OPHTHALMOLOGICAL SIGNS AND LYSOSOMAL ENZYME ACTIVITIES IN PATIENTS WITH POLYSYSTEMIC DISEASES

Summary of Ph. D. Thesis

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ABBREVIATIONS

ACA	anti cardiolipin anticoagulant
AGU	α-glucosidase
AMAN	α-mannosidase
AMC	7-amino-4-methylcoumarin
ANA	antinuclear antibodies
BGA	β-galactosidase
CATH B	cathepsin B
CATH H	cathepsin H
CATH D	cathepsin D
CD	cluster differential
DFP	diisopropyl fluorophosphate
DPP I	dipeptidyl-peptidase I
DPP II	dipeptidyl-peptidase II
FITC	fluorescein isothyocyanate
GCU	β-glucuronidase
HEX	β-hexosaminidase
HLA-DR	human leukocyte antigen
KCS	keratoconjunctivitis sicca
LA	lupus anticoagulant
MHC	major histocompatibility
4-MU	4-methylumbelliferyl
PMNL	polymorphonuclear leukocytes
RA	rheumatoid arthritis
RF	rheumatoid factor
SLE	systemic lupus erythematosus
SS	Sjögren`s syndrome
TPP I	tripeptidyl-peptidase I

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1. INTRODUCTION

Sjögren`s syndrome (SS), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) are members of the family of systemic autoimmune diseases, in which the immune response is activated to recognize a limited variety of ubiquitously expressed autoantigens, and during which several target tissues may become damaged.

Features of SS may be found in almost every autoimmune rheumatic disease, including RA, SLE, scleroderma and others. The development of secondary SS associated with RA occurs on a different genetic background (HLA-DR4).

Early diagnosis and appropriate treatment are essential for optimal management of SS, and affect the patients` general well being and quality of life. At present, there are no specific diagnostic tests for SS and no universally accepted diagnostic criteria.

Many outcome parameters have to be recorded in order to assess the response for the treatment in clinical studies of RA. Only few laboratory markers, such as C-reactive protein or erythrocyte sedimentation rate correlate well with the inflammatory activity of RA. Since both parameters are frequently elevated in case of other conditions e.g. infections, there is currently no surrogate marker that specifically reflects the degree of joint inflammation. This is of importance, since the interobserver variability in assessing the clinical status of RA patients can be considerable.

Most autoantibodies are not specific for SLE and might be produced non-specifically as a result of polyclonal B cell activation. An ideal test would be specific, sensitive and have a high positive predictive value, and it should reflect disease activity, correlate with organ involvement, or predict relapse. No test or test panel can currently perform all these tasks because some of the clinical features of SLE are not antibody mediated.

Lysosomal enzymes seem to play an important role in many physiological and pathological processes. Alterations in their activity have been observed in a great variety of disorders, like inflammation process or neoplasias. Previous studies have also demonstrated increased activities of lysosomal hydrolaseses in the serum of patients with RA, SLE, dermatomyositis or psoriasis. We measured the activity of lysosomal enzyme activities in patients with adenocarcinomas of the gastroesophageal junction and the squamous cell carcinomas of the lower third of the esophagus. Our results and other studies have called our attention to compare the progression of the well known ophthalmic observations in polysystemic diseases to the lysosomal enzyme activities in leukocytes which were used for the diagnosis of other type of diseases.

2. CONCEPTUAL BACKGROUND

2.1 Sjögren`s syndrome

Sjögren's syndrome (SS), one of the most common autoimmune diseases, is characterized by progressive lymphocytic and plasma cell infiltration of the salivary and lacrimal glands leading to xerostomia and xerophthalmia, respectively. Other tissues and organs may also be affected with multiple extraglandular manifestations such as arthritis, cutaneous vasculitis, renal tubular acidosis, and chronic pulmonary disease. The most common form of ocular involvement is immune mediated noninfectious inflammation of the lacrimal glands and ocular surface resulting in decreased tear production and inflammatory changes on the ocular surface, known as SS associated keratoconjunctivitis sicca (KCS). Some common signs of KCS are conjunctival injection, mucous strands, filamentary keratitis, tear film debris, and a punctate keratopathy. Advanced KCS may lead to serious complications, including keratitis, symblepharon, pannus, corneal thinning, and ceration with perforation.

2.2 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease primarily affecting the synovial membrane and leading to joint damage and joint destruction. Apart from the potentially destructive joint manifestations of the disease, it is also characterized by systemic features. Rheumatoid arthritis is associated with numerous ophthalmic signs and symptoms. The most significant complications include KCS, keratitis, sclerokeratitis, scleritis, scleromalacia perforance, and uveitis. Rapid detection and early treatment decreases the ocular complications and increases the overall quality of the life of patients.

2.3 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic generalized inflammatory connective tissue disease with characteristic autoantibodies primarily affecting the skin, musculoskeletal system, serous membranes, kidneys, central nervous system (CNS), and cells of the blood. Acute exacerbations of disease activity are followed by periods of remission. About 80%-90% of SLE patients are women. KCS, with or without xerostomia and retinopathy are the most common ocular manifestations. Involving the retina, cotton-wool spots and retinal hemorrhages are the most frequently reported findings, but retinal edema, hard exudates, microaneurysms, and vascular tortuosity have also been noted. Lupus retinopathy can occur as an independent manifestation of the underlying disease process. Central retinal vein occlusion has also been reported in patients with SLE, but appears to be less common than arterial occlusive disease.

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List of abstract directly related to the subjects of the Thesis:

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2.4 Lysosomal enzymes

Human polymorphonuclear leukocytes (PMNL) play a fundamental role in many inflammatory diseases. Their proinflammatory activity is exerted trough the release of different chemical mediators, such as preformed mediators, including several lysosomal enzymes and de novo synthesized mediators, like oxygen-free radicals.

Lysosomal enzymes are common in human body fluids, such as serum, urine, and tears. Some studies have also revealed the lysosomal enzyme activities in aqueous humor and subretinal fluid, but so far no study has investigated the specific activities of lysosomal enzymes from the leukocytes of patients with SS, RA, and SLE.

Specific activity of some lysosomal enzymes have been demonstrated in the anterior segment of the eye, in ciliary body, corneal epithelium, stormal keratocytes, endothelium, as well as lens epithelium.

3. AIMS

Based on previous studies, we hypothetized that

- the lysosomal glycosidase and protease activities in leukocytes may provide information in the role of tissue damage by enzymes in polysystemic autoimmune disease,
- proteinases and glycosidases from leukocytes might represent potential markers of the disease activity.

In order to get answer for the hypotheses above, patients with primary SS, RA, and SLE were chosen for our investigations.

The aims of this study were:

- the ophthalmic examinations of patients in the three groups of autoimmune diseases and controls,
- to measure the activities of lysosomal glycosidases and proteases in leukocytes of the patients in the three groups of autoimmune diseases and controls,
- to find a potential correlation between the ophthalmic signs and activity values of lysosomal enzymes in patients of the three autoimmune diseases,
- to determine whether activities of enzymes in leucocytes might be markers for monitoring the progression of SS, RA, and /or SLE.

4. PATIENTS AND METHODS

4.1. Patients

Thirty-eight patients with primary SS (1 male, 37 female) with a mean age of 53 years; thirty persons (5 males and 25 females), mean age 56 years, who were registered as RA patients; thirty-seven SLE patients (1 male and 36 females) with a mean age of 45 years, and 36 healthy subjects (12 males and 24 females) with a mean age of 50 years were selected randomly for this study.

The control group was without any autoimmune or other documented disease. SS, RA and SLE patients were subdivided into 3 groups based on the duration of the illness: patients were diagnosed 1) less than 5 years, 2) 5-10 years, and 3) more than 10 years prior to enrollment. In cases of SS, the first group had nine, the second group had seven, and the third group had twenty-two patients. In cases of RA: fourteen, ten, and six patients were in the 1st, 2nd, and 3rd groups, respectively. Sixteen SLE patients were in the first, ten in the second, and eleven in the third group.

4.2. Ophthalmic examinations

Patients with SS, RA, and SLE and the control subjects underwent standard ophthalmologic examinations including visual acuity tests, intraocular pressure with applanation tonometer, slit-lamp biomicroscopic examination of the anterior segment of the eye using slit-lamps, and ophthalmoscopic examination with direct ophthalmoscope and 90 D lense. The patients` perimetry was carried out by TAP and Goldmann perimeter. Additionally, all subjects were evaluated for KCS using the Schirmer I test (without any anesthetics), break-up time test (BUT), and rose bengal score measurements. The ocular tests were considered positive as follows: wetting of ≤5 mm of the paper strip in 5 minutes on the Schirmer I test, BUT ≤10 seconds, and rose bengal score of ≥4 on the von Bijsterweld scoring system.

4.3. Sample preparation for enzymatic assay

Blood samples were taken using Vacutainer® cell preparation tubes containing sodium citrate. For leukocyte purification, a heparin-dextran solution was added to the blood according to Johnson et al.. After incubation at room temperature for 1–2 hours, the upper layer was removed and centrifuged at 870 x *g* for 10 minutes. The pellet was resuspended in 1 mL normal saline plus 3 mL distilled water and centrifuged at 515 x *g* for 8 minutes. The pellet was free of red blood cells, and was resuspended in 4 mL normal saline and centrifuged at 515 x *g* for 8 minutes. After repetition of the last step, the leukocytes were homogenized in 0.15 M NaCl in 0.1% Triton-X 100 with a “Brinkman” Polytron homogenizer.

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8. SUMMARY AND CONCLUSIONS

- Significantly increased lysosomal enzyme activities (proteases and glycosidases) were found in leukocytes from patients who had been suffering from SS for more than 5 years, but less than 10 years.

- The changes in lysosomal enzyme activities indicate that these enzymes might play a role in SS-associated tissue injury. The activities of glycosidases, which are important in glycoprotein breakdown, and proteases, DPP I and TPP I, which play a role in proteolytic and in collagen degradation, were elevated.

- In the latter stages of SS (>10 years), the presence of ophthalmic signs may be a consequence of the long-lasting tissue injury caused by elevated lysosomal enzymes.

- We can conclude from our results that elevated levels of two glycosidases (AGU and HEX) and some lysosomal proteases (DPP I, CATH B and CATH D), but not TPP I or DPP II, are associated with RA. Lysosomal cysteine and aspartate peptidases may be responsible for the elevated level of protein destruction in RA.

- All of the enzymes showed higher elevation among those RA patients who had keratitis, except for DPP I, CATH B and CATH D, while those patients who had any kind of corneal degeneration showed high elevation of CATH B.

- Since CATH B showed the highest enzyme activity increase in the three groups of RA patients investigated, it may provide a reliable marker for monitoring the progression of RA.

- In cases of patients with SLE, there were almost the same number of patients with atrophy of the optic nerve in the second (5-10 years) and in the third group (≥10 years) and higher elevation of enzyme activities was found among these cases. All of the glycosidases and the proteases, but CATH H and CATH D show the progression of the disease.

- We suggest that the measurement of lysosomal enzyme activities in leukocytes and ophtalmic observations will be of great value for further evaluation of these enzymes as a prognostic marker in polysystemic autoimmune diseases and tissue damage.

The homogenized material was then centrifuged, and the supernatant was used for the enzyme assays and protein determination.

4.4. Measurement of enzyme activities

Glycosidase activities were measured with the use of 4-methylumbelliferyl (4-MU) substrates. Protease assays with 7-amino-4-methylcoumarin (AMC) substrates or FITC-hemoglobin were carried out as described previously. Samples of 5, 10 or 25 µL were diluted 2-, 4-, and 8-fold with 0.15 M NaCl-0.1% Triton-X 100. Duplicates from each dilution were transferred to 96-well microtiter plates. The enzyme reaction was initiated by addition of a solution containing a substrate in buffer, and the plates were incubated at 37 °C for 30–180 min. Assays involving 4-MU and AMC substrates were stopped by adding 100 µL 0.5 M glycine-NaOH buffer at pH 10.5, or 100 µL 0.1 M monochloroacetic acid in 0.1 M acetic acid at pH 4.3. The amount of 4-MU or AMC liberated at the assay is proportional to the enzyme activity; the unit for both assays is nmol/h/mg protein. The substrates were obtained from Sigma (St. Louis, MO, USA), except for the DPP I substrate (H-Gly-Arg-AMC) and the cathepsin L inhibitor (Z-Phe-Phe-diazomethylketone) for the CATH B assay, which were obtained from Bachem Bioscience Inc. (King of Prussia, PA, USA).

The emission (λ_{ex} 360 nm, λ_{em} 460 nm) was measured with a Cytofluor II, Fluorescence multi-well plate reader (CytoFluor 4000, PerSeptive Biosystems, Inc., Framingham, MA, USA). The activities were normalized to the protein content in the sample measured according to the method of Lowry et al..

4.5. Statistical analysis

Data are presented as mean±SE. Statistical significance was assessed by the Student *t* test and the Kruskal-Wallis one-way ANOVA on ranks. The results were considered significant, if $p \leq 0.05$.

4.6. Ethics

This study was conducted in accordance with the Declaration of Helsinki. This medical research was subject to ethical standards that promote respect for all human beings and protect their health and rights. It conformed to generally accepted scientific principles based on a thorough knowledge of scientific literature, other relevant sources of information, and on adequate laboratory experimentation. The experimental protocol was approved by the ethical review committee of the University of Szeged. The right of research subjects to safeguard their integrity was always respected.

5. RESULTS

5.1. Sjögren`s syndrome (SS)

5.1.1 Ophthalmological signs of patients with SS

Filamentary keratitis (32%) and a decreased tear production (55%) were more marked in the third group of SS patients (who were sick for more 10 years), than that of in the second group (5-10 years) with 14% and 29%, respectively. Cupping of the optic disc was only found in the third group (≥10 years).

We also found that all of the SS patients had KCS and one patient had conjunctival degeneration (pinguecula), too. The next observed ophthalmological signs did not differ with the duration of the disease: corneal degeneration (4 patients), cataract (4 patients), atrophy of the optic nerve (26 patients), and visual field defects (12 patients). In two cases, we found maculopathy, and one patient exhibited macular degeneration.

5.1.2 Lysosomal enzyme activities in leukocytes of patients with SS

In all the SS patients, significantly increased activities of the glycosidases BGA, AMAN, GCU, HEX; and the proteases CATH B, DPP I, TPP I, and CATH D were found as compared with the controls.

In the first group (SS was recognized less than 5 years prior to our investigations), the activities of BGA, AMAN, GCU, HEX, CATH B and DPP I were increased significantly as compared to the controls. In the second group (5-10 years of sickness), all of the measured lysosomal enzyme activities were elevated as compared both to the controls and to the first SS group, except for two enzymes: CATH B, which showed elevated values only when compared to the control group and AGU, which activity was not higher than that of the controls. In the third group (≥10 years), the enzyme activities were less than in the second group, but the activities of AMAN, GCU, and HEX remained significantly elevated compared to the control values.

5.1.3 Association between lysosomal enzyme activities and ophthalmologial signs of patients with SS

The Schirmer I test, performed according to precious studies was the most accurate among all of the published functional tests. Enzyme activities increased to a greater degree in subjects with SS whose Schirmer I test was >5 mm in 5 min (n = 18) than in those patients whose Schirmer I test was ≤5 mm in 5 min (n = 20). DPP I, AMAN, GCU, and HEX activities were significantly increased in case of subjects whose tear production was ≤5 mm in 5 min, as compared to the control group. Direct correlation has been found between the activity of CATH B and D and the Schirmer I test (data not shown). Additional studies should be conducted to determine the reason for this relation.

concluded in agreement with Florakis et al. that this symptom is not related to the age of the patients.

In every individual with RA, we found significant correlation between the presence of keratitis and elevation of specific activities of the measured glycosidases, but BGA and DPP II. On the other hand, CATH B also showed elevated activity, but the elevation did not correlate with the observed keratitis (Figure 5). CATH B and CATH D showed significant elevation among those patients who had any other kind of corneal degeneration. There were only three patients who had keratitis and also three who had corneal degeneration, so a study of larger populations is needed to confirm the prognostic importance and clinical relevance of this factor in RA.

KCS is the most common ocular manifestation of SLE, but visual morbidity is usually due to retinal and neuro-ophthalmic manifestations of the disease. Ocular manifestations of lupus are significant in that they reflect extraocular involvement of the disease. Their presence should alert the clinician to the likely presence of extraocular disease activity. Less patients had atrophy of the optic nerve (30%) in the third group (≥10 years) than in the second one (5-10 years) (40%). All of the enzymes showed higher elevation among those patients who had optic nerve atrophy, except for CATH H and CATH D. This means, that all of the glycosidases and the proteases, but CATH H and CATH D could show the progression of the disease in case of SLE.

information about the role of these enzymes in the progress of the disease. AGU, AMAN, GCU, HEX (glycosidases), and CATH D showed moderate increase of the activity. In contrary, the elevation of DPP I and CATH B activity was much more emphasized. They were the highest in the group of patients that were sick more than 10 years. DPP II and TPP I and the cysteine protease CATH H activities did not change during the observation period, as we predicted by the substrate specificity.

In contrast to a previous study, our investigations demonstrated that in cases of patients with SLE, all of the lysosomal enzymes being investigated showed significant increase except for CATH H. BGA, CATH C, and DPP I. showed the highest enzyme activity increase in the second group, which was similar in case of SS patients. The elevation of enzyme level in cases of GCU and HEX was similar in the three groups of patients. TPP I, CATH B and CATH D had the highest enzyme activities in the first group of patients, who were diagnosed less than 5 years before the investigation. The other enzymes did not show any characteristic pattern.

6.3 Relation between ophthalmic findings and elevated lysosomal enzyme activities

The reduction in tear secretion noted in subjects with SS of ≥10 years' duration was consistent with the aforementioned results, as the increased release of proteolytic and glycolytic enzymes in the lacrimal glands could cause degradation of the glandular extracellular matrix, thus leading to this effect

Filamentary keratitis, a severe ophthalmologic sign in SS patients, was also seen mostly in subjects with SS of ≥10 years' duration. However, enzyme activities were higher among patients who did not have this ophthalmic disorder, except in the cases of AGU, CATH B, and CATH D (Figure 3). Thus, lysosomal enzyme activities in the leukocytes of subjects with SS appeared to follow the state of the disease in the first 10 years. The relatively lower elevations of activities in patients with SS of ≥10 years' duration as compared to those who were sick for 5-10 years were not indicative of recovery from the disease, as evidenced by the relatively higher incidence of keratitis in this group. Rather, it suggests that enzyme activity cannot increase any further because of the slower protein synthesis in this stage of the disease; a similar phenomenon is seen with late-stage starvation and cancer. In our study, the mean age in the first group (ill for <5 years) was 50 years, and in the second (5-10 yesrs) and third group (>10 years), it was 57 years of age. The findigs of Florakis et al. showed that the cupping and atrophy of the optic disc are a consequence of the disease and are not correlated with the age of the patients. We found atrophy of the optic disc only in the third group. Since there is not much difference between the mean age of the groups, we

Excessive corneal dryness may lead to the appearance of mucous threads attached to the cornea, a condition known as filamentary keratitis. Lysosomal enzyme elevations versus controls were lower in patients with SS who had filamentary keratitis (n = 27) compared with those subjects who did not (n = 11) with the exception of AGU, CATH B, and CAH D, which were mildly elevated.

The specific enzyme activities and some of the ophthalmologic findings depended on the duration of SS, but they were independent on the severity of the disease.

5.2. Rheumatoid arthritis (RA)

5.2.1 Ophthalmological signs of patients with RA

We found that 16 patients had KCS which is the most frequent ophthalmological symptom of RA. Distorsion of the episcleral blood vessels, cataract, angiopathy, atrophy of the optic disc and visual defect were found at 5, 9, 8, 10, and 5 patients, respectively. Keratitis, corneal degeneration, corneal ulcer and maculopathy were found at three, while pseudoexfoliation and conjunctival degeneration were found at two patients.

Keratitis (22%) were more common in the second group (5-10 years), while corneal degeneration occurred mainly both in the second (5-10 years) and in the third group (≥10 years) (10%-10%).

5.2.2 Lysosomal enzyme activities in leukocytes of patients with RA

Significantly increased activities were found in case of AGU, HEX, DPP I, CATH B and CATH D calculated with the results of all patients in the three groups, while BGA, AMAN, CATH H, DPP II and TPP I did not show any significant changes.

Significantly decreased lysosomal enzyme activities were found in case of BGA in the first group (who were diagnosed with RA less than five years before our investigations); but in case of AMAN, GCU, HEX, CATH H, DPP II, and TPP I the decrease was not significant. The enhancement in the enzyme activities was the highest in the third group (who were sick for more 10 years) with the exception of BGA. CATH B showed the greatest enzyme activity increase in all of the 3 groups of patients. The activities of CATH B were 7 times, 4 times, and 8 times higher in the first, second, and third group, respectively than in the control group.

5.2.3 Association between lysosomal enzyme activities and ophthalmologial signs of patients with RA

Figure 5 demonstrates the correlation between the presence of keratitis and the specific enzyme activities. All of the enzymes showed higher elevation
while those patients who had any kind of corneal degeneration showed high elevation of CATH B.

5.3. Systemic lupus erythematosus (SLE)

5.3.1 Ophthalmological signs of patients with SLE

We found that 18 patients had KCS, 4 and 5 had corneal- and conjunctival degeneration, respectively, 6 had cataract, 10 had angiopathy of the fundus, 7 had atrophy of the optic nerve, 4 had visual field defect, and 1 had retinal branch artery occlusion.

Both optic neuropathy and retinal occlusive disease can result in optic atrophy. We investigated the occurance of the optic nerve atrophy in the three groups of patients. The number of patients with atrophy of the optic nerve was less (30%) in the third group ($\geq$10 years) than in the second one (5-10 years) (40%).

5.3.2 Lysosomal enzyme activities in leukocytes of patients with SLE

Significantly increased activities of the glycosidases and proteases were found in all SLE patients as compared to the controls with the exception of CATH H. In all of the SLE patients, significantly increased activities of the glycosidases (AGU, BGA, AMAN, GCU, HEX), and the proteinases (CATH C, DPP I, TPP I, CATH B, CATH D) were found, as compared with controls ($p \leq 0.05$). In cases of AGU and AMAN, the enzyme activities decreased in the second group (SLE was diagnosed more than 5, but less than 10 years prior to our investigations), and than increased again in the third group (> 10 years). In cases of GCU and HEX, the same amount of enzyme level elevation was shown in all three groups. TPP I and CATH B had the highest enzyme activities in the first group of patients, who were diagnosed less than 5 years before our investigations. BGA, CATH C, DPP I and II, and CATH D showed the highest elevation in the second group of SLE patients.

5.3.3 Association between lysosomal enzyme activities and ophthalmologial signs of patients with SLE

All of the enzymes showed higher elevation among those patients who had optic nerve atrophy, except for CATH H and CATH D.

5.4. Comparison of the results of the three polysystemic autoimmune diseases

Significant changes were found in specific lysosomal enzyme activities in leukocytes from patients of the three groups of investigated polysytemic autoimmune diseases compared to the healthy controls. We found, that in the cases of patients with SS, the highest enzyme activity elevations were observed in the second period of SS (5-10 years). Whereas, most of the enzyme activities were lower in the third group of SS patients ($\geq$ 10 years) than in the second group.

In occurrence of RA, contrary to the results of SS, the increase in the enzyme activities was the highest in the third group (who were sick for more than 10 years) with the exception of BGA.

In case of patients with SLE, we found that the activity changes of lysosomal enzymes during the progression of the disease showed a high variety. Similar pattern in the elevation of activities of BGA, DPP I, and CATH H has been observed in the patients with SS.

6. DISCUSSION

6.1 Ophthalmological signs in patients of the three polysystemic autoimmune diseases

Many ophthalmological signs were seen even in the first groups of patients diagnosed with SS, RA, or SLE who were sick less than 5 years. Different signs were characteristic for the investigated three polysystemic autoimmune diseases, except for KCS which was the most frequent ophthalmological symptom in each disease. Most of the observed ophthalmological signs differ with the duration of the disease. Interestingly, less patients had corneal degeneration, cataract, atrophy of the optic nerve, and visual field defects in case of SS and atrophy of the optic nerve in case of RA and SLE in the third group ($\geq$10 years) than in the second one (5-10 years).

6.2 Changes in lysosomal enzyme activities

Changes in lysosomal enzyme activities may result in impairment of phagocytic and endocytic activities, inadequate extracellular matrix turnover, and remodeling, which suggest that lysosomal enzyme activities might be involved in the pathogenesis of autoimmune diseases. Therefore, we supposed that activities of lysosomal enzymes might also show correlation with ophthalmic signs of the disease.

We found that acid glycosidases, except for AGU, showed 2- to 3-fold higher elevation of activities than that of the control values. These were the greatest increases among the lysosomal enzymes mesured in the leukocytes of individuals with SS. These enzymes play a role in catabolism of glycoproteins to monosaccharides and amino acids. All of the six enzymes studied were extremely hyperactivited in subjects with SS of 5-10 years' duration. We experienced that the proteases with the most elevated activities in SS are TPP I and DPP II.

In cases of RA patients we found that the activities of two lysosomal cysteine proteases (CATH B and DPP I), two glycosidases (AGU and HEX), and an aspartic protease (CATH D) were elevated, but these elevations can not give