

## Interplay of Interleukin -1 $\beta$ , Nitric Oxide and Glycosaminoglycan in Synovial fluid obtained from Total knee replacement Patients may explain erosive Osteoarthritis

PriyaKulkarni<sup>1</sup>, Soumya Koppikar<sup>1</sup>, Dhanashri Ingale<sup>1</sup>, ShantanuDeshpande<sup>2</sup> and Abhay Harsulkar<sup>1</sup>.

<sup>1</sup>Interactive Research School for Health Affairs (IRSHA), BharatiVidyapeeth Deemed University Medical College Campus, Pune-Sarata Road, Pune 411043.

<sup>2</sup>Department of Orthopaedics, Bharati Hospital, Pune-Satara Road, Pune 411043.

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**ABSTRACT:** Synovial fluid (SF) analysis provides a snap-shot of various transactions between synoviocytes and chondrocytes. Using SFs from 'Total knee replacement' patients, we attempted evaluation of Interleukin -1 $\beta$  (IL-1 $\beta$ ), nitric-oxide (NO), Nitrate/Nitrite and Glycosaminoglycans (GAG), which play an important role in synovitis and cartilage degradation. Two knee Osteoarthritis patients of Kellgren-Lawrence Grade-4 are presented here. SF obtained (patient1 – bilateral, patient2 – right knee) by arthrotomy was analysed for IL-1 $\beta$ , Nitrate/Nitrite, NO and GAG by ELISA, Griess reaction and DMMB method respectively. Higher IL-1 $\beta$  (23.18 $\pm$ 5.5, 41.36 $\pm$ 2.3 and 89.09 $\pm$ 17.8pg/ml, respectively) was found in all samples, when compared with the normal values obtained from published literature (9.30 – 30.10pg/ml). NO (patient1L- 22.4 $\pm$ 0.0, patient1R- 15.9 $\pm$ 0.3 and patient2- 62.36 $\pm$ 0.6 $\mu$ M/ml) and its metabolites Nitrate/Nitrite levels were, patient1L- 23.34 $\pm$ 0.1, patient1R- 20.50 $\pm$ 0.5 and patient2- 77.66 $\pm$ 1.5 $\mu$ M. Remarkably high GAG was measured in all samples (460.83 $\pm$ 12.0, 310.83 $\pm$ 42.8 and 1570.0 $\pm$ 24.9pg/ml respectively). In the present study, correlation of GAG, NO and IL-1 $\beta$  could be observed in SF with respect to knee inflammation and Osteoarthritis severity. High GAG values in all the samples were indicative of cartilage loss to the greater extent. SFs from both knees of the same patient showed the difference in IL-1 $\beta$  and NO, suggesting their role in local inflammation.

**KEYWORDS:** Glycosaminoglycans, IL-1  $\beta$ , Knee Osteoarthritis, Nitric Oxide, Synovial fluid.

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### I. INTRODUCTION

Osteoarthritis (OA), a degenerative disease, is associated with reduced quality of life and amounts to enormous familial and social burden (1). OA pathology revolves around altered or impaired cytokine signalling (2) particularly IL-1 $\beta$  and TNF- $\alpha$ , leading to synovitis, an inflammatory condition, which destabilizes the process of cartilage remodelling leading to cartilage loss. Cartilage being aneural and avascular depends upon nutrients, enzymes, cytokines and growth factors available through SF (3). We here report investigations on two elderly ladies diagnosed having bilateral knee OA; at 'stage 4' on Kellgren & Lawrence grading (KL), a terminal stage of disease and operated for Total Knee Replacement (TKR). The patients were at equal radiological staging and their blood parameters were comparable. This enabled us to estimate markers retained in SF throughout the chain of events culminating towards the total cartilage loss. It is worth mentioning here that the patients were on anti-inflammatory medications for long periods, which might have affected the levels of inflammatory mediators (4).

**The clinical findings of the studied patients are summarized as follows:**

#### Patient 1

A 58 years old lady with complaints of pain and walking difficulty since 4 years revealed a tenderness of both knees joints on physical examination. Her left knee joint range of motion (ROM) was 0-90<sup>0</sup> while right knee joint ROM was 0-110<sup>0</sup>. The lady had a history of Ischemic Heart Disease and had undergone coronary artery bypass graft surgery in 2005. Her recent 2D-echo suggests mild aortic stenosis. The BMI was calculated as 22.89 kg/sq.m. The full leg standing radiograph showed a typical OA, Varus deformity and joint space reduction. A plain knee X-ray (standing) indicates sub-chondral sclerosis and medial osteophyte formation of right knee joint and thus diagnosed with tri-compartmental bilateral degenerative OA.

#### Patient 2

A 63 years lady undergone TKR of left knee in July 2011, had complaints of difficult and painful movement of right knee since last 4 years. Her BMI was 31.47 kg/sq.m. The lady was hypertensive and on regular uses of

chlorthalidone 6.25mg once a day. On her physical examination, stiffness of the right knee joint was noted. Left Ventricular Distolic Dysfunction was detected with normal 2D-echo (Ejection Factor 65%). Patient's knee radiographs showed a complete absence of joint space, lateral tibial-subluxion and Varus deformity indicating degenerative tri-compartmental OA thus suggestive for planned TKR.

## II. MATERIAL AND METHODS

### SF collection

Arthrotomy was carried out by most sterile way for collecting SFs. Both patients had standard midline incision and medial para-patellar approach while performing TKR. After minimal arthrotomy, the SF aspirated with 20cc of syringe and transported into sterile container. The protocol was approved by Institutional Ethical Committee (BVDU/MC/56). The obtained SFs were assayed for NO, nitrate/nitrite, GAG and IL-1 $\beta$  levels.

### Biochemical Assays

NO levels were measured as described (5). The Nitrate/Nitrite activities were determined by using a kit (Cayman, Michigan, USA) following manufacturer's instructions. GAG levels were measured by dye-binding assay, using 1,2-dimethylmethylene blue (DMMB) with chondroitin sulphate as standard (6). The SF samples were quantified for IL-1 $\beta$  using ELISA kit in plate format (Abnova, Walnut, CA) according to the manufacturer's instructions.

## III. RESULT AND DISCUSSION

Routine investigations of blood and SF were found within normal range (Table No.1). Samples Patient1L (left-knee), Patient1R (right-knee) and Patient2 (right-knee) were collected from patient1 and patient2 respectively. Higher values of IL-1 $\beta$  were obtained compared with control non-OA levels from published literature; the lowest was 23.18 $\pm$ 5.5 pg/ml (Patient1L). IL-1 $\beta$  is a pro-inflammatory, catabolic cytokine involved in initiation and progression of OA (7). Kokebie et al (8) has estimated 9.30–13.10pg/ml, as a standard range for IL-1 $\beta$  in non-OA subjects. In the present report, the highest IL-1 $\beta$  measured was in patient2 (89.09 $\pm$ 17.8 pg/ml); a case of tri-compartmental OA with tibial-subluxion, and varus deformity, one of the worst forms of OA found in practice. Interestingly, sample patient1L and 1R, although were from the same individual revealed different yet higher levels of IL-1 $\beta$ . This might relate to local insults, potentially leading to acute inflammation. Elevated IL-1 $\beta$  may trigger activation of matrix metallo-proteinases and other enzymes resulting cartilage degradation. It also has ability to induce pro-inflammatory mediators including cytokines, chemokines and angiogenic factors proliferating local hematopoietic cells (4). Higher IL-1 $\beta$  in all these samples was suggestive of acute synovial inflammation. NO is well-known as stress-response of cells mediated by iNOS, which is linked to cartilage degradation. NO potentiates IL-1 $\beta$  (and *vice versa*), worsen existing inflammation, inhibits collagen and proteoglycan synthesis and enhanced apoptosis (9). This is supported by current observations where higher IL-1 $\beta$  and higher NO were noted, which correlates with cartilage loss as indicated by GAG values. As an exception, patient1R showed higher IL-1 $\beta$  (41.36 $\pm$ 2.3 pg/ml) and lower NO (15.9 $\pm$ 0.3  $\mu$ M/ml), which could be the effect of long-term use of NSAIDs. Interestingly, patient1R also showed osteophytes, which correlates lower NO levels. NO may augment bone gain by inhibiting resorbing actions of prostaglandins, which may promote osteophyte formation(9). Vodovotz et al (10) associated TGF- $\beta$  in osteophyte formation, which is NO antagonist (11).

Increased GAG levels specify loss of aggrecan, which is a predominant proteoglycan found in articular cartilage (12). The physiological role of these molecules is not well understood however, evidence suggests that they may be involved in initiation of inflammatory response. Free GAG is strongly associated with allergy and inflammation and is co-released with histamine upon cellular degranulation(13). Highest GAG value in patient2 1570.0 $\pm$ 24.9  $\mu$ g/ml in sample was measured comparing to normal GAG value of 13.1  $\mu$ g/ml (14), which suggest cartilage loss to greater extend. In patient1R despite of high IL-1 $\beta$ , comparative lower level of GAG was observed (IL-1 $\beta$  41.36 $\pm$ 2.3 pg/ml and 310.83 $\pm$ 42.8  $\mu$ g/ml respectively).

## RESULT TABLES

Table no 1. Pathological investigations

Parameters	Patient1	Patient2	Normal Values
<b>Blood Analysis</b>			
Sodium	13 mmol/L	34 mmol/L	135-145
Potassium	4.3 mmol/L	4 mmol/ L	3.5-4.9
Chlorine	106 mEq/L	103 mEq/L	96-110
Urine (routine & microscopic)	NL	NL	None
Blood sugar level (BSL)	206 mg/dl (routine)	91mg/dl (FF) & 100 mg/dl (pp)	Routine: 80-110 Fasting: 90-130 PP: < 180
Blood urea level (BUL)	2.5 mg/dl	26 mg/dl	7-21
Serum Creatinine	0.63 mg/dl	1.10 mg/dl	0.6-1.4
Haemoglobin (Hb)	12 g/dl	12 g/dl	12.6-16.5
Total Leukocyte Count (TLC)	8190 /mm <sup>3</sup>	8600 / mm <sup>3</sup>	4500-10,000
Platelet count	245000 / mm <sup>3</sup>	323000 / mm <sup>3</sup>	150-400
<b>Synovial fluid Analysis</b>			
	Right Knee	Left Knee	
Clarity/Appearance	Slightly turbid	Slightly turbid	Slightly turbid
Colour	Pale yellow	Pale yellow	Pale yellow
Total nucleated cells	Nil	Nil	Nil
Gram stain	Nil	Nil	Nil
Culture	Nil	Nil	Nil
Total protein (g/dL)	4.60	5.10	3.60
LDH (IU/L)	182	78	35
Glucose (mg/dL)	100	120	35
Crystals	Nil	Nil	Nil
			70-110

Table No.2 Pro-inflammatory marker levels observed in SFs

Biomarkers	Patient1L	Patient1R	Patient2	Normal values
IL-1 $\beta$ Levels (pg/ml)	23.18 $\pm$ 5.5	41.36 $\pm$ 2.3	89.09 $\pm$ 17.8	9.30 -13.10 (8).
GAG Levels ( $\mu$ g/ml)	460.83 $\pm$ 12.0	310.83 $\pm$ 42.8	1570.0 $\pm$ 24.9	13.1 (14).
NO Levels ( $\mu$ M/ml)	22.4 $\pm$ 0.0	15.9 $\pm$ 0.3	62.36 $\pm$ 0.6	--
Nitrate/ Nitrite levels ( $\mu$ M)	23.34 $\pm$ 0.1	20.50 $\pm$ 0.5	77.66 $\pm$ 1.5	--

#### IV. CONCLUSION

In conclusion, the analysis of SFs presented here revealed prevalence of pro-inflammatory factors like IL-1 $\beta$  and NO and its presence at the terminal stage of disease may explain persistent pain and swelling seen in these patients. This interplay of inflammatory mediators might have triggered cartilage erosion that linked grade-4 degeneration seen on X-ray and higher GAG levels. These clinical and biochemical correlations helped to understand natural history of disease progression, although the results need to be confirmed on a larger data set.

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