



Correlation of serum Dickkopf-1 content with bone destruction, inflammatory response and oxidation reaction in patients with gouty arthritis

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ABSTRACT

Objective: To study the correlation of serum Dickkopf-1 (DKK-1) content with bone destruction, inflammatory response and oxidation reaction in patients with gouty arthritis.

Methods: A total of 40 patients with acute gouty arthritis who were treated in our hospital between 2013 and 2016 were selected as the group A of the study, 56 patients with asymptomatic hyperuricemia who were treated in our hospital during the same period were selected as the group B of the study, and 60 healthy volunteers who received physical examination in our hospital during the same period were selected as the control group of the study. The serum was collected to detect the contents of DKK-1, bone destruction indexes, inflammatory response indexes and oxidation reaction indexes. **Results:** Serum DKK-1, TRACP5b, RANKL, β -CTX, PGE2, sICAM-1, sVCAM-1, sCD14, MDA, 8-OHdG and 3-NT levels of group A and group B were significantly higher than those of control group while SOD and GSH-Px levels were significantly lower than those of control group; serum DKK-1, TRACP5b, RANKL, β -CTX, PGE2, sICAM-1, sVCAM-1, sCD14, MDA, 8-OHdG and 3-NT levels of group A were significantly higher than those of group B while SOD and GSH-Px levels were significantly lower than those of group B; serum DKK-1 level was positively correlated with TRACP5b, RANKL, β -CTX, PGE2, sICAM-1, sVCAM-1, sCD14, MDA, 8-OHdG and 3-NT levels, and negatively correlated with SOD and GSH-Px levels.

Conclusion: Abnormally elevated DKK-1 in patients with gouty arthritis can induce articular bone destruction as well as inflammatory response and oxidative stress response activation.

1. Introduction

Gout is a kind of metabolic disease caused by purine metabolism disorders and uric acid excretion dysfunction, it is basically characterized by persistent hyperuricemia, and chronically elevated blood uric acid can cause saturated urate to be separated out from extracellular fluid and precipitate within the tissue organs, which causes gouty arthritis, gouty nephropathy and uratoma formation[1,2]. Gouty arthritis is the aseptic inflammation caused by

urate deposition in joint space, synovium of joint, articular cartilage and other parts, and the inflammatory response activation and stress reaction activation have played a crucial role in the development and change of disease. Gouty arthritis will not only cause the aseptic inflammation of the joint, but also lead to the joint bone damage, and severe cases will affect the function of the joint and increase the risk of disability[3,4]. Dickkopf-1 (DKK1) is the inhibitory molecule of Wnt signaling pathway in DKKs family, which can inhibit the differentiation of osteoblasts mediated by Wnt signaling pathway, so as to affect bone synthesis and increase the risk of osteoporosis. In the following studies, we specifically analyzed the correlation of serum DKK1 levels with bone destruction, inflammatory response and oxidative reaction in patients with gouty arthritis.

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2. Research subjects and methods

2.1 General information of research subjects

A total of 40 patients with acute gouty arthritis who were treated in our hospital between 2013 and 2016 were selected as the group A of the study, and all patients were diagnosed clearly with gout and admitted to hospital for acute gouty arthritis; 56 patients with asymptomatic hyperuricemia who were treated in our hospital during the same period were selected as the group B of the study, and all patients were with blood uric acid higher than 416 mmol/L and without history of gouty arthritis attack. Patients combined with osteoporosis, rheumatoid arthritis, osteoarthritis and bone tumors were excluded. 60 healthy volunteers who received physical examination in our hospital during the same period were selected as the control group of the study. Group A included 23 men and 17 women that were 36-58 years old; Group B included 30 men and 26 women that were 33-55 years old; control group included 36 men and 24 women that were 35-60 years old. There was no significant difference in general data among the three groups ($P>0.05$).

2.2 Research methods

2.2.1 Serum sample collection

3 mL of peripheral venous blood was collected from group A before treatment, 3 mL of peripheral venous blood was collected from group B group after the diagnosis of hyperuricemia, 3 mL of peripheral venous blood was collected from control group during physical examination, and the blood was centrifuged to separate the upper clear serum and store it at $-80\text{ }^{\circ}\text{C}$.

2.2.2 Serum index detection

Serum specimens were taken, enzyme-linked immunosorbent assay kit was used to detect DKK-1, TRACP5b, RANKL, β -CTX, PGE2, sICAM-1, sVCAM-1 and sCD14 levels, and

radioimmunoprecipitation kits were used to detect the contents of MDA, 8-OHdG, 3-NT, SOD and GSH-Px.

2.3 Statistical methods

SPSS 21.0 software was used for variance analysis of the differences in measurement data among the three groups, the correlation between two measurement data was by Pearson test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Serum DKK-1 level

Serum DKK-1 contents of group A, group B and control group were $(2.95\pm 0.35)\text{ }\mu\text{g/L}$, $(1.62\pm 0.20)\text{ }\mu\text{g/L}$ and $(0.87\pm 0.10)\text{ }\mu\text{g/L}$ respectively. Analysis of serum DKK-1 contents among three groups of subjects was as follows: serum DKK-1 levels of group A and group B were significantly higher than that of control group, and serum DKK-1 level of group A was significantly higher than that of group B. Differences in pair-wise comparison of serum DKK-1 levels were statistically significant among three groups of subjects ($P<0.05$).

3.2 Serum bone destruction index levels

Analysis of serum bone destruction indexes TRACP5b (ng/mL), RANKL (pg/mL) and β -CTX (ng/mL) levels among three groups of subjects was as follows: serum TRACP5b, RANKL and β -CTX levels of group A and group B were significantly higher than those of control group, and serum TRACP5b, RANKL and β -CTX levels of group A were significantly higher than those of group B. Pearson test showed that serum DKK-1 level in patients with gouty arthritis was positively correlated with TRACP5b, RANKL and β -CTX levels.

Table 1.

Comparison of serum TRACP5b, RANKL and β -CTX.

Groups	n	TRACP5b	RANKL	β -CTX
Group A	40	$3.94\pm 0.52^{*\&}$	$189.32\pm 22.35^{*\&}$	$1.38\pm 0.16^{*\&}$
Group B	56	$2.42\pm 0.34^{\#}$	$121.24\pm 14.59^{\#}$	$0.87\pm 0.11^{\#}$
Control group	60	1.57 ± 0.20	78.65 ± 9.31	0.42 ± 0.06

[#]: compared with indexes of control group, $P<0.05$; [&]: compared with indexes of group B, $P<0.05$.

Table 2.

Comparison of serum PGE2, sICAM-1, sVCAM-1 and sCD14.

Groups	n	PGE2	sICAM-1	sVCAM-1	sCD14
Group A	40	$18.76\pm 2.32^{*\&}$	$194.57\pm 23.57^{*\&}$	$223.51\pm 28.87^{*\&}$	$53.32\pm 6.72^{*\&}$
Group B	56	$10.35\pm 1.51^{\#}$	$121.36\pm 14.62^{\#}$	$146.22\pm 17.64^{\#}$	$37.15\pm 5.52^{\#}$
Control group	60	6.54 ± 0.74	67.54 ± 8.75	89.33 ± 11.37	20.34 ± 3.31

[#]: compared with indexes of control group, $P<0.05$; [&]: compared with indexes of group B, $P<0.05$.

Table 3.

Comparison of serum MDA, 8-OHdG, 3-NT, SOD and GSH-Px.

Groups	n	MDA	8-OHdG	3-NT	SOD	GSH-Px
Group A	40	12.52±1.47* ^{&}	2.35±0.31* ^{&}	35.92±5.59* ^{&}	76.86±9.35* ^{&}	59.67±7.72* ^{&}
Group B	56	7.85±0.93 [†]	1.52±0.17 [†]	24.12±3.29 [†]	113.52±12.58 [†]	89.42±10.35 [†]
Control group	60	4.57±0.62	0.78±0.11	16.73±2.03	164.43±20.35	127.64±14.63

[†]: compared with indexes of control group, $P < 0.05$; [&]: compared with indexes of group B, $P < 0.05$.

3.3 Serum inflammatory response index levels

Analysis of serum inflammatory response indexes PGE2 (ng/mL), sICAM-1 (pg/mL), sVCAM-1 (pg/mL) and sCD14 (ng/mL) levels among three groups of subjects was as follows: serum PGE2, sICAM-1, sVCAM-1 and sCD14 levels of group A and group B were significantly higher than those of control group, and serum PGE2, sICAM-1, sVCAM-1 and sCD14 levels of group A were significantly higher than those of group B. Pearson test showed that serum DKK-1 level in patients with gouty arthritis was positively correlated with PGE2, sICAM-1, sVCAM-1 and sCD14 levels.

3.4 Serum oxidation stress index levels

Analysis of serum oxidation stress indexes MDA ($\mu\text{mol/L}$), 8-OHdG (ng/mL), 3-NT (nmol/L), SOD (U/mL) and GSH-Px (U/mL) levels among three groups of subjects was as follows: serum MDA, 8-OHdG and 3-NT levels of group A and group B were significantly higher than those of control group while SOD and GSH-Px levels were significantly lower than those of control group, and serum MDA, 8-OHdG and 3-NT levels of group A were significantly higher than those of group B while SOD and GSH-Px levels were significantly lower than those of group B. Pearson test showed that serum DKK-1 level in patients with gouty arthritis was positively correlated with MDA, 8-OHdG and 3-NT levels, and negatively correlated with SOD and GSH-Px levels.

4. Discussion

Gouty arthritis is a joint lesion caused by abnormal metabolism of purine and continuous increase of blood uric acid levels, and the aseptic inflammation of local joints is the most prominent pathologic feature of gouty arthritis. Continuous increase of blood uric acid levels will increase the separation out of uric acid and form urate crystals, and the crystal deposition in joint space, synovium of joint, articular cartilage and other parts can cause aseptic inflammation, and thus result in clinical symptoms of joint pain[5]. Gouty arthritis is not only with the aseptic inflammation of the joints, but also with the continuous destruction of articular bone, but the specific mechanism is still not elucidated. DKK-1 is the inhibitor of osteogenesis signaling pathway Wnt signaling pathway in the DKKs family, which can inhibit the activation of Wnt signaling pathways

to hinder the osteoblast differentiation and maturation, thus affect the bone synthesis process mediated by osteoblasts and participate in the occurrence of osteoporosis[6-8]. In the study, the analysis of the changes in serum DKK-1 contents between patients with gouty arthritis and patients with hyperuricemia showed that serum DKK-1 levels of group A and group B were significantly higher than that of control group, and serum DKK-1 level of group A was significantly higher than that of group B. This indicates that during the development of gout, the increase of the blood uric acid content will result in the increase of DKK-1 secretion and the increase of DKK-1 secretion is involved in the occurrence of gouty arthritis.

Bone destruction process involves the change in the balance of osteoblast and osteoclast, and DKK-1 effect on osteoblast differentiation can also cause the enhancement of osteoclast activity, and thereby mediate bone matrix degradation and bone destruction[9]. TRACP5b and RANKL are the active molecules synthesized and secreted by osteoclasts, and they participate in the degradation of bone matrix[10,11]; β -CTX is the product of collagen degradation in bone matrix and can reflect the activity of osteoclasts[12,13]. In the study, analysis of the changes in serum bone destruction index contents between patients with gouty arthritis and patients with hyperuricemia showed that serum TRACP5b, RANKL and β -CTX levels of group A and group B were significantly higher than those of control group, and serum TRACP5b, RANKL and β -CTX levels of group A were significantly higher than those of group B. This shows that in the development of gouty arthritis, the activity of osteoclasts is significantly enhanced and can mediate bone damage in joints. Further analysis of the correlation between serum DKK-1 and bone destruction indexes in patients with gouty arthritis showed that serum DKK-1 level was positively correlated with TRACP5b, RANKL and β -CTX levels. It means that the excessively secreted DKK-1 in patients with gouty arthritis has a promoting effect on osteoclast differentiation and maturation, and can enhance the osteoclast activity to cause the joint bone destruction in patients with gouty arthritis.

Aseptic inflammation is an important pathological feature of the acute onset of gouty arthritis, and the secretion of multiple inflammatory mediators is significantly increased in this process[14,15]. PGE2 is an inflammatory mediator produced when COX-2 catalyzes arachidonic acid metabolism, and it has strong algogenic activity; sICAM-1 and sVCAM-1 are soluble forms of adhesion molecules ICAM-1 and VCAM-1, which can promote inflammatory cells to infiltrate in local joint and mediate inflammatory reaction activation amplification; sCD14 is a ligand for TLRs, and can activate the inflammatory response

mediated by TLRs. In the study, analysis of the changes in serum inflammatory response index contents between patients with gouty arthritis and patients with hyperuricemia showed that serum PGE2, sICAM-1, sVCAM-1 and sCD14 levels of group A and group B were significantly higher than those of control group, and serum PGE2, sICAM-1, sVCAM-1 and sCD14 levels of group A were significantly higher than those of group B. This shows that in the development and change of gouty arthritis, the inflammatory response is significantly activated and the inflammatory mediator secretion significantly increases. Further analysis of the correlation between serum DKK-1 and inflammatory response indicators in patients with gouty arthritis showed that serum DKK-1 level was positively correlated with PGE2, sICAM-1, sVCAM-1 and sCD14 levels. This indicates that the excessively secreted DKK-1 in patients with gouty arthritis has promoting effect on the secretion of inflammatory mediators, and can mediate the aseptic inflammation of the joint by increasing the secretion of inflammatory mediators.

During the acute attack of gouty arthritis, the injury of joint is not only associated with the activation of aseptic inflammation, but also associated with the massive generation of oxygen free radicals and the activation of oxidative stress response[16]. The massively generated oxygen free radicals in the joint cavity can cause the peroxidation of lipid, nucleic acid and protein to lead to the joint damage, and also generate the corresponding oxidation products MDA, 8-OHdG and 3-NT. In addition, the massive generation of oxygen free radicals can also cause the massive consumption of antioxidant enzymes such as SOD and GSH-Px. In the study, analysis of the changes in serum oxidative stress response index contents between patients with gouty arthritis and patients with hyperuricemia showed that serum MDA, 8-OHdG and 3-NT levels of group A and group B were significantly higher than those of control group while SOD and GSH-Px levels were significantly lower than those of control group, and the changes in above oxidative stress indexes of group A were more significant than those of group B. This indicates that during the development of gouty arthritis, the oxidative stress response is significantly activated, and the production of oxidative products and the consumption of antioxidant enzymes both increase significantly. Further analysis of the correlation between serum DKK-1 and oxidative stress reaction indexes in patients with gouty arthritis showed that serum DKK-1 level was positively correlated with MDA, 8-OHdG and 3-NT levels, and negatively correlated with SOD and GSH-Px levels. It means that the excessively secreted DKK-1 in patients with gouty arthritis has promoting effect on the production of oxygen free radicals, and can increase the production of oxygen free radicals to significantly increase the formation of oxidation products and the consumption of antioxidant enzyme and cause oxidative stress damage of the joints.

Based on the discussion and analysis above, it is believed that the abnormal increase of serum DKK-1 level is closely related to the acute attack of gouty arthritis, and promoting osteoclast differentiation, causing bone destruction and activating inflammatory response and oxidative stress reaction are the molecular pathways for DKK-1 to be involved in development and change of gouty arthritis.

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