

PROFILE AND ASSOCIATION OF CIRCULATING SURVIVIN LEVEL WITH SEVERAL INDICATORS OF KNEE OSTEOARTHRITIS

Elysanti Dwi Martadiani¹, Sianny Herawati²

¹Medical Staff of Diagnostic Radiology, Udayana Faculty of Medicine/Sanglah General Hospital, Denpasar-Bali, Indonesia

²Medical Staff of Clinical Pathology, Udayana Faculty of Medicine/Sanglah General Hospital, Denpasar-Bali, Indonesia

ABSTRACT

Background: Chondrocyte apoptosis has been known playing a role in knee osteoarthritis (KOA) pathomechanism. Survivin is a survival protein which is believed to be involved in cellular division and prevention of cell death. Unfortunately, there are lack of studies exploring the role and profile of survivin in osteoarthritic knee joints.

Objectives: To study about the profile of circulating level of survivin and its correlations with several indicators related to primary KOA.

Materials and Methods: This is a cross-sectional study in primary KOA. Subjects are consecutively collected. Anamnesis, physical and laboratory examination, also knee radiographs were performed to determine KOA severity, knee pain severity, the presence of other joint OA instead of KOA, overweight, diabetes mellitus (DM), hyperuricaemia and OA medication. Plasma level of survivin was measured with ELISA method. The correlations between high plasma survivin level with KOA severity, knee pain severity and the presence of overweight were determined separately using Spearman's correlation tests. Multivariate linier regression test was done to find out the association of high plasma survivin level with all variables related to knee OA ($\alpha=0.05$).

Results: There were no significant correlation between high plasma survivin level with KOA severity or knee pain severity ($p>0.05$), although an inverse non-significant correlation showed by KOA severity ($r= -0.03$) and knee pain severity ($r= -0.08$) with high plasma survivin level. Only overweight and high plasma survivin level gave a significant positive correlation ($r=0.36$; $p=0.03$). Gender, presence of other joint OA, DM, hyperuricaemia, OA medication showed insignificant association with high plasma survivin level ($p>0.05$).

Conclusion: In primary KOA patients, knee OA severity and knee pain severity showed no significant correlation with high plasma survivin level, but overweight had a significant positive correlation with high plasma survivin level.

Keywords: plasma survivin level, knee osteoarthritis

Profil dan Hubungan Tingkat Survivin dalam Sirkulasi dengan Beberapa Indikator Osteoartritis Lutut

ABSTRAK

Latar Belakang: Apoptosis kondrosit telah diketahui memainkan peran pada patomekanisme osteoartritis lutut (*knee osteoarthritis* – KOA). Survivin merupakan suatu protein *survival* yang dipercaya terlibat dalam pembelahan sel dan pencegahan kematian sel. Sayangnya, hanya sedikit penelitian yang mempelajari peran dan profil dari survivin pada osteoartritis sendi lutut.

Tujuan: Mempelajari profil dari survivin yang bersirkulasi dan korelasinya dengan beberapa indikator terkait KOA primer.

Bahan dan Cara: Penelitian ini merupakan suatu studi *cross-sectional* pada KOA primer. Subjek secara konsekutif diikuti dalam penelitian. Anamnesis, pemeriksaan fisik dan laboratorium, serta foto polos lutut dilakukan untuk menentukan keparahan KOA, keparahan nyeri lutut, adanya OA sendi lain selain KOA, berat badan lebih, diabetes mellitus (DM), hiperurisemia dan pengobatan OA. Tingkat survivin plasma diukur menggunakan metode ELISA. Korelasi antara tingkat survivin plasma yang tinggi dengan keparahan KOA, keparahan nyeri lutut, dan adanya berat badan lebih diukur secara terpisah menggunakan *Spearman's correlation test*. *Multivariate linier regression test* dilakukan untuk menentukan hubungan antara tingkat survivin plasma yang tinggi dengan semua variabel yang terkait OA lutut ($\alpha=0.05$).

Hasil: Tidak ada korelasi signifikan antara tingkat survivin plasma yang tinggi dengan keparahan KOA atau keparahan nyeri lutut ($p>0.05$), walaupun terdapat korelasi terbalik non-signifikan yang ditunjukkan oleh keparahan KOA ($r=-0.03$) dan keparahan nyeri lutut ($r=-0.08$) dengan tingkat survivin plasma yang tinggi. Hanya kelebihan berat badan dan tingkat survivin plasma yang tinggi yang memberikan korelasi positif signifikan ($r=0.36$; $p=0.03$). Jenis kelamin, adanya OA sendi lain, DM, hiperurisemia, pengobatan OA menunjukkan hubungan yang tidak signifikan dengan tingkat survivin plasma yang tinggi ($p>0.05$).

Kesimpulan: Pada pasien KOA primer, keparahan OA lutut dan keparahan nyeri lutut tidak menunjukkan korelasi yang signifikan dengan tingkat survivin plasma yang tinggi, tetapi kelebihan berat badan memiliki korelasi positif signifikan dengan tingkat survivin plasma yang tinggi.

Kata Kunci: tingkat survivin plasma, osteoarthritis lutut

INTRODUCTION

Knee osteoarthritis (KOA), a most common form of OA, is a chronic degenerative joint disease, which is consisted of progressive disturbance and loss of joint cartilage, excessive bony growth and soft tissue changes surround the joint. This disease causes pain, loss of joint function and disability. Etiology of OA has not been completely understood, although several OA risk factor sare known involving in the cartilage degeneration.

Structural changes in OA cartilage manifest as cartilage fibrillation, fissure, ulceration and finally loss

of cartilage thicknes.¹ Changes in cartilage structure and periarticular bone causes modification of the articular surface contour.² Together with subchondral bone remodelling, those changes consequently influence the joint biomechanic and increase progressivity of cartilage break down.³

Knee radiograph allows visualization of bony changes in KOA. Bony changes in KOA include osteophyte formation as a result of reparative process in the low-stress area that often locates in peripheral site of the joint; subchondral bone sclerosis or eburnation as an effect of remodelling process; subchondral cyst or pseudocyst as a consequence of bony contusion that initiates microfracture which allows penetration of sinovial fluid into the bone; and joint space narrowing as a result of cartilage thinning.^{4,5} Radiographically, severity of KOA is determined using Kellgren and Lawrence classification, which is based on osteophyte, joint space narrowing, subchondral sclerosis and joint deformity.⁶

Speak about cartilage thinning or loss in OA, apoptosis or programmed cell in chondrocyte has an important role in the biomolecular pathomechanism of cartilage degradation. In physiologic condition, apoptosis in cartilage is unusual, due to the the existence of maintenance of metabolic homeostasis and chondrocyte adhesion to extracellular matrix proteins.^{7,8} But in OA, there is increasing influence of proapoptotic mechanicsm together with metabolic factors.⁹ Several studies found that apoptosis process in OA was antagonized by the initiation of various molecular antiapoptotic mechanisms.^{10,11}

Survivin (encoded by BIRC5), a 16.8 KDa protein, is the smallest member of family of inhibitor of apoptosis protein (IAPs).¹² It is comprised of one N-terminal baculovirus IAP repeat (BIR) domain and a long C-terminal-helix coiled region.⁹ Survivin is believed to be involved in cellular division and prevention of cell death. Some interactions happen between survivin with many proteins, regulators, transcriptional networks and modifiers, therefore survivin is considered as a key molecule expanding multiple parallel signalling in cellular homeostasis.¹³

It is well-known that expression of survivin is greatly up-regulated in malignancy and it is being involved in the important factors of tumour progression (for example cell proliferation, elusion of apoptosis, resistance to growth-inhibitory signals and angiogenesis).¹⁴ Recent studies reported that survivin also had important role in differentiation, growth, and

regeneration of healthy tissues including hematopoietic stem cells.¹⁵ Some studies investigated the role of survivin in non-oncologic musculoskeletal disease, such as rheumatoid arthritis (RA). High levels of survivin mRNA and protein have been reported in the inflamed synovial membrane in RA^{13,16,17}, synovial fluid¹⁸ and peripheral blood samples.¹⁹ In contrast to RA, the role of survivin in osteoarthritic joints is still unclear. Although apoptosis process is greatly involved in joint cartilage loss, there were only limited studies exploring about survivin and knee osteoarthritis (KOA). Based on those conditions, this study was performed to explore about the profile of circulating level of survivin in primary KOA.

MATERIAL AND METHODS

This research is a cross sectional study to investigate the correlations between plasma survivin level with several indicators related to primary KOA. Research subjects were primary knee OA patients who visited Geriatric Ambulatory Ward of Sanglah General Hospital, Denpasar, Bali, during six months observation period. Subjects were collected consecutively. Inclusion criteria are bilateral primary KOA patients, aged ≥ 50 year-old, who had comparable degree of KOA severity between right and left knee. Exclusion criteria included patients with symptoms of knee synovitis, concomitant tumor or malignancy and RA.

Independent variable was the level of plasma survivin. Dependent variables were KOA severity, knee pain severity, the presence of other joint OA, overweight, gender, diabetes mellitus (DM), hyperuricaemia and OA medication. Anamnesis using questionnaire was done to get information about knee pain or pain of other joint, OA medication, and history of suffering from tumor, RA or DM. Physical examination was performed to evaluate knee range of movement (ROM), the presence of visible or palpable tumor and the presence of knee synovitis (pain together with joint swelling and disturbance of knee ROM). Primary knee OA diagnosis was based on the American College of Rheumatology (ACR) criteria 1986.²⁰ Knee radiograph was performed using weight bearing anterior-posterior and lateral projection with extended knee, interpreted by a radiologist. KOA grading for its severity was determined from knee radiograph using the Kellgren and Lawrence (K&L) classification²¹ and subsequently classified as early (K&L grade I-II) and advance stage (K&L grade III-IV). Radiograph was performed for patient with clinically suspicion of OA in other joint (such as shoulder, hand, spine, hip, or ankle

OA). Knee pain was evaluated using visual analogue scale (VAS) for each knee, which ranged from no pain until severe pain (scale 0–100 mm). Body weight and height were measured to calculate body mass index (BMI), and classified as overweight if BMI 25-30 kg/m² and obese if BMI >30 kg/m². If subject completed the criteria of overweight or obese, we include them into overweight group.

Peripheral blood sampling was done to evaluate serum urate concentration, plasma fasting glucose, and also to get plasma sample for survivin measurement. Hyperuricemia if serum urate concentration is more than 7 mg/dl. Diabetes mellitus was determined based on WHO criteria (2006). Plasma survivin measurement was using human survivin kit (Quantikine, R&D System®, Inc., USA), which used quantitative sandwich ELISA using monoclonal antibody specific for human survivin. We divided plasma survivin level into high and low level, based on its concentration above or under the median value.

The correlations between KOA severity, knee pain severity and the presence of overweight with high plasma survivin level were determined separately using Spearman's correlation tests. Multivariate linier regression test was done to find out the association of high plasma survivin level with gender, presence of other joint OA, DM, hyperuricaemia, and OA medication ($\alpha = 0.05$).

Before this study started, research ethical clearance was agreed by the local ethical committee (Department of Research and Development, Faculty of Medicine Udayana University-Sanglah General Hospital Denpasar).

RESULT

There were 37 subjects with primary KOA who participated in this study, consisted of 6 males (16%) and 31 females (84%). Table 1 showed about subject characteristic.

From all of the subjects, mean plasma survivin level was 37.76 ± 72.2 pg/mL. Median value of the plasma survivin level was 7.5 pg/mL. There were 19 subjects (51%) had low plasma survivin level, and 18 (49%) subjects had high plasma survivin level.

Statistic analysis showed that there was no

significant correlation between KOA severity or knee pain severity with high plasma survivin level ($p > 0.05$, respectively). Significant correlation ($p = 0.03$) was shown by overweight and high plasma survivin level (Table 2).

Table 1. Subject Characteristic

Parameter	Mean \pm SD	n (%)
Sex: Male		6 (16%)
Female		31 (84%)
Age (year): Mean \pm SD	63.14 \pm 7.46	
Knee pain: Mild-moderate		15 (41%)
Severe		22 (59%)
Having OA medication: Yes		2 (5%)
No		35 (95%)
Comorbid diseases: DM		5 (14%)
Hyperuricemia		3 (8%)
Suffered from other joint OA		7 (19%)
BMI (kg/ m ²)	26 \pm 3.66	
Overweight (BMI > 25 kg/ m ²)		20 (54%)
KOA severity: Early stage (K&L I-II)		20 (54%)
Advance stage (K&L III-IV)		17 (46%)

*K-L: Kellgren Lawrence Scale

Table 2. Correlation between KOA severity, knee pain severity and overweight with plasma survivin level

Plasma Survivin Level	Correlation Coefficient (Spearman)	p value
Indicator		
KOA severity	-0.03	0.86
Knee pain severity	-0.08	0.65
Overweight	0.36	0.03*

*: significant ($p < 0.05$)

Table 3. Association between high plasma survivin level with several variables related to KOA

Variable	B	CI	p value
Knee OA severity	-0.91	0.06-2.84	0.36
Knee pain severity	-1.85	0.01-1.82	0.14
Overweight	3.16	1.59-348.78	0.02*
Gender	2.42	0.49-251.65	0.13
Presence of other joint OA	-0.06	0.09-10.01	0.96
DM	-0.46	0.08 -4.88	0.66
Hyperuricemia	2.56	0.36 -475.96	0.16

*: significant ($p < 0.05$); B : constanta; CI :Confidence Interval

Whilst multivariate linier regression test applied to confounding variables (KOA severity, knee pain severity and overweight with involvement of gender, presence of other joint OA, DM, hyperuricaemia, and OA medication as confounding factors), it demonstrated that there was no significant association between

confounding variables with high level of plasma survivin, but overweight was still had significant correlation with high plasma surviving level (Table 3)

DISCUSSION

Osteoarthritis is an interaction of several systems consists of biomechanical, biochemical, enzymatic and molecular. In OA, degradation process of cartilage matrix is more dominant than synthesis, as a consequence of failure to maintain cartilage homeostasis.^{22,23}

Chondrocyte is programmed to respon direct biomechanic disturbance received by the joint. Physiochemical changes in cell morphology, membrane cell deformity, and also the fluid flow changes in the connective tissue that caused by mechanical loading will be detected by chondrocyte mechanoreceptors (e.g. integrin, mechanosensitive ion channel and connexin). Activation of these mechanoreceptors stimulates intracelular signal cascades which regulate gene expression, protein changes and tissue remodelling.^{3,24,25} Mechanical loading also activates stress-activated ion channels (SACs), one of chondrocyte's membrane receptor, results in stimulation of interaction of SACs with integrin. This interaction causes intracelular calcium flux, thus increases intracelular calcium concentration, and afterward stimulates mitochondria to release apoptosis inducing factor (AIF) and cytochrome C. Release of cytochrome C causes activation of caspase-9 and caspase-3, the mediators of apoptosis, which ends as chondrocyte apoptosis.^{23,25} Integrin activation also stimulates intracelular signaling pathway, which stimululates chondrocyte to produce proinflammatory citokines, chemokines, proteolytic enzymes, eicosanoid agents and nitrite oxide, that subsequently initiates catabolic and anabolic imbalance in chondrocyte and causes cartilage matrix degradation eventually.²³

Survivin, is a bifunctional protein that regulates cell division and suppresses apoptosist. It acts as a potent inhibitor of apoptotic cell death through inhibition of caspase activity, and protects cells as a stress response factor against unfavourable environments.²⁶ Survivin is highly expressed during embryonic development and may be important in tissue homeostasis and differentiation. Among IAP family member, surviving exhibits the most restricted expression in adult tissue but has been identified in several apoptosis-regulated fetal tissue.²⁷

So far, there are limited data explain about correlation of survivin and KOA. To our knowledge, this is the first study evaluates about correlation about circulating survivin level in KOA, although we could not provide evidence of significant correlation between survivin level and KOA severity. Even though not significant statistically, there are some interesting finding from our result. First, about tendency of the inverse correlation between KOA severity ($r = -0.03$, $p > 0.05$) with high level of plasma survivin. Lechler *et al.*, (2011) reported that antiapoptotic protein survivin is re-expressed in osteoarthritic human cartilage and primary human chondrocytes, but it was not detectable in macroscopically and microscopically unaffected cartilage of osteoarthritic knee joints, and suppression of survivin resulted in inhibition of cell cycle progression and sensitization toward apoptotic stimuli *in vitro*.⁹ Our result opens a new possibility that survivin may 'protect' cartilage from apoptosis process so that its level runs inversely with KOA severity as a manifestation of knee cartilage breakdown, but these hypothesize has to be investigated further.

The other concern about our result is the trend of inverse non significant correlation between knee pain severity and high plasma survivin level ($r = -0.08$; $p > 0.05$) in KOA. It offers a hypothesis that survivin may decrease knee pain through some signal transducer mechanism. One of pain-related mediator associated with OA is prostaglandine, especially prostaglandine E2 (PGE2). It has been implicated in the pain signaling²⁸, cartilage erosion²⁹ and inflammation associated with OA.³⁰ Several studies reported that PGE2 regulates survivin expression.^{31,32} If further study could prove the role of PGE2 in survivin expression in KOA, it may develop a new perspective about using of antiprotaglandines for decreasing knee pain and increasing survivin for preventing further degradation of joint cartilage.

A significant positive correlation was demonstrated by overweight with high plasma survivin level ($r = 0.36$; $p = 0.03$). High BMI is well known as a risk factor for KOA development. One of possibility that explains pathogenesis of OA and overweight is via leptin.^{33,34} Leptin is a hormon that firstly synthesized by adipocyte and has strong relationship with the amount of adipose tissue and BMI. Leptin may increase production of proinflammatory factor in cartilage through induction of production of nitrite oxide, PGE2, interleukin-6 and interleukin-8.³⁵ Several studies found that leptin increases the expression of survivin in breast cancer.^{36,37} Those may explain the relationship between overweight, leptin and survivin. In KOA, we assume that

there may be a role of leptin in regulation of survivin, thus it needs to be advancedly studied.

Limitation of this study is we did not investigate the cartilage tissue directly to know about the exact level of survivin in the OA tissue, as a consideration that survivin level in peripheral blood could be influenced by many factor, thus persuades its circulating level. Further studies are needed in order to explain about biomolecular pathways between survivin and KOA.

CONCLUSION

In primary KOA patients, knee OA severity and knee pain severity showed no significant correlation with high plasma survivin level, but overweight had a significant positive correlation with high plasma survivin level.

DISCLOSURE

This article has been presented as a free paper presentation in 17th Congress of ASEAN Association of Radiology (AAR), on 8-9 May 2015.

ACKNOWLEDGMENT

We thank the staff of Radiology Department, Sanglah General Hospital for their support, and also the knee OA patients for their participation.

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