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Original article

AGE-RELATED CHANGES OF CHONDROITIN 6-SULFATE WF6 EPITOPE LEVEL IN NORMAL HUMAN SERUM

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Abstract

Objective To investigate the level chondroitin sulfate WF6 epitopes in healthy populations by age-related variation.

Material and method Fasting-morning serum samples were collected during April 2003-2005. The volunteers were checked for free from diseases of the joints, bones, liver, endocrine system, or any other chronic disorders, and none of them were currently taking any medication known to modify arthritic diseases or influence joint metabolism. All of the samples were divided into seven groups depending on their age, and chondroitin 6-sulfate was quantitated by a competitive immunoassay using the monoclonal antibody, WF6.

Results The mean level of serum chondroitin sulfate in the 6thand 7th group(aged more than 55 years old) had a significant difference to that in the other groups(p<0.05).

Conclusion The striking level of chondroitin sulfate in the 6thand 7th group, which were aged older than 55 years, correlated with the prevalence of osteoarthritis. Since the samples were taken from non-symptomatic osteoarthritis populations, this epitope might be the earliest sign of cartilaginous change in osteoarthritis disease. **Chiang Mai Med Bull 2006;45(4):139-144.**

Keywords: chondroitin sulfate WF6 epitopes, age-related and biomarker

Osteoarthritis (OA) disease is a problem that has been faced by mankind for a long time. In the last two decades, there have been many recommendations for new groups of medication and modern methods of treatment for delaying the disability of patients before

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undergoing arthroplasty. Symptomatic slow acting drugs are developing a basis on the knowledge of biochemical change of the synovial joint. Although reported results compared classical medications, there has never been an ideal quantitative result of treatment. As the novel surgical technique in sport medicine includes cartilagenous repair, which comprises the mosaic repaired technique and cartilage transplantation, the method in monitoring the outcome is still an important problem.

Development of the chondrogenic marker is an innovation that monitors the degradable process of cartilage in both primary and secondary osteoarthritis, and evaluates the effectiveness of symptomatic slow acting drugs and any new surgical cartilaginous interventions.

The first biologically credible biochemical marker of cartilage follower Modified Koch's postulates. The second is regularly found in patients with OA of the joint, and the third from normal values by defining age appropriate to change in the state of OA, and marker change where applicable.[1] Several studies reported a quantitative change of biochemical markers and some institutes have tried to find an ideal biochemical marker for an effective way to monitor cartilaginous pathology and interventions[2-7]

This study established an assay for the investigation of chondroitin sulfate (CS) epitopes in serum samples of patients with joint disease. A novel monoclonal antibody, WF6, recognized a native epitope in CS chains. The mAb-WF6 is credible in biochemical methodology, and linked with the chondroitin-6-sulfate, which is the richest glycoaminoglycan in the extracellular martrix of hyaline cartilage tissue. According to a study in an animal model, the levels of serum chondroitin sulfate WF6 epitopes in iatrogenic induced osteoarthritis (hydrocortisone succinate intra-articulary injection) were higher in rabbits than in the control group.[8] Peansukmanee reported a significantly higher serum chondroitin sulfate WF6 epitope level in osteoarthritis horses than in non-osteoarthritis ones.[8] The extended study of chondroitin sulfate WF6 epitope level in humans poses the challenge of developing this antibody for clinical use. The objective of this study was to quantify the chondroitin sulfate WF6 epitope level in healthy humans by age-related variation.

Material and method

Fasting-morning serum samples were collected at the Chiang Mai University Hospital (Maharaj Nakorn Chiang Mai Hospital) during April 2003-2005, and they were divided into seven different groups. The first group comprised children aged below fifteen years, who were admitted to the Pediatric Unit of the Orthopaedics Department. They were checked for freedom from articular conditions. Their serum was taken at the time of intravenous saline administration before going to the operating room. The second to fifth group comprised healthy blood donors. They were checked for free from diseases of the joints, bones, liver, endocrine system, or other chronic disorders and none of them were currently taking any medication known to modify arthritic diseases or influence joint metabolism. The sixth and seventh group were healthy subjects who had come for an annually check up at the out-patients department. Serum samples were assayed by a competitive immunoassay with monoclonal antibody WF6, which recognized native epitopes in chondroitin sulfate chains without chondroi-tinase ABC digestion.

Statistical analysis

The age-related serum chondroitin sulfate WF6 levels of 1st -7th group were analyzed statistically by the Kruskall Wallis and ANOVA test to determine their difference.

Table 1. Number of subjects in each group classified by age

Group	Age range	Number of sample	M/F
1	0-15	21	13/8
2	16-25	35	17/18
3	26-35	30	15/15
4	36-45	34	17/17
5	46-55	30	15/15
6	56-65	29	14/15
7	>66	32	12/20

M = male, F = female

Results

Table 1 The number of subjects in each group, with age and sex determined.

Table 2 The quantitative data of serum chondroitin sulfate WF6 epitope concentration (ng/mL).

Figure 1 The level of serum chondroitin sulfate WF6 epitope concentration (ng/mL). Statistical analysis showing the mean level of each group.

There was no significant difference in mean level of serum chondroitin sulfate WF6 epitope levels between groups 1, 2, 3, 4 and 5. The 6th and 7th groups were significantly different to groups the 1, 2, 3, 4 and 5.

Discussion

The serum chondroitin sulfate epitope levels of the 1st to 5th group had no significant difference between each other, but they were significantly different to the 6th and 7th group. The striking level of chondroitin sulfate in the 6th and 7th group, which was aged over 55 years old, correlated with the prevalence of osteoarthritis from a previous report.[9] The prevalence of osteoarthritis in humans older than fifty years old is more than fifty percent and this group will present symptoms when they reach sixty years old. Since the samples were taken from non symptomatic osteoarthritis populations, the rising chondroitin sulfate epitope level in the last two groups might be the earliest sign of cartilaginous change in osteoarthritis disease. The serum chondroitin sulfate level in the first group, aged under 15 years, was at a higher level than the in the other groups, except for the last two. The possibility of a high serum chondroitin sulfate WF6 level in the first group created a greater cartilaginous component ratio per body weight and cartilagenous metabolism than in adults.[2] The difference of vascular anatomy, such as the epiphyseal arterial system, in which the chondroitin sulfate can mobilize in serum, was absent in adults.[10] The higher level of chondroitin

Table 2. Quantitative data of serum chondoitin sulfate WF6 epitope concentration (ng/mL)

Group	1	2	3	4	5	6*	7*
Mean	2984.79	2589.32	928.37	1329.16	1467.73	6849.94	7513.16
SD	2294.43	2704.66	1337.47	1369.70	1141.78	4857.43	5909.46
Max	8,359.00	89,408.71	3412.95	43,144.38	4,508.60	16,839.00	232,231.10
Min	26.05	10.85	25.60	11.90	139.40	47.80	95.30

*significant difference from other groups

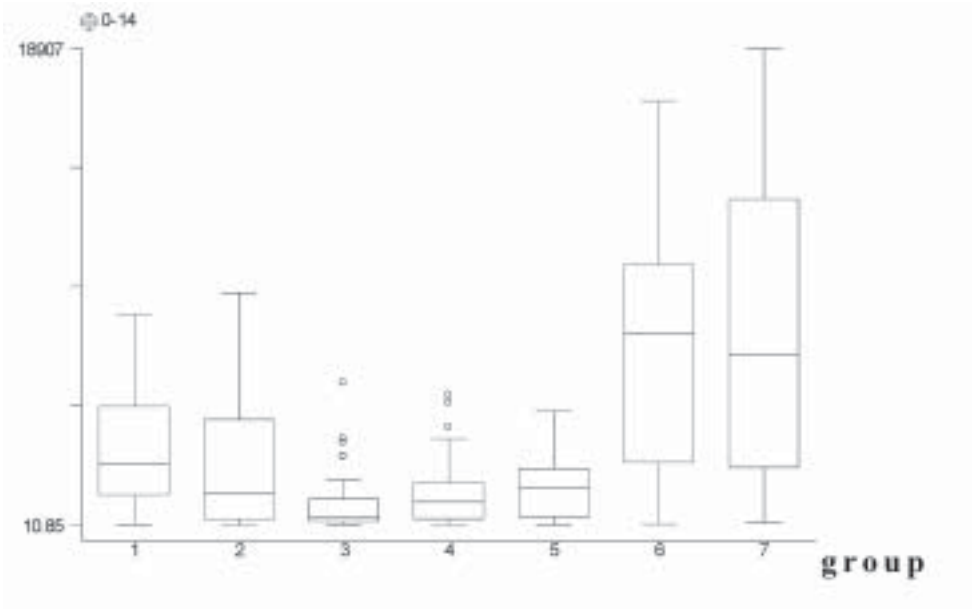

Figure 1. The level of serum chondroidtin sulfate WF6 epitope concentration (ng/mL). Statistic analysis showing the mean level of each group.

sulfate in the first group was not significantly different from the 2nd,3rd,4th and 5th group, possibly due to the smaller sample size.

As age is the strongest risk factor for primary osteoarthritis,[11] this study divided the samples into groups depending on the physical change of humans due to age. The first group comprised children and adolescents at puberty. The second group consisted of teenagers and early adults and the third to fifth groups were made up of adults who were sub divided into three groups. In the sixth group, samples gradually started to change in articular cartilage, and they presented over 50% prevalence of osteoarthritis disease in the 50 year-old population. In the last group, most of the people presented clinical symptoms of osteoarthritis, and those older than 65 years showed manual or pedal osteoarthritis on radiographs in 90% of woman and almost 80% of men.[9,11]

The circulating level of chondroitin sulfate WF6 epitopes is a balance of several factors; the release from tissue, the uptake within the lymphatics and their removal from serum by the liver once they have entered the circulation. Serum detection of WF6 may, therefore, be influenced by other factors that compromise liver uptake or kidney excretion of chondroitin sulfate proteoglycan metabolites, and care was taken to exclude any samples with other non-joint related pathology from this study.[12] Whether chondroitin sulfate WF6 epitopes relate to disease activity or progression will need to be evaluated in a more detailed longitudinal study.

Conclusion

Chondroitin sulfate WF6 epitope is the metabolic chondrogenic marker that can monitor the catabolism of cartilage in serum.[12,13]

It might be a catabolic biomarker that can be used for early diagnosis of osteoarthritis and may be the marker for measurement of responses to treatment after further detailed studies.

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การศึกษาระดับสารคอนดรอยตินซัลเฟต WF6 อิพิโทปในกระแสเลือดมนุษย์แบ่งแยกตามระดับอายุ

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บทคัดย่อ

วัตถุประสงค์ เพื่อศึกษาปริมาณสารคอนดรอยตินซัลเฟต WF6 อิพิโทปในแระแสเลือดมนุษย์ในอาสาสมัครที่มีสุขภาพดีโดยแบ่งแยกตามระดับอายุ

วิธีการศึกษา ทำการเจาะเลือดของอาสาสมัครที่งดน้ำและอาหารตั้งแต่ช่วง เดือนเมษายน พ.ศ. 2547-2548. อาสาสมัครที่ได้รับเลือกเข้าในโครงการวิจัยนั้นจะได้รับการตรวจร่างกายและตรวจทางห้องปฏิบัติการว่าไม่มีประวัติโรคข้อ, โรคตับ, โรคทางต่อมไร้ท่อ หรือโรคเรื้อรังชนิดอื่นๆ และอาสาสมัครทุกคนไม่ได้มีประวัติการทานยาปรับสมดุลโรคข้อใด ตัวอย่างเลือดจำนวน 5 มิลลิลิตร จะได้รับการแบ่งเป็นเจ็ดกลุ่มย่อยตามระดับอายุ จากนั้นนำตัวอย่างเลือดส่งไปห้องปฏิบัติการเพื่อส่งตรวจสารคอนดรอยตินซัลเฟต WF6 อิพิโทป

ผลการศึกษา ค่าเฉลี่ยของระดับปริมาณสารคอนดรอยตินซัลเฟต WF6 อิพิโทปในกระแสเลือดของอาสาสมัครในกลุ่มที่ 6 และ 7 (อายุมากกว่า 55 ปี) มีความแตกต่างอย่างมีนัยสำคัญกับค่าเฉลี่ยของระดับปริมาณสารคอนดรอยตินซัลเฟต WF6 อิพิโทปในกลุ่มตัวอย่างอื่นๆ ($p<0.05$)

สรุปผล ค่าเฉลี่ยของระดับปริมาณสารคอนดรอยตินซัลเฟต WF6 อิพิโทปในกระแสเลือดของอาสาสมัครที่สูงขึ้นมากในกลุ่มที่ 6 และ 7 นั้น มีความสัมพันธุ์กับความชุกของโรคข้อเสื่อมที่ยังไม่มีอาการในประชากรในระดับที่ได้เคยมีรายงานในการศึกษาก่อนหน้านี้ และเนื่องจากตัวอย่างเลือดได้นำมาจากอาสาสมัครที่ไม่มีอาการข้อเสื่อมเช่นกันนั้นหมายความว่าระดับปริมาณสารคอนดรอยติน-ซัลเฟต WF6 อิพิโทปอาจจะเป็นสารที่มีความไวในการให้การวินิจฉัยภาวะโรคข้อเสื่อมในผู้สูงอายุได้ **เชียงใหม่เวชสาร 2549;45(4):139-144.**

คำสำคัญ: chondroitin sulfate WF6 epitopes, age-related and biomarker