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The Quantitative Changes of the Plasma Fibronectin (FN) with Aging

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ABSTRACT. Using an enzyme linked immunosorbent assay (ELISA), we investigated the changes in the amount of human plasma fibronectin (FN) with aging. The increase of plasma FN concentration with aging was slight up to about 50 years of age. Between 60 and 100 years there was a marked increase of plasma FN concentration with aging. The plasma FN concentration from three patients with Werner's syndrome showed higher value than the average of their age group.

Key words : ELISA — Fibronectin — Aging

The biological effects of fibronectin (FN) include the promotion of cellular adhesion, cellular migration, wound healing and maintenance of cell structure.[1] There are two types of FNs. The one called cellular FN is widely present in the connective tissue and the basement membrane in the body,[2] and it is also known as the large external transformation sensitive protein (LETS),[3] the cell surface protein (CSP),[4] and the cell adhesion factor (CAF).[5] It is composed of two or more 240,000 to 245,000 molecular weight subunits held together by disulfide bonds.[1] The another one is called plasma FN, which is known as the cold insoluble globulin (CIG).[6] It is composed of two 230,000 to 235,000 molecular weight subunits.[1] Yamada *et al.*[7] reported that cellular FN and plasma FN are similar but not identical and both FNs are indistinguishable by immunological methods. The relationship between cellular FN and plasma FN is not yet understood.

The values of plasma FN concentration in various diseases have been reported,[8-10] but there have been few reports concerning its changes with aging. We suppose that the changes of plasma FN with aging are a very important area of research on pathophysiology in various diseases as well as on the wound healing mechanism.

The quantification of plasma FN made by the immune diffusion method,[11] the electroimmunoassay[12] and the lasernephelometry[13] has been reported in the literatures. But there are few reports on the quantification of plasma FN using an enzyme linked immunosorbent assay (ELISA). The purpose of this study is to examine and estimate the plasma FN concentration in various ages using an ELISA.

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MATERIALS AND METHODS

1. *Collection of samples*

Plasma samples were obtained from hospitalized patients in our Medical School Hospital and from students and staff of our school. Plasma was taken on 3.8% citrate (0.5 ml for 4.5 ml whole blood), being prepared by centrifuging the blood at 2,000 rpm for 15 min at room temperature. It was stored at −20°C until use and thawed at 37°C. This storage has been shown not to alter the plasma FN value.[12,13]

2. *Enzyme Linked Immunosorbent Assay (ELISA)*

a) Materials

Antihuman FN IgG fraction from goats and horse radish peroxidase (HRP) coupled to goat antihuman FN were purchased from Cappel Laboratories (U.S.A.). Human plasma FN used as a control was obtained from Bethesda Research Laboratories (U.S.A.). The enzyme substrate for HRP was 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)(ABTS), which was purchased from Medical Biological Laboratories (Japan). Microtiter plates were obtained from Dynatech (West Germany).

b) Coating of plates for ELISA

Antihuman FN IgG fraction was diluted with 10 mM carbonate buffer (pH 9.6), and 100 μl of the solution were added to each well in a flat-bottomed microtiter plate (Fig. 1-1) and incubated at 37°C for two hours. The plate was then washed three times with phosphate-buffered saline containing 0.05% Tween 20 (PBS/T). Then egg-albumin was diluted with 10 mM carbonate

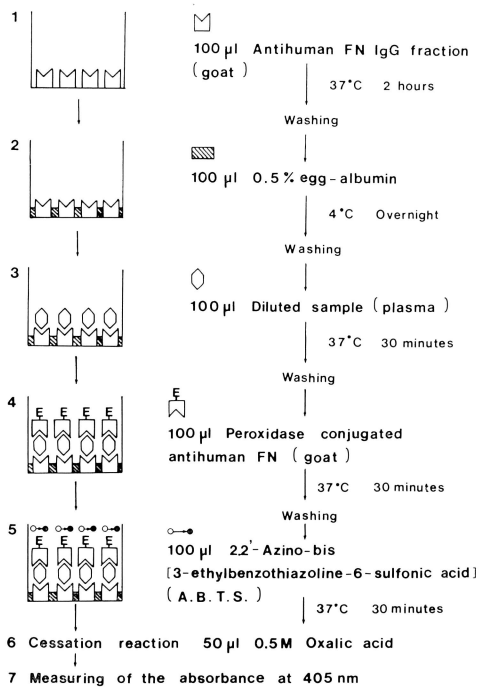

Fig. 1. Diagram of enzyme linked immunosorbent assay.

buffer at a final concentration of 0.5%, and 100 μl of the solution was added to each well and allowed to adsorb overnight (Fig. 1-2) at 4°C. Following this, the plate was washed three times with PBS/T. Plasma (sample) were diluted with PBS, and 100 μl of each of them was added to each well (Fig. 1-3) and incubated at 37°C for 30 minutes. After the plate was washed again three times with PBS/T, 100 μl of peroxidase conjugated antihuman FN at an appropriate dilution was added (Fig. 1-4) and incubated at 37°C for 30

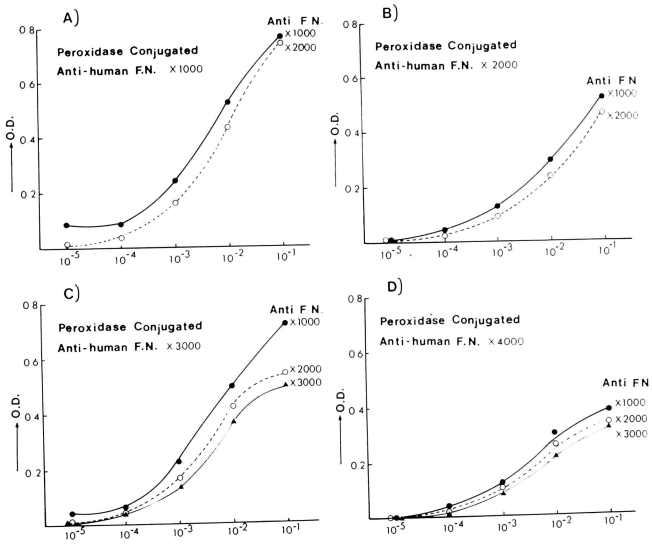

Fig. 2. The combinations of 1st (antihuman FN) and 2nd (peroxidase conjugated antihuman FN) antibodies for quantification of plasma FN concentration.

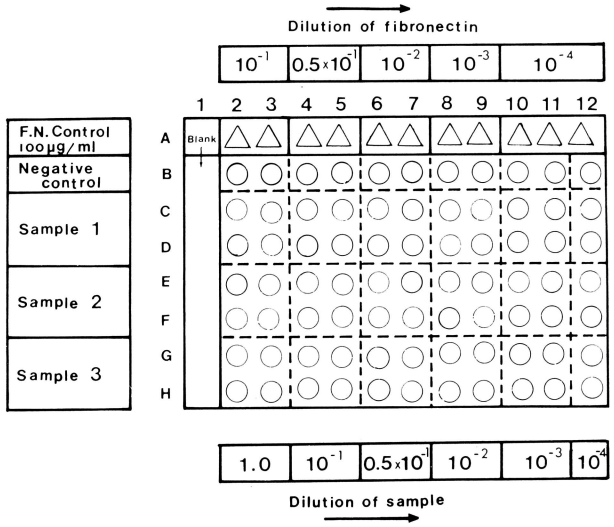

Fig. 3. Constitution of the microtiter plate for quantification of plasma FN concentration.

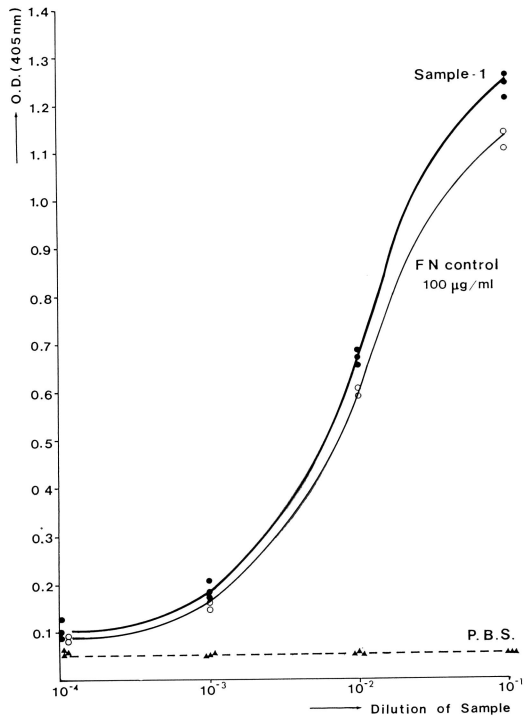

Fig. 4. Dilution-absorbance curves for sample plasma and control plasma FN.

minutes. The plate was washed three times with PBS/T. Then 100 μl of ABTS, the enzyme substrate for HRP, was added to each well (Fig. 1-5) and incubated at 37°C for 30 minutes. ABTS becomes a radical cation with HRP and produces a blue-green color. The enzyme reaction was stopped with 50 μl of 0.5 M oxalic acid (Fig. 1-6). The absorbance of the products of the enzyme reaction was measured using a micro-ELISA autoreader, which reads the absorbance of the solution within the microtiter wells at 405 nm.

c) Quantification of plasma FN of the sample plasma

1) The appropriate combination of concentration of the first (antihuman FN) and the second antibody (peroxidase conjugated antihuman FN)

We made a pilot study in order to ascertain the appropriate combination of concentration of the first and second antibody for obtaining the best chromophore. Figure 2 shows the results of this pilot study. We considered that Figure 2-A showed the most effective chromophore among others. The appropriate concentrations of the first and second antibodies were regarded as 1:2000 and 1:1000, respectively.

2) Constitution of the microtiter plate for the quantification of plasma FN concentration

In order to obtain dilution-absorbance curves of the control FN (100 μg/ml) and the sample plasma, we constituted the microtiter plate as shown in Figure 3. The second line (B) in the Figure 3 was PBS used as the negative control. We read the absorbance with micro-ELISA autoreader and drew up the dilution-absorbance curves (Fig. 4), which were used to calculate the

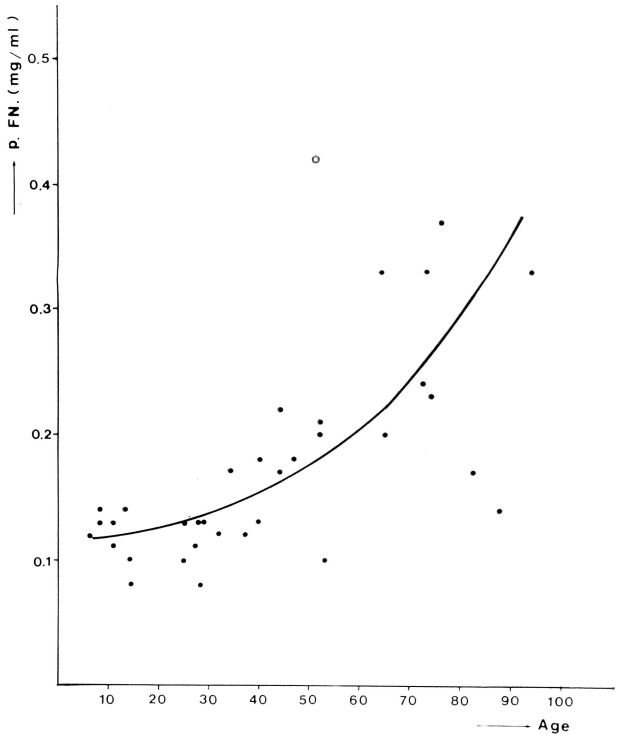

Fig. 5. The changes of plasma FN with aging. Open dots show Werner's syndrome. The correlation coefficient (r) is 0.72 (significant at $p<0.01$).

FN concentration in the sample plasma.

RESULTS

The summary of our observation on plasma FN concentration is shown in Table 1. Plasma FN concentration increased slightly up to about 50 years of age. Between 60 and 100 years there was a strong increase of plasma FN with age, up to the value more than the double of the average value found below 30 years (Table 2). The correlation coefficient between age and plasma FN concentration was 0.72 (Fig. 5).

We have also quantified the plasma FN from three patients with Werner's syndrome. Their plasma FN concentration was approximately three times higher than the average value of their age qroup (Tables 2, 3).

DISCUSSION

According to the previous reports,[1,6,14] the plasma FN concentration is about 0.3 mg/ml. Recently Matsuda *et al.*[11] have reported that the concentration of plasma FN was 26.4±4.1 mg/dl in twenties, 29.6±4.4 mg/dl in thirties, 36.8±6.0 mg/dl in forties and 36.6±9.3 mg/dl in fifties using the immune

TABLE 1. Summary of the observations on plasma fibronectin with aging.

No.	Age	Sex	FN (mg/ml)	No.	Age	Sex	FN (mg/ml)
1	6	F	0.12	18	40	F	0.13
2	8	F	0.14	19	40	M	0.18
3	8	F	0.13	20	44	M	0.17
4	11	F	0.11	21	44	F	0.22
5	11	F	0.13	22	47	F	0.18
6	13	F	0.14	23	52	F	0.21
7	14	F	0.08	24	52	M	0.20
8	14	F	0.10	25	53	M	0.10
9	25	M	0.13	26	64	F	0.33
10	25	M	0.10	27	65	F	0.20
11	27	M	0.11	28	72	F	0.24
12	28	M	0.13	29	73	F	0.33
13	28	M	0.08	30	74	M	0.23
14	28	M	0.13	31	76	F	0.37
15	32	F	0.12	32	82	M	0.17
16	34	M	0.17	33	88	M	0.14
17	37	M	0.12	34	94	M	0.33

TABLE 2. The average value of plasma fibronectin concentration in young and old human population.

Age	Number of samples	Plasma fibronectin (mg/ml±S.D.)
0 to 29 years	14	0.12±0.02
30 to 59 years	11	0.16±0.04
60 to 100 years	9	0.26±0.08

TABLE 3. The value of plasma fibronectin concentration in Werner's syndrome.

Age	Sex	Plasma fibronectin (mg/ml)
32	F	0.52
36	F	0.50
51	F	0.42

diffusion method. Labat-Robert *et al.*[13] have reported that the average value of plasma FN was 27.39±12.96 mg/dl between 0 and 15 years and 111.36± 26.14 mg/dl between 70 and 90 years using the laser nephelometric procedure. And Eriksen *et al.*[12] have reported that the plasma FN concentration was dependent on both sex and age. The plasma FN concentration in males was significantly higher than in females and the concentration increased with age. Although it seems difficult to simply make a comparison because of the difference in the quantification methods, our results are in conformity with those obtained

by Matsuda *et al.*[11] and Eriksen *et al.*[12]

The data obtained from the quantification of plasma FN in Werner's syndrome show the higher plasma FN concentration than the average value of their age group (Fig. 5). It is thought that these data coincide with the early expression of aging in this syndrome.

Recent peptide mapping and monoclonal antibody studies show that at least three different regions exist between cellular FN and plasma FN. All three regions of apparent structural difference can be mapped to the interior of the molecule, which appears to rule out the hypothesis that plasma FN is derived from cellular FN by proteolytic processing.[15,16] Nevertheless, the relationship between cellular FN and plasma FN is not yet understood. Therefore it is difficult at present to refer to the cause of the increase of plama FN concentration in old human population.

Chandrasekhar and Millis[17] have reported that FN from aged human skin fibroblasts was defective in promoting cellular adhesion. Labat-Robert *et al.*[13] have suggested that the deficient quality of "old" cellular FN was compensated by an overproduction of plasma FN in the aging human body. Currently we are examining the qualitative changes of cellular FN and plasma FN with aging.

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