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Original Research Article

IDENTIFIED AND MOLECULAR CHARACTERIZATION OF THE PATHOLOGY DISORDERS IN FROZEN SHOULDER PATIENTS OF SOUTH INDIANS, INDIA

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ABSTRACT

Introduction: Frozen shoulder is a common disease which causes significant morbidity. Frozen shoulder is a common shoulder disorder characterized by pain and gradual loss of active and passive glenohumeral motion that occurs in 2–5% of the general population. However, the etiology and pathophysiologic mechanisms that lead to the development of frozen shoulder are poorly understood, and there is no consensus regarding optimal treatment.

Materials and Methods: Total number of 850 patients were initially recruited during a one year period between January 2014andJanuary 2015;we treated 70 patients with a diagnosis of primary frozen shoulder resistant to conservative treatment by manipulation under anaesthetic and arthroscopic release of the rotator interval, at a mean time from onset of 5 months.

Result and Discussion: Biopsies were taken from this site and histological and immunocytochemical analysis was performed to identify thetypes of cell present. The tissue was characterised by the presence of fibroblasts,proliferating fibroblasts and chronic inflammatory cells. The infiltrate of chronic inflammatory cells was predominantly made up of mast cells, with T cells, B cells and macrophages also present.

Conclusion: In conclusion, arthroscope biopsy material from the rotator interval in patients with frozen shoulder revealed immunocytochemical evidence of both chronic inflammation and proliferative fibrosis, supporting the theory that this is both a chronic inflammatory and a fibrotic condition.Together with the presence of high vascularity and nerve tissue, this inflammation and fibrosis explains why frozen shoulder is such a painful and stiff condition.

KEY WORDS: Frozen Shoulder Patients, Immunocytochemical, Manipulation, Rotator interval.

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INTRODUCTION

Primary frozen shoulder is a common, severely debilitating condition with a prevalence of between 2% and 5% [1-3]. It is frequently difficult to manage. The diagnosis is made on clinical grounds. A set of diagnostic criteria were initially described by Codman in 1934 [4] and still hold true today. They include pain in the shoulder which comes on slowly and is felt at the insertion of the deltoid, inability to sleep on the affected side, atrophy of the spinatus, and little in the way of local tenderness. There is restriction of both active and passive movement, with painful and restricted elevation and external rotation. The pain is 'very trying', but the patient is able to continue with daily habits and routines. Bridgman J and co-authors said that the radiographs of the shoulder appear normal. Stiffness may also occur after fracture or in association with joint diseases such as osteoarthritis (OA); this is referred to as a secondary frozen shoulder [1-4].

Neer C and co-authors evaluated that the pathological disorders of frozen shoulder patients remains unclear, with information usually derived only from recalcitrant cases. Arthroscopy and open exploration of the frozen shoulder have increased our understanding of both the macroscopic and microscopic appearances. Neviaser J and co-authors confirmed that the pathology affects the glenohumeral capsular tissue and is particularly localised to the coracohumeral ligament in the rotator interval [5]. Analysis of this tissue has shown inflammatory changes [6,7], fibrosis and proliferative myelofibrosis [8]. This process may be cytokine mediated [9,10].

Diagnosis, in both primary and secondary settings, is based on clinical examination and medical history. A key alerting feature is restriction of shoulder movement in all directions – passive and active range of movement. Blood tests, radiography and ultrasound are usually normal and not routinely required unless history or physical examination suggests the need to rule out other pathologies, for example if rheumatoid arthritis or osteoarthritis is suspected. Frozen shoulder is commonly managed in the primary care setting. In a UK study of patterns of referral and diagnosis of shoulder conditions it was estimated that 22% of patients were referred to secondary care, up to 3 years following initial presentation, although most referrals occurred within 3 months [11,12]. There is little evidence available on referral patterns in relation to frozen shoulder specifically [13-15].

The effectiveness of physiotherapy following discharge from hospital lacks evaluation. The uncertainty regarding effectiveness subsequently makes it difficult for commissioning organisations, health care practitioners and patients to make decisions regarding rehabilitation and service provision in the INDIA varies widely. The purpose of this thesis was three-fold.

Firstly, to evaluate existing evidence regarding post discharge physiotherapy exercises. Secondly, to contribute to the evidence base for this under-researched, but increasingly common, area of physiotherapy practice by developing and evaluating a new physiotherapy intervention. Thirdly to assess the feasibility of achieving blind outcome assessment in a pragmatic rehabilitation trial.

The main aim of our study was to identify and molecular characterization of the cell type or types involved in the pathological disorders of frozen shoulder, using new and previously unused immunocytochemical techniques and antibodies, in the hope that this may lead to earlier and more effective treatment of this often debilitating condition.

MATERIALS AND METHODS

Biopsy material from the rotator interval was obtained at arthroscopy from 22 patients with a diagnosis of primary frozen shoulder and subjected to histological and immunocytochemical analysis. The diagnosis of primary frozen shoulder was made according to Codman's criteria. All the patients were initially managed nonoperatively with intra-articular injection of local anaesthetic and steroid. There were 22 who failed to respond to conservative management and underwent a manipulation under anaesthesia, of whom ten were men and 12 were women, with a mean age of 53 years (41 to 66). The mean time from the onset of symptoms was 15 months (3 to 36). In 64% (14 of 22) arthroscopy was undertaken less than 12 months from onset of the condition. All patients had symptomatic pain and stiffness at the time of biopsy. There were five diabetics, three of whom were non-insulin dependent. The diagnosis of frozen shoulder was confirmed arthroscopically and a release of the tissue of the rotator interval performed. A biopsy was taken from this site and the material fixed in formalin and embedded in paraffin.

The blocks were cut into 5 µm sections using a Leica RM2135 microtome (Leica Microsystems UK, Milton Keynes, United Kingdom), heat adhered on to Snowcoat X-tra pre-cleaned micro slides (Surgipath, Winnipeg, Canada) and stored at 37°C. The slides were stained with haematoxylin and eosin, toluidine blue and congo red for histological analysis. Immunocytochemical analysis was performed with antibodies directed against CD45 (Leukocyte common antigen (LCA)), CD3 (T cells), CD20 (B cells), CD68 (macrophages), Lyve 1 (lymphatics), S100 (neural marker), PC10 and MIB1 (proliferative cell markers), vimentin, ásmooth muscle actin and calponin (F-actin and tropomyosin).

The sections of mounted tissue were de-waxed in xylene and rehydrated through decreasing alcohol concentrations. Endogenous tissue peroxidase was blocked with a hydrogen peroxide solution. Antigen retrieval is antibody dependent and was performed according to the manufacturer's instructions (DakoCytomation DK-2600, Glostrup, Denmark). Two main techniques were used: heat-induced epitope retrieval and enzymatic pre-treatment with trypsin. Visualisation was performed using the Autostrainer Plus (DakoCytomation) and the Chemate EnVision kit (DakoCytomation). This kit contains horseradish peroxidase conjugated secondary antibody on a polymer backbone, concentrated 32 32 diaminobenzidine chromogen and substrate buffer. After labelling with the primary antibody, the secondary antibody horseradish peroxidase-labelled polymer binds to the primary antibody. Bound horseradish peroxidase converts the diaminobenzidine into an insoluble brown precipitate visible on light microscopy. Standard laboratory positive control slides were used for all stains to confirm the success of the technique.

Table 1: Results for immunocytochemical and histological staining of biopsy material from the frozen shoulder.

S.No	Antibody	Cell type	Result*	No. of Patient positive (%) (n = 35)
1	CD45LCA	Leukocytes	+++	21
2	CD3	T cells	++	34
3	CD20	B cells	+++	41
4	CD68	Macrophages	+ +	34
5	Smooth muscle/Desmin	Myofibroblasts	+++	12
6	Lycel	Lymphatic endothelium	+++	13
7	S100	Neural tissue	+++	14
8	Vimentin	Fibroblasts	++	12
9	MIBI	Proliferating cells	+	11
10	Mast cell tryptase	Mast cells	+++	14

All the slides were examined under light microscopy and the presence of labelled cells was documented and tabulated. Absence of staining was documented as a negative result (-) and the presence of staining as a positive result (+) using a scale based on the number of cells per high-power field (×140), (+) = 1 to 4, (++) 5 to 10, (+++) = > 10, (++++) = > 100.

* result, number of cells counted per high-power field × 400; -, none +, 1 to 4; ++, 5 to 10; +++, > 10; ++++, > 100 † on some occasions, fragility of the tissue led to failure of the technique, resulting in n < 22 for some tests ‡ LCA, leucocyte common antigen

RESULTS

Histological analysis of the biopsy material showed evidence of scattered chronic inflammation, mast cell infiltrate and an absence of amyloid (Table 1). Immunocytochemistry confirmed an inflammatory infiltrate with significant positive staining for CD45 (LCA). Staining with CD3, CD20, CD68 and mast cell tryptase identified these cells to be predominantlymast cells, macrophages T cells and B cells. Inflammatory cells were identified in 35 patients (95%) The CD34 antibodies stained strongly positive, identifying high vascularity in 15 patients (68%). Lyve 1 and S100 antibody staining was frequently positive, demonstrating the presence of lymphatic

(90%, 18 of 20, where on two occasions fragility of the tissue led to failure of the technique) and nervous tissue (77%, 17 of 22), respectively. Staining with vimentin, a fibroblast marker, showed the majority of the cell population to be fibroblasts; 95% MIB1 (Ki 67) and proliferating cell nuclear antigen stained strongly, illustrating active proliferation of the fibroblasts. Desmin, ásmooth-muscle action and calponin (smooth muscle cell markers) were usually stained negative outside the vessel wall, indicating very little evidence of myofibroblast cells.

DISCUSSION

Since the first description of this condition nearly 150 years ago, 16 limited progress has been made in identifying the underlying mechanisms involved.1 Studies of cadavers and at open surgery and arthroscopy have identified the anatomical area predominantly involved in frozen shoulder tobe the rotator interval, which contains the coracohumeral ligament. [9,17-19] Biopsy material was therefore taken from this region for this study.

A great deal of work has been directed at the microscopic pathology of frozen shoulder and three schools of thought have emerged: first, that it is an inflammatory process,10,11 second, a fibrotic process,[12,13,17,20] and third [14], an inflammatory process with subsequent reactive capsular fibrosis. Frozen shoulder is a painful, stiff condition which often responds to intra-articular steroids. Macroscopically the capsular tissue is thickened, inflamed and congested in appearance [17,19,21]. These clinical and macroscopic features support the pathological findings of both inflammation and fibrosis. Characteristically, pain precedes stiffness in frozen shoulder, which suggests an evolution from inflammation to fibrosis. This study supports this theory by finding histological evidence of both an inflammatory cell infiltrate and fibrosis. Histological analysis showed a frequent scattered inflammatory infiltrate which was confirmed with immunocytochemistry using a variety of antibodies not previously used in the study of frozen shoulder. Analysis with CD3, CD20, CD68 and mast cell tryptase antibodies identified these cells to be predominantly mast cells, macrophages and T cells, confirming this to be a chronic inflammatory cell infiltrate. The presence of these immune system cells and of mast cells has not previously been seen in frozen shoulder. The CD34 antibodies stained strongly positive, identifying high vascularity resulting from angiogenesis. This vascularity is a characteristic macroscopic finding and is entirely consistent with the presence of chronic inflammation.

The pathology of frozen shoulder has been shown in other studies to be similar to that of Dupuytren's disease, but little evidence of a myofibroblast population,using the smooth muscle cell markers Desmin, ásmoothmuscleaction and calponin, was found in this study [13].

The absence of myofibroblasts may indicate that the patients in this study were in the early, inflammatory stage of the condition, rather than the more chronic recalcitrant patients examined in other studies. No differences in cell population were noted as regards the longevity or severity of the symptoms in this study, which may be a result of the small sample size. Mast cells regulate fibroblast proliferation both *in vitro* and *in vivo*.

The presence of mast cells in the biopsies leads us to postulate that these chronic inflammatory cells may be the cellular intermediary to the fibroblast proliferation. The presence of T and B cells may mean that this mast cellmediated proliferative fibrosis is an immunomodulated response. Further investigation is required to evaluate these cellular interactions more clearly. Differences in the cellular make-up were sought in the diabetic patients, but none were found, possibly as a result of the small sample size. Not every patient was stained with every antibody, as certain antibodies were interchangeable, such as proliferating cell nuclear antigen and MIB1 and ásmooth-muscle actin and Desmin. In certain patients tissue failure during the enzymatic or heat treatment process of antibody retrieval occurred with inadequate residual tissue precluding retesting in these patients. This explains the apparent disparity of number for some antibodies in Table 1.

This study provides an original contribution to knowledge in frozen shoulder and has important implications for enhancing clinical practice. The findings suggest that a hospital based exercise class produced a rapid recovery with a minimum number of visits to the hospital. Physiotherapy could also be considered to optimize speed of recovery of frozen shoulder. The Constant score, OSS and HADS are recommended in the management of frozen shoulder. Finally, GPs and physiotherapists require training in the clinical diagnostic accuracy of frozen shoulder. The need for further research in this area is emphasized.

CONCLUSION

In conclusion, arthroscope biopsy material from the rotator interval in patients with frozen shoulder revealed immunocytochemical evidence of both chronic inflammation and proliferative fibrosis, supporting the theory that this is both a chronic inflammatory and a fibrotic condition. Together with the presence of high vascularity and nerve tissue, this inflammation and fibrosis explains why frozen shoulder is such a painful and stiff condition. The presence of T and B cells suggests that the pathology may be immunomodulated. Mast cells, which are known to be involved in regulating fibroblast proliferation, may be the cellular intermediary between the chronic inflammation and fibrosis.

Conflicts of interest: None

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