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Relationship between Pentosidine and Pyridinoline Levels in Human Diabetic Cataract Lenses

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Summary **The relationship between the levels of two different crosslink compounds, pentosidine and pyridinoline, in human diabetic cataract lenses was investigated to elucidate the pathogenic mechanism of diabetic cataract. Subjects were classified into diabetes mellitus (DM) group and non-DM group according to the presence or absence of DM. The levels of the crosslink compounds were determined using high-performance liquid chromatography and spectrofluorometry after acid hydrolysis. In the non-DM group the pentosidine level was significantly and positively correlated with the pyridinoline level and age. In the DM group the pentosidine level was not significantly correlated with either pyridinoline level or age. Pyridinoline levels and age were not significantly correlated in either group. The increase in crosslink compounds due to glycation and the relationship between the compounds are changed in DM lenses.**

Key Words**: pentosidine, pyridinoline, crosslink, glycation, lens**

Introduction

Pentosidine is one of the advanced glycation endproducts (AGEs) formed by the glycation of proteins. Pentosidine was first isolated from the dura matter of the brain by Sell and Monnier [*1*], as a crosslinking compound with fluorescent properties in which a lysine and arginine residue are crosslinked with a pentose. It has been regarded as one of the senescent crosslinks that increase with aging.

Pyridinoline is a pyridinium crosslink compound belonging to the 3-hydroxypyridinium family, which was originally isolated from bovine Achilles tendon by Fujimoto *et al.* [*2*] in 1977. It plays an important role in the retention of fiber structure by contributing to the stabilization of the collagen molecular chain, and is considered to be a nonreducible mature crosslink of collagen that is formed under normal conditions. Pyridinoline is found most abundantly in bone but also in most other tissues including tendons, fascias, and arteries, and is known to be distributed mainly in types I and II collagen. Because it is excreted through the kidney as collagen breaks down, it is widely used clinically as a marker for bone metabolism to reflect bone resorption [*3*]. It has been clarified that urinary pyridinoline increases with bone growth, declines after 20 years of age, and tends to increase again after 50 years of age. But it has also been reported that pyridinoline levels in joint cartilage and the vitreous are not influenced by aging [*4*, *5*]. An animal study showed that a trace amount of collagen is also contained in the lens [*6*] though crystallin is the major protein of the lens. While pentosidine is synthesized disorderly and is known to change the properties of collagen and other compounds [*7*], pyridinoline is one of the mature crosslinks whose synthesis is controlled enzymatically.

Pentosidine and pyridinoline are non-disulfide crosslinks that exhibit non-tryptophan autofluorescence. Both pyridinoline and pentosidine are chemically extremely stable fluorescent substance and their simultaneous measurement

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Table 1. HPLC measurement conditions

Gel-filtration column:	TKS precolumn PW (4.6 mm × 3.5 cm)
ODS reversed-phase column:	TSK-GEL ODS-80T (4.6 mm × 15 cm)
Mobile phase:	50 ml/l acetonitrile containing 30 mmol/l HFBA
Flow rate:	1.0 ml/min
Sample injection volume:	160 µl

method using high performance liquid chromatography (HPLC) and fluorescent detector has been established by Takahashi *et al.* [*8*, *9*]. We have already reported the presence of these crosslinks in the vitreous of patients with diabetes mellitus (DM) [*5*], and changes in the pentosidine levels in the lenses of diabetic patients [*10*]. It is known that the pentosidine levels in lenses increase with aging, but to our knowledge there have been no published reports on the changes in pyridinoline levels in lenses. Because there is very little metabolic turnover of proteins in the lens, it is possible that pyridinoline accumulation in the lens is different than in other organs. If the levels of two substances which have totally different nature and formation process are measured at the same time, fluctuations in the ratio of both substances may reflect some pathology or condition in which these substances are involved. In this study we measured the levels of these two crosslink substances in cataractous lenses of diabetic patients and non-diabetic patients, and analyzed the correlation between these two types of crosslinks and the correlation between their levels and age in DM and non-DM cataractous lenses.

Materials and Methods

Lens samples were obtained from 29 patients who underwent cataract surgery at the Tsukuba Hashimoto Optical Clinic, and who were at the same stage according to Emery's classification. Informed consent was obtained from all patients. The subjects were classified into the DM group ($n = 12$, 60.0 ± 10.9 years) and non-DM group ($n = 17$, 66.6 ± 7.71 years) by the presence or absence of DM according to the diagnostic criteria of Japan Diabetes Society. Patients with a serum creatinine level higher than 1.3 mg/dl were excluded from this study as it is known that pentosidine levels are abnormally high in patients with nephropathy. Cataractous lens material was obtained by ultrasonic emulsification and aspiration, thus only the cortex and the nucleus but not the lens capsule were included in the samples for the present study. All samples were collected and stored at −30°C until analysis.

One ml of lens sample was mixed with an equal volume of 12 mol/l HCl, and hydrolyzed under nitrogen at 110°C for 20 h in a sealed glass tube. Hydrolysates were filtrated with membrane filters with a pore size of 0.45 µm (DISMIC-25 cs; Toyo Roshi, Tokyo, Japan). The filtrate (200 µl) was mixed with 5 ml of water, evaporated under reduced pressure with a TC-8 concentrator (Taitec, Tokyo, Japan), and the residue was dissolved in 200 µl of 1% n-heptafluorobutyric acid (HFBA).

Pentosidine and Pyridinoline were quantified using HPLC and spectrofluorometry (Ex/Em: 307/390 nm) according to the method of Takahashi *et al.* [*8*, *9*] (Table 1, Figs. 1 and 2).

Relationships between the pentosidine level and the pyridinoline level, the pentosidine level and age, and the pyridinoline level and age were analyzed using regression analysis and a p value <0.05 was considered significant.

Results and Discussion

In the lenses of the non-DM group, pentosidine levels were significantly and positively correlated with pyridinoline levels ($r = 0.545$, $p = 0.0223$) and age ($r = 0.590$, $p = 0.0112$) (Fig. 3). Increased levels of pentosidine due to aging are considered to be one of the factors for cataract development. The observed correlation between pyridinoline and pentosidine suggests that there is a link between their production processes, or that pentosidine crosslinks are added to fibers and capsules in which pyridinoline is already functioning, which lowers the metabolism of the fiber and the cell to lengthen the half-life.

Pyridinoline levels in the lens which tended to increase with age may reflect gradually deteriorating metabolism of pyridinoline, but it might also be because fiber cells accumulate in the center of the lens with aging to form the nucleus, and the weight and volume of the nucleus increase with aging, a phenomenon that is characteristic to the lens.

In the lenses of the DM group, pentosidine levels were not significantly correlated with either pyridinoline levels ($r = 0.572$, $p = 0.0511$) or age ($r = -0.088$, $p = 0.792$) (Fig. 4). No significant correlation was observed between any combination of pentosidine levels, pyridinoline levels, and age in the DM group. Pentosidine and pyridinoline levels were correlated ($r = 0.572$, $p = 0.0511$) but the significance was lost. It has been reported that the levels of pentosidine in the lenses of diabetic patients are elevated due to the hyperglycemia and oxidative stresses [*11*], but it is unlikely that

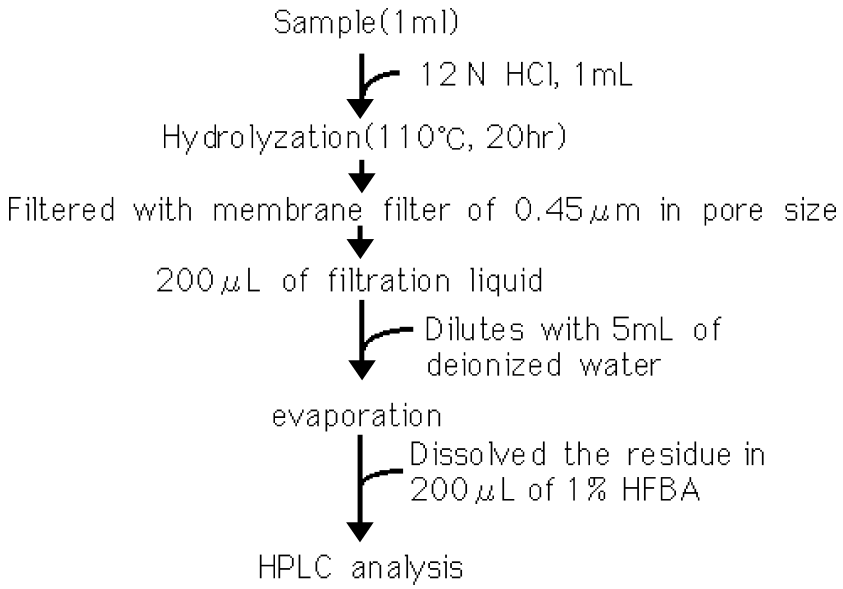

Fig. 1. Method for measurement of pentosidine and pyridinoline.

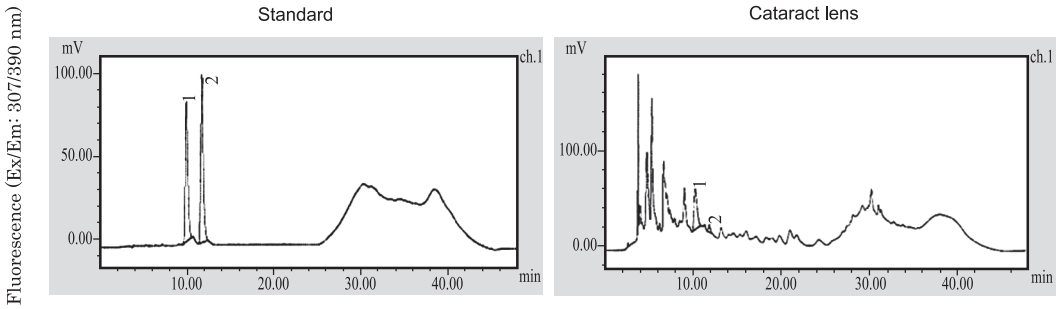

Fig. 2. Chromatograms of pentosidine and pyridinoline: standard materials and cataractous lens samples (1-pyridinoline, 2-pentosidine).

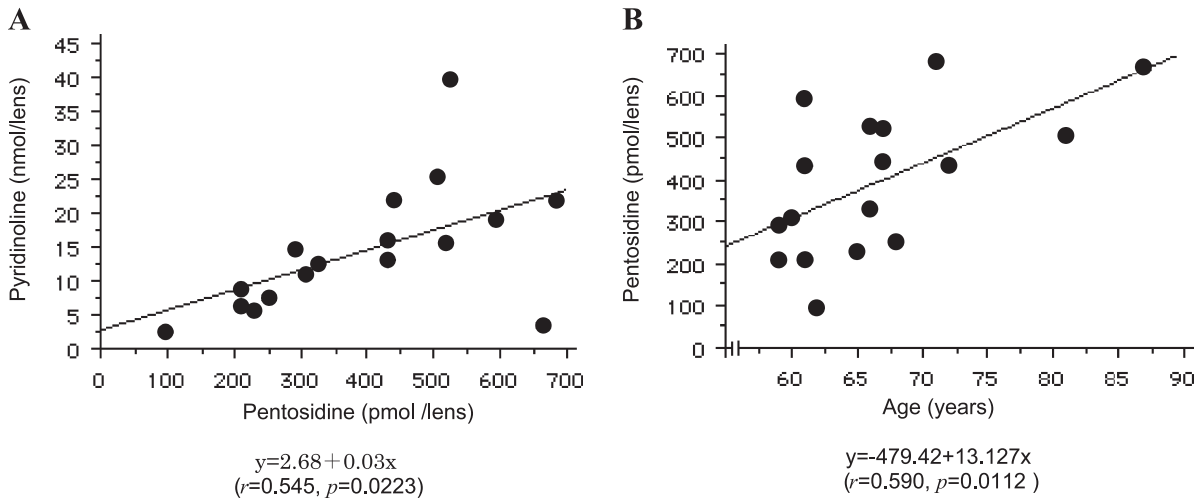

Fig. 3. Potential relationships in the non-DM group. A: pentosidine and pyridinoline level show positive correlation, $r = 0.545$, $p = 0.0223$ and B: pentosidine level and age also show a significant positive correlation, $r = 0.590$, $p = 0.0112$.

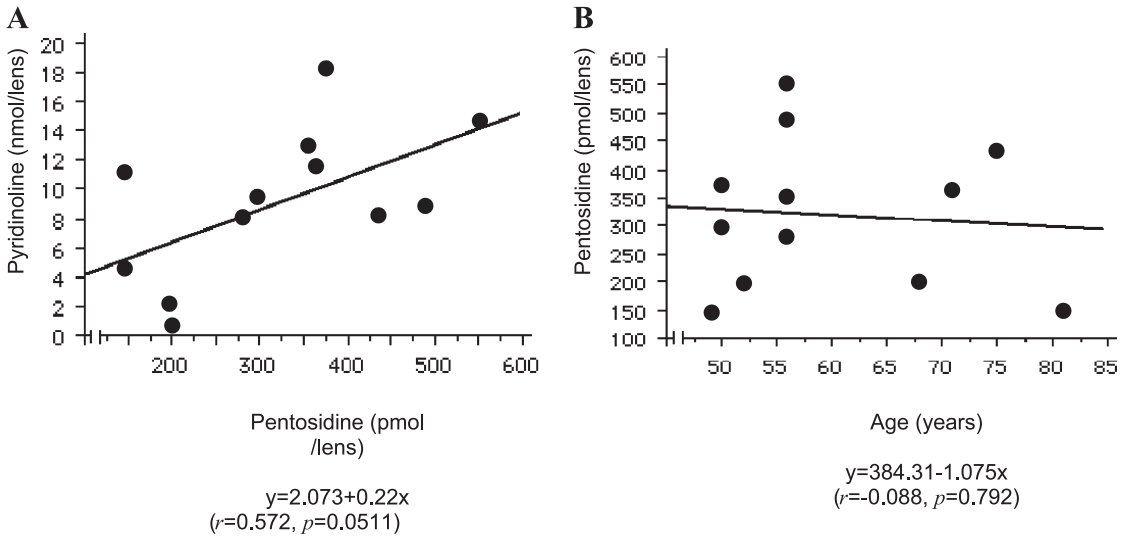

Fig. 4. Potential relationships in the DM group. A: pentosidine and pyridinoline level depicts a positive correlation, $r = 0.572$, $p = 0.0511$ and B: pentosidine level and age does not exhibit any relationship, $r = 0.088$, $p = 0.792$.

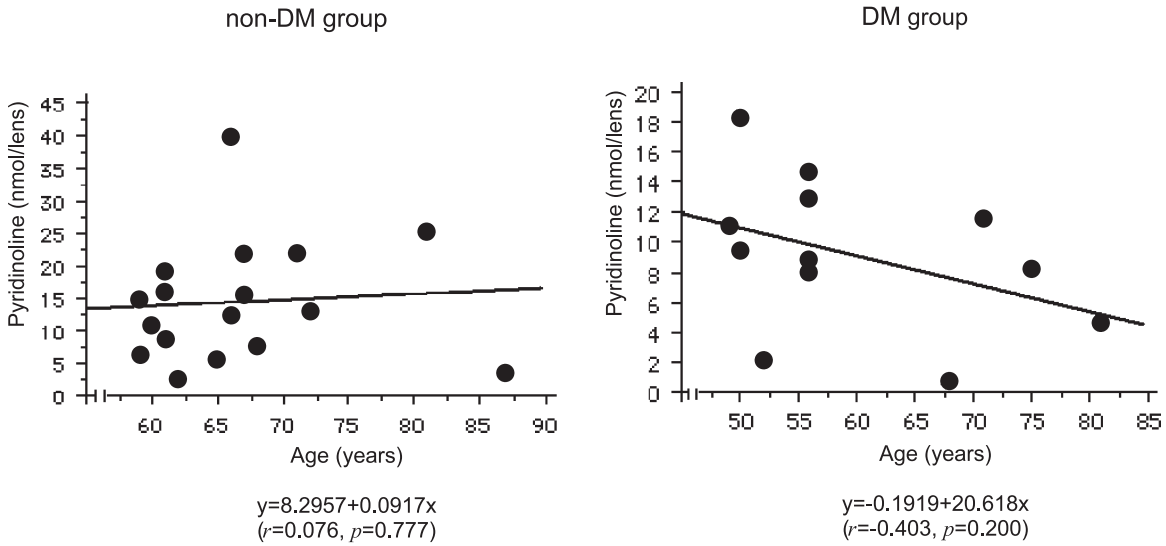

Fig. 5. Relationship between pyridinoline levels and age. non-DM group shows no relationship, $r = 0.076$, $p = 0.777$ and DM group depicts a negative correlation, but it is not significant, $r = 0.403$, $p = 0.200$.

production of pyridinoline is affected by blood sugar. Thus, it is inferred that in the lenses of diabetic patients the balance between pentosidine and pyridinoline is disrupted due to the disorderly increase of pentosidine by enhanced glycation. We plan to conduct further studies involving more patients in the future to clarify this.

Pyridinoline levels and age were not significantly correlated in either group (Fig. 5). In this study, the source of the detected pyridinoline was not clear. Because the sample did not contain lens capsules, it was inferred that pyridinoline crosslinks in the lens exist between anterior capsule and epithelial cells, between posterior capsule and fiber cells, between fiber cells, or between proteins like crystalline in the fiber cells of the cortex or in the nucleus, and is likely to function to maintain and stabilize nuclear structure and to maintain transparency as a trace component.

It is thought that the increase of AGE-related crosslinking compounds like pentosidine leads to the formation of proteins with massive molecular weight through the formation of crosslinks between water-soluble proteins, which increases the amount of insoluble proteins. These insoluble proteins are thought to cause light scattering and be involved

in the development of cataract. Increase in light scattering through the formation of crosslinks by AGEs was proven in an experimental model of α-crystalline aggregation through its glycation induced with methylglyoxal [*12*]. Also, it has been reported that the fluorescence of insoluble proteins remarkably increases in the cataractous lens with aging [*13*].

Also, it is very exciting from the viewpoint of the research of lens autofluorescence [*14*] that this substance, which exhibits autofluorescence at the wavelength relatively close to that of pentosidine, was discovered in the lens.

If we can clarify the relationship between such pathology and these substances by measuring their levels in lenses of cases with clear history of diabetes or clear glycemic control status or of nephropathy cases with large amount of AGE accumulation in the future, it may lead to a discovery of a new index that shows the accumulation status of AGEs *in vivo*. The question of how these crosslink compounds undergo changes in the lens, where protein metabolism is extremely low, will merit further research.

Conclusion

This is the first study that measured and reported the level of pyridinoline, a mature crosslink, in human lenses. Significant correlations were observed between pentosidine and pyridinoline levels and between pentosidine level and age in the lenses of subjects without DM. However, no such correlations were found in the lenses of patients with DM.

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