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Use of Collagen Breakdown Products in the Diagnosis of Some Connective Tissue Diseases

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Abstract. Collagen is the most abundant protein in the extracellular matrix and connective tissue. There are more than 20 different types of collagen each with a basic structure consisting of triple helix made of three polypeptide chains. Functions of collagen include tissue scaffolding, providing mechanical strength, tensile stiffness etc. During rapid growth and in diseases like arthritis, osteoporosis, paget's disease, metastatic bone disease and renal osteodystrophy, the extent of collagen degradation is quite extensive. Collagen breakdown products released in the body fluids during diseases can be used to measure the rate of collagen breakdown. Collagen breakdown products which could be used as disease markers include pyridinium crosslinks, pyridinoline (hydroxylysyl pyridinoline), deoxypyridinoline (lysyl pyridinoline), peptide-bound cross linked C-telopeptide, peptide-bound crosslinked N-telopeptide and hydroxyproline .

The clinical role of these biochemical markers could include early diagnosis of diseases, monitoring disease progression, monitoring the therapeutic progress, predicting the disease outcome and selection of optimal therapy.

Introduction

Collagen is the most abundant protein in the extracellular matrix and connective tissue. Collagens are considered to be a group of proteins with a characteristic molecular structure . There are more than 20 types of collagen with triple helix made of three polypeptide chain as their basic structural unit [1, 2] .The triple helix (tropocollagen) is right-handed helix composed of three α-chains which might be identical (homotrimers) as in collagen type II, III, VII, VIII and X or others with two or more different types of chains (heterotrimers) as in collagen type I, IV, V, VI, IX and XI. Each of the three α-chain is left-handed helix with a pitch of 18 amino acid per turn [2, 3].

The three polypeptide chains are held together by hydrogen bonds between the chains. The amino acid sequence in α-chain is a repeating tripeptide unit, Gly-X-Y, where X is often proline and Y is often 4-hydroxyproline or hydroxylysine, resulting in triple helical domains of 300nm in length which correspond to about 1000 amino acids. Glycine residue is the smallest amino acid in every third position of polypeptide chain, positioned in the center of the triple helix. This allows a close packaging along the central axis of the molecule. Proline and 4-hydroxyproline ring structure cause kinks in the polypeptide chain which facilitate the formation of the helical conformation of each α-chain (Fig. 1) [1, 4]. Hydroxyproline and hydroxylysine residues in collagen arise by hydroxylation of proline and lysine during post translational modification by prolyl and lysyl hydroxylase respectively. The hydroxyl group of hydroxylysine may be glycosylated [1].

Collagen fibrils are supramolecular assemblies consisting of triple-helical collagen molecules associated in a variety of ways to provide different degrees of tensile strength. The α-chains of collagen molecules and the fibrils are cross-liked by unusual types of covalent bonds involving lysine, 5-hydroxylysine or histidine that are present at few of X and Y positions. These links create uncommon amino acid residues such as dehydrohydroxylysinon or leucinon [4].

Collagens can be classified into fibril-forming collagens, fibril-associated collagens (FACIT), net-work-forming collagens, anchoring fibrils, transmembrane collagens, basement membrane collagens and others based on structure and supramolecular organization (Table 1)[2] .

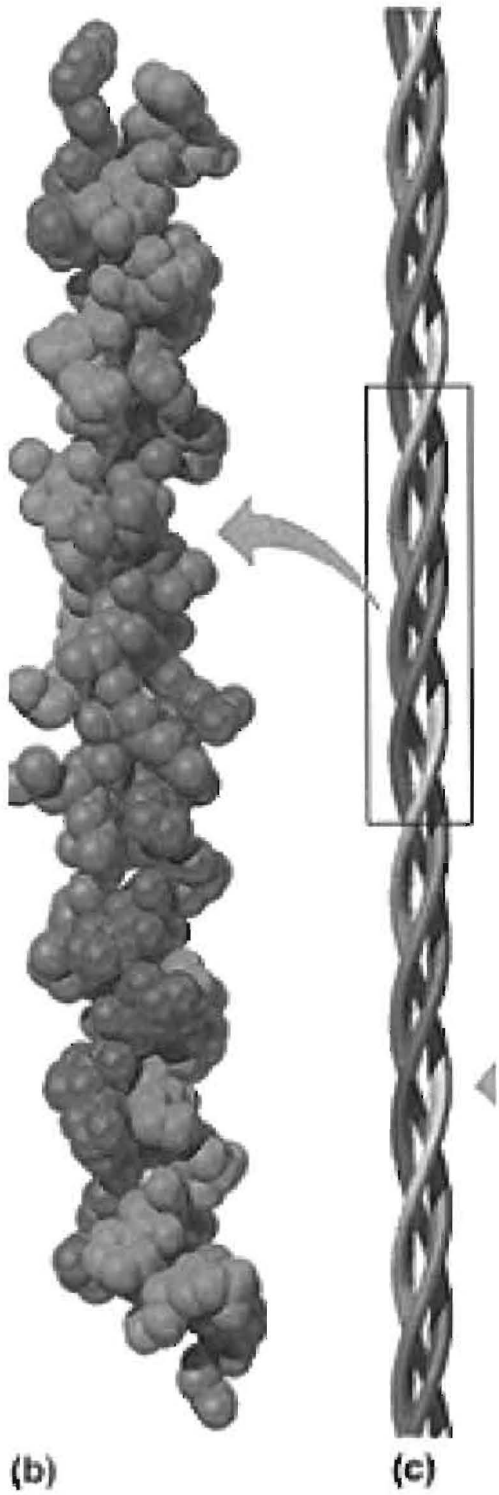

Fig. 1. Collagen fibrils at right have a characteristic banding pattern, reflecting the regularly staggered packing of the individual collagen molecules in the fibril [1].

Fibril Forming Collagens

These are the collagen type I, II, III, V and XI . These are the most abundant and widespread family forming about 90% of total collagen. These collagens have the ability to assemble into highly orientated supramolecular aggregates [2, 4].

Type I collagen form 90% of organic mass of bone, and major collagen of tendons, skin, ligaments, cornea and many interstitial connective tissues. The collagen type I triple helical fibres are mostly incorporated into composite containing either type III or type V collagen. Type I collagen provide tensile strength, stiffness in particular after calcification and in bone give biomechanical properties [2].

Type II collagen is the predominant component of hyaline cartilage. It is also present in the corneal epithelium, notochord, nucleus pulposus of intervertebral discs and embryonic epithelial-mesenchymal transition. Compared to type I , type II collagen contain higher content of hydroxylysine as well as glucose and galactose residues. Alternative splicing of type II collagen pre-mRNA results in two forms of the α1 (II)-chain. Type IIA variant, the embryonic form is found in perchondrogenic mesenchyme, osteophytes, perichondrium, vertebrae and chondrogenic tumors. Type IIB is a characteristic marker of mature cartilage [2].

Type III collagen is an important component of reticular fibres in the interstitial tissue of lungs, liver, dermis, spleen and vessels. This often contributes to mixed fibrils with type I collagen and is abundant in elastic tissues [2].

Type V and XI, in combination with each other appear in various tissues. They share similar biochemical properties. Type V functions as core structure of fibrils with type I and III collagen. It contributes to organic bone matrix ,corneal stroma and interstitial matrix of muscle, liver, lungs, and placenta. Type XI collagen is present largely in articular cartilage with type II collagen [2].

Basement Membrane Collagens

Basement membranes Collagen is a collagen type IV and is found in the basement membranes [2].

Microfibrillar Collagen

Microfibrillar collagen is a collagen type VI. This collagen aggregates in to filaments in extracellular matrix and forms an independent microfibrillar network in virtually all connective tissue, except bone [2].

Anchoring Fibrils

Anchoring fibrils are found in skin, dermal–epidermal junctions, oral mucosa and cervix [2].

Table 1. Various collagen types as they belong to the major collagen families [2].

Type	Molecular composition	Genes (genomic localization)	Tissue distribution
Fibril-forming collagens			
I	[a1(I)]2a2(I)	COL1A1(17q21.31q22) COL1A2 (7q22.1)	bone, dermis, tendon, ligaments, cornea
II	[a1(II)]3	COL2A1 (12q13.11 – q13.2)	cartilage, vitreous body, nucleus pulposus
III	[a1(III)]3	COL3A1 (2q31)	skin,vessel wall, reticular fibres of most tissues (lungs, liver, spleen, etc.)
V	a1(V),a2(V),a3(V)	COL5A1 (9q34.2– q34.3)	lung, cornea, bone, fetal membranes; together with type I collagen
XI	a1(XI)a2(XI)a3(XI)	COL11A1 (1p21) COL11A2 (6p21.3) COL11A3 = COL2A1	cartilage, vitreous body
Basement membrane collagens			
IV	[a1(IV)]2a2(IV); a1–a6	COL4A1 (13q34) COL4A2 (13q34) COL4A3 (2q36– q37) COL4A4 (2q36– q37) COL4A5 (Xq22.3) COL4A6 (Xp22.3)	basement membranes
Microfibrillar collagen			
VI	a1(VI),a2(VI),a3(VI)	COL6A1 (21q22.3) COL6A2 (21q22.3) COL6A3 (2q37)	widespread:dermis, cartilage,placenta, lungs, vessel wall, intervertebral disc
Anchoring fibrils			
VII	[a1(VII)]3	COL7A1 (3p21.3)	skin, dermal– epidermal junctions; oral mucosa, cervix,
Hexagonal network-forming collagens			
VIII	[a1(VIII)]2a2(VIII)	COL8A1 (3q12– q13.1) COL8A2(1p34.3–p32.3)	endothelial cells, Descemet's membrane
X	[a3(X)]3	COL10A1(6q21– q22.3)	hypertrophic cartilage
FACIT collagens			
IX	a1(IX)a2(IX)a3(IX)	COL9A1 (6q13) COL9A2 (1p33– p32.2)	cartilage, vitreous humor, cornea
XII	[a1(XII)]3	COL12A1 (6q12– q13)	perichondrium, ligaments, tendon
XIV	[a1(XIV)]3	COL9A1 (8q23)	dermis, tendon, vessel wall, placenta, lungs, liver
XIX	[a1(XIX)]3	COL19A1 (6q12– q14)	Human rhabdomyosarcoma
XX	[a1(XX)]3		corneal epithelium, embryonic skin, sternal cartilage, tendon
XXI	[a1(XXI)]3	COL21A1(6p12.3–11.2)	blood vessel wall
Transmembrane collagens			
XIII	[a1(XIII)]3	COL13A1 (10q22)	epidermis, hair follicle, endomysium, intestine, chondrocytes, lungs, liver
XVII	[a1(XVII)]3	COL17A1 (10q24.3)	dermal– epidermal junctions
Multiplexins			
XV	[a1(XV)]3	COL15A1 (9q21– q22)	fibroblasts, smooth muscle cells, kidney, pancreas,
XVI	[a1(XVI)]3	COL16A1 (1p34)	fibroblasts, amnion, keratinocytes
XVIII	[a1(XVIII)]3	COL18A1 (21q22.3)	lungs, liver

Hexagonal Network-Forming Collagen

Hexagonal network-forming collagens are collagen type X and VIII .Type X collagen is component of hypertrophic cartilage in the fetal and juvenile growth plate, in ribs and vertebrate and has a role in endochondral ossification and matrix calcification .Type VIII collagen, is homologous to type X collagen structure but has different function and distribution. This network-forming collagen is produced by endothelial cells and assembles in hexagonal lattices (eg. in the Decrement's membrane of cornea) [2].

Fibril-Associated Collagens (FACIT Collagen)

Fibril-associated collagens are collagens type IX, XII, XIV, XVI, XIX and XX. The structures of these collagens is interrupted by short non-helical domains and the trimeric molecules are associated with the surfaces of various fibrils. Type IX collagen located along the surface of type II collagen fibrils in antiparallel direction. Type XVI collagen is found in hyaline cartilage and skin and is associated with type II collagen. Type XII and type XIV collagens molecules are associated with type I collagen in skin, perichondrium, periosteum, tendons, lung, liver, placenta and vessel walls. The functions of these collagen and type XIX, XX collagen is poorly understood [2].

Transmembrane Collagens

Transmembrane collagens are collagen type XIII and XVII. These types of collagen even span cell membranes as hexagonal network collagen forming collagen and component of epidermis, hair follicle, endomysium, intestine, chondrocytes, lungs, liver and dermal– epidermal junctions [2].

Multiplexins

Multiplexins are collagen type XV, XVI and XVIII . Collagen type XV is found in fibroblasts, smooth muscle cells, kidney, pancreas. Type XVI collagen is found in hyaline cartilage and skin and is associated with a subset of the collagen "type II fibers" . Type XVIII collagen is present in lungs and liver [2].

Collagen fibers are highly stable with half-lives as long as several months .The breakdown or remodeling of collagen fibrils depends on collagenases which are a part of matrix metalloproteinases family. It occurs normally in tissues in response to growth or injury. However, during rapid growth and in disease states, such as arthritis, cancer, and chronic nonhealing ulcers, there is extensive degradation of collagen. The collagen breakdown products are released in the body fluids and can be used to measure the rate of collagen breakdown [5, 6].

The measurement of collagen/collagen breakdown products have the potential to emerge as promising clinical tools. These may include early diagnosis of various diseases, monitoring disease progression, monitoring the therapeutic progress, predicting the disease outcome and selection of optimal therapy.

Collagen Formation Markers

Type I collagen constitutes about 90% of organic bone matrix. It is synthesized as procollagen containing C-and N-terminal extension peptides. Products of type I collagen synthesis include procollagen type I C-terminal peptide and procollagen type I N-terminal peptide. In the extracellular matrix, these terminal peptides are cleaved as procollagen type I C-terminal peptide and procollagen type I N-terminal peptide which can be measured in serum as markers of bone formation. The standard analytical methods for measurement of procollagen type I C-terminal peptide and procollagen type I N-terminal peptide is immunoassay [7].

Markers of Bone Resorption

Pyridinium cross-links: Each molecule of type I collagen is composed of two α1(I)chains and one α2(I) chain that forms a triple-helical strand. Many collagen strands are joined together by cross-links located in between the nonhelical ends of one collagen molecule and the helical region of another molecule. The two cross-links are pyridinoline and deoxypyridinoline formed by oxidation of lysine and hydroxylysine residues. Deoxypyridinoline is found only in bone and dentine and is considered as specific bone marker. During bone resorption deoxypyridinoline is released into the circulation and urine either as free form or bound to N- or C-terminal telopeptide of type I collagen [7].

Cross-linked telopeptides: during bone resorption short fragments at both N-and C-terminal domains of collagen molecules are cleaved by osteoclasts and enter the circulation. These fragments are still cross-linked through pyridinoline or deoxypyridinoline and are called telopeptides. Cross-linked telopeptides are specific and sensitive markers of bone resorption [7].

Hydroxyproline and hydroxylysine are two types of hydrolyzed amino acids unique to collagen, which are formed intracellularly during the posttranslational phase of collagen synthesis [7]. Since collagen contains appreciable amounts of hydroxyproline, estimation of urinary hydroxyproline has been used

as a measure of the daily turnover of collagen if the dietary sources of hydroxyproline are first reduced to a minimum. Since bone contains about 40 per cent of the total body collagen, determinations of the 24 h urinary hydroxyproline output have proved to be of value in the clinical study of bone diseases [8].

In blood and urine, hydroxyproline occurs in three forms: as free hydroxyproline and combined in peptides or in proteins. In urine it is mainly (90 per cent) in the peptide form while in blood it is mainly (80 per cent) in the protein form (hypro-protein) [8].

If the patient is on a diet sufficiently low in collagen, the 24 hour output provides a useful index of the turnover of bone matrix, complementing the evidence on mineral turnover provided by estimations of serum calcium, phosphate, and alkaline phosphatase [8].

Diseases

Arthritic disease

Rheumatoid arthritis and osteoarthritis - One of the major clinical manifestation of these diseases are an abnormal and degraded articular cartilage leading to loss of its physical properties accompanied by joint pain and loss of mobility of the patient. Osteoarthritis is a chronic disease characterized by progressive destruction of articular cartilage and subchondral bone, has multiple etiologies which includes joint trauma and genetic mutation in fibrillar collagen genes. Rheumatoid arthritis is an autoimmune disorder characterized by persistent joint inflammation, polymorphonuclear cell infiltration and synovial hyperplasia resulting in cartilage loss and joint deformity. In joint disease the rate of degradation of matrix often exceeds the rate of synthesis. As a consequence the tissue becomes thin and mechanically weak resulting in the structural damages associated with osteoarthritis and rheumatoid arthritis[9].

The increased catabolic rate causes the fragments of the extracellular matrix to be released into circulation where they can be quantified as a measure of cartilage turnover. Main components of articular cartilage are fibril forming collagen type II which constitute over 60% of cartilage's dry weight.The potential use of these biochemical markers in the diagnosis and monitoring of osteoarthritis and rheumatoid arthritis has been the focus of much research over the last 2 decades.The synovial fluid, serum and urine are the main site of markers of collagen type II metabolites, but routine measurements have been focused on serum and urine.

In the collagen type II, covalent cross links joining two telopeptides to the α-helical region of a third collagen molecule serves to link individual collagen monomers into a rigid fibrilar network. Among the main cross link types are pyridinoline and deoxypyridinoline.

In earlier study [9], a glycosylated form of the pyridinoline cross link was proposed to be a more specific marker of synovial extra cellular matrix and elevated level of this marker has been reported in osteoarthritis .

Degradation fragments derived from the C-telopeptide part of collagen type II is also a marker of cartilage degradation which are elevated in rheumatoid arthritis and osteoarthritis patients [10,11] .

Osteoporosis

This is the most common metabolic disease of bone and occurs mostly in females. Aging is major risk factor because approximately 10% of skeletal mass is lost per year after 35 to 40 years of age. Other risk factors include a positive family history, alcohol abuse, smoking [1].

The bone resorption markers which increase in osteoporosis are pyridinium cross-links, cross-liked telopeptides, hydroxyproline and hydroxylisine. The two cross links are pyridinoline and deoxypyridinoline formed by oxidation of lysine and hydroxylysine residues. Pyridinoline is not specific to the bone and it is present in several types of tissues, including bone, cartilage, ligaments, and vessels. Deoxypyridinoline, in contrast, is found only in the bone and dentine and is considered relatively specific bone marker. In the process of bone resorption, osteoclasts cleave cross-linked type I collagen, releasing pyridinoline and deoxypyridinoline into the circulation and is excreted in urine as free form or bound to N- or C-terminal telopeptide of type I collagen. The production of pyridinoline and deoxypyridinoline is not influenced by the diet and therefore can be of diagnostic use .

Cross-linked telopeptides are short fragments af both N- and C- terminal domains of collagen molecules. These fragments are still cross-linked through pyridinoline or deoxypyridinoline and are called telopeptides. They are specific and sensitive markers of bone resorption.

Hydroxyproline and hydroxylysine are two types of amino acids unique to collagen, which are formed intracellularly during the posttranslational phase of collagen synthesis. They are considered nonspecific markers of bone turnover, although measurement of urinary hydroxyproline has been widely used a bone resorption marker [7] .

Paget's disease

Paget's disease of bone is a chronic localized metabolic bone disorder, characterized by increased bone turnover, bone hypertrophy, and abnormal bone structure. Its clinical features include pain, deformity, fracture, deafness, neurological symptoms and neoplastic degeneration [12]. Diagnosis of Paget's disease and assessment of disease activity is usually performed by bone scintigraphy and radiographs. These techniques are sensitive for diagnosis but are generally unsuitable for monitoring short-term response to treatment. Biochemical markers of bone turnover constitute a fast and noninvasive technique assessing bone disease activity and response to therapy. Bone turnover is severely dysregulated in Paget's disease, with an increase in local remodeling and modeling of bone, mirrored by significant increases in the serum and urinary levels of bone turnover markers [13].

High levels of urinary hydroxyproline are found in Paget's disease of bone. Determinations are more often carried out for the control of treatment than for diagnosis, as for example in the control of calcitonin therapy for Paget's disease. In this disease the rates of both bone formation and bone destruction are greatly increased; on treatment with calcitonin, osteoclastic bone resorption is reduced, leading usually to clinical improvement and a slow fall in urinary hydroxyproline and serum alkaline phosphatase [8].

Bone resorption parameters are a variety of biochemical tests reflecting bone matrix resorption and provide good indices of disease activity [14]. For many years, measurement of 24-h or second-morning void urinary hydroxyproline/creatinine has been used successfully as an index of bone collagen resorption. More specific tests of bone collagen resorption have been developed in recent years. These include urinary and serum deoxypyridinoline, N-telopeptide and C-telopeptide which are not affected by dietary intake [15,16]. Many of these tests provide a more immediate index of response to therapy of Paget's disease compared with indices of osteoblastic activity.

Metastatic Bone Disease

This is a complication of many solid tumors, it is especially common in breast, prostate, lung, bladder and thyroid carcinomas [17]. Biochemical markers of bone metabolism specifically reflect bone resorption or bone formation rates and are strongly affected by the processes active in metastatic bone involvement.

Some reports have evaluated the usefulness of galactosyl-hydroxylysine in patients with metastatic bone disease. In patients with breast cancer, this marker seems to be useful in the diagnosis of established bone metastases [18].

Renal Osteodystrophy

This is the spectrum of bone disorders that develops in patients with chronic renal failure and continues to be a major long-term complication which is associated with high rates of morbidity [18]. The ideal biochemical marker of bone turnover should be unique to bone, reflect total skeletal activity and well correlate with histomorphometric findings and results of radiocalcium kinetics.

Type I collagen cross-linked telopeptide levels were correlated directly with total alkaline phosphatase, bone alkaline phosphatase and with several histomorphometric indices of bone turnover. Type I collagen cross-linked telopeptide is a useful marker in renal osteodystrophy [19].

Pyridinoline cross-links of collagen exist in two chemical forms, namely hydroxylysyl pyridinoline (or pyridinoline) and lysyl pyridinoline (or deoxypyridinoline). There excretion in urine has been validated as an excellent marker in several metabolic bone diseases [20].

Other Disease States

In endocrine diseases , increased levels of thyroxine, parathormone, or growth hormone in serum are accompanied by increased urinary hydroxyproline outputs. High levels are also found inconsistently in some connective tissue diseases. In children, the more rapid and variable growth rate is paralleled by much higher and more variable hydroxyproline excretion. A "hydroxyproline index" (hydroxyproline/creatinine × weight in kg) has been used as a test of growth when assessing the treatment of malnourished children .

The free hydroxyproline, in urine may be used to determine if the patient has been on a low collagen diet which is very sensitive to dietary collagen [8]. Earlier studies [5, 6] have also shown that serum and urinary hydroxyproline could be used to show the damage to collagen by heavy metals like mercuric chloride.

Conclusion

Collagen is the most abundant tissue protein in our body and is affected in many diseases. Its breakdown products could be used as one of the many biochemical markers in the diagnosis/monitoring of many diseases.

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