

Correlation of the Osteogenic Protein-1 (OP-1) with Age, Cartilage metabolic Markers and Antioxidants in the Osteoarthritic Patients of Sikkim

SONAM CHODEN BHUTIA¹, T.A. SINGH², MINGMA LHAMU SHERPA³

ABSTRACT

Background: Age and oxidative stress have been implicated as the main causes of the cartilage damage in osteoarthritis (OA). The osteogenic protein-1(OP-1), a member of the bone morphogenetic family (BMP), plays a major role in cartilage repair.

Aim: To correlate the OP-1 in the synovial fluid with the age and antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx) and uric acid (UA) in the blood and with the cartilage metabolic markers like hyaluronic acid (HA) and keratan sulphate (KS) in the synovial fluid of osteoarthritic patients.

Method: Seventy five osteoarthritic patients were taken up for the study. Heparinized blood samples were collected for the

estimation of SOD, GPx and UA. Synovial fluid was aspirated for the estimation of HA, KS and OP-1. OP-1 was estimated by using a polyclonal antibody (anti-OP-1) which was produced against OP-1 in the mouse ascitic fluid by sandwich ELISA.

Result: Age and UA showed a significant correlation with OP-1. On the other hand, the correlations between OP-1 and the antioxidants (SOD and GPx) and the cartilage metabolic markers (HA and KS) were insignificant in osteoarthritic patients.

Conclusion: The study suggests about the important role of OP-1 in cartilage aging and degeneration, which may help us in understanding the potential for the therapeutic interventions in the treatment of cartilage degenerative processes.

Key words: Osteogenic protein-1, Antioxidants, Cartilage metabolic markers

INTRODUCTION

Osteoarthritis (OA) is characterized by the degradation of articular cartilage, which causes pain and disability [1], among which knee OA is the most common form [2]. The degradation of the articular cartilage is mainly caused by excess loading, aging, and a metabolic imbalance in the tissues [3-5]. The molecules derived from the cartilage, which are present in the synovial fluid, may be a markers of the biosynthetic changes or of degradative changes [6]. Keratan sulphate (KS) and Hyaluronic acid which are present in the synovial fluid have been studied as cartilage metabolic markers [7]. Reactive oxygen species have been seen to play a role in the pathogenesis of OA by causing oxidative damage which is generated by the cells within the joints [8]. To prevent the ROS toxicity, our body possess well co-ordinated antioxidant systems like SOD and GPx and non enzymic antioxidants like UA. The regeneration and repair of the cartilage have become one of the major obstacles in the current orthopaedics. OP-1, which is known as the Bone morphogenetic protein -7 (BMP-7), belongs to the transforming growth factor (TGF- β) superfamily, plays a crucial role in the cartilage repair and the maintenance of the articular cartilage integrity [9]. Many biochemical markers have been studied for monitoring the cartilage destruction [10,11]. The relationship between OP-1 in the synovial fluid and the antioxidants in the blood and the cartilage metabolic markers in the synovial fluid among the osteoarthritic patients, has not been studied much.

The present study aimed at finding the correlation between OP-1 and age, the cartilage metabolic markers and the antioxidants in osteoarthritic patients.

MATERIAL AND METHODS

This study was conducted in the Department of Biochemistry, Sikkim Manipal Institute of Medical Sciences (SMIMS), India.

Patient Selection

Seventy five clinically diagnosed patients with primary knee osteoarthritis, who were of the age group of 40–80 years, were selected from among the patients who attended the Sir Thutop Namgyal Memorial Hospital (STNM) and the Central Referral Hospital (CRH), Gangtok, India. Informed consents were obtained from the participants prior to the study.

Inclusion Criteria

The patients were selected according to the criteria which was described by the American College of Rheumatology [12].

Exclusion Criteria

Smokers, alcoholics, diabetics, hypertensives and the patients who were suffering from any other systemic diseases, which included the patients with post traumatic osteoarthritis, were excluded from the study.

The Sample Collection and Processing

Heparinized venous blood samples were collected and a part of it was centrifuged and the plasma was used for the estimation of UA (Uricase Method, RFCL, India) [13]. The other part of the venous blood was haemolyzed and it was used to estimate the activity of SOD by using the Ransod kit and the activity of GPx by using the Ransel kit (Both were obtained from the Randox Laboratory Ltd, UK) [14,15]. The levels of the antioxidants were analyzed in a semi-auto analyzer (mod: CHEM-5 plus V2, Transasia, ERBA, India). The synovial fluid was aspirated and centrifuged to remove the debris and the supernatant was used for the estimation of HA and KS by doing a sandwich enzyme linked Immunosorbent (ELISA) (Blue Gene Biotech Co Ltd, Shanghai, China) [16]. Their levels were read in an ELISA reader (RFCL, India).

OP-1 ESTIMATION

OP-1 was estimated by using anti-OP-1(IgG) which was produced against OP-1 in the mouse ascitic fluid in our laboratory, as was described by Cheiramaraj et al., [17], and anti-OP-1(sc-9305, Santa Cruz, USA) which was conjugated with horse radish peroxidase (HRP, Bangalore Genie, Bangalore, India), by Sandwich ELISA, in the synovial fluid of osteoarthritic patients. To calculate the concentrations of OP-1 in the synovial fluid, a standard curve was prepared by using known concentrations of OP-1 (78.4 ug/ml) which were diluted in TBS/Tween at concentrations which ranged from 0 ng/ml to 250 ng/ml.

STATISTICAL ANALYSIS

SPSS, version 16 was used for the statistical analysis. Pearson's correlation was used to study the correlation between OP-1 and age, the cartilage metabolic markers and the antioxidants in osteoarthritic patients. A p value which was <0.05 was taken as significant in our study.

RESULT

The correlation between OP-1 in the synovial fluid and the antioxidants (SOD, GPx, UA) in the blood and the cartilage metabolic markers (HA, KS) in the synovial fluid and the ages of the osteoarthritic patients have been shown in the [Table/Fig-1, 2 and 3].

n=75	OP-1 (ng/ml)	
	r-value	p value
SOD(u/ml)	- 0.067	0.567°
GPx(u/l)	0.139	0.235°
UA(mg/dl)	0.237	0.041*

[Table/Fig-1]: Correlation between OP-1 in the synovial fluid and antioxidants (SOD, UA, GPx) in the blood of osteoarthritic patient. p< 0.05 considered significant*, not significant°

n=75	OP-1	
	r value	p value
HA (ng/ml)	0.78	0.507°
KS(ng/ml)	0.65	0.581°

[Table/Fig-2]: Correlation between OP-1 and the cartilage metabolic markers in the synovial fluid of osteoarthritic patient p< 0.05 considered significant*, not significant°

n=75	OP-1	
	r value	p value
Age	-0.283	0.041*

[Table/Fig-3]: Correlation between OP-1 in the synovial fluid and age of osteoarthritic patients P<0.05 significant, Significant*

In the study, OP-1 showed a negative significant correlation with the age (r= -0.283). While the correlation between OP-1 and Uric acid (r=0.237) was positively significant, GPx, HA and KS (r=0.139, 0.78 and 0.65) showed an insignificant positive correlation. Whereas the correlation between OP-1 and SOD (r= - 0.067) was negatively insignificant.

DISCUSSION

The presence of OP-1 was documented for the first time in the human synovial fluid [7]. OP-1 has been implicated to have a wide range of anabolic and anti-catabolic activities [9]. In this study, the correlation between OP-1 and the age of the osteoarthritic patients was found to be negatively significant. This could be the result of the increase in the cartilage aging and degeneration [18 -20], which had led to the low level of OP-1, thus contributing to

the development of osteoarthritis. On the other hand, OP-1 in the synovial fluid showed a significant positive correlation with the UA in the blood of osteoarthritic patients, which may indicate the genetic and hormonal influence [21, 22] of uric acid.

We could not find a significant correlation between OP-1 in the synovial fluid with the antioxidants (SOD, GPx) in the blood and with the cartilage metabolic markers (HA and KS) in osteoarthritic patients. This may be due to the limitations of our study, where the antioxidant assay and OP-1 were studied in different body fluids. In the future, further studies would be required to correlate the antioxidants, the cartilage metabolic markers and OP-1 in the blood and the synovial fluid, to get a true picture about the relationship between these parameters in OA.

CONCLUSION

The results of our study suggest the important role of OP-1 between aging and OA. The relationship of OP-1 with the age and UA may help us in understanding the mechanisms of the development and the progression of OA, which may pave way for developing different preventive and therapeutic strategies.

REFERENCE

- [1] Carr AJ. Beyond disability: measuring the social and personal consequences of osteoarthritis. *Osteoarthritis cartilage*. 1999; 7: 230-38.
- [2] Haq SA, Davatchi F. Osteoarthritis of the knees in the COPCORD world. *International Journal of Rheumatic Disease*. 2011; 14: 122-29.
- [3] Aigner T, Haag J, Martin J, Buckwalter J. Osteoarthritis: aging of matrix cells-going for a remedy. *Current Drugs Targets* 2007; 8:325-31.
- [4] Aigner T, Gerwin N. Growth plate cartilage as developmental model in osteoarthritis research potentials and limitations. *Current Drug Targets* 2007; 8:377-85.
- [5] van der Kraan PM, Blaney Davidson EN, van den Berg WB. A role for age-related changes in TGF beta signaling in aberrant chondrocyte differentiation and osteoarthritis. *Arthritis Research and Therapy*. 2010; 12 :201-14.
- [6] Thonar EJ-MA, Manicourt D-H. Noninvasive markers in Osteoarthritis. In *Osteoarthritis: Diagnosis and Medical/Surgical Management*. 3rd edition. Edited by Markowitz R, Goldberg V, Vowell DS, Altman RD, Buckwalter J. Philadelphia: PA and WB Saunders; 2001;293-313.
- [7] Chubinskaya S, Frank BS, Michalska M, Kumar B, Merrihew CA, Thonar EJ, et al. Osteogenic protein 1 in synovial fluid from patients with rheumatoid arthritis or osteoarthritis: relationship with disease and levels of hyaluronan and antigenic keratan sulphate. *Arthritis Res Ther*. 2006; 8:R73.
- [8] Henrotin Y, Deby-Dupont G, Deby C, Franchimont P, Emerit I. Active oxygen species, articular inflammation and cartilage damage. *EXS*. 1992;62:308-22.
- [9] Rueger DC, Chubinskaya S. BMPs in articular cartilage repair. *Bone Morphogenetic Proteins: Regeneration of Bone and Beyond*. Edited by Vukicevic S, Sampath KT. Basel: Birkhauser Verlag AG; 2004: 109-32.
- [10] Reijman M, Hazes Jm, Bierma-Zeinstra SM. A Hs marker for osteoarthritis: cross-sectional and longitudinal approach. *Arthritis Rheum*. 2004; 50:2471-8.
- [11] Sugimoto H, Yamada H, Terada N. Intraarticular injections of high molecular weight hyaluronan for osteoarthritis of the knee-predication of effectiveness with biological markers. *J Rheumatol*. 2006; 33: 2527-31.
- [12] Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of Osteoarthritis: Classification of osteoarthritis of the knee. *Arthritis Rheum*. 1986; 29:1039-49.
- [13] Trinder P. *Ann. Clin. Biochem*. 1969; 6:24-27.
- [14] Woolliams JA, Wiener G, Anderson PH, Mc Murray CH. *Research in Veterinary Science*. 1983; 34:253-56.
- [15] Paglia D E, Valentine W N, *J Lab Clin Med*. 1967; 70:158.
- [16] Shanghai Bluegene Biotech CO., Ltd. Human Hyaluronic acid Elisa kit and Human keratan sulphate Elisa kit. Cat number-E01H0004, E0590HU. lot #20110815.
- [17] Cheiramaraj K, Reddy M V R, Harinath B C. Diagnostic use of polyclonal antibodies raised in mouse ascitic fluid in bancroftian filariasis. *J Immunoassay*. 1990;11: 429-44.
- [18] Chubinskaya S, Hurtig M, Rueger DC. OP-1/BMP-7 in cartilage repair. *Int Orthop*. 2007 December; 31:773-81.
- [19] Chubinskaya S, Kumar B, Heretis K, Merrihew C, Rueger DC, Kuettner KE. Age-related changes in cartilage endogenous osteogenic protein-1 (OP-1). *Biochim Biophys Acta*. 2002; 1588 :126-34.
- [20] Merrihew C, Kumar B, Heretis K, Rueger DC, Kuettner KE, Chubinskaya S. Alterations in endogenous osteogenic protein-1 with degeneration of human articular cartilage. *J Orthop Res*. 2003; 21:899-907
- [21] Lawrence JS. Rheumatism in population. *Proc R Soc Med*. 1977; 70 (6): 448-9.
- [22] Spector TD, Champion GD. Generalized osteoarthritis: a hormonally mediated disease. *Ann Rheum Dis*. 1989;48: 523-7.

PARTICULARS OF CONTRIBUTORS:

1. Lecturer, Department of Biochemistry, Sikkim Manipal Institute of Medical Sciences, Gangtok-737102, Sikkim, India.
2. Professor & Head, Department of Biochemistry, Sikkim Manipal Institute of Medical Sciences Gangtok-737102, Sikkim, India.
3. Associate Professor, Department of Biochemistry, Sikkim Manipal Institute of Medical Sciences, Gangtok-737102, Sikkim, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Sonam Choden Bhutia,

Lecturer, Department of Biochemistry, Sikkim Manipal Institute of Medical Sciences, 5th Mile, Tadong, Gangtok-737102, Sikkim, India.

Phone: 09735974048, E-mail: sonamcho07@hotmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Dec 12, 2012**

Date of Peer Review: **May 14, 2013**

Date of Acceptance: **May 15, 2013**

Date of Publishing: **Aug 01, 2013**