J Shoulder Elbow Surg (2016) 25, 981–988

Journal of Shoulder and Elbow Surgery

www.elsevier.com/locate/ymse

Advanced glycation end products in idiopathic frozen shoulders

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Background: The pathophysiologic mechanisms behind proliferation of fibroblasts and deposition of dense collagen matrix in idiopathic frozen shoulder remain unclear. Accumulation of advanced glycation end products (AGEs) with cross-linking and stabilization of collagen has been hypothesized to contribute to this pathophysiologic process. This study investigated whether the immunoreactivity of AGEs is higher in patients with idiopathic frozen shoulder than in the control groups.

Methods: Shoulder capsule samples were collected from 8 patients with idiopathic frozen shoulder, 6 with unstable shoulders (control 1), and 8 with rotator cuff tears (control 2). The samples were hematoxylin and eosin stained and analyzed by immunohistochemistry using antibodies against AGEs. Immunoreactivities were rated in a blinded fashion from none (0) to strong (3). Immunohistochemical distribution within the capsule was noted.

Results: Frozen shoulder patients had greater frequency and severity of self-reported pain ($P = .02$) than rotator cuff tear patients and more restricted range of motion in all planes ($P < .05$) than patients of the instability and rotator cuff tear groups. Hematoxylin and eosin–stained capsular tissue from frozen shoulder showed fibroblastic proliferation, increased numbers of adipocytes, and increased subsynovial vascularity. Immunoreactivity of AGEs was stronger in frozen shoulder capsules (2.8) than in instability (0.3; $P = .0001$) and rotator cuff tear (1.1; $P = .016$) capsules.

Conclusion: This study highlights a potential role for AGEs in the pathogenesis of frozen shoulder. The overexpression of AGEs may explain the fibroblastic proliferation and deposition of collagen matrix in idiopathic frozen shoulder.

Level of evidence: Basic Science Study; Histology

Keywords: Frozen shoulder; advanced glycation end products; immunoreactivity; stiff shoulder; fibroblastic proliferation; adhesive capsulitis

The study was conducted in accordance with ethics approval from the Human Research Ethics Committee—Central Network, South East Health (HREC/96/55, HREC/14/130).

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1058-2746/$ - see front matter © 2016 Journal of Shoulder and Elbow Surgery Board of Trustees.
http://dx.doi.org/10.1016/j.jse.2015.10.015

Frozen shoulder, also known as adhesive capsulitis, is a common shoulder condition of unknown etiology characterized by severe pain, stiffness, and restricted active and passive shoulder motion. Estimates of the incidence of frozen shoulder reported from shoulder clinics range from 2% to 5%.[4,25] Women aged between 40 and 60 years are the most commonly affected group.

The disease typically lasts between 2 and 3 years and can be divided into 3 phases: a painful inflammatory phase with progressive stiffness (10-36 weeks); a stiff phase when the pain gradually decreases but the range of motion in all planes becomes severely restricted (4-12 months); and a recovery phase, which involves the gradual spontaneous improvement of shoulder function (5-26 months).[22] However, Shaffer et al[25] and Hand et al,[9] in their long-term (averaging 7 and 4 years, respectively) follow-up studies, found that 40% to 50% of nonoperatively managed frozen shoulder patients did not regain their baseline function and still had ongoing residual pain.

Our current understanding of the underlying etiology and pathogenesis of frozen shoulder is limited. Histologic studies[5,23] have demonstrated fibroblastic and myofibroblastic proliferation in dense types I and III collagen matrix in the shoulder capsule. Capsular fibrosis and contracture have been suggested to stiffen the shoulder capsule and to restrict range of motion. The pain of frozen shoulder was suggested to relate to neurogenesis and capsular inflammation, supported by the presence of increased numbers of chronic inflammatory cells with higher levels of inflammatory cytokines and angiogenesis.[10,28] However, the etiologic mechanisms behind proliferation of fibroblasts and deposition of dense collagen matrix in shoulder capsules of frozen shoulder patients are still unclear.

Diabetic patients are more likely than nondiabetic patients to develop frozen shoulder. Bridgman[4] described the strong association between diabetes and frozen shoulder on observing 11% incidence among 800 diabetic patients compared with 2% incidence among 600 nondiabetic patients. Arkkila et al[2] found high prevalence of frozen shoulder in type 1 (10%) and type 2 (22%) diabetic patients. A multivariate regression analysis in a case-control study by Wang et al[27] has confirmed that diabetes is an independent predictor of adhesive capsulitis ($P = .005$; odds ratio, 3.05).

Accumulation of advanced glycation end products (AGEs) with cross-linking and stabilization of collagen has been hypothesized to contribute to the higher incidence of frozen shoulder in diabetic patients.[12,15] AGEs are thought to form because of the nonenzymatic reaction of the ketone group on reducing sugars with free amino groups on proteins, resulting in progressive rearrangement, dehydration, and condensation.[26] The formation of AGEs progressively increases with normal aging. However, because of the long-standing hyperglycemic state in diabetes mellitus, glucose forms covalent adducts with the plasma proteins through a nonenzymatic process known as glycation and thus accelerates accumulation of AGEs.[26] AGEs attract monocytes and macrophages that release inflammatory cytokines that coordinate degradation and removal of senescent material with replacement by new tissue components.

To our knowledge, no previous studies considered the potential contribution of AGEs in frozen shoulder. However, previous studies have identified a contribution of AGEs in the pathogenesis of diabetic retinopathy, diabetic nephropathy, and diabetic cardiomyopathy. Treatment of retinal glial cells with 16% and 32% AGEs resulted in dose- and time-dependent immunocytochemical expression elevations of basic fibroblast growth factor in the culture medium ($P < .05$).[1] This suggested the role of AGEs in promoting fibroblast proliferation by enhancing production of basic fibroblast growth factor in diabetic retinopathy. The accumulation of AGEs on vessels facilitated cross-linking of collagen and increased resistance to proteolysis, which aided in fibrosis of the vessels and reduced arterial compliance in diabetic cardiomyopathy.[3] Furthermore, interaction of AGEs with RAGE, the receptor for AGEs, contributed to the development of atherosclerosis by activating adhesion molecules and proinflammatory cytokines and growth factors.[24] The expression of extracellular proteins including types I and IV collagens, fibronectin, and laminin as well as expression of profibrotic cytokines and growth factors, including transforming growth factor β1, platelet-derived growth factor B, and connective tissue growth factor, was increased by AGEs in a dose- and time-dependent manner in diabetic nephropathy.[8] Furthermore, AGEs decreased the expression of matrix metalloproteinases secreted by mesangial cells and increased expression of tissue metalloproteinase inhibitors in diabetic nephropathy.[19,29] Decreased activities of matrix metalloproteinases and increased activities of tissue inhibitors of matrix metalloproteinase leading to disruption of homeostasis between synthesis and degradation of extracellular matrix components also have been suggested as one of the pathogenic mechanisms of capsular fibrosis in frozen shoulder.[6,11,17]

We hypothesized that immunoreactivity of AGEs in frozen shoulder capsules will be higher compared with the control shoulder capsules.

This study was primarily designed to investigate whether AGEs contribute to the pathogenic mechanism of frozen shoulder by determining AGE immunoreactivity in human specimens and to describe the immunohistochemical distribution of antigens to AGE antibodies in regions of the frozen shoulder capsule at the microscopic level.

Materials and methods

Study design

A prospective case-control study was conducted at the Orthopaedics Research Institute, St. George Hospital, and the National Day Surgery, Sydney, NSW, Australia, during a 9-month period.