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The Distribution of YKL-40 in Osteoarthritic and Normal Human Articular Cartilage

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YKL-40, also called human cartilage glycoprotein-39, is a major secretory protein of human chondrocytes in cell culture. YKL-40 mRNA is expressed by cartilage from patients with rheumatoid arthritis, but is not detectable in normal human cartilage. The aim was to investigate the distribution of YKL-40 in osteoarthritic (n = 9) and macroscopically normal (n = 5) human articular cartilage, collected from 12 pre-selected areas of the femoral head, to discover a potential role for YKL-40 in cartilage remodelling in osteoarthritis. Immunohistochemical analysis showed that YKL-40 staining was found in chondrocytes of osteoarthritic cartilage mainly in the superficial and middle zone of the cartilage rather than the deep zone. There was a tendency for high number of YKL-40 positive chondrocytes in areas of the femoral head with a considerable biomechanical load. The number of chondrocytes with a positive staining for YKL-40 was in general low in normal cartilage. The present findings, together with previous observations, suggests that YKL-40 may be of importance in cartilage remodelling/degradation of osteoarthritic joints.

Key words: cartilage, human cartilage glycoprotein-39 (HC gp-39), immunohistochemistry, osteoarthritis, YKL-40

Osteoarthritis is a slowly progressive disease of unknown etiology resulting in loss of normal joint function due to articular cartilage breakdown. Multiple pathogenetic mechanisms are implicated and the disease process in the osteoarthritic joint involves an alteration in the normal balance of synthesis and degradation, not only of the articular cartilage but also of the subchondral bone and of the synovial membrane (1 – 3). The cartilage matrix metabolism is affected by both biomechanical factors and enzymatic pathways. In osteoarthritis several different proteases (metalloproteinases, serine proteases, and thiol proteases) degrade the cartilage matrix (4); the metalloproteinases are thought to be the central mediators of matrix degradation. Free radicals released from chondrocytes probably also contribute to the extracellular cleavage of articular cartilage matrix molecules (4). However, the details of the mechanism involved in the osteoarthritic pathogenesis are not known.

YKL-40[§], also called human cartilage glycoprotein-39 (HC gp-39) or 38-kDa heparin binding glycoprotein, is a chitin and heparin binding glycoprotein that is a member of a protein family related in sequence to bacterial chitinases but without chitinase activity (5 – 8). While the function of YKL-40 is not yet known, the tissue distribution of the protein suggests that it could function in tissue remodelling (5 – 14). The gene has been sequenced, but promoter analysis has not been described (15). Verheijden *et al*. have shown that HC gp-39 contains several DR4 peptide binding motifs and that HC gp-39 may be a target of the immune response in rheumatoid arthritis (16). When HC gp-39 was injected into BALB/c mice a chronic relapsing arthritis was found, and inhalation of the protein led to tolerance of antigen-specific T-cells and in suppression of HC gp-39 induced arthritis.

In vitro studies have shown that YKL-40 is one of the most abundant proteins secreted by human chondrocytes in primary culture (6, 7) and it is also secreted by human synovial cells (11), macrophages (8, 14, 17), and neutrophils (18). Hakala *et al*. (6) could not demonstrate mRNA-expression of YKL-40 in normal new-born or adult human cartilage. However, high levels were detected in articular cartilage from patients with rheumatoid arthritis. The regulation of YKL-40 is scantily evaluated; the secretion of YKL-40 by human chondrocytes *in vitro* is not influenced by the insulin-like growth factor-1 while the transforming growth factor-β reduces the release of YKL-40 to barely detectable levels (6), and the secretion of YKL-40 by human synovial cells *in vitro* is unaffected by the interleukine-1 and the tumor necrosis factor-α (11).

YKL-40 in serum and synovial fluid can be

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[§]The protein has been termed YKL-40 from its molecular weight (40 kDa) and the one letter code for its three NH2-terminal amino acids (tyrosine, lysine, and leucine) (9).

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measured by radioimmunoassay (5). We have found increased levels of YKL-40 in serum and synovial fluid from patients with rheumatoid arthritis or severe osteoarthritis of the knee joint compared to normals (5, 19) and further a relation between the level of YKL-40 in serum and synovial fluid are reported with 10 fold higher values in synovial fluid than in serum (19). The levels of YKL-40 in synovial fluid from an arthritic joint may originate from the articular cartilage (chondrocytes), the synovial membrane (synovial cells, macrophages), and polymorphonuclear leukocytes.

The object of the present immunohistochemical study was to analyse the detailed zonal and topographical distribution of YKL-40 in cartilage from osteoarthritic and macroscopically normal human femoral heads in order to identify differences in the staining of YKL-40 that could suggest a potential role for YKL-40 in the pathophysiology in cartilage remodelling in osteoarthritis.

Materials and methods

This study was part of a larger project involving articular cartilage and bone (20) and was approved by the local ethical committee.

Osteoarthritic cartilage: Samples of femoral head articular cartilage were obtained from 9 patients (all Caucasians. Seven females with a mean age of 72 years, range 39 – 85 years; and two males, 68 and 71 years) undergoing replacement surgery of the hip due to idiopathic osteoarthritis. All femoral heads contained areas with denuded bone. According to the macroscopic evaluation system of Collins and McElligott (21) the overall grades of osteoarthritis of the hip joints were III and IV. However, most of the samples were from areas where the cartilage appeared only partially affected or even macroscopically normal. The patients were informed verbally and in writing about the project, and all gave their consent.

Normal cartilage: Samples of macroscopically normal femoral head articular cartilage with Collins/McElligott grade 0 (21) were obtained from five subjects (all Caucasians. Two females, 66 and 82 years; and three males with a mean age of 78 years, range 72 – 88 years) at the time of autopsy and within 24 hours of death. None of the subjects had a previous clinical history of inflammatory or non-inflammatory joint disease or of chronic systemic inflammatory disease.

Collection of articular cartilage: Standardised samples of cartilage were collected from up to twelve topographically different areas of each femoral head: The anterior, posterior, lateral and medial aspects of perifoveal, central, and peripheral areas (Figure 1). The samples were stored in cryovials at $-80^{\circ}C$ until immunohistochemical analysis could be performed.

Only 75 cartilage sections from the 9 osteoarthritic femoral heads were evaluated; 33 sections were not scored due to inadequate quality of the sections, e.g. tissue folds (n = 11), or due to loss of sections (n = 18) either during the staining procedure or because many areas contained only denuded bone and hence no cartilage specimens were collected. In some cases (n = 4) only the middle and/or the deep zone of the cartilage section could be scored. A total of 53 sections from the 5 macroscopically normal femoral heads were evaluated; Seven sections were not scored due to inadequate quality (n = 4) or because of loss of section during the staining procedure (n = 3). Table I shows the number of sections suitable for immunohistochemical analysis.

Immunohistochemical staining for YKL-40: Four μm thick cryostat cartilage sections were cut perpendicular to the surface of the articular cartilage and mounted on glass slides (Super Frost + /Plus, Menzel-Gläser, Germany). The cartilage cryostat sections were acetone fixed at room temperature for 15 minutes. The immunohistochemical procedure was performed using a Shandon Sequenza (Life Science International, Basingstoke, U.K.) to prevent the cartilage sections from floating off and to achieve consistency of staining. The affinity purified polyclonal YKL-40 antibody was demonstrated by avidin/biotinylated horseradish peroxidase staining technique (ABComplex) as follows: Coverplates (Life Science International, Basingstoke, U.K.) and glass slides were fastened with Tris Buffered Saline (TBS; 0.05 M/0.15 M NaCl) and the sections were washed twice for 5 min with TBS. Non-specific binding was blocked by incubation for 10 min at room temperature with TBS containing 20% (v/v)

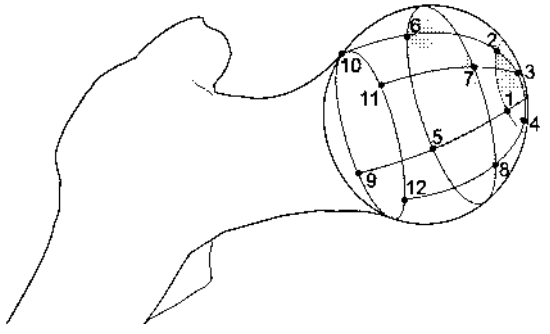

Fig. 1. From each femoral head standardised samples of cartilage were collected from twelve topographical preselected areas: the anterior, posterior, lateral, and medial aspects of perifoveal, central, and peripheral areas. *Perifoveal*: 1 = anterior, 2 = lateral, 3 = posterior, 4 = medial; *Central*: 5 = anterior, 6 = lateral, 7 = posterior, 8 = medial; *Peripheral*: 9 = anterior, 10 = lateral, 11 = posterior, 12 = medial.

Table I. The numbers of cartilage sections described according to the topographic location of the section and to the superficial, middle, and deep zone of each section from the 9 patients with osteoarthritic cartilage and the 5 age matched controls with macroscopically normal cartilage.

	Osteoarthritic cartilage			Controls Macroscopical normal cartilage		
	Superficial	Middle zone	Deep	Superficial	Middle zone	Deep
Topographical numbers						
Perifoveal:						
1	5	5	4	4	4	4
2	8	8	7	4	4	4
3	6	6	6	4	4	4
4	5	5	5	5	5	5
Central:						
5	5	7	5	4	4	4
6	6	7	4	4	4	4
7	7	7	6	4	4	4
8	6	7	6	5	5	5
Peripheral:						
9	6	6	6	4	4	4
10	7	7	7	5	5	5
11	7	7	6	5	5	5
12	7	7	7	5	5	5

The topographical numbers are presenting sample location on the femoral head (see Figure 1).
Perifoveal: 1=anterior; 2=lateral; 3=posterior; and 4=medial.
Central : 5=anterior; 6=lateral; 7=posterior; and 8 =medial.
Peripheral: 9=anterior; 10=lateral; 11=posterior; and 12 =medial.

normal swine serum (DAKO X901, Copenhagen, Denmark). Thereafter incubation for 30 min at room temperature with an affinity-purified rabbit polyclonal antiserum against human YKL-40 used at a protein concentration of 0.033 g/l diluted in TBS with 20% (v/v) normal swine serum. The rabbit anti human YKL-40 antibodies used in these studies were purified from antiserum by affinity chromatography using a Sepharose support with covalently attached purified human YKL-40. The antibodies were eluted by 100 mM glycine (pH 2.5). The specificity of the affinity purified polyclonal antibodies used in the immunohistochemical analysis was tested by Western blotting of material from conditioned serum free media from human articular cartilage explants after 5 days in culture. The antibodies reacted with a single 40 kDa band in the same position as YKL-40 (personal observation). The human YKL-40 used for immunisation and for affinity purification of antibodies was purified from the serum free conditioned medium of MG-63 cells by heparin affinity chromatography followed by gel filtration over Sephacryl S-300 HR, as described elsewhere (5). Nonimmune rabbit polyclonal IgG (DAKO X936) was used as control in the same IgG concentration of 0.033 g/l diluted in TBS with 20% (v/v) normal swine serum. Sections were washed twice for 5 min with TBS and then incubated for 30 min at room temperature with a swine anti rabbit IgG (DAKO E0353) used in a dilution of 1:400 in TBS with 20% (v/v) normal swine serum. Sections were washed twice with TBS. Antibody binding was visualised by incubation for 30 minutes with a complex of avidin and biotinylated horseradish peroxidase (ABComplex, DAKO 0355) and staining for 20 min with AEC (3-amino-9-ethylcarbazole) staining kit (SIGMA AEC101). The sections were counterstained with Mayer's hematoxylin and mounted in Glycergel Mounting Medium (DAKO C563). Positive staining was recognised as a dark red color associated with cell membrane and/or cytoplasm.

Microscopic evaluation: The full section of intact cartilage from articular surface to tidemark was divided into quarters: The ¼ of the full section of the cartilage, which includes the surface, was named the superficial zone; the ½ of the full section of cartilage below the superficial zone the middle zone; and the ¼ of the full section of cartilage below the middle zone and above the tidemark the deep zone. The calcified zone of articular cartilage was not included in the evaluation. Occasionally, the superficial layer was covered by a fibrous pannus which was not included in the zonal division of the section (20). The osteoarthritic cartilage sections were

evaluated for the presence of surface fibrillation/fissures and clusters/clones. In 81% of the sections these osteoarthritic histological changes were observed. Because inter- and intra observer reproducibility of immuno-histochemical scoring are rather low (22), we have chosen a rather crude, but reproducible, scoring system (Keld Ostergaard, unpublished observation). Minor variation in the staining of YKL-40 with respect to topography or zones may have been missed due to the immunohistochemical scoring system utilised. Each cartilage section was microscopically examined twice by one observer and scored for the presence of YKL-40 positive chondrocytes in the superficial, middle, and deep zone in a scale as follows: Score 0 = all chondrocytes negative; score 1 = more negative chondrocytes than positive chondrocytes; score 2 = positive and negative chondrocytes of approximately equal number; score 3 = more positive chondrocytes than negative chondrocytes; score 4 = all chondrocytes positive.

Statistical analysis: The statistical analysis was done with SPSS® (Statistical Package for the Social Science) Software. Comparison of the number of YKL-40 positive chondrocytes between the different zones of articular cartilage was calculated by Wilcoxon Matched-pairs Signed Ranked Test. Comparison of the number of YKL-40 positive chondrocytes of each zone between the 12 topographical locations was calculated by the Friedman two-way ANOVA test for all normal femoral heads. P values less than 0.05 were considered to be significant.

Results

Osteoarthritic cartilage sections: Light micrographs of immunohistochemical staining of YKL-40 by chondrocytes from articular cartilage from osteoarthritic femoral heads are illustrated in Figure 2. YKL-40 staining is found in the cytoplasm of the chondrocytes but not in the cartilage matrix as shown in a cartilage section from the superficial zone of location no. 6 (central and lateral) (Figure 2a). YKL-40 positive chondrocytes were mainly found in the superficial zone of the cartilage.

A cartilage section from the deep zone of location no. 12 (peripheral and medial) demonstrates that YKL-40 positive chondrocytes were also found in the deep zone of the cartilage (Figure 2b). The YKL-40 staining exhibited a granular distribution in the cytoplasm of the chondrocytes as shown in Figure 2c illustrating two chondrocytes from the superficial zone of a cartilage section from location no. 6 (central and lateral). Figure 2d illustrates the granular distribution of YKL-40 in a chondrocyte in the deep zone of the cartilage from location no.12 (peripheral and medial). A cartilage section from location no. 5 (central and anterior) illustrating both YKL-40 positive and YKL-40 negative chondrocytes in the superficial zone of cartilage covered with pannus is shown in Figure 2e. Chondrocytes in clusters/clones and in areas of excessive surface fibrillation showed a similar pattern of YKL-40 staining as isolated chondrocytes and chondrocytes in other areas of the same section.

The zonal distribution of YKL-40 positive chondrocytes in osteoarthritic cartilage is presented in Table II. In sections with histological changes, YKL-40 positive chondrocytes were found in 82% in the superficial zone, 81% in the middle zone, and in 62% of the sections in the deep zone. In the osteoarthritic cartilage sections without histological changes, YKL-40 positive chondrocytes were found in only 57% in the superficial zone, 57% in the middle zone, and in 45% in the deep zone. When positive, the sections scored from 1 (< 50% YKL-40 positive chondrocytes) to 4 (100% YKL-40 positive chondrocytes). In the sections with histological changes, the number of YKL-40 positive chondrocytes in the superficial zone (median score 1, range 0 – 4) and middle zone (median score 1, range 0 – 4) were significantly higher than that in the deep zone (median score 1, range 0 – 3) of the cartilage (superficial zone vs. deep zone $p = 0.007$; middle vs. deep zone $p = 0.000$, Wilcoxon Matched-pairs Signed

Table II. Zonal distribution of YKL-40 in articular cartilage from osteoarthritic or macroscopically normal femoral heads.

	SCORE				
	0	1	2	3	4
Osteoarthritic cartilage (with histological changes)					
Superficial zone	18%	44%	23%	12%	3%
Middle zone	19%	40%	29%	11%	1%
Deep zone	38%	30%	21%	11%	0%
Osteoarthritic cartilage (without histological changes)					
Superficial zone	43%	43%	7%	7%	0%
Middle zone	43%	57%	0%	0%	0%
Deep zone	55%	45%	0%	0%	0%
Controls, Macroscopic normal cartilage					
Superficial zone	77%	23%	0%	0%	0%
Middle zone	79%	21%	0%	0%	0%
Deep zone	92%	8%	0%	0%	0%

The values shown represent the percentage of the sections with a given score out of the total number of sections described in each zone. Score 0 = all chondrocytes YKL-40 negative; score 1 = more YKL-40 negative chondrocytes than YKL-40 positive chondrocytes; score 2 = YKL-40 positive and negative chondrocytes of approximately equal number; score 3 = more YKL-40 positive chondrocytes than YKL-40 negative chondrocytes; and score 4 = all chondrocytes positive.

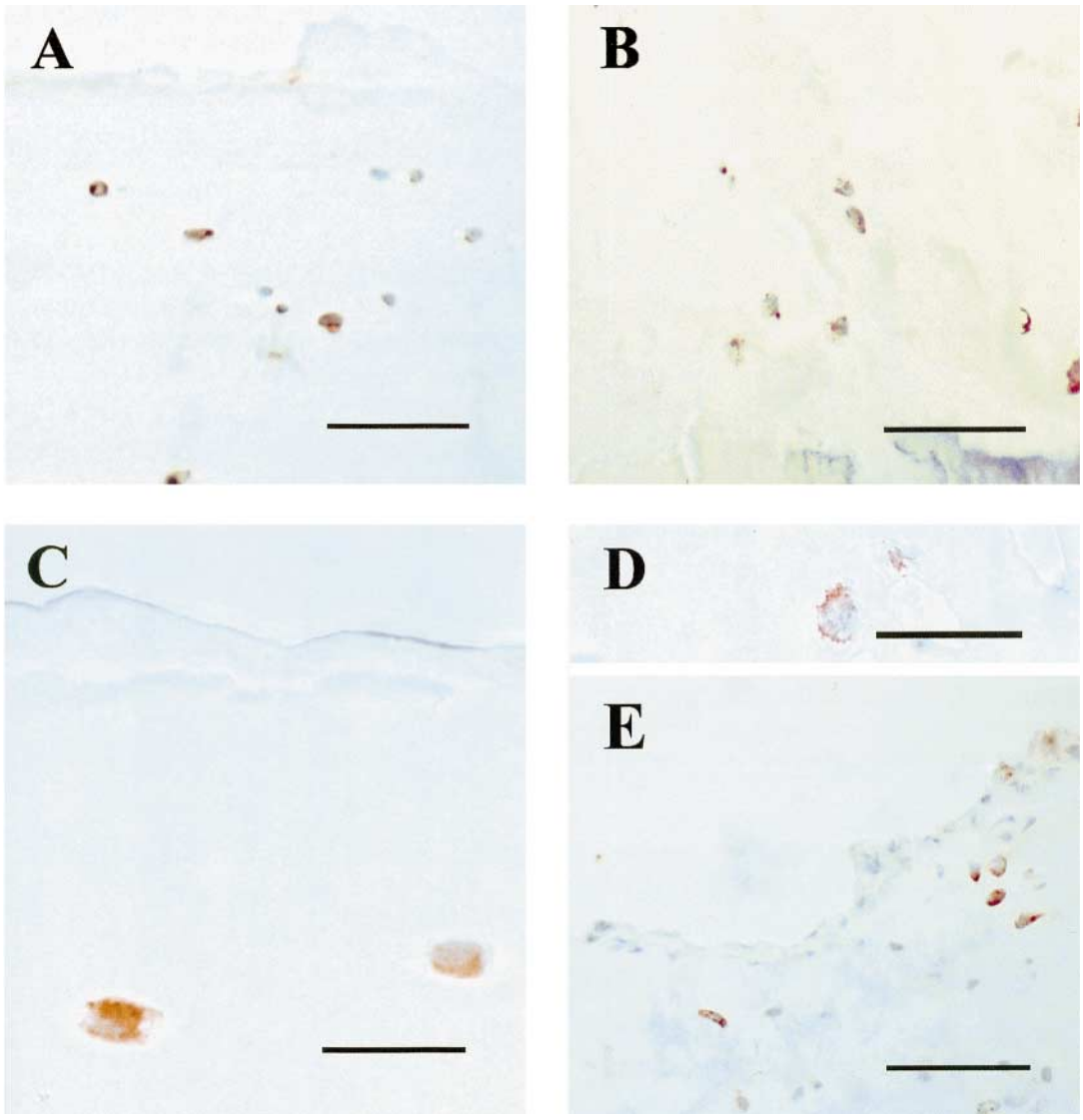

Fig. 2. Light micrographs of immunohistochemical staining of YKL-40 by chondrocytes from articular cartilage from osteoarthritic femoral heads. **A)** Cartilage section from location no. 6 (superficial zone) (see Figure 1) illustrating YKL-40 staining in the chondrocytes. YKL-40 positive chondrocytes were mainly found in the superficial zone of the cartilage (bar = 50 μm). **B)** Cartilage section from location no. 12 (deep zone): YKL-40 positive chondrocytes were also distributed in the deep zone of the cartilage, but the level of the YKL-40 staining was less prominent compared to chondrocytes in the superficial zone (bar = 50 μm). **C)** The micrograph shows a granular distribution of YKL-40 in the cytoplasm of a chondrocyte from the superficial zone of a cartilage. Section from location no. 6 (central and lateral) (bar = 20 μm). **D)** Chondrocyte from the deep zone of a section from location no. 12 illustrating the granular distribution of YKL-40 (bar = 20 μm). **E)** Cartilage section from location no. 5 (central and anterior) illustrating both YKL-40 positive and YKL-40 negative chondrocytes in the superficial zone of cartilage covered with pannus (bar = 50 μm).

Ranks Test). No significant difference was found between the YKL-40 staining in chondrocytes from the superficial and middle zone ($p = 0.463$, Wilcoxon Matched-pairs Signed Ranks Test). In the sections without histological changes, no significant difference was found between the number of YKL-40 positive chondrocytes in the superficial zone (median score 1, range 0 – 3), the middle zone (median score 1, range 0 – 2), and the deep zone (median score 0, range 0 – 1) of the cartilage (superficial zone vs. deep zone $p = 0.096$; middle vs. deep zone $p = 0.083$, superficial zone vs. Middle zone $p = 0.206$, Wilcoxon Matched-pairs Signed Ranks Test).

The topographical distribution of YKL-40 in cartilage with histological changes from osteoarthritic femoral heads is shown in Table III. In the superficial zone of the cartilage of the femoral head, most locations had a median score of 1 (range 0 – 4), and the highest scores were found at location 2 (perifoveal and lateral, median score of 2) and at location 6 and location 1 (central and lateral/perifoveal respectively, median score 1.5). Locations

Table III. Topographical distribution of YKL-40 positive chondrocytes in osteoarthritic cartilage with histological changes and macroscopically normal cartilage from femoral heads. Values are given as median scores (and ranges) of the sections at each topographical location and zone.

	Osteoarthritic cartilage with histological changes			Controls Macroscopical normal cartilage		
	Superficial	Middle zone	Deep	Superficial	Middle zone	Deep
Perifoveal:						
1	1.5 (1–3)	1 (1–2)	2 (1–2)	0 (0–1)	0 (0–1)	0 (0–1)
2	2 (0–4)	2 (0–3)	1.5 (0–3)	0 (0–1)	0	0
3	1 (0–3)	1 (0–3)	0 (0–3)	0.5 (0–1)	0.5 (0–1)	0 (0–1)
4	0.5 (0–2)	0.5 (0–2)	0 (0–2)	0 (0–1)	0	0
Central:						
5	1 (0–2)	0.5(0–1)	0.5 (0–1)	0	0	0
6	1.5 (1–4)	2 (1–4)	1 (0–3)	0 (0–1)	0 (0–1)	0
7	1 (1–2)	1 (1–2)	1 (0–1)	0 (0–1)	(0–1)	0
8	1 (0–1)	1 (0–2)	0.5 (0–2)	0	0	0
Peripheral:						
9	1 (0–2)	2 (0–2)	0 (0–2)	0	0	0
10	1 (0–3)	2 (0–3)	1 (0–3)	0 (0–1)	1 (0–1)	0 (0–1)
11	1 (0–2)	1 (0–3)	1 (0–2)	0 (0–1)	0 (0–1)	0 (0–1)
12	1 (0–3)	2 (0–3)	1 (0–3)	0 (0–1)	0 (0–1)	0

The topographical numbers are presenting sample location on the femoral head (see Figure 1). Perifoveal: 1=anterior; 2=lateral; 3=posterior; and 4=medial. Central : 5=anterior; 6=lateral; 7=posterior; and 8=medial. Peripheral: 9= anterior; 10=lateral; 11=posterior; and 12=medial.
Score 0=all chondrocytes YKL-40 negative; score 1=more YKL-40 negative chondrocytes than YKL-40 positive chondrocytes; score 2=YKL-40 positive and negative chondrocytes of approximately equal number; score 3=more YKL-40 positive chondrocytes than YKL-40 negative chondrocytes; and score 4=all chondrocytes positive.

2 and 6 were also the sites with the greatest frequency of YKL-40 positive chondrocytes in the superficial zone when the results were evaluated as the highest average score per section rather than as the highest median score, with an average score of 2.3 for location 2 and 2 for location 6 followed by 1.75 for location 1. In the middle zone, the highest median score were 2 and found at location 2, location 6, location 9, location 10 and at location 12, and the highest average scores were found for location 6 (average score 2.2) and location 2 (average score 1.7). In the deep zone, the highest median score was found for location 1 (median score 2) and location 2 (median score 1,5) and the highest average scores were found for location 2 (average score 1.7), location 1 (average score 1.67), and location 6 (average score 1.33).

The actual topographical position of the different numbered locations in the femoral head, with shading to illustrate the relative number of YKL-40 positive chondrocytes at each location in the superficial zone in osteoarthritic cartilage with histological changes is shown in Figure 3a. The locations with the highest number of YKL-40 positive chondrocytes, location 1 (perifoveal and anterior), location 2 (perifoveal and lateral) and location 6 (central and lateral), are areas of the femoral head with high biochemical load (23, 24).

Normal cartilage sections: The zonal distribution of YKL-40 positive chondrocytes in osteoarthritic cartilage is presented in Table II. YKL-40 positive chondrocytes were found in only 23% of the sections in the superficial zone, 21% sections of the middle zone and 8% of the sections in the deep zone, and none of the YKL-40 positive sections achieved a score higher than 1 (more YKL-40 negative chondrocytes than YKL-40 positive chondrocytes). The number of YKL-40 positive chondrocytes in the superficial zone (median score 0, range 0–1) and middle zone (median score 0, range 0–1) was higher than in the deep (median score 0, range 0–1), although the trend was less pronounced than in the osteoarthritic cartilage (superficial zone vs. deep zone $p<0.05$; middle zone vs. deep zone $p<0.05$, Wilcoxon Matched-pairs Signed Ranks Test). No significant difference was found in the number of YKL-40 positive chondrocytes between the superficial and middle zone of the cartilage ($p<0.7$, Wilcoxon Matched-pairs Signed Ranks Test).

The topographical distribution of YKL-40 positive chondrocytes in the macroscopically normal cartilage from femoral heads of the control subjects is shown in Table III and Figure 3 B. There was no overt variation in the frequency of YKL-40 positive chondrocytes with respect to the topographic site in any of the zones of the cartilage (superficial zone

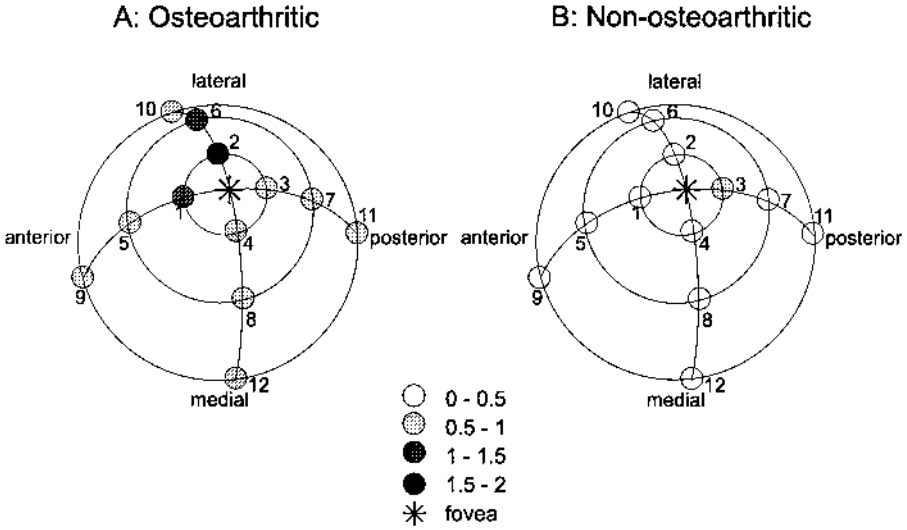

Fig. 3. The relation between topography on the femoral head and YKL-40 staining by chondrocytes in the superficial zone of **A)** osteoarthritic articular cartilage with histological changes, and **B)** macroscopically normal articular cartilage. A tendency for topographical variation with high number of YKL-40 positive chondrocytes (median score >1) was found at the perifoveal and anterior, perifoveal and lateral areas as well as at the central and lateral areas (see figure 1; location no. 1, 2 and 6) of osteoarthritic femoral heads. These are locations with a considerable biomechanical load. No overt variation was found in the number of YKL-40 positive chondrocytes in cartilage from macroscopically normal femoral heads.

$p<0.9$; middle zone $p<0.8$; deep zone $p<0.9$ all non-significant; Friedman Two-way ANOVA). Most locations had a median score 0 (range 0 – 1) in the superficial and middle zone, but a score 0.5 and 1 was found at location no. 3 (perifoveal and medial) and at location no. 10 (peripheral and lateral), respectively.

Discussion

The present immunohistochemical study of cartilage from femoral heads shows that YKL-40 is localised in the cytoplasm of chondrocytes. Demasking of YKL-40 by enzyme digestions might have revealed YKL-40 in the cartilage matrix. A negative result would, however, not exclude the presence of the antigen, as it might have been diluted or bound in a way preventing its detection. It is known that chondrocytes secrete YKL-40 into culture medium (6); therefore the existence of YKL-40 also in the articular intercellular matrix may be hypothesised. Chondrocytes located in the superficial and middle zone of the osteoarthritic articular cartilage had a greater frequency of YKL-40 staining than chondrocytes in the deep zone of osteoarthritic cartilage and also than chondrocytes in all zones from macroscopically normal cartilage. Our study suggested not only a zonal variation but also a tendency for topographical variation in YKL-40 expression; i.e. chondrocytes with a positive staining of the protein was found to be pronounced in areas lateral to fovea of the femoral head, an area known to be associated with especially high biomechanical load (23, 24). This indicates that the expression of YKL-40 may be a result of changes in both the biomechanical and biochemical environment of the chondrocytes.

A low intra- and interobserver reproducibility and a questionable validity of the original histological-histochemical grading system for osteoarthritic articular cartilage has recently been reported (22, 25). Therefore, we have not attempted to correlate the YKL-40 staining in chondrocytes from the femoral heads with a histopathological score obtained through such a grading system (26) or a modified system (27). We could not observe any difference in the staining pattern in chondrocytes in clusters/clones or in chondrocytes localised to areas of excessive surface fibrillation/fissures compared to isolated chondrocytes or chondrocytes in other areas of the same section. However, osteoarthritic cartilage sections with histological changes, i.e. surface fibrillation/fissures and clones/clusters, exhibited more YKL-40 positive sections than osteoarthritic cartilage sections without histological changes.

The present immunohistochemical findings of a higher number of chondrocytes with positive staining for YKL-40 in osteoarthritic cartilage suggest that YKL-40 may be of importance in the pathophysiology of cartilage remodelling taking place in osteoarthritis, although it can not be ruled out that the changes are caused by concurrent phenomena. That YKL-40 could play a role in cartilage remodelling, is also supported by the fact that YKL-40 mRNA can be detected in rheumatoid arthritic cartilage but not in normal cartilage (6). However, we found a sparse

expression of YKL-40 positive chondrocytes in some sections with macroscopically normal cartilage. Hakala et al. (6) could not detect mRNA YKL-40 expression from newborn or adult cartilage. The macroscopically normal cartilage in our study originated from older subjects with a mean age of 76 years. This may explain the difference between the findings of Hakala et al. and ours. Recently, we have analysed normal articular cartilage from young adults and could not find any chondrocytes with positive staining of YKL-40 (personal observation).

The metalloproteinases are believed to be the primarily mediators of articular cartilage matrix degradation (4). An increased expression and protein content of neutrophil collagenase (MMP-8) (28) and of interstitial collagenase (MMP-13) (29) in osteoarthritic cartilage compared to normal cartilage has been described. The expression was found to be associated with increased cleavage by collagenase(s) of type II collagen in osteoarthritis (30). Studies using *in situ* hybridisation have shown that expression of interstitial collagenase (MMP-1) (31) and MMP-8 (28, 32) were mainly detectable in chondrocytes in the superficial layer and upper region of the middle layer of osteoarthritic and normal cartilage. The present study has demonstrated that YKL-40 staining is found in chondrocytes in the same cartilage zones of the osteoarthritic cartilage as the metalloproteinases.

The physiological function of YKL-40 is unknown. YKL-40 is a chitin-binding lectin (8), but has no chitinase activity, probably because of the lack of glutamate in position 141 (6–8). Chitin, a polymer of β-1,4-N-acetylglucosamine, is one of the most abundant polysaccharides in nature and is found in the exoskeletons of insects, in shells of crustaceans, and fungal cell walls, but not found in vertebrates (33). Although YKL-40 lacks chitinase activity, there is a high level of amino acid sequence identity between YKL-40 and chitinases, particularly in the sequence regions that are thought to be involved in substrate binding in bacterial chitinases (6, 7, 34). This suggests that YKL-40 may be capable of recognising specific glycan structures in addition to chitin. Therefore the putative glycan binding activity of YKL-40 may target specific carbohydrate moieties on the cell surface or on other proteins for purposes such as the activation or destruction of these carbohydrate moieties during tissue remodelling. The pattern of YKL-40 expression and that of the other members of family 18 glycosyl hydrolases expression in tissues, shows that they are all found in situations where a considerable amount of tissue remodelling occurs (6–14,35,36). We have recently found that YKL-40 is a matrix protein of specific granules in human neutrophils (18). These granules contain a variety of proteins (37) that may be of significance in tissue remodelling, some of which are also expressed by cartilage e.g. MMP-8, gelatinase (MMP-9), and urokinase type plasminogen activator. YKL-40 released from the specific granules of the neutrophils could also be of importance in degradation of the extracellular matrix, for example in rheumatoid arthritis and other inflammatory diseases. Previously, we have reported that YKL-40 is detected in high levels in the synovial fluid from patients with rheumatoid arthritis and osteoarthritis (5, 19). It is not known if YKL-40 secreted by chondrocytes contribute significantly to the level of YKL-40 in synovial fluid, since YKL-40 is also secreted by synovial cells, macrophages, and neutrophils, all of which are found in an arthritic joint.

Assuming that the secretion of YKL-40 in neutrophils (18) and macrophages (14, 15, 17) is associated with a role in tissue remodelling/degradation, the synthesis of YKL-40 by chondrocytes could also indicate an importance of the protein in remodelling/degradation of the extracellular matrix of cartilage in osteoarthritic joints.

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