Abstract. – OBJECTIVE: To investigate the relationship between the gene polymorphism of osteoprotegerin (OPG) and bone mineral density (BMD) in hemodialysis patients.

PATIENTS AND METHODS: A total of 147 patients with end-stage renal disease (ESRD) who were admitted to the Weifang People’s Hospital for maintenance hemodialysis between January 2014 and December 2015 were enrolled. Peripheral blood was collected from the subjects for assay of the polymorphism of A163G and G1181C loci on the OPG gene. The measurements of the levels of RANK, RANKL, TNF-α, IL-6, PINP, CTX-I, CTX-II and TRACP5 in the isolated serum were taken.

RESULTS: For the polymorphism of A163G locus on the OPG gene, the BMDs of left femoral neck and lumbar posterior L1-L4 of the AA genotype were significantly higher than those of the AG and GG genotypes. There was no significant difference in comparison of BMDs at the forearm (distal 1/3) between the AA genotype and AG and GG genotypes. No significant differences were found in the comparison of BMDs at all sites between AG and GG genotypes. The serum level of RANKL of the AA genotype was significantly higher than levels of AG and GG genotypes, but the levels of RANK, TNF-α, IL-6, PINP, CTX-I, CTX-II and TRACP5 were prominently lower than those levels of AG and GG genotypes. For the polymorphism of G1181C locus on the OPG gene, the BMDs of left femoral neck and lumbar posterior L1-L4 of the CC genotype were significantly higher than the BMDs of GG and GC genotypes. There was no significant difference in the comparison of BMDs at the forearm (distal 1/3) between the CC genotype and GG and GC genotypes. No significant differences were found in the comparison of BMDs at all sites between GG and GC genotypes. The serum level of RANKL of the CC genotype was significantly higher than the level of GG and GC genotypes. However, the levels of RANK, TNF-α, IL-6, PINP, CTX-I, CTX-II and TRACP5 were prominently lower than those levels of GG and GC genotypes.

CONCLUSIONS: The polymorphisms of A163G and G1181C loci on the OPG gene were correlated with the BMD of hemodialysis patients. The genotype AA of A163G and genotype CC of G1181C were identified as the protective factors for BMD.

Key Words: Osteoprotegerin, Hemodialysis, Gene polymorphism, Bone mineral density.
OPG-RANK-RANKL gene and the osteoporosis of patients with ESRD receiving hemodialysis. Thus, in this research, we focused on the relationship between bone mineral density (BMD) and the polymorphism of two important gene loci, A163G and G1181C, to provide references for the early screening of patients with high-risk factors and the prophylaxis of osteoporosis.

**Patients and Methods**

**Patients**

A total of 147 patients with ESRD who were admitted in the Weifang People’s Hospital for maintenance hemodialysis between January 2014 and December 2015 were enrolled. Inclusion criteria were as follows: 1) patients who had received hemodialysis for at least 3 months; 2) patients with no heart failure, bone fracture or pulmonary infection in 3 months. The subjects were informed of the research items and agreed to assay their BMD. Exclusion criteria were as follows: 1) patients with bone marrow neoplasms or any other tumors; 2) patients who were long-term bedridden; 3) patients with connective tissue diseases or cirrhosis; 4) patients who had been administrated psychotropic drugs or gonadal hormones. In the study, there were 78 males and 69 females with an average age of 60.93 ± 7.94 years and average hemodialysis duration of 45.68 ± 7.84 months.

**Polymorphism of OPM Detected by MALDI-TOFMS**

Genomic DNA was extracted from peripheral blood samples collected from the fasting subjects before hemodialysis. PCR primer and SNuPE (single nucleotide primer extension) were designed for A163G and G1181C, two loci with single nucleotide polymorphism. Genomic DNA samples were taken with PCR amplification, and then products were analyzed by agarose gel electrophoresis (AGE) to identify the genotype. The genotypes of A163G included AA, AG and GG, while the genotypes of G1181C included GG, GC and CC.

**Detection of Serum Biochemical Indexes of Bone Metabolism**

Peripheral blood samples collected from the fasting subjects before hemodialysis were centrifuged after standing for 3 minutes, and the serum was separated. Then, the serum levels of IL-6 (interleukin-6), TNF-α (tumor necrosis factor α), RANK, RANKL, PINP (procollagen I N-terminal peptide), CTX-I (type I collagen cross-linked C-terminal telopeptide), CTX-II (type II collagen cross-linked C-terminal telopeptide) and TRACP5b (tartrate-resistant acid phosphatase 5b) were assayed with the ELISA (enzyme-linked immunosorbent assay) kits (Be-yotime Biotechnology Co., Ltd., Jiangsu, China). All procedures were strictly compliant with the following instructions of the kits: 1) standard curves were drawn according to the absorption values of standard protein at a wavelength of 450 nm; 2) the absorption value of the substance to be assayed was read at the wavelength of 450 nm; 3) the assayed values were substituted into the standard curves, by which the content was calculated.

**Measurements of BMD**

The BMDs of the left femoral neck, lumbar poster anterior L1-L4 and the BMD at the forearm (distal 1/3) were measured by dual-energy X-ray absorptiometry, or DXA (Institute of Heavy Ion Physics of Beijing University, Beijing, China).

**Statistical Analysis**

Data were recorded and statistically processed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Comparison between groups was performed using One-way ANOVA test followed by Post Hoc Test (Least Significant Difference). Differences with \( p < 0.05 \) were considered statistically significant.

**Results**

**Distribution of Polymorphism of A163G and G1181C**

For the polymorphism of A163G, there were 9 subjects with AA genotype (6.12%), 58 subjects with AG (39.46%) and 80 with GG (54.42%). For the polymorphism of G1181C, there were 83 subjects with GG (56.46%), 42 with GC (28.57%) and 22 with CC (14.97%).

**Analysis of the Relationship Between BMD and Polymorphism of A163G and G1181C**

For the polymorphism of A163G locus on the OPG gene, the BMDs of left femoral neck and lumbar poster anterior L1-L4 of the AA genotype were significantly higher than those of AG and GG genotypes. There was no significant difference in the comparison of BMDs at the forearm (distal 1/3) between the AA genotype...
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and AG and GG genotypes. No significant differences were found in the comparison of BMDs at all sites between AG and GG genotypes. For the polymorphism of G1181C locus on the OPG gene, the BMDs of left femoral neck and lumbar poster anterior L1-L4 of the CC genotype were significantly higher than those of GG and GC genotypes. There was no significant difference in the comparison of BMDs at the forearm (distal 1/3) between the CC genotype and GG and GC genotypes. No significant differences were found in the comparison of BMDs at all sites between GG and GC genotypes (Table I).

### Comparison of Serum Levels of Relevant Substances in Passage of RANK/RANKL

For the A163G gene locus, the serum level of RANKL of the AA genotype was significantly higher than those levels of AG and GG genotypes, but the levels of RANK, TNF-α and IL-6 were prominently lower than those levels of AG and GG genotypes. For G1181C gene locus, the serum level of RANKL of the CC genotype was significantly higher than those levels of GG and GC genotypes. However, the levels of RANK, TNF-α and IL-6 were prominently lower than those levels of GG and GC genotypes (Table II).

### Comparisons of Serum Levels of Bone Turnover Markers

For the A163G gene locus, serum levels of PINP, CTX-I, CTX-II and TRACP5 of AA genotype were significantly lower than those levels of AG and GG genotypes. For the G1181C gene locus, serum levels of PINP, CTX-I, CTX-II and TRACP5 of the CC genotype were significantly lower than those levels of GG and GC genotypes (Table III).

### Discussion

As a new member of the TNF receptor superfamily, OPG plays an important role in the regulation of bone metabolism. The expression level of the OPG gene in patients with osteoporosis decreases significantly. There is no widely accepted understanding of the influences of polymorphism of the OPG gene on the BMD. In this study, we found the BMDs in the left femoral neck and lumbar poster anterior L1-L4 of AA and CC genotypes were relatively higher, indicating a relationship between the BMD of patients re-

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**Table I.** Analysis of the relationship between BMD and polymorphism of A163G and G1181C (g/cm²).

<table>
<thead>
<tr>
<th>SNP Locus</th>
<th>Genotype</th>
<th>Left femoral neck</th>
<th>Lumbar posetoanterior L1-L4</th>
<th>The forearm (distal 1/3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A163G</td>
<td>AA</td>
<td>1.03±0.14*</td>
<td>0.98±0.15*</td>
<td>0.59±0.07</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>0.77±0.08</td>
<td>0.69±0.08</td>
<td>0.61±0.07</td>
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<tr>
<td></td>
<td>GG</td>
<td>0.83±0.10</td>
<td>0.74±0.09</td>
<td>0.55±0.08</td>
</tr>
<tr>
<td>G1181C</td>
<td>GG</td>
<td>0.76±0.08</td>
<td>0.66±0.08</td>
<td>0.62±0.08</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>0.72±0.09</td>
<td>0.61±0.05</td>
<td>0.67±0.09</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1.18±0.15ab</td>
<td>1.08±0.22ab</td>
<td>0.59±0.06</td>
</tr>
</tbody>
</table>

Note: *referred to p < 0.05 compared with AG genotype; # referred to p < 0.05 compared with GG genotype; refered to p < 0.05 compared with GG genotype; *referred to p < 0.05 compared with GC genotype.

**Table II.** Comparison of serum levels of the relevant substances in the passage of RANK/RANKL.

<table>
<thead>
<tr>
<th>SNP locus</th>
<th>Genotype</th>
<th>RANKL (ng/ml)</th>
<th>RANK (ng/ml)</th>
<th>TNF-α (ng/ml)</th>
<th>IL-6 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A163G</td>
<td>AA</td>
<td>146.92±20.14*</td>
<td>34.59±5.22*</td>
<td>7.95±0.92*</td>
<td>127.95±16.79*</td>
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<tr>
<td></td>
<td>AG</td>
<td>76.75±9.33</td>
<td>58.66±8.64</td>
<td>20.33±3.14</td>
<td>248.69±36.48</td>
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<tr>
<td></td>
<td>GG</td>
<td>80.14±9.82</td>
<td>60.31±8.35</td>
<td>19.62±2.55</td>
<td>260.14±32.52</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>73.15±8.58</td>
<td>57.79±8.68</td>
<td>18.68±2.74</td>
<td>120.34±17.58</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>75.52±9.14</td>
<td>59.14±7.95</td>
<td>19.33±3.09</td>
<td>255.28±31.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>156.68±22.58ab</td>
<td>36.67±5.68ab</td>
<td>8.58±1.02ab</td>
<td>262.32±38.94ab</td>
</tr>
</tbody>
</table>

Note: *referred to p < 0.05 compared with AG genotype; # referred to p < 0.05 compared with GG genotype; refered to p < 0.05 compared with GG genotype; *referred to p < 0.05 compared with GC genotype.
receiving hemodialysis and the gene polymorphism of A163G and G1181C. Conversely, there were no differences in BMD at the forearm (distal 1/3) among different genotypes. However, no differences in BMDs were found in the forearm (distal 1/3) of different genotypes for changes in BMDs caused by gene polymorphism. This might be partially compensated for by a lower BMD and more frequent activities in the distal end of forearm.

OPG, as the important part of the OPG/RANKL/RANK molecular regulation system, would cause changes in BMD by its influences on bone resorption and formation through the regulation of the balance status between osteoblasts and osteoclasts. RANKL, synthesized by osteoblasts, would promote the differentiation, maturation and proliferation of osteoclasts to accelerate the process of bone resorption by reacting with the RANK distributed on the surface of osteoclasts. OPG, secreted by bone marrow stromal cells, would compete with RANKL for binding RANK to suppress the differentiation and maturation of osteoclasts, and would directly act on the osteoclasts to accelerate their apoptosis. The OPG/RANKL/RANK passage is regulated by multiple inflammatory factors. Under the micro-inflammatory state in patients receiving hemodialysis, significant increases have frequently been found in the expression levels of various inflammatory factors such as IL-6 and TNF-α. This would stimulate the osteoblasts and osteoclasts to synthesize the RANKL and RANK respectively to promote the maturation of osteoclasts and absorption of bone. In this study, we found that serum levels of RANKL of AA and CC genotypes were significantly higher, but the levels of RANK, TNF-α and IL-6 were prominently lower, indicating that the higher expression of RANKL was related to enhanced activity of osteoblasts. In contrast, the lower expression of RANK was related to suppression of the activity of osteoclasts.

We found that the AA genotype of A163G locus and the CC genotype of G1181C would also indirectly regulate the RANK/RANKL expression by alleviating the micro-inflammatory state and reducing the secretion of IL-6 and TNF-α. Thus, polymorphism of OPG would affect the changes in BMD of patients receiving hemodialysis through the regulation of RANK/RANKL activity.

The serum levels of bone metabolic substances such as PINP, CTX-I, CTX-II and TRACP5 would reflect the activity of osteoblasts and osteoclasts. PINP is used to assess the activity of bone turnover: the higher the content of PINP, the more active the bone turnover. CTX-I and CTX-II are the degradation products of type-I and type-II collagen of osseous tissue, reflecting the activity of osteoclasts and the degree of bone resorption. TRACP5, secreted by osteoclasts, participates in the degradation of the substrate in calcium and phosphate mineralization in bone matrix to accelerate the process of bone resorption. A research has confirmed the significant increases in serum levels of bone metabolic substances such as PINP, CTX-I, CTX-II and TRACP5 in patients with osteoporosis. In this study, we found that the serum levels of PINP, CTX-I, CTX-II and TRACP5 in patients with AA and CC genotypes of A163G and G1181C loci were relatively low, suggesting that the polymorphism of OPG could regulate the bone turnover.

**Conclusions**

We observed that the polymorphisms of A163G and G1181C loci on the OPG gene were correlated with the BMD of patients receiving hemodialysis.
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The authors declare no conflicts of interest.

Conflict of interest

References


