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Impact of rs1126616 Gene Polymorphism of Osteopontin in Kidney Stone Formation

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Abstract

Kidney stone is a complex disease resulting from environmental as well as hereditary factors and principally composes of approximately 75% calcium oxalate (CaOx) crystals, which are formed through a multi-step process. Osteopontin gene was identified by isolating cDNA from cultured rat osteosarcoma cells in 1986. *Osteopontin* gene located on human chromosome 4 (4q22.1). This gene encodes several non-collagenous bone and dentin proteins. In this study, 93 normal control samples and 92 kidney stonedisease samples that were grouped according to the types of kidney stone disease and clinical characteristics of patients, including gender and average age were observed with restriction fragment length polymorphism (RLFP) technique.58 patients and 43 controls displayed the “C or G” / “C or G” genotype, 48 patients and 34 controls displayed T / “G or C” genotype. Since there is no appropriate restriction enzyme to recognize the G or C nucleotide in that position, it could not be possible to discriminate the C-G nucleotides. Additionally no T/T genotype was observed. The results werefound statistically significant by chi-square test ($p < 0.05$). In conclusion,the analysis of the polymorphism showed that OPN gene might be a risk factor for kidney stone formation, and suggesting that genetic polymorphisms in OPN gene modify individual susceptibility to nephrolithiasis.

Keywords: Kidney stone, osteopontin, polymorphism, PCR-RLFP

Introduction

Kidney stone disease is a complex disease and a worldwide health problem that affects almost all population (1). The kidney stone can be classified into four types; calcium oxalate (CaOx) crystal stones, uric acid stones, struvite stones and cystine stones (1). It mainly composes of about 75% calcium oxalate crystals, which are formed through a multi- step process (1). Understanding the epidemiology of kidney stones disease is important to determine the significance of the disease at a community level, the associations and risk factors for individuals and the likeliness of stone recurrence (1,2). It may present at any age, onset is more common in young and middle-aged adults (2).The risk factors of kidney stone is a complex multi- factorial

disease resulting from interactions between hereditary nature and environmental (2). Several factors increase the risk for developing kidney stone diseases, including dehydration and inadequate fluid intake, decreased urinary volume, certain chemical levels in the urine that are too high such as oxalate, calcium and uric acid or too low citrate and magnesium, and several medical conditions such as medullary sponge kidney, reflux, renal tubular acidosis and urinary tract infections, genetic abnormalities also increases the risk (1).

several previous analysis of kidney stone reported many macromolecules, organics and inorganics are known to inhibit stone formation, including Tamm-Horsfall, glycosaminoglycans, Calgranulin, Bikunin and Osteopontin (2).

Osteopontin (OPN) also called urinary stone protein, secreted phosphoprotein 1 (SPP1), bone sialoprotein, and early T lymphocyte activation 1 (ETA1) (7). *Osteopontin* gene was identified by isolating cDNA from cultured rat osteosarcoma cells by Oldberg et al. (3) in 1986. *Osteopontin* gene located on human chromosome 4q22.1 (4). This gene encodes several non-collagenous bone and dentin proteins (4). *Osteopontin* comprise of 7 exons, the human *osteopontin* gene sequence spans approximately 9 kb and the open reading frame consists of 942 nucleotide from the start codon in exon 2 to the stop codon in exon 7 (5). Osteopontin regulate normal bone resorption and inhibition of urinary crystallization and may prevent the renal stone formation by decreasing the growth and aggregation of CaOx crystals (6). OPN plays an important role in physiological calcified regions such as bone, dental calculus, and otolith and pathological calcified regions such as calcified cancer cells, kidney stones, and arteriosclerosis (5).

The aim of this study, molecular investigation of the relationship between previously identified *Osteopontin* gene polymorphism rs1126616 with nephrolithiasis and kidney stone disease by polymerase chain reaction and restriction fragment length polymorphism analysis (RFLP). Since kidney stone formation is a multifactorial disorder, to our professional opinion, the determination of the role of rs1126616 polymorphism in stone disease might help to provide the most appropriate approaches to the patients.

MATERIAL AND METHODS

The samples were collected from the laboratory department at Zheen International Hospital, Erbil, Iraq. A total of 185 samples were analyzed. The study included 93 healthy control and 92 CaOx stone formers (sporadic) that were analyzed. The blood samples were stored at -20˚C until further analysis. Informed consent was taken from all participants and the study was approved by local ethics committee. Approval number: 05.02.2013/53.

Molecular analysis

Molecular genetics analysis was carried out on genomic DNA extracted from whole blood using the salting-out method and stored at 20˚C. rs1126616 gene polymorphism of osteopontin was genotyped by polymerase chain reaction (PCR) and endonuclease digestion. Polymerase chain reaction amplification (Applied Biosystem) was conducted using a gradient thermal cycler device in a 25 ml reaction mixture in PCR tubes containing 2µL DNA template, 1.5µL dNTP, 0,125 µL Taq DNA polymerase and 2µL Taq Enhancer MgCl2, 2.5µL 10X PCR buffer Ammonium sulfate (NH4)2SO4, 14.875µL dH2O and 1µL of forward and reverse primers. The nucleotide sequences of the forward and reverse primers were employed; 5’-ATTCTTCATTTGTGCCGTGA -3’ and 5’- TTTTGGGGTCTACAACCAGC -3’, respectively. The cycling conditions consisted of initial denaturation at 94˚C for 3 min, 35

cycles of denaturation 94˚C for 30 sec, annealing at 57˚C for 30 sec and extension 72˚C for 30 sec, and final extension 72˚C for 4 min. The 356 bp PCR length products to *osteopontin* gene were separated on a 2.0% agarose gel pre-stained with ethidium bromide. Genotyping was carried using the AluI restriction enzyme as manufactured. µL of PCR product was mixed with U AluI and appropriately buffered and incubated at 37˚C during a 16-h period. The genotypes were separated on a 2.0 % agarose gel electrophoresis stained with ethidium bromide and visualized under ultraviolet light (Figure 1). Statistical analysis was carried out using Chi square (X^2) test. Significance was assumed for values $p \leq 0.05$.

Results

In this study the rs1126616 gene polymorphism of *Osteopontin* was analyzed via restriction fragment length polymorphism and separated by agarose gel electrophoresis and stained with ethidium bromide. As a result of RFLP analysis;58 patients and 43 controls displayed the "C or G" / "C or G" genotype, 48 patients and 34 controls displayed T / "G or C" genotype. Since there is no appropriate restriction enzyme to recognize the G or C nucleotide in that position, it could not be possible to discriminate the C-G nucleotides. Additionally no T/T genotype was observed. The Figure 1 shows the RLFP result of rs1126616.

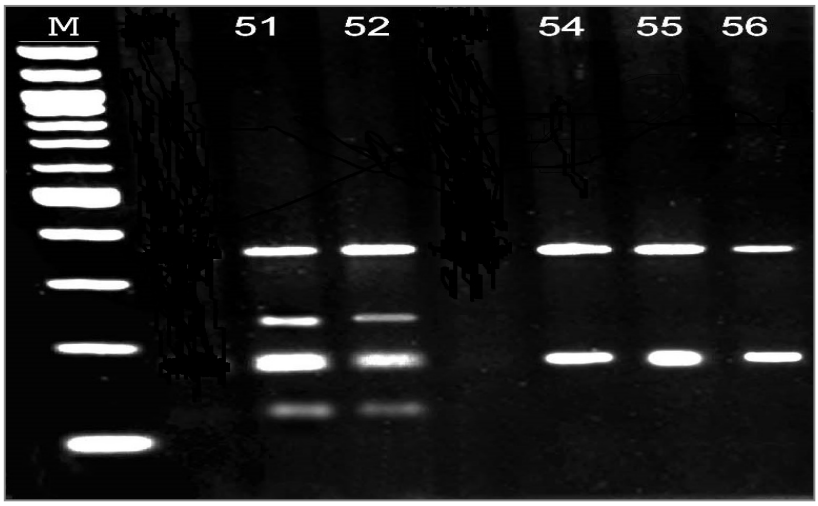

Figure 1. The DNA fragments after restriction enzyme cleavage. Markers: 100 bp (left). Samples 51 and 52 display T / "C or G" genotype; samples 54, 55 and 56 display "C or G" / "C or G" genotype.

The RLFP result was obtained from 92 samples of kidney stone disease and 93 samples of normal controls. In this study, the results were statistically significant (p =0,0317, Chi Square test). The Figure 2 indicates the statistical result.

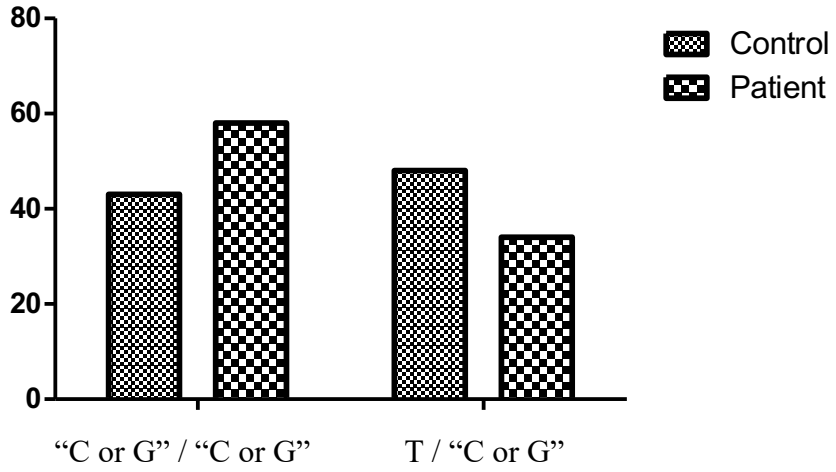

Figure 2. Statistical analysis result of rs1126616 in kidney stone disease.

Disscusion

The purpose of this study, molecular investigation of the association between rs1126616 single nucleotide polymorphism of *Osteopontin* gene and kidney stone formation by restriction fragment length polymorphism analysis (RFLP).

Chemical composition analysis of urinary stones has indicated that calcium oxalate stones are the most common and occur in approximately 75% to 80% of cases (8). On the basis of frequency of occurrence, renal stones are most prevalently composed of calcium oxalate (CaOx). The CaOx crystals generally form in the renal tubules and exposure of renal epithelial cells to CaOx crystals results in increased synthesis of urinary proteins including osteopontin (OPN) (9). OPN is a highly phosphorylated glycoprotein originally identified in bone and has an arginine-glycine-aspartate amino acid sequence functioning as an adhesion motif of the protein to integrins and CD44 (10,11). OPN is synthesized as an 32 kDa protein, but due to extensive posttranslational modifications such as phosphorylation, glycosylation and proteolytic processing, its apparent molecular mass ranges from 45 to 75 kDa (12). OPN was not only identified as the main organic component in the urinary stone matrix but also had remarkably increased expression in the distal tubular cells in the stone-forming rat (12).

Single nucleotide polymorphisms (SNP) of the human *OPN* gene has been reported to be associated with many diseases (13). Especially, Gao and colleagues (13) have investigated 61 polymorphisms and evaluated four haplotypes among these polymorphisms (13). Two of these haplotypes have been identified as risk factors for kidney stone diseases (14). They have tried to shed a light to understand the mechanism of how the *OPN* gene can change the structure of OPN molecule (11).

There are only a limited number of published studies related to OPN gene polymorphism and kidney stone disease (14). A single nucleotide polymorphism of the human OPN gene (A9402G, in exon 7) was reported to be associated with the risk for urinary stone formation (5). One study reported a polymorphism (C/T genotype, Ala250Ala, in exon 7) in the OPN gene, which has a significantly higher incidence in patients with recurrent stone formation or familial nephrolithiasis (15). Gokhan et al. (15) identified two SNPs in osteopontin gene were detected in kidney stone disease, both nucleotide substitutions arise in the osteopontin coding region.

In this study, we report that a silent polymorphism, C6982T; rs1126616, in exon 7 is significantly associated with kidney stone formation. The T/T genotype or T allele carrier for rs1126616 had an increased kidney stone risk compared with those of the C or G-allele carriers for rs1126616 (16).

Latterly, expression researches showed. that oxalate and CaOx crystals influence the expression of several genes (including ONP) in renal tubular cells which subsequently modify the attachment, growth and aggregation of CaOx crystals (17). Data from qPCR demonstrated a significant up-regulation of OPN mRNA in the treatment group (17).

Cultured mouse kidney cortical cells secrete osteopontin, a bone matrix protein that is also found in urine (18). Osteopontin is associated with cell proliferation/ tumorogenesis and also inhibits kidney stone mineral crystal growth (18). Using antibodies raised against osteopontin isolated from the culture medium, previous work reported localized osteopontin in normal rat kidney (18).

In conclusion, the analysis of each polymorphism showed that OPN gene might be a risk factor for kidney stone formation, and suggesting that genetic polymorphisms in OPN gene modify individual susceptibility to nephrolithiasis. Further studies are necessary to verify these findings in different ethnic groups.

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