Plasma level of Osteopontin does not respond to total replacement Surgery in patients with severe Primary knee/Hip Osteoarthritis[☆]

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ARTICLE INFO

Article history:

Received 5 March 2017

Accepted 22 June 2017

Available online 23 June 2017

Keywords:

Osteopontin OPN

Osteoarthritis OA

Total joint replacement surgery

ABSTRACT

Objective: To test the response of Osteopontin levels in patients with knee/hip Osteoarthritis undergoing a replacement surgery.

Method: Plasma samples of 30 (16 with knee Osteoarthritis and 14 with hip Osteoarthritis) patients have been collected before and 3 month after a knee/hip replacement in order to investigate the above stated aim of this study.

Result: Osteopontin levels did not decrease after 3 months of the surgery.

Conclusion: Osteopontin levels showed no significant reduction and therefore do not respond to replacement treatment.

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1. Introduction

Osteoarthritis (OA) is known as a disorder of the articular cartilage and subchondral bone in conjunction with a thinning out and wear of the cartilage resulting in a decreased compliance of the subchondral bone.¹ Mostly, OA has been considered as a “wear and tear disease”. However, it is now believed as an inflammatory disease associated with the growth of blood vessels from the subchondral bone into the articular-cartilage phase. Therefore researchers focused their studies on the evaluation of proinflammatory mediators and cytokines especially in the synovium, articular-cartilage and subchondral bone of OA.² Although the complete etiology of OA is still poorly understood there is evidence that several biochemical factors are involved in the immunopathogenesis. Serum oligomeric matrix protein and tenascin-C in synovial-fluid have been found to correlate to the radiographic severity/progression of OA.³ Osteoarthritis is mediated through increased production of pro-inflammatory and catabolic mediators in the cartilage. Interleukin-1 β (IL-1 β) is predominantly involved in both the initiation and progression of OA.⁴

Osteopontin, which is considered as a member of Small integrin-binding-ligand-N-linked glycosylated-proteins, is present in the extracellular matrix of mineralized tissues, at sites of inflammation.⁵

OPN is a cytokine which is expressed by activated T-cells, macrophages and dendritic-cells and is upregulated in state of inflammation. OPN is involved in angiogenesis, apoptosis, inflammation, Regulation of mineral growth and bone resorption.⁶ In addition, OPN is considered to be a multifunctional marker which is also expressed in other chronic inflammatory and autoimmune diseases.⁷ It is also elevated in knee-OA, both in plasma and synovial fluids,^{8–10} and involved in the pathogenesis of OA, leading to progressive degeneration of the cartilage.¹¹ However, no marker has shown any sign of response towards the joint-replacement surgery other than Coll 2-1 levels in serum which decreased to normal level after the joint-replacement surgery.¹²

Therefore the role of chemokines and inflammatory markers in the pathogenesis of arthritis and their potential therapeutic target are subject of recent research.^{13–15} The paper aims at testing the course of plasma OPN levels in patients with severe isolated knee/hip OA undergoing joint-replacement (Plasma samples are being collected before and 3-month after surgery), its relation to the severity and using it as a follow-up investigative marker in comparison with the chronicity of OA.

[☆] The protocols and analysis were approved by the Ethical Board by the faculty of medicine of the RWTH Aachen University (EK 231/14).

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2. Methods

The sample consists of 30 patients of which 16 patients symptomatic severe idiopathic (Kellgren & Lawrence¹⁸ score ≥ 3) (K&L), knee- and 14 with hip OA), 18 females & 12 males; (mean age 69.31 ± 7.62 & 72.21 ± 7.36 respectively). The sample has been collected in order to fulfill the checklist of American College of Rheumatology criteria for the classification of the osteoarthritis.^{16,17} Patients with other inflammatory or autoimmune diseases like rheumatoid arthritis, gout, and systemic-lupus-erythematosus were excluded. The indication for total joint-replacement therapy has been based on the patient's history, clinical and radiological examinations. This procedure has been applied for all the sample patients. The patient underwent conservative therapy and reported severe pain during daily activities, night, and reduced walking distance <500-m. Blood samples were diluted with EDTA in the preparation. Stored fresh frozen plasma OPN (-30°C) was assessed (one week before and 3 month after the operation) by ELISA according to the manufactures instructions using the ELISA kit (Catalog Number DOST00, SOST00P, DOST00) Quantikine ELISA. Standards of recombinant human Osteopontin plasma samples were added to 96-well microtiter plates precoated with rabbit polyclonal antibody against Osteopontin and incubated for 1 h at room temperature. The wells were then washed seven times with washing buffer and incubated for 30 min at 4°C with a horseradish peroxidase-labeled-mouse monoclonal-antibody to human Osteopontin. Afterwards, substrate solution was added to each well, and the plate was incubated for 30-min at room temperature in the dark. Finally, the reaction was stopped with the stop-solution. Then absorbance was measured at 450-nm using automated-microtiter plate-reader. The Osteopontin concentration was calculated by the standard-curve & sensitivity was 3.3 ng/ml.

Plasma OPN levels were measured before and after surgery to assess its possible correlation to responsiveness to treatment.

3. Results

Demographic and laboratory characteristics of the study population are shown in Table 1. Plasma OPN levels in referenced normal values: ranged from 49.2–175 ng/ml with a mean level of 94.8 ± 24.9 ng/ml. Pre-Operative evaluation of plasma OPN level among knee OA patients showed that plasma OPN levels in knee OA group ranged from 212.2 ng/ml to 457.6 ng/ml with a mean level of 305.85 ± 55.31 ng/ml as shown in Table 2. Pre-Operative evaluation of plasma OPN level among hip OA patients showed that plasma OPN levels in hip OA group ranged from 162.3 ng/ml to 396.9 ng/ml with a mean level of 303.06 ± 64.90 ng/ml as shown in Table 3. The Comparison between levels of OPN in patients (pre-operatively) and normal values revealed a statistically high significant difference as shown in Table 4. Comparison between levels of OPN pre-operatively and postoperatively in all patients revealed a statistically high significant difference as shown in Table 5 and Fig. 1. Comparison between reference normal values of

Table 1
Age and sex.

	Knee O.A	Hip O.A	P			
	53.3%	46.7%				
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD		
Age (years)	57.0–85.0	69.31 ± 7.62	58–87.0	72.21 ± 7.36	0.17	NS
Sex	Female	Male	Female	Male		
Numbers	9	7	9	5	0.25	NS
	6.25%	43.75%	64.3%	35.7%		

HS = highly significant, p = level of significance, SD = standard deviations, NS = non significant.

Table 2

Comparison between levels of OPN in knee O.A patients (pre-operatively) and normal values revealed a statistically high significant difference.

	Pre-operative Knee O.A		Normal references		P	Sig
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range		
Plasma OPN	305.85 ± 55.31	212.2–457.6	94.8 ± 24.9	49.2–175	0.000	HS

Table 3

Comparison between levels of OPN in hip O.A patients (pre-operatively) and normal values revealed a statistically high significant difference.

	Pre-operative hip O.A		Normal references		P	Sig
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range		
Plasma OPN	303.06 ± 64.90	162.3–396.9	94.8 ± 24.9	49.2–175	0.000	HS

Table 4

Comparison between levels of OPN (pre-operatively) in all patients and normal values revealed a statistically high significant difference.

	Pre-operative		Normal references		P	Sig
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range		
Plasma OPN	307.88 ± 60.32	162.–457.6	94.8 ± 24.9	49.2–175	0.000	HS

Table 5

Comparison between levels of OPN pre- and post-operatively revealed a statistically high significant difference.

	Pre-operative	Post-operative	p	Sig
plasma OPN (Mean $\pm$ SD)	305.85 ± 55.97	434.2 ± 144.85	0.000	HS

OPN and levels of OPN pre-operatively and postoperatively in all patients revealed a statistically high significant difference as shown in Table 6 and Fig. 2.

4. Discussion

The study of this paper has been designed to analyze OPN plasma levels before and 3 months after knee and hip replacement. However, considering the fact that, Coll2-1 levels showed normal values 3 months after total knee and hip replacement in patients with OA.¹² The study showed no statistically significant difference in age/sex difference between plasma OPN levels in patients with knee OA and patients with hip OA ($p = 0.17$ and 0.25 respectively).

The determination of whether plasma OPN levels have increased has been analyzed by comparing the plasma OPN levels in patients with knee/hip OA to the normal reference. As expected OPN levels (mean 307.88 ± 60.32 ng/ml) in the osteoarthritis patients were significantly higher when compared to the normal references (mean 94.8 ng/ml ± 24.9) ($P = 0.000$).

Wang and Denhardt stated that up-regulation of OPN leads to the induction of pro-inflammatory cytokines production like IL1B, TNF, ccl2 and cxcl. Furthermore it also has an effect on the nuclear factor kappa P pathway activation.⁶ These pro-inflammatory mediators are being produced by the synovial membrane, and diffuse into the cartilage through synovial fluid which leads to chondrocytes activation. Activated chondrocytes produce catabolic factors like proteases and other pro-inflammatory cytokines and thereby lead to further cartilage degeneration.¹¹

Moreover, Yagi et al.¹⁹ found that cartilage in advanced OA had a higher OPN mRNA expression than cartilage with minimal OA.

Fig. 1. The Comparison between pre-joint replacement and post-operative in all OA patients as regards mean plasma OPN levels.

Table 6

Comparison between levels of OPN pre- & post-operatively and normal references revealed a statistically high significant difference.

	Pre-operative	Post-operative	Normal references	P	Sig
Plasma OPN (Mean ± SD)	307.88 ± 60.32	407.10 ± 125.97	94.8 ± 24.9	0.000	HS

The plasma OPN in knee patients (preoperatively) ranged from 212.2–457.6 ng/ml with a mean of 305.55 ± 55.97 ng/ml. Plasma OPN after the knee replacement (3 month) has significantly increased ($P=0.001$) ranging from 254.9–829 with mean of 434.2 ± 144.85 ng/ml.

In addition, the plasma OPN in hip patients (preoperatively) ranged from 162.3–396.9 with mean of 303.06 ± 64.9 ng/ml. Similar to what has been analyzed with knee replacement the plasma OPN in hip patients has significantly increased ($P=0.001$) after the hip replacement (3 months) ranging from 230 to 549.9 ng/ml with mean of 376.14 ± 90.69 ng/ml.

These above mentioned plasma values of OPN after total knee and hip replacement do indicate that this marker should not be used as a short time marker in the follow-up examination. Such results were more or less coherent with²⁰ statements proclaiming that serum OPN does not indicate responsiveness to therapeutic treatment for patients with Rheumatoid arthritis. According to (242) OPN levels in synovial fluids increased after 1 month of anterior cruciate ligament (ACL) reconstruction surgery. Also after rupture of the ACL isolated or combined with a meniscus tear OPN increased for 6 months.²³ These results are coherent with^{24,25} indicating that OPN is an early response marker to stress signals.

Study groups vs Normal referced value

Fig. 2. The Comparison between the mean of OPN levels in normal values, pre-Operative and post-operative (3 months) plasma OPN levels (all patients).

In our study, monitoring of OPN levels has been done by collecting plasma one week before and 3 months after the joint replacement. The selection of monitoring OPN levels 3 months after surgery relies on the results of Deberg et al., who analyzed the change in serum level of nitrated form of collagen type II, Coll2-1NO2 in knee and hip osteoarthritis patients. Serum changes have been monitored pre and post operatively after 3 and 12 months respectively. Coll2-1NO2 levels elevated after 3 months and remained at the same level after 1-year.¹² Type II collagen is the major structural protein in cartilage, making up approximately 50% of the extracellular cartilage matrix. Type II collagen-derived fragments have been extensively investigated as potential markers of cartilage remodeling in OA and rheumatoid arthritis (RA).²¹

Therefore a possible reason for the increase in plasma OPN 3 month after knee/hip replacement surgery could be related to a release from bone, cartilage or synovium during operation. This phenomenon might be explained by Yamaga et al.²² and Lohmander et al.,²³ who stated that OPN levels correlate with the severity of articular cartilage damage in lateral tibial plateau and ACT rupture respectively, which is coherent with^{24,25} indicating that OPN is an early response marker to stress signals. However, further studies need to be conducted in order to clarify the above mentioned explanations and results (follow up frame of 12 month or more).

5. Limitations

The study includes several limitations. First, the paper excluded a comparison of pre-surgery OPN levels of knee/hip OA patients with a control group (aged-matched healthy subjects). Most of the patients were around 71 years old. It is rather difficult implementing an aged matched healthy control group to the study due to the fact that most of the patients in that age suffer to some degree from knee or hip OA. Second, plasma OPN samples were only collected 1 week before and 3 months after the operation, which demonstrates a short-time follow-up investigation in comparison with the actual chronicity of the disease. However the study still provides information regarding the OPN level in blood samples of patient before and after joint replacement.

6. Conclusion

Plasma OPN levels correlate with knee/hip OA severity but do not decrease after (3 months) total joint replacement surgery. Further studies should be done to investigate OPN levels 12 months after the knee/hip replacement surgery in patients with knee/hip OA.

Conflict of interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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