Markers of Laminin and Type I Collagen in Sera of Patients with Sickle Cell Disease

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ABSTRACT

Sickle cell disease commonly causes avascular necrosis of ht skeletal system. Repeated episodes of sickling of the erythrocytes lead to repeated episodes of vaso-occlusion which affects the bone, spleen and other organs. Recently developed assay methods, which are based on the measurement of some of the fragments of procollagen or collagen molecule or parts of non-collagenous basement proteins are used to assess the connective tissue metabolism in patients with this disorder. The levels of carboxyterminal propeptide of type I procollagen, carboxyterminal telopeptide of type I collagen, and fragment P1 of laminin (PICP, ICTP, and laminin P1 respectively) in serum were measured by radioimmunoassay in patients with sickle cell disease. The concentrations of PICP, ICTP (both ug/l), and laminin (U/ml) were statistically significant in the study group than in the control group ($p < 0.001$, 0.003, and 0.02, respectively). The upper limits of the reference ranges (i.e. mean ± 2 SD of control) for PICP, ICTP, and laminin were exceeded by 7/20 (35%), 8/20 (40%), and 14/20 (70%) of patients respectively. The biomarkers of type I collagen (PICP and ICTP) had positive correlation. There was no correlation between serum laminin and PICP or ICTP levels in the patient group.

Conclusions: These findings indicate that serum markers of type I collagen and laminin may be useful as valuable non-invasive indices of extracellular matrix in bone, spleen or other tissues in sickle cell disease.

Key words: Sickle cell disease; carboxyterminal propeptide of type I procollagen;.

INTRODUCTION

Recently, several assay methods have been introduced that allow assessment of the activity of connective tissue metabolism in patients by analyzing blood samples and other body fluids[1,2]. These assay techniques are based on the measurement of some of the enzymes of collagen synthesis[3-5], fragments of procollagen molecules[6] or collagen molecules[7-9], or known parts of non-collagenous basement proteins[10,11]. The most studied bone biochemical marker is type I collagen[12]. Thus, the methods of assay widely used are: the carboxyterminal propeptide of type I procollagen and the carboxyterminal telopeptide of type I collagen[12]. Carboxyterminal propeptide of type I procollagen (PICP) is partially set free during the synthesis of collagen, before the collagen molecules assemble to form fibrils[12]. Carboxyterminal telopeptide of type I collagen (ICTP) is the widely used for the assessment of bone resorption or degradation[12].

Type I collagen accounts for about 90 per cent of the organic matrix of mineralized bone, while several non-collagenous proteins including laminin make the rest[13]. This collagen is also the most abundant type in the supportive structures of soft tissues[12]. Type I collagen is synthesized by the osteoblasts[2,12]. During collagen type I synthesis the carboxyterminal and aminoterminal of the procollagen molecules are split off as intact fragments extracellularly and released into the bloodstream before extracellular fibril formation[2,12,14]. The measurement of serum concentrations of the procollagen fragments reflects the amount of mineralized bone[2,12,14]. Immunoassays for the determination of the serum PICP are commercially available[2,12,14].

It is known that diseases such as rheumatoid arthritis, multiple myeloma, and metastatic carcinoma to the bone are associated with elevated degradation of type I collagen in the bone[15]. The non-helical terminal regions, the amino- and the carboxyterminal telopeptide region of type I collagen are sites for the cross-linkage to he helical region of an adjacent collagen molecule by pyridinoline[2,12,15]. During bone degradation or resorption, these cross-linking peptides are released into the bloodstream. The carboxyterminal telopeptide of type I collagen (ICTP) is released as an intact fragment and it is measured by serum radioimmunoassay[2,12,15]. The released aminoterminal telopeptide of type I collagen is also measured but in urine with an enzyme immunoassay method[16].

Laminin is a major non-collagenous glycoprotein of basement membrane. The presence of breakdown

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products of laminin in serum is indicative of the normal turnover of basement membranes[17,18]. The most widely used biomarker for monitoring laminin metabolism in human beings is the laminin P1 fragment and the serum assays of this marker has proved very useful in monitoring of patients with malignant disorders and liver disease[19].

Sickle cell disease is known to be the commonest cause of avascular necrosis of the bone[20-24]. Most of the bone infarcts in these patients are found in areas supplied by end arteries, especially the head of the long bones such as femur and humerus[24], although other bones are also affected. It is also known that the disease affects small arteries and capillaries. It is generally accepted that painful episodes and the end organ damage associated with sickle cell disease are caused by microvascular occlusion and tissue ischaemia resulting from complex interactions between the sickle erythrocytes, endothelium, platelets, plasma clotting factors, and certain mediators of inflammation[25].

The aim of the present study is to assess the serum concentrations of the biochemical markers of type I collagen and laminin and see how this disease which affects the blood vessels, capillaries, and most organs of the body including the skeletal system influences these biochemical markers.

MATERIALS AND METHODS

Reference individuals and patients sera were obtained from:

a. 20 apparently healthy persons (9 males and 11 females), age range 20 – 52 years (median 30 years). None of the persons had a history serious disease or was taking drugs known to affect bone metabolism or the blood vessels. The control had no history of fractures. All control subjects had haemoglobin AA.
b. 20 patients (9 males and 11 females) age range 18 to 51 years (median 30 years). The diagnosis of sickle cell disease was based on the haemoglobin pattern on cellulose acetate electrophoresis. Family studies were consistent, where available. The patients were classified into the following according to their genotypes:
   i. SS-Sickle cell anaemia- 15 patients
   ii. Sβ- Sickle cell thalassaemia - 2 patients,
   iii. SC- Sickle cell haemoglobin C disease- 3 patients

The clinical history and routine laboratory tests showed that none of the patients had liver disease, renal or inflammatory disease and none showed symptoms of these complications during the time blood samples were taken. All blood samples were taken prior to therapy. Sera were separated from the blood clot by centrifugation and the sera were stored at -20°C until analyzed.

The study protocol was approved by the local Ethics Committee and ever patient and control subject gave his or her informed consent.

Assays of laminin concentration in serum: Serum laminin concentration was measured by a radioimmunoassay (CIS, Diagnostics, KK, Groupe ORIS). Briefly, patient or control subject serum and anti-laminin P1 were mixed, followed by addition of ^{125}I-labelled laminin P1. After incubation, bound radioactivity was precipitated by a second antibody directed against rabbit IgG and then centrifuged. The supernatant was decanted and the ^{125}I activity present in the precipitate is measured in the gamma scintillation counter. The serum level of laminin was expressed in units per milliliter (U/ml). The CV between duplicates was 3.5%.

Determination of carboxyterminal propeptide of procollagen type I in serum (PICP): The concentration of the serum PICP was determined using the radioimmunoassay from Incstar Corporation (Stillwater, MN, USA). All analyses were done in duplicate. Serum concentration of PICP was expressed in microgram per liter (µg/l). The intra- and inter-assay CV were 3.8% and 6.5% respectively.

Assay of carboxyterminal telopeptide of collagen type I (ICTP): Serum ICTP level was measured by a radioimmunoassay kit from Incstar Corporation (Stillwater, MN., USA). All analyses were done in duplicates. The concentration of ICTP in serum was expressed in microgram per liter(µg/l). The intra-assay coefficient of variation (CV) was 6.1% and the inter-assay CV was 7.5%.

Statistical analysis: The results are presented as means±SD. Median values and range are included. The mean±SD are compared, as applicable, by ANOVA and Student's t-test. The correlations were determined by linear regression analysis. Statistical significance was acknowledged when $p \leq 0.05$.

RESULTS

The concentrations of PICP, ICTP (both ug/l), and laminin (U/ml) were statistically significant in the study group than in the control group ($p < 0.001$, 0.003, and 0.02, respectively) (Table 1). The upper limits of the reference ranges (i.e. mean+2SD of control) for PICP, ICTP, and laminin were exceeded by 7/20 (35%), 8/20 (40%) and 14/20 (70 %) of the patients respectively. There was a positive correlation between the serum markers of type I collagen ($r=0.54$, $p<0.01$). No significant correlation was found between serum laminin level and other biochemical determination such as PICP and ICTP.

Table 1: Summary of the biochemical markers in control group and patients with sickle cell disease

Biochemical Markers	Control Group			Sickle cell disease			P value
	Mean±SD*	Median	Range	Mean±SD*	Median	Range	
Carboxyterminal propeptide of type I procollagen (PICP, µg/l)	135.8±34.4	128.7	77.8-198.4	230.6±134.9	187.1	48-560	<0.0001
Carboxyterminal telopeptide of type I collagen (ICTP, µg/l)	2.8±0.6	2.8	1.8-4.0	5.0±3.4	3.8	1.6-12.1	<0.003
Laminin (U/ml.)	1.3±0.05	1.3	1.2-1.3	1.6 ± 0.02	1.7	1.3-1.9	<0.02

SD*= Standard Deviation

DISCUSSION

The determination of metabolites of type I collagen, the main collagen in bone, is very useful in monitoring bone turnover in many diseased conditions. Several studies have demonstrated that ICTP, a degradation product of mature type I collagen fibres, reflects bone resorption in several metabolic disorders of the bone, rheumatoid arthritis, and malignant tumours with bone metastases[26]. In postmenopausal women or osteoporosis, high values of ICTP have been found[13,15,16,27]. In malignant diseases of the skeleton high values of both metabolites have been demonstrated in multiple myeloma and prostate carcinoma metastatic to skeleton[26].

In the present study, PICP, which reflects synthesis of type I collagen was moderately increased when compared to ICTP, the metabolite of type I collagen breakdown, probably reflecting the lower bone formation activity in sickle cell disease. There was a significant positive correlation between serum ICTP and PICP levels.

Basement membranes are subject to metabolic turnover leading to the release of antigen related to laminin and other proteins into the bloodstream. Elevated serum concentrations of laminin P1 fragment have been demonstrated in various disorders with active tissue remodeling, such as alcoholic liver disorder with cirrhosis[18,28], diabetes micro-angiopathy[17,19], and progressive systemic sclerosis[1]. The changes in the serum concentration of the human laminin P1 antigens in any conditions seem to be relatively small[29]. But these changes were statistically significant when groups are compared. Serum laminin did not correlate with the biomarkers of the type I collagen. It is important to interpret the result with caution, because circulating laminin is heterogeneous consisting of intact as well as various proteinase-resistant fragments. Laminin fragments may be taken up and degraded by the liver endothelial and liver parenchymal cells[28]. But none of the patients under study had liver disease. It has also been demonstrated that small amounts of laminin are taken up by the spleen[30]. Thus, the serum level of laminin-related antigen depends not only on the amount of laminin released into the bloodstream but also on the extent of uptake and degradation. It is very important to serum laminin and spleen size in sickle cell disease since the spleen is severely affected by this disorder. In sickle cell disease the spleen undergoes acute enlargement trapping a significant proportion of he red blood cells, causing a precipitate fall in haemoglobin concentration and the risk of death from peripheral circulatory failure[31].

Despite the above reservations increased serum laminin concentrations in patients with sickle cell disease may reflect ongoing basement membrane metabolism. Furthermore, our recent study found increased serum laminin and aminoterminal propeptide of type III procollagen (PIIINP) in patients with sickle cell haemoglobinopathies, which reflects bone marrow fibrogenesis and angiogenesis[32]. In sickle cell haemoglobinopathies vascular occlusion involves both microcirculation and macrocirculation. Vascular occlusion in the microcirculation causes the acute painful episodes, which is a hallmark this disease. Vascular occlusion in the macrocirculation is associated organ failure[33].

In conclusion, our findings show that there are changes in the metabolism of laminin and type I collagen in sickle cell disease. The presence of early alterations in the markers of type I collagen and laminin might contribute to early detection and selection of those patients who need more accurate treatment and follow-up of the changes in the skeletal system, spleen and other organs.

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