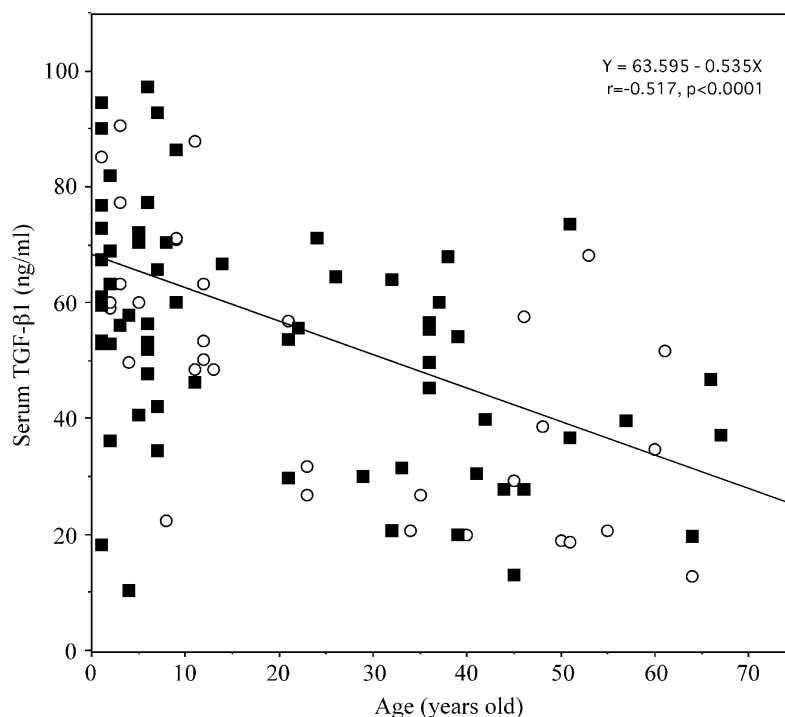


Fig. 1. The correlation between serum TGF-β1 level and age. Serum TGF-β1 level of healthy subjects; 55 children (1–14 years old) and 44 adults (21–67 years old), open circles for females ( $n = 35$ ), and closed squares for males ( $n = 64$ ).

( $n = 55$ ), whereas the mean serum TGF-β1 values of adults was  $40.3 \pm 17.7$  ( $n = 44$ ). The serum TGF-β1 of children was significantly higher than that of adults ( $p < 0.01$ ).

As far as we know, there has been no report investigating the control value of serum TGF-β1 covering all age ranges. Young et al. reported that the serum TGF-β1 level was not correlated with the age [18], however, their study involved blood donors aged from 17 to 69 years old. The lack of information for ages below 17 is the reason our results differed from Young et al. In the range corresponding to Young's report in our result, there was no significant correlation between age and the level of serum TGF-β1 ( $p = 0.1386$ ). On the other hand, Rosensweig et al. reported the plasma TGF-β1 level showed a negative correlation with age [19]. Serum TGF-β1 level might change with age as does the plasma TGF-β1 level, although the serum sample contains the TGF-β1 secreted by platelets during the clotting process.

The levels of some cytokines vary with age. For example, the serum level of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) [16], and the serum soluble IL-2 receptor both decrease with age [16,20,21], whereas the serum IL-6 level in older subjects is significantly higher than

that in younger subjects [17,18]. These age-dependent changes in cytokines suggest that they play important roles in regulation, maturation and differentiation during early childhood [22].

In our data covering a wide life span, there was no significant difference in the level between males and females (male;  $53.8 \pm 20.6$  ng/ml,  $n = 64$ , female;  $49.0 \pm 21.6$  ng/ml,  $n = 35$ ). No information on sex difference has been reported elsewhere, either.

As stated before, serum/plasma TGF-β1 level has been measured in patients with several diseases, such as tumors [6–11,23–25], systemic sclerosis [12], Alzheimer's disease [13], and renal allograft transplantation [14]. However, there is insufficient information for pediatrics. We are currently examining the correlation between the serum TGF-β1 level and pediatric patients with kidney diseases, such as focal glomerular sclerosis and nephritis.

#### 4. Conclusion

We determined the serum TGF-β1 level of healthy Japanese children as a control value by means of ELISA specific for human TGF-β1. It was found that the serum

TGF- $\beta$ 1 level of children (0–14 years old) was significantly higher than that of adults (over 15 years old). Thus, it is recommended that when the serum TGF- $\beta$ 1 levels of patients are evaluated, the values should be compared with those of age-matched controls.

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