RESEARCH ARTICLE

Association of MMP-9 gene polymorphisms with nephrolithiasis patients

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Background: Nephrolithiasis is one of the causes which lead to chronic kidney disease (CKD). Matrix metalloproteinases (MMPs) are endopeptidases degrading extracellular matrix which correlate with the pathogenesis of atherosclerosis. The current study was designed to analyze the association of (R279Q, C1562T) polymorphism of MMP-9 with nephrolithiasis patients.

Methods: Genotyping of MMP-9/R279Q and of MMP-9/C1562T polymorphism were carried out by PCR-based restriction digestion method. Serum level of MMP-9, oxidative stress marker, MDA, and uric acid were measured in patients and control.

Results: Allele frequencies of the MMP-9/C1562T polymorphism for C and T allele were 71.25% and 28.75% in patients, 87.08% and 12.92% in control respectively. The homozygote TT was more frequent in the nephrolithiasis patients group, while T allele frequency was significantly higher in the nephrolithiasis patients group than in the control group. The patients with CT and TT genotype showed a significant increase in serum MMP-9, Total Oxidant Status (TOS), Oxidative Stress Index (OSI), Malondialdehyde (MDA), and uric acid when compared to CC genotype in patients with nephrolithiasis. The R279Q polymorphism site with regard to the relationship with nephrolithiasis was not significant.

Conclusion: The result indicates that patients with TT genotype had an increased risk of stones. Also, the results demonstrate that TT allele of the C1562T polymorphism in the MMP-9 gene is related with an increase of oxidative stress in nephrolithiasis patients and may possibly impose a risk for cardiovascular diseases in patients with TT genotype of MMP-9.

KEYWORDS

malondialdehyde, MMP-9 gene polymorphism, nephrolithiasis, total antioxidant status

1 | INTRODUCTION

Nephrolithiasis is a common chronic disorder associated with painful stone episodes.¹ It can cause post-renal acute kidney injury via obstruction of urinary outflow, often associated with rapid deterioration in renal function.² Kidney stone is one of main disorders of the urinary tract.³ Kidney stone may have various compositions which include, in order of decreasing frequency: calcium oxalate, uric acid, calcium phosphate, struvite, and cystine.⁴ The MMPs are structurally

related, zinc dependent.⁵ The MMP-9 degrades extracellular matrix proteins, cytokines, and chemokine to regulate physiological and pathophysiological tissue alteration.^{6,7} MMPs are provided with descriptive names, depending on substrate specificity.⁸ MMP-9 is a gelatinase, in which 92-kDa collagenase (IV) is secreted from various cells including fibroblasts, lymphocytes, endothelial cells, neutrophils, and macrophages.⁹ Several studies showed that imbalance in the activity of MMP-9 would lead to vascular disease in patients with kidney failure.¹⁰⁻¹² Furthermore, MMP9 is highly expressed in the weak

regions of atherosclerotic plaques and may be causally involved in plaque rupture.¹³⁻¹⁵ The increase in plasma concentrations of MMP9 may be a predictor of cardiovascular disease risk and plasma MMP9 concentrations are elevated in patients with acute myocardial infarction.^{16,17} Other studies showed that there was an increased risk for coronary heart disease in patients with kidney stones due to increased risk for CKD.^{18,19} Thus, the current study proposed that: two functional polymorphisms in the MMP-9 gene promoter region (C1562T; rs3918242, and Q279R; rs17576) might affect the alterations in MMP-9 levels linked with nephrolithiasis and the alteration in plasma MMP-9 concentrations might found in patients with nephrolithiasis. Besides, the relationship between genetic variations of the MMP-9 gene with oxidative stress in nephrolithiasis patients was examined.

2 | MATERIALS AND METHODS

2.1 | Patients collection and samples storage

Patients suffering from major infections like T2DM, diabetic nephropathy, heart disease, history of alcohol intake, taking potent antioxidant, smokers, and pregnant females were excepted in the current study. One hundred twenty patients with nephrolithiasis and one hundred twenty healthy as control were involved in this study. All the patients and controls were subjected to the same clinical examination. Furthermore, all the patients and controls were also subjected to the same biochemical test and urine test and were given the ultrasound and X-ray test.

Six milliliters of blood samples were collected from patients and control and separated into two parts. The first was stored at -20°C for DNA analysis. The other part was allowed to clot and the serum obtained was used to measure serum MMP-9 and other biochemical

parameters were stored at -20°C . The study was supported by the Institutional Ethics Committee [IIUM/305/14/11/2/IREC 300].

2.2 | Genotyping MMP-9/R279Q and MMP-9/C1562T polymorphism

2.2.1 | DNA extraction

The genomic DNA was extracted by using the standard salting out method.²⁰ Extracted DNA was used as a template, the promoter region of MMP-9/R279Q and MMP-9/C1562T were amplified by PCR. Primers sequences are presented in Table 1. Final volume of reaction was 25 μL [12.5 μL 2 $\times$ Power TaqPCR Master Mix (Pomoga Corporation, Madison, WI, USA), 1 μL each primer (10 pmol/ μL), and 1 μL genomic DNA]. The restriction enzyme *SphI* and *SmaI* (Promega, USA) were used to digest the PCR products of R279Q and C1562T polymorphism sites at 37°C for 16 hours followed by 2% agarose electrophoresis (100 V, 60 minutes). The MMP-9/R279Q allele produced a 277 base pair (bp), 181 and 96 bp. MMP-9/C1562T allele was produced a 436 bp fragment, 242 and 194 bp (Figure 1).

2.3 | Measurement of oxidative stress index (OSI), total oxidant status (TOS), total antioxidant status (TAS), MMP-9, malondialdehyde (MDA), and other biochemical parameters

Serum of TAS, TOS, and OSI of were determined by the methods developed by Erel.²¹⁻²³ Serum MMP-9 was measured by ELISA (Cusabio Biotech Com.). The modified method of Satoh was used to measure the MDA concentration.²⁴ Serum urea, creatinine cholesterol, and uric acid were done by commercial kits.

TABLE 1 The PCR conditions and primers for the MMP-9/R279Q and MMP-9/C1562T genes

Polymorphism Primers	Initial denature	Denature	Annealing	Extension	Cycles	Final extension
MMP-9/R279Q						
F:5'-ATGGGTCAAAGAACAGGA-3' R:5'-GGTAGACAGGGTGGAGG-3'	5 min, 95°C	30 s, 94°C	30 s, 58°C	30 s, 72°C	30 cycles	7 min, 72°C
MMP- 9/C1562T						
F:5'-GCCTGGCACATAGTAGGCCC-3' R:5'-CTTCCTAGCCAGCCGGCATC-3'	5 min, 95°C	30 s, 94°C	30 s, 64.5°C	45 s, 72°C	35 cycles	10 min, 72°C

FIGURE 1 Genotyping of the MMP-9/R279Q and MMP-9/C1562T. (A) MMP-9/R279Q: 1, AA genotype; 2, 4, AG genotype; 3, GG genotype (B) MMP-9/C1562T: M, molecular marker; 1, 4, 5 and 7, CC genotype; 2, 3 and 6, CT genotype; 8, CT genotype

2.4 | Statistical analysis

The polymorphisms were tested for confirmation with Hardy-Weinberg expectations in the nephrolithiasis patient and control groups. Differences in demographic characteristics, selected variables and frequencies of the genotypes, alleles of the two MMP-9 polymorphisms between groups were evaluated using the chi-square test (for categorical variables) and ANOVA for comparison the biochemical parameters between groups. The association between MMP-9 variant genotypes and nephrolithiasis risk was estimated by odds ratios (ORs) and their 95% confidence intervals (CIs) using univariate analysis. The factors associated with nephrolithiasis were evaluated using multifactor logistic regression. All data were analyzed using SPSS for Windows 20.0 (SPSS Inc, Chicago, IL, USA).

3 | RESULTS

3.1 | MMP-9/R279Q and MMP-9/C1562T allele frequency and genotype distribution

Table 2 showed the distribution of genotypes and the prevalence of alleles in the nephrolithiasis group and the control group. The frequencies of CC, CT, and TT of MMP-9/C1562T polymorphism were 58.33%, 25.83%, and 15.84% in the nephrolithiasis group, and 77.50%, 19.17%, and 3.33% in the control group. The homozygote TT was more frequent in the nephrolithiasis group, and allele frequencies of the MMP-9/C1562T polymorphism for C and T allele were 71.25% and 28.75% in patients group, 87.08% and 12.92% in control, respectively, $P < .0001$. The MMP-9 gene R279Q polymorphism showed non-significant change in allele distribution and genotypes between study groups ($P > .05$), Table 2.

3.2 | Biochemical study in nephrolithiasis patients and control groups

There was not much difference observed between age, urea, creatinine, uric acid, and cholesterol between patients and control groups. MMP-9 displayed a significant increase in patient groups than in control groups as shown in Table 3. Besides, there was also a significant difference of serum TAS, TOS, OSI, and MDA in patients in comparison to control.

In Table 3, Serum MMP-9 showed a significant increase in patients than control. The subjects with TT and CT genotype showed higher levels of MMP-9 than subjects with CC genotypes. Also, a significant difference was found in subjects when divided according genotypes

TABLE 3 Age and biochemical parameters in serum of nephrolithiasis and control groups

Characteristics	Patients (n=120)	Control (n=120)	P-value
Age (y)	48.42±8.33	49.27±7.01	.393
Urea (mmol/L)	4.66±0.98	4.49±0.86	.155
Creatinine (μmol/L)	82.78±11.46	82.08±6.34	.559
Uric acid (μmol/L)	323.32±65.32	310.12±68.23	.127
Cholesterol (mg/dL)	194.41±20.23	189.31±21.12	.057
MMP-9 (ng/mL)	148.05±74.37	113.10±40.71	.00001
TAS (mmol. Trolox Eq/L)	1.68±0.42	2.03±0.49	.00001
TOS (μmol. H2O2 Eq/L)	18.98±3.01	13.60±1.71	.00001
OSI (arbitrary unit)	10.81±3.90	6.18±2.01	.00001
MDA (nmol/mL)	3.78±0.53	1.23±0.25	.00001

TABLE 2 Genotypes distribution and allele frequencies of the MMP-9/R279Q and MMP-9/C1562T polymorphism between patients and control groups

MMP-9 polymorphisms	Nephrolithiasis patients [n=120 (%)]	Control group [n=120 (%)]	OR (95% CI)	P value
MMP-9/R279Q				
AA	60 (50.00)	58 (48.33)	1	
AG	38 (31.67)	41 (34.17)	0.896 (0.507-1.584)	.705
GG	22 (18.33)	21 (17.50)	1.013 (0.504-2.036)	.971
AG+GG	60 (50.00)	62 (51.67)	0.935 (0.564-1.552)	.796
A allele	158 (65.83)	157 (65.42)	1	
G allele	82 (34.17)	83 (34.58)	0.892 (0.674-1.431)	.923
MMP-9/C1562T				
CC	70 (58.33)	93 (77.50)	1	
CT	31 (25.83)	23 (19.17)	1.791 (0.961-3.336)	.064
TT	19 (15.84)	4 (3.33)	6.311 (2.055-19.379)	.0004
CT+TT	50 (41.67)	27 (22.50)	2.460 (1.403-4.314)	.001
C allele	171 (71.25)	209 (87.08)	1	
T allele	69 (28.75)	31 (12.92)	2.720 (1.701-4.351)	.00001

TABLE 4 Distribution of serum MMP-9 in patients group according to genotype

Characteristic	MMP-9/R279Q genotype			MMP-9/C1562T genotype				
	AA [n=60]	AG [n=38]	GG [n=22]	P value	CC [n=70]	CT [n=31]	TT [n=19]	P value
MMP-9 (ng/mL)	128.33±44.70	131.56±78.20	135.48±78.25	.898	132.11±49.22	167.18±41.13	190.35±74.50	.00001
TAS (mmol Trolox Eq/L)	1.66±0.33	1.70±0.40	1.68±0.41	.871	1.57±0.36	1.26±0.40	1.00±0.33	.00001
TOS (μmol H ₂ O ₂ Eq/L)	18.95±2.30	18.52±2.24	20.00±2.25	.054	17.80±2.10	21.95±2.34	28.87±2.23	.00001
OSI (arbitrary unit)	8.45±3.33	8.75±3.00	9.67±3.12	.312	10.05±3.22	11.98±3.20	17.05±3.89	.00001
Cholesterol (mg/dL)	191.12±16.85	195.29±16.40	198.16±15.80	.186	196.90±18.40	208.23±17.65	222.93±12.25	.00001
MDA (nmol/mL)	3.20±0.43	3.30±0.47	3.45±0.48	.084	3.20±0.44	4.50±0.41	5.40±0.49	.00001
S.Uric acid (μmol/L)	328.40±55.35	331.40±50.65	330.68±60.64	.962	324.20±57.55	350.25±67.00	365.75±65.00	.015

in patients group in Table 4. Besides, the AA, AG, and GG genotypes showed no significant differences in patients group.

A substantial decrease of serum TAS was shown in patients in comparison to control in Table 3. Serum OSI and TOS were significantly increased in patients than in control as shown in Table 3. The subjects with TT and CT genotype showed lower levels of TAS and higher levels of TOS and OSI than subjects with CC genotypes. On the other hand, a significant difference was found in subjects when divided according genotypes in patients group in Table 4. The AA, AG, and GG genotypes showed no significant differences in patients group.

Serum MDA increased significantly in nephrolithiasis patients compared to control ($P<.01$; Table 3), while no significant difference was shown in cholesterol level between the groups. From Table 3, there was also an significant increase in serum uric acid patients group when compared to control. As for Table 4, a significant difference was found in subjects when divided according genotypes in patients group. However, non-significant differences were shown between AA, AG, and GG in patients group.

A significant correlation was detected between MMP-9 with oxidative stress markers, and uric acid in patients with nephrolithiasis, while no significant correlation was observed in control, Table 5.

4 | DISCUSSION

The present results come to an agreement with another study which hypothesized that nephrolithiasis and urinary calculus have been connected with high concentration of uric acid in the blood.²⁵ However, the increase in uric acid in the present study is not significant, while other studies showed that elevated uric acid is an independent risk factor for kidney disease in the general population.^{26,27}

Individuals may not develop the same type of disease despite being exposed to similar environmental and lifestyle factors. For that reason, inherited factors played a main role in the development of diseases. In contrast, there was no previous study conducted that linked MMP gene polymorphism with nephrolithiasis. The result showed the increase of serum MMP-9 concentration significantly in patients

TABLE 5 Representation of Pearson's correlation values for patients and control groups

Correlative variables	Pearson's correlation values patients group	Pearson's correlation values for control group
	r-value	r-value
MMP-9 vs TOS	.73 ^a	.11 ^c
MMP-9 vs TAS	-.65 ^b	-.14 ^c
MMP-9 vs OSI	.69 ^b	.12 ^c
MMP-9 vs MDA	.75 ^a	.12 ^c
MMP-9 vs uric acid	-.62 ^b	.10 ^c

^aSignificant value at $P<.01$.

^bSignificant value at $P<.05$.

^cNon-significant value.

compared to control group. MMP-9 activity and concentrations is relied on its expression levels and functional genetic polymorphisms in the MMP-9 gene.^{28–30} Another study exhibited that MMP9 is essential for in vivo nephron mass creation and protection of kidney function.³¹ The study assumed that two functional polymorphisms of MMP-9 gene (R279Q; rs17576 and C1562T; rs3918242) may possibly affect the variations in MMP-9 levels related with nephrolithiasis.

The result of the present study revealed that C1562T alleles of the MMP-9 gene are associated in patients within nephrolithiasis. On the other hand, no relationship was found between R279Q polymorphism and nephrolithiasis, and these results were not demonstrated in previous studies. As the results indicated, MMP-9 gene polymorphisms can alter MMP-9 concentrations and increase cardiovascular complications in nephrolithiasis. Actually, the MMP-9/C1562T polymorphism results in the damage of a nuclear repressor binding site of protein which lead to higher MMP-9 mRNA and protein levels once the T allele is extant.³² The MMP-9/R279Q polymorphism is found in the gelatinase-specific fibronectin type II domain, which causes the formation of amino acid in the catalytic domain, and the change in MMP-9 activity.³³ The R279Q allele was found to be connected between high level of MMP-9 and myocardial infarction and cardiovascular disease.³⁰ Moreover, other studies did not found any correlation between R279Q polymorphism with the risk of cardiovascular disease.^{34,35}

Several studies published indicated the status of oxidative stress in different disorders that escorted with chronic kidney disease.^{36–38} On top of that, other studies noted that lipid peroxidation causes tissue damage due to the development of different diseases like atherosclerosis, and is related to the increase of cardiovascular mortality and morbidity in CKD.^{39,40}

This study demonstrated that the serum concentration of TOS, MDA, OSI TAS and uric acid statistically differed in patients with nephrolithiasis. On the other hand, the research has also shown significant correlation between serum concentration with different oxidative stress and uric acid. Furthermore, the results suggest that MMP-9 is one of the factors that mediate the relationship between nephrolithiasis and atherosclerosis. On top of that, in CKD arterial stiffening increases cardiac afterload and left ventricular hypertrophy, reduces coronary artery perfusion and myocardial ischemia, but increases pulse pressure that promotes atheroma formation and vascular remodeling.⁴¹ MMP-9 is secreted via inflammatory cells in the adventitia or smooth muscle cells.⁴² In our study, a significant difference was found in the serum concentration of MMP-9 between nephrolithiasis patients and control group. Moreover, the significant increase was also found in the value of this biomarker within MMP-9/C1562T genotype T/T, TG and AG GG types in patients with nephrolithiasis.

The current study conclude that MMP genetic polymorphisms affect MMP variations in nephrolithiasis with the TT genotype for the C1562T polymorphism is showing to high serum MMP-9 levels. This result may possibly indicate a group of patients that will have worse cardiovascular prognosis. Finally, the study of genetic polymorphisms could help select a specific high-risk population, who may particularly benefit from targeted antioxidant strategies.

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