Chondroitin sulfate epitope (WF6) and hyaluronic acid as serum markers of cartilage degeneration in patients following anterior cruciate ligament injury

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Abstract

Serum chondroitin sulfate epitope (WF6) and hyaluronic acid (HA) levels were determined to be of clinical relevance to an anterior cruciate ligament (ACL) injury. This cross-sectional study recruited participants from two distinct groups. Group A was comprised of 74 healthy controls, and group B consisted of 33 ACL injury patients. Serum samples were taken and assayed by a competitive immunoassay with monoclonal antibody WF6. Serum HA was also determined by an ELISA-based assay using biotinylated HA-binding proteins. Both groups A and B shared similar values of age, body mass index, white blood cell count and percentage of polymorphonuclear cells. ESR levels were also shown to be within normal limits. The serum WF6 epitope levels of group B were significantly higher than those of group A, whereas serum HA levels were not different between the two groups. The serum WF6 epitope level is more sensitive to changes in articular cartilage due to a non-inflammatory instability condition than the serum HA level, and should prove to be one of the most promising assays for early post-traumatic arthritis detection.

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1. Introduction

Post-traumatic osteoarthritis is a common outcome in young patients following injuries from either vehicle or sport accidents. Reconstructive surgery may not correct all of the problems that lead to the associated pathological conditions.1 To assess the subclinical changes, cartilage markers have been developed; these represent a major advance in the early detection and monitoring of the progression of cartilage pathology.2,3 Serum WF6 epitope was characterised and shown to recognise a specific pattern of sulfation in chondroitin 6-sulfate, which was variably expressed in the extracellular matrix of hyaline cartilage.4,5 There was an over-express of chondroitin sulfate proteoglycan in pathological conditions involving accumulation and turnover of the extracellular matrix.5 Serum hyaluronic acid (HA) level also has been widely studied and characterised, particularly for its biological role in arthritis conditions.4–6

Herein, we are interested in investigating the properties of the monoclonal antibodies WF6 and HA in association with changes of the extracellular matrix molecules of articular cartilage in anterior cruciate ligament (ACL) injury patients. This study also further characterises the markers of clinical relevance in post-traumatic patients.

2. Methods

This cross-sectional study recruited participants from two distinct groups between April 2003 and April 2005.
All participants consented to enter the study, and were admitted into hospital the night before taking a blood sample. All procedures were approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University. Group A was comprised of healthy controls and used similar criteria to group B, which consisted of ACL injury patients. The inclusion criteria was: a history of isolated ipsilateral traumatic knee injury; a definitive intra-operative diagnosis of ACL rupture; an age range between 16 and 45 years; being free from other diseases of the joints, bones, liver, and endocrine system, or from other chronic disorders. The exclusion criteria was: multiple ligament injury; associated articular injury; multiple-trauma patients; those currently taking any medication known to modify arthritic diseases or influence joint metabolism. Fasting morning serum was taken at 6:00–7:00 a.m., while the participants remained supine after wake-up. Serum samples were assayed by a competitive immunoassay with the monoclonal antibody WF6, as well as by an ELISA-based assay using biotinylated HA-binding proteins.4,5 Body mass index (BMI), white blood cell (WBC) count, percentage of polymorphonuclear (PMN) cells, and erythrocyte sedimentary rate (ESR) were analysed by using Student’s t-test. To evaluate the quantitative data of serum markers, we performed a non-parametric Wilcoxon rank-sum test. All data was considered significant when \( p < 0.05 \). The correlation of serum WF6 levels and the time from injury to the operative day was analysed by Pearson’s correlation coefficient test. A receiver-operating characteristic (ROC) curve was constructed to establish the diagnostic cutoff level of serum biological markers in order to discriminate anterior cruciate injury patients from healthy controls.4,5

### 3. Results

Both groups shared similar values of age, BMI, WBC count and percentage of PMN. Erythrocyte sedimentation rate results differed significantly between the two groups, but were determined to be within clinically normal range, as demonstrated in Table 1. A physical examination of group B patients revealed no signs of inflammation. The serum WF6 epitope level of group B was significantly higher than that of group A, whereas serum HA levels were not different between the two groups (Fig. 1). There were also no significant differences in serum WF6 epitope levels between participants with an ACL tear and those with an ACL tear with meniscus injury. There was no correlation between the time from injury to operative day and the rising of serum WF6 epitope levels, as shown by a regression analysis study (data not shown). For the ROC curve of serum WF6 epitope, the cutoff value was 1500 ng/ml for discriminating between groups A and B, thus providing the highest accuracy for diagnosis of an ACL injury.

### 4. Discussion

Serum WF6 epitope demonstrated the significant biochemical changes in chronic instability conditions, compared to healthy age-match controls. This finding corresponds with previous studies of human articular cartilage change following rupture of the ACL.2,3 The circulating serum WF6 epitope presents a low level in normal conditions4–6; and the quantity of chondroitin 6-sulfate in cruciate ligament, meniscus and synovial tissues is less than that in articular cartilage.6 Therefore, the increase of serum WF6 epitope released from the extracellular matrix of articular cartilage is due to the instability condition. This increase is lacking in ACL/ACL with meniscus injury patients without associated articular cartilage damage. For the cutoff value of serum WF6 epitope in clinical use, this study selected an optimal value of 1500 ng/ml, depending on the screening purpose of this marker. On the other hand, the cutoff value could rise to 3500 ng/ml with a high specificity purpose: that is, specificity of 81.1% and sensitivity of 54.5%. The area under the curve for serum WF6 epitope was 0.68 (95% confidence interval).
Serum HA level has been studied widely for various conditions. For arthritic conditions, HA is an important component of synovial fluid, which is mainly produced by synoviocytes. It is constantly cleaned and recirculated by the lymphatic system. Serum HA presents at low concentrations in normal conditions, and rises markedly in inflamed joints, correlated with disease activity. It is shown to rise not only in arthropic conditions, but also in chronic liver conditions which cause an increase in HA accumulation. Rising serum HA levels are influenced by several factors, including posture, eating and morning activities. Thus, we carefully selected all participants using strict criteria, and used a similar phlebotomy technique for both groups.

The analysis of serum HA levels in group B showed no significant difference from those of the control group. Therefore, serum HA is not deemed as suitable for detecting the changes of instability condition as is the serum WF6 epitope. This finding illustrates that a chronic instability condition can lead to gradual changes in articular cartilage. However, instability within a limited period, as in this model, may not coincide with the actual time required to develop osteoarthritis. A determination of what degree of instability, and for how long, will cause irreversible change and thereby lead to post-traumatic arthritis still requires additional supporting information.

Since serum WF6 epitope level was only recently introduced as a method of studying post-traumatic injury patients, the small sample size limits the ability to find relationships of statistical significance between pathological severity and biological marker levels. However, this result will benefit future studies by further characterising the clinical use of serum WF6 epitope, whether in the screening, monitoring or prognosis of post-traumatic joint patients after advanced surgical procedures. Biological markers are of considerable interest in identifying better criteria for diagnosis and improved methods for monitoring disease activity and progression. There have been many advances in medical approaches for halting the irreversible changes caused by osteoarthritis; however, they still lack the sensitive tools for addressing the treatment results. This study not only demonstrates the high sensitivity of serum WF6 epitope for detecting articular conditions, but also reveals some interesting aspects regarding instability conditions and post-traumatic osteoarthritis changes which merit further exploration.

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References


