

# SERUM $\beta_2$ -MICROGLOBULIN CONCENTRATION CORRELATES WITH URINARY CONCENTRATIONS OF TYPE 1 COLLAGEN CROSS-LINKED N-TELOPEPTIDES AND DEOXYPYRIDINOLINE IN RHEUMATOID ARTHRITIS

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**Background:** Determination of serum  $\beta_2$ -microglobulin concentration, an invasive procedure, has been advocated for monitoring patients' response to treatment in rheumatoid arthritis. The object of this study was to find out if serum  $\beta_2$ -microglobulin concentration correlated with urinary excretions of type 1 collagen cross-linked N-telopeptides (NTx) and deoxypyridinoline (Pyrilinks-D) in rheumatoid arthritis (RA).

**Subjects and Methods:** Using chemiluminiscent assay, serum  $\beta_2$ -microglobulin concentrations were estimated in 25 female patients with active RA, 25 female with inactive disease, and 25 age-matched healthy female controls. Concentrations of NTx and Pylilinks-D were also determined by immunoabsorbent assays in spot urine samples from these subject groups.

**Results:** The serum concentration of  $\beta_2$ -microglobulin in patients with RA ( $7.45 \pm 2.10$  mg/L) was significantly higher ( $P < 0.001$ ) than the concentrations in patients with inactive disease ( $3.33 \pm 0.76$  mg/L), or than in normal healthy controls ( $2.74 \pm 0.52$  mg/L). Similarly, in patients with active RA, the spot urinary concentrations of NTx ( $123.08 \pm 25.53$  nmol BCE/mmol creatinine) and Pylilinks-D ( $15.08 \pm 3.29$  nmol/mmol creatinine) were significantly higher ( $P < 0.01$ ) than those in patients with inactive disease ( $58.42 \pm 12.65$  nmol BCE/mmol creatinine and  $10.10 \pm 2.43$  nmol/mmol creatinine, respectively). In patients with active RA, serum concentration of  $\beta_2$ -microglobulin correlated positively with spot urinary NTx concentrations ( $r = 0.9910$ ,  $P = 0.0001$ ), and Pylilinks-D concentration ( $r = 0.6177$ ,  $P = 0.001$ ).

**Conclusion:** In patients with active RA, the spot urinary concentrations of NTx and Pylilinks-D correlated positively with serum  $\beta_2$ -microglobulin. Therefore, the estimations of these urinary markers may take the place of serum  $\beta_2$ -microglobulin estimation in monitoring the patient's response to treatment in rheumatoid arthritis.

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**Key Words:** Serum  $\beta_2$ -microglobulin, N-telopeptides, deoxypyridinoline, rheumatoid arthritis.

$\beta_2$ -microglobulin, a polypeptide with a molecular weight of 11.7 KD, is produced and secreted by both T and B lymphocytes.<sup>1,2</sup> It is, therefore, found in serum and many other biological fluids.<sup>3</sup> It forms part of the light chain of the HLA class I molecule,<sup>4,6</sup> and its amino acid sequence shows close homology with the constant regions of immunoglobulin heavy and light chains.<sup>7,8</sup> Increased serum  $\beta_2$ -microglobulin concentrations have been reported in connective tissue diseases,<sup>9,10</sup> and there is a report associating serum  $\beta_2$ -microglobulin concentration with disease activity in rheumatoid arthritis.<sup>11</sup> However, there is no report of any association between serum  $\beta_2$ -microglobulin and urinary excretions of markers of bone metabolism in rheumatoid arthritis. The purpose of this

study was to find if there was any association between serum  $\beta_2$ -microglobulin concentration and urinary excretions of type 1 collagen cross-linked N-telopeptides (NTx) and deoxypyridinoline (Pylilinks-D) in patients with rheumatoid arthritis (RA). If there was any association, then the estimation of these urinary metabolites which involve noninvasive techniques might be preferable to the estimation of serum  $\beta_2$ -microglobulin, which involves an invasive method.

## Subjects and Methods

We studied 50 selected ambulant female patients who fulfilled the American College of Rheumatology (formerly The American Rheumatism Association) criteria for rheumatoid arthritis.<sup>12</sup> Their ages ranged from 28 to 45 years (mean  $\pm$  SD =  $40.28 \pm 6.07$  years). All patients were receiving at least one disease-modifying antirheumatic drug, including methotrexate, hydroxychloroquine, sulfasalazine and imuran. None of the patients was on steroids and none had liver or renal disease or extra-articular manifestation of RA. The activity of RA was

TABLE 1. Demographic, clinical and laboratory features of patients with rheumatoid arthritis (RA) and controls.

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Features	Controls (n=25)	Patients with active RA (n=25)	Patients with inactive RA (n=25)
Age (years)	40.87±8.45	41.19±8.31	40.94±7.48
Disease duration (years)	–	9.4±3.8	7.1±3.69
Morning stiffness (minutes)	–	80±37.0	25±12.0
Tender joint count	–	7.36±1.08	3.4±1.2
Swollen joint count	–	5.48±1.12	1.5±1.0
ESR (mm/hr)	–	38.2±9.5	16.4±1.8
Steroid use	–	None	None
DMARDs used	–	Yes	Yes
Extra-articular manifestations	–	None	None

All patients are female. Data are presented as the mean±standard deviation (SD); DMARDs=disease-modifying antirheumatic drugs.

assessed with the following indices: swollen joint count (range 0-66); tender joint count (range 0-69); duration of morning stiffness (minutes); patient's assessment of pain by the visual analog scale (VAS range 0-100 mm); patient's and physician's global assessment of disease activity (0 = asymptomatic, 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe); and Westergren erythrocyte sedimentation rate (ESR) in mm/hr. Patients with active disease (n=25) were defined by the presence of at least three of the following six criteria: six or more tender joints; three or more swollen joints; morning stiffness lasting more than 30 minutes; and Westergren ESR of more than 30 mm/hr. Disease activity should also have been present in the patient for at least four weeks. The controls selected were 25 normal, healthy age-matched female subjects who were not on any medication. Informed consent was obtained from each subject. Table 1 summarizes the demographic and clinical data of the study groups.

#### Collection of Urine and Blood Samples

Spot urine samples were collected from each patient and control subject in the daytime, avoiding the first morning urine. Aliquots of urine samples were kept without additives at -70°C until analysis. A blood sample was collected from each patient and control at the time of urine collection and serum was separated and kept frozen at -70°C.

#### Determination of Urinary NTx

The Osteomark kit (Ostex International Inc., Seattle, WA, USA), an enzyme-linked immunoabsorbent assay kit for the measurement of cross-linked N-telopeptides of type I collagen (NTx) in human urine, was used for determination of urinary NTx. The manufacturer's instructions for the assay were followed. Duplicate measurements were performed for each urine sample and the results were expressed as nanomoles (nmol) of bone

collagen equivalent (BCE) per millimole (mmol) urinary creatinine measured on a Hitachi 911 autoanalyser. The intra-assay and inter-assay coefficients for NTx were 12.65% and 14%, respectively.

#### Determination of Urinary Pyrilinks-D

The Pylilinks-D assay (Metra Biosystems Inc., CA, USA), which is a competitive immunassay in a micro titer strip well format utilizing a monoclonal antideoxy-pyridinoline (Dpd) antibody coated on the strip to capture Dpd was used to determine the urinary concentrations of Pylilinks-D. The manufacturer's instructions for the assay were followed. Dpd in the urine samples competed with conjugated Dpd-alkaline phosphatase for the antibody and the reaction was detected with a pNPP substrate. Duplicate measurements were performed for each urine sample and the results were expressed as nmol Dpd/mmol creatinine measured on a Hitachi 911 autoanalyser. The intra-assay and inter-assay coefficients of variations were 4.8% and 4.6%, respectively.

#### Determination of Urinary Creatinine Concentration

Urine creatinine was measured in each sample routinely on the Hitachi 911 autoanalyser.

#### Determination of Serum $\beta_2$ -Microglobulin Concentration

This was done using the Immulite automated analyser (Diagnostic Product Corporation, Los Angeles, CA, USA) designed for the quantitative measurement of  $\beta_2$ -microglobulin in serum and urine. Briefly, the Immulite  $\beta_2$ -microglobulin automated analyser is a solid phase, two-site chemiluminescent immunometric assay. The solid phase, a polystyrene bead enclosed within an immulite test unit, is coated with a polyclonal antibody specific for  $\beta_2$ -microglobulin. The sample and alkaline phosphatase-conjugated monoclonal antibody were incubated for 30 minutes at 37°C in the test unit with intermittent agitation.  $\beta_2$ -microglobulin in the sample formed an antibody sandwich complex. Unbound conjugate was then removed by a centrifugal wash, after which substrate was added and the test unit incubated for a further 10 minutes. The bound complex, measured by the immunometer, was proportional to the concentration of  $\beta_2$ -microglobulin in the sample. The intra-assay and inter-assay coefficients of variations were 5.6% and 8.5%, respectively.

#### Determination of Serum Type I Collagen Carboxyterminal Telopeptide (ICTP) Concentration

The Incstar (Incstar Corporation, Minnesota, USA) 125<sub>I</sub> RIA kit for the quantitative determination of carboxyterminal telopeptide of type I collagen in human serum was used. The Incstar ICTP assay is an equilibrium

TABLE 2. Concentrations (mean±SD) of serum  $\beta_2$ -microglobulin, ICTP, and spot urine NTx and Pylilinks-D in rheumatoid arthritis (RA).

Parameters	Patients with active RA (n=25)	Patients with inactive RA (n=25)	Healthy control	Significance
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Serum $\beta_2$ -microglobulin (mg/L)	7.45±2.10	3.37±0.76	2.74±0.52	a, b
Serum ICTP ( $\mu$ g/L)	9.52±1.56	4.08±0.96	3.43±1.03	a, b
Urinary NTx (nmol BCE/mmol creatinine)	123.08±25.53	58.42±12.65	53.37±10.65	a, b
Urinary Pylrilinks-D (nmol/mmol creatinine)	15.08±3.29	10.10±2.43	9.73±2.52	a, b

a=significant difference between active RA and controls; b=significant difference between active and inactive RA.

TABLE 3. Correlations between serum  $\beta_2$ -microglobulin and ICTP concentration and spot urinary concentrations of NTx and Pylrilinks-D in patients with active rheumatoid arthritis.

Parameter	Urine NTx (nmol BCE/mmol creatinine)	Urinary Pylrilinks-D (nmol/mmol creatinine)	ESR (mm/hr)
Serum $\beta_2$ -microglobulin (mg/L)	n=25; r=0.9910; P=0.0001	n=25; r=0.6177; P=0.001	n=25; r=0.8860; P=0.0001
Serum ICTP ( $\mu$ g/L)	n=25; r=0.5200; P=0.008	n=25; r=0.4903; P=0.01	n=25; r=0.6203; P=0.001

immunoassay. Briefly, samples were incubated with the  $^{125}$ I ICTP tracer and ICTP primary antibody for two hours at 37°C, after which a precipitated second antibody complex was added to separate the bound from free tracer. The assay was then centrifuged and decanted after a 30-minute incubation at room temperature. The bound tracer in the pellet was counted with a gamma counter. Counts were inversely proportional to the amount of ICTP present in each sample. The intra-assay and inter-assay coefficients of variations were 5.7% and 7.2%, respectively.

#### Statistical Analysis of Data

Simple descriptive statistics (mean, standard deviation and coefficients) were used in the statistical analysis of data. Analysis of variance (ANOVA) was used to determine the differences in mean values between the studied groups. Spearman's correlation was used to evaluate the type of association between the variables. These statistical analyses were done on computer with statistical package SPSS (version 6).

### Results

The mean  $\pm$  standard deviation (SD) serum concentration of  $\beta_2$ -microglobulin in patients with active RA (7.45±2.10 mg/L) was significantly higher ( $P<0.001$ ) than the concentration in patients with inactive disease (3.33±0.76 mg/L), and normal healthy controls (2.74±0.52 mg/L). The difference between the values for normal healthy controls and patients with inactive disease was not statistically significant ( $P<0.05$ ). Table 2 shows that in patients with active disease, spot urinary concentrations of

NTx (123.08±25.53 nmol BCE/mmol creatinine and 10.10±2.43 nmol/mmol creatinine) and Pylrilinks-D (15.08±3.29 nmol/mmol creatinine) were significantly higher ( $P<0.01$ ) than those of inactive disease (58.42±12.65 nmol BCE/mmol creatinine and 10.10±2.43 nmol/mmol creatinine, respectively). Similarly, the serum ICTP concentration in patients with active disease (9.52±1.56  $\mu$ g/L) was significantly higher ( $P<0.01$ ) than the values in patients with inactive disease (4.08±0.96  $\mu$ g/L), and that of healthy normal controls (3.43±1.03  $\mu$ g/L).

Table 3 shows that the serum  $\beta_2$ -microglobulin concentration in patients with active RA correlated positively with urinary NTx concentration ( $r=0.9910$ ,  $P=0.0001$ ) and urinary Pylrilinks-D concentration ( $r=0.6177$ ,  $P=0.001$ ). Although serum ICTP concentration correlated positively with urinary NTx ( $r=0.5200$ ,  $P=0.008$ ) and Pylrilinks-D ( $r=0.4903$ ,  $P=0.013$ ), the positivity of the associations was not as strong as those with serum  $\beta_2$ -microglobulin. These data suggest positive association between serum  $\beta_2$ -microglobulin and spot urinary concentrations of NTx and Pylrilinks-D.

### Discussion

Our findings that patients with active rheumatoid arthritis had significantly higher concentration of serum  $\beta_2$ -microglobulin than healthy controls confirmed the results from previous studies.<sup>9-11,13</sup> Since  $\beta_2$ -microglobulin is produced by lymphocytes whose number is increased in rheumatoid arthritis, the reason for the elevated serum  $\beta_2$ -microglobulin concentration in active rheumatoid arthritis is obvious.

What is new and of practical clinical significance in this study is the finding that serum  $\beta_2$ -microglobulin concentration is positively associated with urinary concentration of type I collagen cross-linked N-telopeptides and deoxypyridinoline in patients with active rheumatoid arthritis. Type I collagen accounts for about 90% of the organic matrix of bone, and is also the major matrix protein in tendons, ligaments and soft connective tissue. The structure of NTx is unique. The N-telopeptide interactions that form pyridinoline are predominantly  $\alpha_1(I)$  to  $\alpha_2(I)$  and  $\alpha_2(I)$  to  $\alpha_2(I)$ , compared with other tissue type I collagen.<sup>14</sup> Its urinary excretion is, therefore, a specific marker for collagen degradation.<sup>15</sup> In a previous study (submitted for publication), we reported that urinary NTx correlated strongly with indices of disease activity in RA. Our present finding that urinary NTx correlated strongly with serum  $\beta_2$ -microglobulin suggests that the mechanism responsible for increased  $\beta_2$ -microglobulin production in RA may also play a part in the increased collagen degradation seen in this disease. The use of serum  $\beta_2$ -microglobulin estimation for monitoring of patient's response to treatment in RA has been advocated.<sup>16</sup> Since urinary NTx was found to be closely associated with serum  $\beta_2$ -microglobulin, its estimation, which involves a non-invasive procedure, might be preferable to the estimation of serum  $\beta_2$ -microglobulin, which involves an invasive method.

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