

## Association of IL-8 -251 A/T Polymorphism and Osteoporosis in Postmenopausal Indonesian Women

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### Abstract

Osteoporosis is a common bone disease known to have a strong genetic component, but the details of the traits and underlying mechanisms remain poorly understood. In postmenopausal women, osteoporosis is characterized by decreased bone mineral density. Interleukin-8 (IL-8) is an inflammatory marker, commonly detected in postmenopausal women with osteoporosis. This cytokine has been suggested to play a role in bone resorption, and is potentially influenced by polymorphisms of the encoding (IL-8) gene. The study aimed to investigate the association of the -251 A/T polymorphism of IL-8 with the risk of osteoporosis in postmenopausal Indonesian women.

Using polymerase chain reaction-restriction fragment length polymorphism analysis, we assessed the status of the IL-8 -251 A/T polymorphism in stored DNA samples of 75 postmenopausal women with osteoporosis and of 25 healthy control subjects. Genotype and allotype comparisons were carried out according to T-score grouping.

Chi-Square analysis revealed that subjects with AT and TT genotypes of the IL-8 -251 A/T polymorphism were at a significant risk of osteoporosis (odds ratio = 4.540, 95%).

The results indicate that the -251 A/T polymorphism of IL-8 is significantly associated with osteoporosis in the tested Indonesian sample population.

Clinical article (J Int Dent Med Res 2020; 13(4): 1527-1530)

**Keywords:** Osteoporosis, bone mineral density, polymorphism, IL-8

**Received date:** 10 January 2020

**Accept date:** 15 March 2020

### Introduction

Osteoporosis is characterized by the deterioration of bone micro-architecture and resulting decrease in bone mass. This leads to increased bone fragility and enhanced risk of fracture.<sup>1</sup> Osteoporosis is a multifactorial disease, meaning that identifying the primary factors is challenging.<sup>2</sup> However, osteoporosis has been proven to be caused by an imbalance in the remodeling process caused by osteoclast hyperactivity and consequent increased new bone formation. The interaction of genetic and environmental factors is one cause of osteoporosis;<sup>3</sup> environmental factors can regulate the expression of key genes and, therefore, influence disease progression, and

both factors are involved in the control of bone turnover.<sup>1</sup> Over the last 30–40 years, osteoporosis has become a recognized health concern and is increasingly common in the current aging populations, as the risk of osteoporosis grows with age. The risk is higher for women than men, and increases dramatically after the onset of menopause.<sup>4,5</sup> According to the International Osteoporosis Foundation, about 200 million women around the world currently suffer from the disease.<sup>6,7</sup>

One common and simple indicator of osteoporosis is a decline in bone mineral density (BMD) to a T-score of -2.5 or less.<sup>8</sup> The pathogenesis of osteoporosis involves increased osteoclast activity and the production of pro-inflammatory cytokines related to estrogen withdrawal in postmenopausal women.<sup>7</sup> Postmenopausal bone loss is caused by increased production of specific cytokines such as interleukin-8 (IL-8) which may directly stimulate osteoclastogenesis and bone resorption.<sup>9</sup> This suggests that the gene encoding IL-8 (*IL-8*) has a potential role in

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osteoporosis, and that the expression of this gene may depend on the polymorphic variant of the individual.

The objective of the present study was to investigate whether the -251 A/T polymorphism of *IL-8* is associated with an increased risk of osteoporosis in postmenopausal Indonesian women.

## Materials and methods

### Subjects

The study used 100 DNA samples extracted from blood serum of 75 postmenopausal women with osteoporosis and 25 healthy (non-osteoporotic) individuals (control subjects) (Table 1). The subjects were recruited with known diagnoses of osteoporosis. We used retrospective or case-control study as the design. The DNA isolation procedures that were used followed previously published protocols.<sup>9</sup> The DNA samples were stored at -20°C in the Oral Biology Laboratory of the Faculty of Dentistry, Universitas Indonesia. This study and all its protocols were approved by the Ethical Committee of the Faculty of Dentistry, Universitas Indonesia.

The BMD of the subjects was measured at the calcaneus using the Sonost 3000 bone densitometer. Osteoporosis was defined as a BMD of <2.5 standard deviations below the mean of the young adult reference; i.e., T-score of <-2.5. Normal status was defined as T-score ≥ -1, and osteopenia as -1 > T-score ≥ -2.5. This performed as part of check-up and ten the results extracted from records for the present study. Subjects with osteoporosis and osteopenia were grouped together.

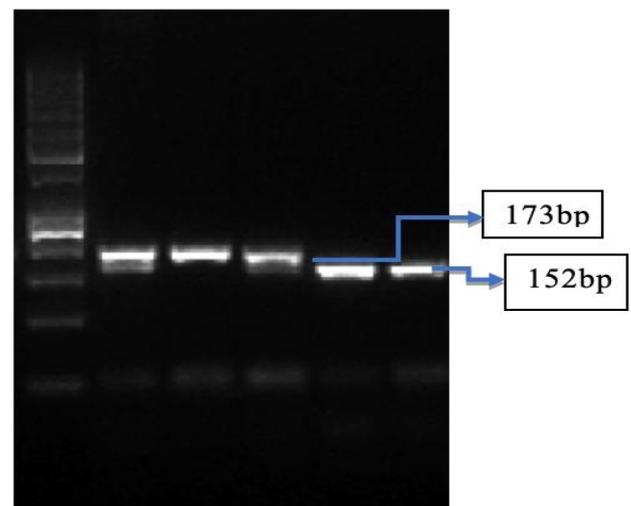
### DNA genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was employed to determine the genotypes of the -251 A/T *IL-8* polymorphisms. The PCR reaction was carried out in a 20-μL reaction mixture containing 10 μL of Master Mix (Thermo Fisher Scientific, USA), 0.4 μL each of forward (5'-CCA-TCA-TGA-TAG-CAT-CTG-TA-3') and reverse (5'-CCACAATTTGGTGAATTATTA-3') primers, and 20 μg of genomic DNA. We performed PCR using a T100 thermal cycler (Bio-Rad, USA) using the following program: initial denaturation at 94°C for 5 min then 35 cycles of 30 s at 94°C, 30 s at 54°C, and 60 s at 72°C, followed by a

final extension step of 72°C for 7 min. For RFLP, the PCR product (173 bp) was digested using the *VspI* restriction enzyme (0.5 U) (Genetics Science, Indonesia) for 16 hours at 37°C. Digestion products were separated by electrophoresis using 3% agarose gels, which were subsequently stained with Gel Red (Biotium Inc., USA). Gels were visualized using the Gel Doc 200 (Bio-Rad). The *VspI* digestion step yields fragments of 173 bp (homozygote AA); 21 bp and 152 bp (homozygote TT); or 21 bp, 152 bp, and 173 bp (heterozygote AT). The 21 bp product is located within the primer and so cannot be discriminated by electrophoresis.

### Statistical analysis

The statistical difference between cases and controls was analyzed using the Chi-squared or Kruskal-Wallis tests to compare T-scores between groups according to genotype and allotype of the -251 A/T polymorphism of *IL-8* and to identify the risk of osteoporosis by using odd ratio. Logistic regression analysis was used to assess the association of polymorphism with the risk of osteoporosis. Statistical significance was assumed at  $p < 0.05$ . All statistical analyses were performed using IBM SPSS Statistics software version 25 (IBM, NY, USA).



**Figure 1.** Representative gel image of *VspI* digestion products. Lane M: 50-bp ladder, lanes 1 to 3: wild type (AA), lanes 4 and 5: mutant homozygote (TT).

## Results

Figure 1 shows representative examples of gel separation of RFLP products prior to

genotyping. The results of PCR-RFLP (Table 1) show that the T allele was more common than the A allele in both groups, and T was more frequent in the osteoporosis than the normal group. The genotype distribution ( $p = 0.715$ ) was consistent with Hardy Weinberg Equilibrium in the osteoporosis group, but not in the control group ( $p = 0.0028$ ). We used logistic regression analysis to identify a statistically significant relation between BMD and polymorphism of Interleukin-8 -251 A/T ( $\chi^2 = 0.1, p < 0.05$ ).

## Discussion

The chemokine family are cytokines which are either be produced by bone cells or have effects on bone cell function.<sup>10</sup> Interleukin-8 is a member of the chemokine family produced in bone cells which promotes osteogenesis during bone formation.<sup>11</sup> Previous studies have shown levels of IL-6 and IL-8 to be increased in sera of patients with osteoporosis, and it has been shown that IL-8 has specific neutrophil chemotactic activity. It has been reported that IL-8 is produced by human osteoblast-like cells, human osteosarcoma MG-63 cells, human bone marrow stromal cells, and human osteoclasts,<sup>12</sup> and it has been suggested that increased production of cytokines such as IL-6 and IL-8 regulates postmenopausal bone loss. However, the mechanisms underlying these processes are not clear, and there are few studies on the relationship between polymorphisms of *IL-8* and the risk of osteoporosis. The present hospital-based case-control study aimed to evaluate this association among postmenopausal Indonesian women. We identified a significant association between the *IL-8* -251 A/T polymorphism and osteoporosis in this population. The differences in genotype and allele distribution between case and control groups indicate that the *IL-8* -251 A/T polymorphism might be related to the development of osteoporosis. Furthermore, our results indicate the TT genotype to be associated with a higher risk of osteoporosis than the AA genotype. To the best of our knowledge, our study is the first to investigate the possible role of polymorphisms of *IL-8* -251 A/T as a risk factor for osteoporosis and to report that such genotype variations do influence the susceptibility to osteoporosis among postmenopausal Indonesian women. Our analysis of the influence of the *IL-8* -251 A/T polymorphism on IL-8 serum levels

revealed median serum levels to be significantly higher among cases than controls. Additionally, serum IL-8 levels were found to be higher among patients carrying the TT genotype than non-carriers. Our data suggest that serum IL-8 levels might be regulated by *IL-8* -251 A/T polymorphisms in patients with osteoporosis.

## Conclusions

The results of our study indicate that the -251 A/T polymorphism of *IL-8* is significantly associated with postmenopausal osteoporosis in Indonesian women.

## Acknowledgements

Financial support for research and publication from The University of Indonesia 2019 to EIA is gratefully acknowledged. All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission.

## Declaration of Interest

The authors report no conflict of interest.

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