Genetic Variants of the Gsr Gene (rs2978663) and the Progression of Osteoporosis

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ABSTRACT

Background: Osteoporosis, demonstrated as an associated disease with more than 30 gene disorders, is a polygenic disorder, and it’s also implicated in bone mineral density (BMD) regulations. Lipid peroxidation and hydrogen peroxide levels significantly increased and vice versa the antioxidant enzymes decreased such as Glutathione S-Reductase GSR was found in female postmenopausal females. Objective: This research was done in order to find out the effect of rs2978663 genotypes on the progression of osteoporosis. Methods: First, blood samples were used to extract DNA for analysis. Molecular examination was achieved using PCR, RFLP, and UV imaging after electrophoresis in an agarose gel, and these results were analyzed by SPSS (version 23). Results: The genotypes differed in healthy people, and the proportions varied, as they were: the highest percentage was represented by the GA genotype (78%), followed by the AA genotype (16%), and the GG genotype (6%). For case samples, the highest percentage was represented by the GA genotype (51%), followed by the AA genotype (30%), and the GG genotype (19%). There are significant associations between GA genotype and restriction of fragility disease. The risk of having osteoporosis was significantly lower in those with the GA genotype (OR = 0.1946; 95% CI = 0.04-0.95; P = 0.03). The A allele frequency of the GSR gene (rs2978663) did not change significantly between study groups (OR: 0.9965, 95% CI: 0.5547-1.7816, P value: 0.9905). Conclusion: Overall, it is safe to say that GSR-int3 (rs-2678663) was shown to have no association with osteoporosis in this research of Iraqi women. Inherent variation in the GSR gene (rs-2678663) is associated with decreased osteoporosis risk.

Keywords: RFLP-PCR techniques, rs2978663, GSR gene polymorphism, ApaLI; SNP.

1. BACKGROUND

Osteoporosis, the most common disease in older persons, is a systematic bone disorder that causes bone disturbances (bone formation and resorption) and reduces bone mass, bone fractures, and bone fragility. Osteoporosis causes serious disability, and mortality and affects all patient’s life aspects (1). Several studies reported that the osteoporosis disease rate had significantly increased. Over 71% of women and 52% of men over the age of 51 in an Iranian cancer research in 2009 had osteoporosis or osteopenia (2). Osteoporosis (low bone mass) or osteopenia cause mineralization. Increased risk of osteoporosis is linked to multiple factors such as smoking, excess vitamin A, alcohol, vitamin D deficiency, inad-
tive oxygen species (ROS) such O2 and H2O2 and the body's ability to remove them. Simply defined, oxidative stress occurs when redox signaling is chaotic (7). In addition to being by-products of cellular aerobic metabolism, exposure to UV or X-rays, and chronic stress, ROS have been found to play an important role in regulating cytokines, cell signaling, growth factor and hormone activities, ion transport, transcription, neuro modulation, apoptosis, and immune modulation (8). Importantly, it helps keep things running smoothly in the immune system, in terms of both defense against infection and the production of T cells (9). The human body has evolved a highly complex and sophisticated antioxidant protection system to shield its cells and organs from ROS, using a wide range of endogenous and exogenous components that work together in a coordinated and mutually beneficial manner to quench the body's supply of free radicals (8). Endogenous antioxidants, which are produced by the body's cells, are one example of a compound that may be either enzymatic or non-enzymatic. Important enzymatic antioxidants include catalase CAT, glutathione S-reductase GSR, superoxide dismutase SOD, glutathione peroxidase GPX, and glutathione reductase GRX (11). Initiation of osteoporosis has been linked to oxidative stress, according to new research (12). This is due to oxidative stress's ability to raise stress risks, reduce the antioxidant defense system's efficacy, and cause an excess of reactive oxygen species (ROS) (12-14). Metabolic bone diseases and the pathogenesis of skeletal system disorders like osteoporosis, which is characterized by low bone mineral density, bone mass reduction, and increased bone fragility, have been linked to an imbalance between osteoblast and osteoclast activity, caused by oxidative stress (12, 15, 16). Bone resorption and the production of osteoclasts are both accelerated by oxidative stress (17). Oxidative stress promotes osteoclast development and increases bone resorption (9). Osteoblast and osteocyte apoptosis is caused by ROS, and these cells are found in the bone matrix and produced by mature osteoblasts, promoting osteoclast-genesis (10), and high ROS levels inhibit or decrease activity and differentiation of osteoblast, thereby mineralization and osteogenesis (11). The enzyme glutathione disulfide reductase (GRS) catalyzes the conversion of glutathione disulfide (GSSG) to glutathione sulfhydryl (GSH), which may scavenge hydroxyl radicals, singlet oxygen, and other electrophiles, thus maintaining a stable reducing environment in the cell. Cellular oxidative equilibrium relies on the GSSG/GSH ratio; cells need to keep their levels of reduced glutathione (GSH) high while keeping their levels of oxidized glutathione disulfide (GSSG) low. This delicate equilibrium is maintained by glutathione reductase, an enzyme responsible for catalyzing the conversion of GSSG to GSH (19). Increased thermal instability and loss of glutathione disulfide reduction activity are hallmarks of the autosomal recessive condition known as inherited glutathione reductase deficiency, caused by SNPs in the gene of GSR. The GSR mutation may cause anomalies in the function and structural conformation of the GSR protein and also generate hereditary glutathione reductase deficiency (20). It is one of about four kinds of SNPs identified as the most associated SNPs with osteoporosis illness. Postmenopausal osteoporosis significantly increased hydrogen peroxide levels and lipid peroxidation while decreasing antioxidant enzyme levels like GPx, SOD, and GT versus healthy people (12). Microarray investigation of an osteoporosis patient's human osteoblast cell line showed considerable differentiation of antioxidant enzyme defense genes including SOD2 and GSR (13). Oxidative stress contributes to bone resorption by inhibiting bone synthesis. Furthermore, antioxidant enzymes have been shown to have a minimal preventive effect in women with osteoporosis (14).

2. OBJECTIVE

This study aimed to figure out if there is any link between polymorphism of the glutathione S-reductase GSR (rs-2978663) gene and postmenopausal women in Iraq.

3. MATERIALS AND METHODS

Population of study

The study included 64 patients with osteoporosis at Al-Imam Al-Hussain Medical-City Hospital, aged a round 21–82 years old (all females); the control group consisted of 36 healthy females (aged 22–72 years old). Samples start being collected between September 2021 and January 2022. Every individual provided a written authentication.

Data processing

The following information was obtained from the patient's questionnaire and case sheet: duration of disease, family history, age, body mass index (BMI), number of abortions, marital status, number of births, menopause, soft drink intake, smoking, and laboratory tests of the patient obtained by the hospital.

Sample collection

From venous blood, about four milliliters were collected and kept in EDTA tubes for genetic analysis (24).

Molecular assay

DNA was extracted from white blood cells via a kit procedure (Genaid, Taiwan). The purity and concentration of DNA were measured using a nanodrop device (Implen, Germany) in the laboratories of the Department of Life Sciences, University of Babylon (25). The primers for the catalase gene were designed depending on the National Center for Biotechnology Information (NCBI) and provided by a company (Macrogen Company, South Korea). The genotypes were detected by PCR as presented in Table 1.

The PCR product was digested with the restriction enzyme ApaLI for 15 minutes at 37 degrees Celsius, as recommended by the manufacturer (Promega, USA). After heating at 65 °C for 30 minutes, the enzyme was rendered inert.

Gel electrophoresis

About 1 gram of agarose gel was dissolved in a 1x TAE buffer (100 ml) under heat, and after adding Red Sword dye to it, this mixture was poured into the gel tray and left to solidify under low temperature for a quarter of an hour. The samples were loaded into the gel and electrophoresed in the presence of a power source (75–80 volts), then photographed under ultraviolet light (26).

Statistical analysis

SPSS version 23 was used for statistical analysis, and a value of p smaller than 0.05 was considered statistically significant. t-test and ANOVA were used for comparisons between groups; the Hardy-Weinberg equation and the chi-square test were used to calculate odds ratios and confidence intervals.
4. RESULTS AND DISCUSSION

After an appropriate amount of highly purified DNA was obtained, the desired region of the gene was amplified. On an agarose gel, the PCR process revealed the presence of one band (569 bp) of the target sequence. The genotyping by RFLP technique showed three genotypes: mutant homozygous (AA, 569 bp); mutant heterozygote (GA, 569, 463, and 106 bp); and wild homozygous (GG, 569, and 106 bp) (Figure 1).

Table 2 shows the distribution of the GSR (rs2978663) gene polymorphism in patients and control groups. Genotype frequencies for the control group were 5.6% (G/G), 77.9% (G/A), and 16.6% (A/A) genotypes, while in the patient group, the genotype frequencies were 18.9% for G/G, 51.5% for G/A, and 29.6% for the A/A genotype. Individual carriers of GA genotypes were less likely to develop osteoporosis [OR = 0.1964, CI 95% = 0.0405–0.9531, P = 0.0434]. Moreover, the A allele frequency of the GSR gene (rs2978663) does not vary significantly between study groups (OR: 0.9965, 95% CI: 0.5547–1.8716, P value: 0.9905), and there are no significant relationships between GSR (rs2978663) genotypes.

This is the first work that unravelles the association between GSR (rs2978663) genetic polymorphisms and osteoporosis patients. Our results show a decreased risk of developing osteoporosis for individuals with the G/A genotype of the GSR (rs2978663) gene. Ten online computational tools used by Yue and Moult revealed four SNPs out of seventeen SNPs identified as the most osteoporosis disease-associated SNPs (28). Hereditary glutathione reductase deficiency may be caused, at least in part, by changes to the GSR gene that alter the protein’s structure and function. In the catalytic cycle of GSR, the Glu-427 polymorphism closest to the rs2978663 gene’s location in intron 3 codes for a protein that plays a crucial role. By removing hydroperoxides, peroxidase, and superoxide and regenerating oxidized GSH, the antioxidant enzyme greatly contributes to oxidative stress in normal, healthy cells (29). Multiple studies (30) have linked reduced CAT, GSR, and SOD activity to osteoporosis in postmenopausal people and mice. There were many oxidative stress-related processes in osteopathy. Reactive oxygen species have a major impact on suppressing signaling during osteoblast genesis (6, 15). In postmenopausal women, BMD levels were significantly associated with the subgroup genotype of a GSR gene polymorphism found in the third intron. This study is the first to show that the rs2978663 GSR gene polymorphism is significantly linked with BMD and to suggest that the A allele of this polymorphism may lead to lower BMD levels in postmenopausal women (16). GSR, GPx, and SOD are antioxidant enzymes produced by bone cells. Their main function is to neutralize free radicals and inhibit the O2 transition to hydroxyl radicals (17). Another study used microarray techniques to investigate the primary osteoblast cell line in humans from osteoporotic women, and the results found a significant change in the expression profile of enzymatic antioxidant defense systems like GSR and SOD2 genes (18). There were several processes involved in osteopathy related to oxidative stress. ROS have a major impact on inhibiting signaling during osteoblast genesis (7, 19).

5. CONCLUSION

This study found that the genetic variation of GSR-int3 (rs2678663) was unrelated to Iraqi women with osteoporosis. The GSR gene polymorphism (rs-2678663) is involved in reducing the risk of osteoporosis.

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