

## VARYING THICKNESS OF TIDE MARK IN ARTICULAR CARTILAGE OF AGEING MALE UNDERGOING OSTEOARTHRITIS IN PAKISTANI POPULATION

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**Objective:** To study increasing thickness of tide mark in articular cartilage of male with advancing age.

**Material and Methods:** Forty samples of articular cartilage of knee joint from male dead bodies of estimated age between 21-60 years, brought to forensic department within six hours of death and undergoing autopsy within 12 hours of death were collected. The samples were grouped as A, B, C and D depending upon the estimated age. The thickness of tide mark was recorded in different age groups.

**Results:** With advancing age, the thickness of tide mark was increased and highly significant statistically (p-value = 0.000).

**Conclusion:** As the age advances the thickness of the tide mark increases in articular cartilage of knee joint of male undergoing osteoarthritic changes & such changes are set in about a decade earlier in Pakistani population.

**Keywords:** tide mark (TM), articular cartilage (AC), osteoarthritis (OA).

### Introduction

Articular cartilage is a specialized connective tissue that covers the ends of the long bones in synovial joints. Its structural, biochemical and metabolic properties are unique in providing the joints with extraordinary resilience, smooth movements and ability to resist enormous physical pressure.<sup>1,3</sup> Articular cartilage follows the path of the bone morphogenesis. Roughly at the beginning of endochondral bone formation in an embryo, articular cartilage also becomes an independent entity. It is separated from the growth plate by the layer of proliferating chondrocytes that develops into mineralized calcified cartilage. This mineralized cartilage present at the bottom of the adult articular cartilage is separated from the upper layers by the tide mark, a microanatomical acellular structure with as yet undefined composition.<sup>4</sup>

The Chondro-osseous junctional region of articular cartilage is very complex and is considered to consist of the deepest layer of non-calcified cartilage that is the tide mark, the layer of calcified cartilage, a thin cement line between the calcified cartilage and subchondral bone.<sup>5</sup>

The tide mark is a clearly defined boundary separating uncalcified from calcified cartilage. It is not a straight line across articular cartilage, but a complex 3D structure and has a distinct microanatomical trilaminar appearance suggesting that this region is more complex and less

understood.<sup>6</sup> It is rich in collagen and contains hyaluronan but appears to lack glycosaminoglycans of conventional proteoglycan. It expresses a very distinctive and limited lectin staining glycoprotein profile, which is due to specific glycoprotein. Biochemical data has shown a high concentration of calcium phospholipid phosphate complexes in the tide mark.<sup>7</sup> Three distinct variations of the collagen framework of the tidemark were identified under scanning electron microscope by Redler I in 1975: (i) a band of randomly arranged compacted fibrils that appeared to be continuous with fibres of the non-calcified and calcified zones. (ii) A band of flattened fibrils running parallel to the undulating surface of the calcified cartilage. (iii) A band of perpendicularly oriented fibrils showing a continuous transition between the non-calcified and calcified zones.<sup>8</sup> The thickness of the single line of tide mark is up to 10 µm and its replication and thickness is taken to be an important feature of osteoarthritis. It is a calcification front advancing in the direction of non-calcified cartilage that reduces the thickness of hyaline cartilage and increases the thickness of calcified cartilage zone.<sup>5</sup> Degeneration of articular cartilage leads to many diseases such as osteoarthritis, a most common age related, non-inflammatory degenerative joint disease and a primary cause of pain and disability.<sup>5,9,10</sup> Knee joint is most commonly involved. The incidence of osteoarthritis is very high and it is only second to ischemic heart disease.<sup>11</sup> It is anticipated that by the

Year 2020 about 18% of the US population will be affected.<sup>12</sup> Nigel Arden (2006) reported that 60% of men living up to seventh decade suffer from knee osteoarthritis<sup>11</sup>. Although the epidemiology of osteoarthritis in the developing world is much less known, the prevalence of osteoarthritis knee in 2008 was 5.8% in India, 9.6% in China, 11.3% in Thailand, 5.1% in Indonesia and 7.5% in Bangladesh.<sup>13</sup> The prevalence of osteoarthritis increases in aged people, and it appears that aged cartilage incur changes which directly predisposes it to the development and progression of osteoarthritis.<sup>14</sup> This study is aimed to observe the thickness of tide mark in ageing articular cartilage indicating severity of osteoarthritis.

**Material And Methods**

A descriptive study in which changes in tide mark of aging articular cartilage were observed and compared with each other. It was conducted in Anatomy Department of King Edward Medical University, Lahore

Forty samples of articular cartilage of knee joint were collected from the autopsies of unknown male dead bodies brought to the Forensic Department of KEMU Lahore, of estimated age between 21 60 years within six hours of death. Dead bodies showing signs of trauma, surgery and gross abnormality and deformity of the knee were not included in the study.

The collected samples of articular cartilage were placed in four groups A, B, C and D depending upon the estimated age of the cadaver.

Group A: Estimated age ranging from 21-30 years

Group B: Estimated age ranging from 31-40 years.

Group C: Estimated age ranging 41-50 years.

Group D: Estimated age ranging 51-60 years.

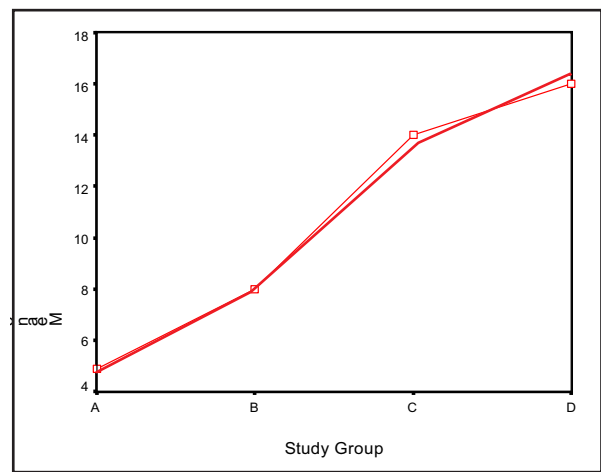
After doing dissection, Knee joint was flexed and 1 cm x 1 cm full thickness chip of articular cartilage was removed from the tibial surface of femoral condyle, 1 cm medial to the medial margin of intercondylar fossa.

The specimens were immediately placed in 10% neutral buffered formalin for 48 hours. Three random tissue samples of articular cartilage from each specimen were taken and processed for paraffin embedding. Five micrometer (5um) thick sections were made on rotary microtome and mounted on clear albumin coated slides. Sections were stained with Hematoxylin and Eosin for routine histological study and Masson's Trichrome stain was used for the evaluations of the histological features such as

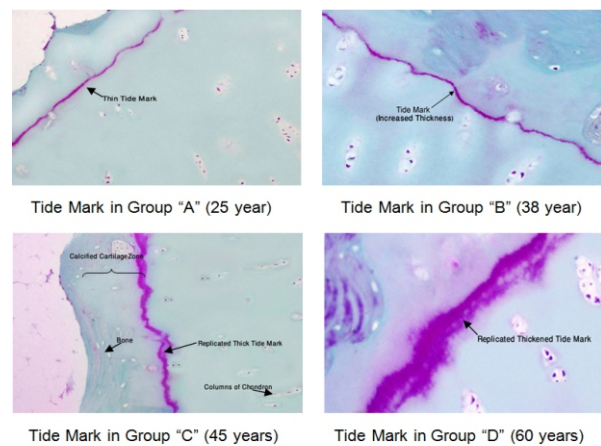
thickness of tide mark and zones of the articular cartilage.

**Results**

The mean thickness of tide mark was  $4.87 \pm 1.90 \mu\text{m}$  in group A,  $8 \pm 1.46 \mu\text{m}$  in group B,  $14 \pm 1.74 \mu\text{m}$  in group C and  $15 \pm 3.16 \mu\text{m}$  in group D. The mean thickness of the tide mark increased from group A to group D and was statistically significant in all study group (p-value = 0.000). Moreover the mean thickness in group A was less as compared to group B, C and D, significant statistically (p-value < 0.05). The mean thickness in group B was also less than group C and group D and was statistically significant (p-value < 0.05). The mean thickness in group C is less in comparison to group D (p-value = 0.04)



**Fig-1:** Mean of thickness and TM and UM.



**Fig-2:** Histomicrograph showing comparison of thickness of tide mark in articular cartilage in different study group (Masson's Trichrome).

**Table-1:** Descriptive analysis of thickness of tide mark and multiple comparison test of thickness in different study groups.

		N	Mean (um)	Std. Deviation	Minimum	Maximum	
<b>Study Grpoups</b>	<b>A</b>	10	4.8750	1.90485	2.50	7.50	
	<b>B</b>	10	8.0000	1.46723	6.25	10.00	
	<b>C</b>	10	14.0000	1.74801	12.00	16.10	
	<b>D</b>	10	15.0000	3.16228	10.00	20.00	
<b>Total</b>		40	10.7188	4.99108	2.50	20.00	
<b>Over all</b>				<b>0.000</b>			
		<b>A vs B</b>	<b>A vs C</b>	<b>A vs D</b>	<b>B vs C</b>	<b>B vs D</b>	<b>C vs D</b>
<b>Pair Wise</b>		0.003	0.000	0.000	0.000	0.000	0.047

## Discussion

This study describes the morphology of ageing male articular cartilage of knee joint and the degenerative changes that are related to the advancing age. In the present study, in calcified cartilage zone, the chondrons in all study groups were either isolated or arranged in groups. These findings were similar to the study of Bhosale and Richardson (2008) and Wang et al (2009) who noted that calcified cartilage zone with its two interfaces, the tide mark and the cement line was a zone with low metabolic activity having a small volume of dispersed cells embedded in calcified matrix<sup>15,16</sup>. It was noted that the thickness of calcified cartilage zone increased with the advancing age. Findings of present study were same as Hayami et al in 2006<sup>17</sup> who observed that thinning of articular cartilage with ageing was due to the surface degradation as well as increase in the thickness of calcified cartilage zone which resulted in reduction of the thickness of hyaline cartilage (upper three zones).<sup>18,19</sup>

In this study the tide mark was clearly visible in Masson's Trichrome stain. When the thickness of tide mark was compared in different study groups it was seen that the thickness increased with age. The mean thickness of tide mark was 4.8750 µm in group A, 8.0000 µm in group B, 14.0000 µm in group C and 15.0000 µm in group D. In addition to that, clear

replications of the tide mark were also observed. So it was noted that increase in the thickness of calcified cartilage zone is at the expense of decrease in the thickness of hyaline cartilage and this is due to the advancement of tide mark in the direction of hyaline cartilage. The duplications of tide mark and its advancement towards non calcified cartilage was also noted by Burr BD in 2011<sup>19</sup> while Oda et al in 2007<sup>18</sup> 71 described that the thickness of upper three zones as well as cellularity decreased with age, the findings that are very similar to this study in which it was observed that the thickness of the calcified cartilage zone and tide mark increased with age.

## Conclusion

Increased thickness of calcified cartilage zone, tide mark and its advancement towards hyaline cartilage are age related degenerative changes which are set in a decade earlier in articular cartilage of Pakistani population.

Factors affecting these changes need to be explored. These findings may act as guideline for modulation of preventive measures and treatment trends.

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