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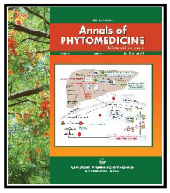

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SIRT1 gene polymorphisms in kidney stone disease

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Abstract

The aim of this study was to investigate the relationship between SIRT1 gene polymorphisms and the presence of kidney stones, as well as the expression of SIRT1 in the serum of patients with kidney stones compared to those without this condition.

In this cross-sectional study, we recruited 100 patients with kidney stones and 100 control subjects without kidney stones. We conducted genotyping for three specific single nucleotide polymorphisms (SNPs)-rs3740051, rs10509291, and rs932658 of the SIRT1 gene using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based on blood samples obtained from the participants. Sirtuin-1 levels were measured using an enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed using SPSS version 21. We assessed the Hardy-Weinberg equilibrium for the alleles. The associations were examined through Chi-square tests, pairwise linkage disequilibrium, and the study of haplotypes created from the three SNPs using SNPStat online and Shesisplus. We found no significant associations between the genotypes of the three SNPs and kidney stone disease ($\chi2=0.633$, $p=0.277$; $\chi2=0.785$, $p=0.5371$; and $\chi2 = 2.74$, $p=0.098$). Additionally, there was no significant connection between the SNPs (rs3740051, rs10509291, and rs932658) and Sirtuin-1 expression ($\chi2=0.2776$, p=0.598; $\chi2=0.2413$, $p=0.6233$; and $\chi2=1.332$, $p=0.248$), respectively. It is noteworthy that serum Sirtuin-1 levels were insignificantly reduced in cases compared to controls ($p=0.233$). The three SNPs of SIRT1 showed weak linkage disequilibrium, indicated by low D' values (0.53, 0.55, and 0.73). The low R^2 values (0.21, 0.1, and 0.06, respectively) did not support the co-inheritance between the alleles. Our study found no substantial association between SIRT1 gene polymorphisms and the presence of kidney stones. However, the observation of reduced serum sirtuin1 levels in cases suggests a potential protective role of SIRT1 in kidney stone formation.

1. Introduction

Nephrolithiasis is one of the most prevalent urological tract problem (Singh *et al.,* 2021; Usha *et al.,* 2023). If left untreated in its early stages, it has been linked to an increased risk of chronic kidney disease (CKD), end stage renal diesease (ESRD) and renal failure (Gudulkar *et al.,* 2020; Singh *et al.,* 2022). In the development of kidney stones, several factors come into play, including inflammation, oxidative stress (OS), abnormalities in crystallization inhibition, irregular mineral metabolism, and the deposition of calcium phosphate in renal tubule or vessel basement membranes (Taylor *et al*., 2015). This plaque buildup can worsen OS and harm tubular epithelial cells. The process is believed to be accelerated by pre-existing renal damage and the natural aging process. Additionally, oxidative and inflammatory responses that occur after plaque accumulation and calcification further contribute to stone formation (Khan *et al*., 2014). Stone formation, along with cellular damage, inflammation, OS, interstitial fibrosis, and intratubular crystal deposition, has been observed at all stages in renal biopsies (Khan *et al*., 2015; Gan *et al*., 2016). The pathophysiology of urinary stone development is intricate, involving environmental, genetic, and metabolic elements (Shadman *et al*., 2017).

In mammals, the yeast Sir2 gene has a counterpart in the Sirtuin family, known as SIRT1. SIRT1 is one of seven different isoforms in the Sirtuin family, known as SIRT1 to SIRT7. All seven isoforms share a common 275-amino-acid catalytic core region and exhibit various subcellular localizations (Chaudhary *et al.*, 2010; Coe *et al.*, 1992). SIRT1 operates as a nicotinamide adenosine dinucleotide (NAD) deacetylase, removing acetyl groups from proteins. It can deacetylate a wide range of substrates in various organs, including the kidney (Covarrubias *et al*., 2021). Additionally, it plays a role in the regulation of gene expression, ageing, metabolism, and cellular energy levels. Given that reduced SIRT1 expression is associated with metabolic diseases like diabetes mellitus in the kidney (Guan *et al*., 2016), the activation of Sirt1 could hold significant therapeutic potential for conditions linked to inflammation or OS (Xie *et al*., 2013). SIRT1 may help prevent oxidative stress, inflammation, and cell death in the kidneys. In a variety of kidney disease models, such as lupus nephritis, chronic kidney disease, acute kidney injury, and diabetic nephropathy, SIRT1 has exhibited renal protective effects. For instance, SIRT1 can reduce oxidative stress in cisplatin-induced kidney injury by increasing catalase production and enhancing mitochondrial quantity and efficiency. Furthermore, in addition to its protective effects on tubular cells, SIRT1 displays antioxidant and anti-apoptosis activity in vascular endothelial cells (Zhang *et al*., 2017). By upregulating SIRT1, a SIRT1 activator can reduce renal fibrosis and damage (Chang *et al*., 2016; Xiao *et al*., 2016).

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Therefore, further investigation is necessary to determine whether SIRT1 could serve as a promising therapeutic target for kidney stones. In recent years, there has been an increasing emphasis on unraveling the genetic foundations of complex human disorders, including nephrolithiasis. To explore the relevance of the SIRT1 gene in nephrolithiasis, this study initially employed a mouse model of CaOx crystal-induced kidney injury. Additionally, genetic variants of the SIRT1 gene have been linked to chronic inflammatory conditions (Shimoyama *et al*., 2011) and cardiovascular disorders (Kalemci *et al.*, 2014).Objectives of the study are: i. Investigate the potential correlation between Sirtuin 1 gene polymorphism and the serum levels of Sirtuin 1 in individuals with urinary oxalate stones. ii. Examine the potential association between SIRT1 gene polymorphisms and the metabolic profile of urinary stone disease.

2. Materials and Methods

2.1 Methodology

The study involved the recruitment of a total of 200 subjects, comprising 100 cases with calcium oxalate kidney stones and 100 healthy controls who were confirmed to be stone-free through ultrasound examinations conducted at the Department of Urology, Justice KS Hegde Charitable Hospital, Mangalore, Karnataka.

The inclusion criteria encompassed individuals aged between 18 and 60 years, of any gender. Exclusion criteria, on the other hand, excluded patients with uric acid and other types of stones from the study.

This study received approval from the Ethics Committee of KS Hegde Medical Academy, NITTE Deemed to be University, and all enrolled subjects provided informed consent.

Blood samples were collected in two types of vacutainers. Two milliliters of EDTA blood were collected for genotyping purposes, while plain blood was collected for subsequent biochemical analyses. These samples were stored at -20°C until they were ready for analysis.

The process of DNA isolation involved bringing the whole blood samples to room temperature, adding 7 ml of RBC lysis buffer to 2 ml of whole blood, and then centrifuging at 3500 rpm for 5 min. After discarding the supernatant, the tube was inverted for half an hour, followed by the addition of 2 ml of WBC lysis buffer and vortexing at high speed. The solution was left to stand at room temperature for 2-4 h until it reached a homogeneous state. This was followed by the addition of 0.7 ml of protein precipitation solution, vigorous vortexing for 20 sec, and centrifugation at 3000 rpm for 10 min. Two milliliters of isopropanol were added to a clean centrifuge tube, the supernatant was discarded into a new labeled tube, and the contents were mixed. The tube was then centrifuged at 3000 rpm for 3 min, and the supernatant was once again discarded. The pellets were washed with 4 ml of 70% ethanol, followed by centrifugation at 3000 rpm for 2 min. The tubes were left to air dry overnight, and finally, 50 µl of Tris EDTA buffer were added, and the DNA was allowed to dissolve. Once dissolved, the DNA was transferred into Eppendorf tubes and stored at -20°C.

For the biochemical investigations, various parameters such as uric acid, creatinine, albumin, blood urea, and calcium were assayed using Agappe kits with a semi-auto analyzer. Additionally, serum Sirtuin-1 and parathyroid hormone (PTH) levels were determined using an enzyme-linked immunosorbent assay (ELISA) from Sincere Biotech.

The genotyping of SIRT1 involved the analysis of three single nucleotide polymorphisms (SNPs); namely, rs10509291, rs3740051, and rs932658, in the extracted DNA. This was accomplished using specific forward and reverse primers, which were selected through Primer 3 Plus software. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and Sanger sequencing.

For SIRT1 rs10509291 polymorphism, the forward primer 5'-TTCATTTCCAACTACGC TATCAA-3' and reverse primer 5'-AAGGCTAGGTTGCAGAGCAG-3' were employed. PCR conditions included an initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The products were subjected to analysis using a genetic sequence analyzer after purification, and the data were subsequently processed using Chromagen software.

SIRT1 rs3740051 polymorphism utilized the forward primer 5'-GGAGGGAATTCACACA CGTT-3' and the reverse primer 5'-CTGGCCTGCCTTAGCCTTGTCT-3'. PCR conditions consisted of an initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The obtained 379 bp fragments were digested with 0.5 µl of Hpa1 restriction enzyme at 37°C overnight and separated on a 3% agarose gel.

SIRT1 rs932658 polymorphism used the forward primer 5'-TTCATTTCCAACTACGCT ATCAA-3' and the reverse primer 5'-AAGGCTAGGTTGCAGAGCAG-3'. PCR conditions were an initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The obtained 286 bp fragments were digested with 0.5 of HpYAV at 37°C overnight and separated on a 3% agarose gel for visualization.

Statistical analysis was conducted using SPSS 23 software. Descriptive statistics included the presentation of mean ± SD for continuous parameters and frequency for categorical variables. The Hardy-Weinberg equilibrium test was applied, and chi-square tests were used to assess the association between genetic polymorphisms and nephrolithiasis. Metabolic parameters were compared between cases and controls utilizing the Mann-Whitney U test. Chi-square analysis was performed to determine the association between gene polymorphisms and serum levels. Pairwise linkage disequilibrium was assessed, including |D'| and R^2 values, and corresponding maps were generated using SNPStat Online and Shesisplus.

3. Results

The median age for both cases and controls was 46 years (ranging from 37 to 55 years) and 42 years (ranging from 26 to 50 years), respectively. The median age did not display a statistically significant difference between cases and controls (p=0.24). In terms of gender distribution, the male-to-female ratio was 62:38 in cases and 78:22 in controls.

For the rs10509291 polymorphism:

- The frequencies of the TT, TA, and AA genotypes were 67%, 26%, and 7% in cases, and 74%, 22%, and 4% in controls, respectively.

- The frequencies of the A-carrier genotypes (TA+AA) and the A allele were higher in cases compared to controls.
- Logistic regression analysis, after adjusting for gender and age, did not observe any significant differences between cases and controls.
- There was no significant distinction in the frequency of T and A alleles between cases and controls. However, the T allele (wild type) was more prevalent in controls, while the mutant allele was more frequent in cases. The analysis of rs10509291 of the SIRT1 gene reveals the predominance of the wild type, homozygous dominant TT genotype in both cases and controls. Mutant alleles were present in approximately one-third of the subjects in both groups (Table 1).

For the rs3740051 polymorphism:

- Genotype and allele distributions of the rs3740051 single nucleotide polymorphism did not exhibit any significant differences between cases and controls.
- The frequencies of the mutant genotypes (AG+GG) were lower in cases compared to controls.
- Consequently, according to the results of logistic regression analysis, the AG genotype showed a risk factor in cases (OR=1.07) but was not statistically significant (p=0.84). The wild type alleles had a higher frequency in both groups (Table 1).

For the rs932658 polymorphism:

- Genotypic and allelic distributions of SNP (rs932658) did not reveal any significant differences between cases and controls.
- The frequencies of the mutant genotypes (AC+CC) were higher in both cases and controls compared to the wild type (AA genotypes).
- The wild allele 'A' was most prevalent in controls.
- According to the results of logistic regression analysis, there was no significant association between this polymorphism and patients with kidney stone disease.

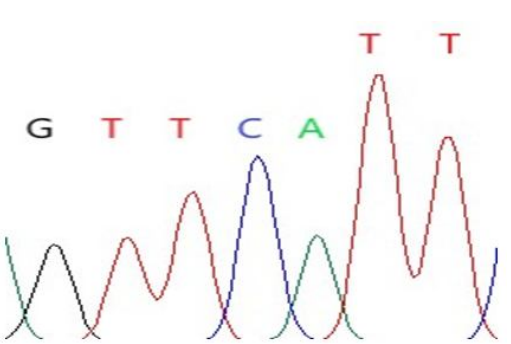
Table 1: Genotypic distribution of the SIRT1 gene variants

1) SIRT1 SNP(rs10509291)	Cases (n=100)	Controls (n=100)	*p* value*	OR,95% CI**	*p* value**
T T	67(65.6%)	74(74.8%)		1.00	0.2
TA	26(27.1%)	22(21.2%)		0.63(0.31-1.26)	
AA	7(7.3%)	4(4%)		0.41(0.11-1.55)	
T T	67(65.6%)	74(74.8%)			
TA+AA	33(34.4%)	26(25.2%)	p=0.277	1.000.58(0.30-1.11)	0.097
T allele					
A allele	16040	17030	p=0.188	1.00	
2) rs3740051					
AA	72(71.9%)	68(68.7%)		1.00	0.84
AG	23(22.9%)	29(28.3%)		1.07(0.54-2.11)	
GG	5(5.2%)	3(3%)		0.66(0.14-3.10)	
AA	72(71.9%)	68(68.7%)		1.001.00	
AG+GG	28(28.1%)	32(31.3%)	p=0.5371	(0.52-1.91)	1
A allele	167	165	p=0.7901		
G allele	33	35			
3) rs932658					
AA	19(18.8%)	29(29.3%)		1.00	0.24
AC	77(77.1%)	70(70.7%)		0.60(0.30-1.22)	
CC	4(4.2%)	1(1%)		0.25(0.03-2.57)	
AA	19(18.8%)	29(29.3%)		1.00 0.59	
AC+CC	81(81.2%)	71(71.7%)	p=0.098	(0.29-1.19)	0.14
A allele	115	128	p=0.183		
C allele	85	72			

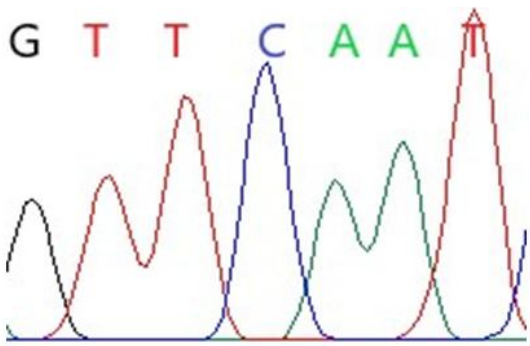
*Chi-square test.

**Logistic regression analysis after adjustment with age and sex.

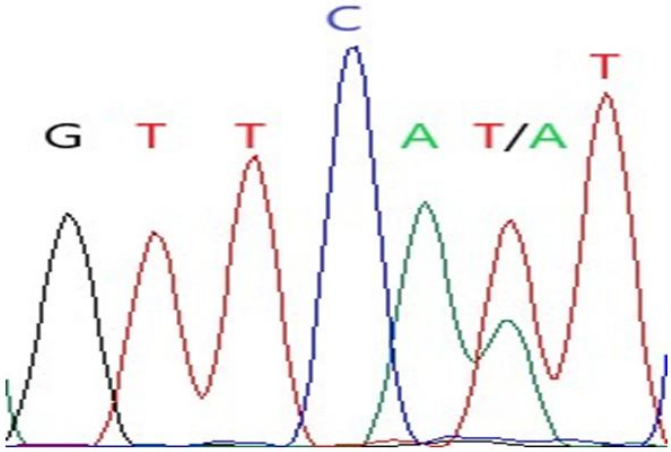
The genotyping results of the SNPs are depicted in Figures 1-3.



A) Graphical representation shows an wild type (T/T genotype)



B) Graphical representation shows an mutant type (A/A genotype)



C) Graphical representation shows an carrier type (T/A genotype)

Figure 1: Sequencing result of rs10509291.

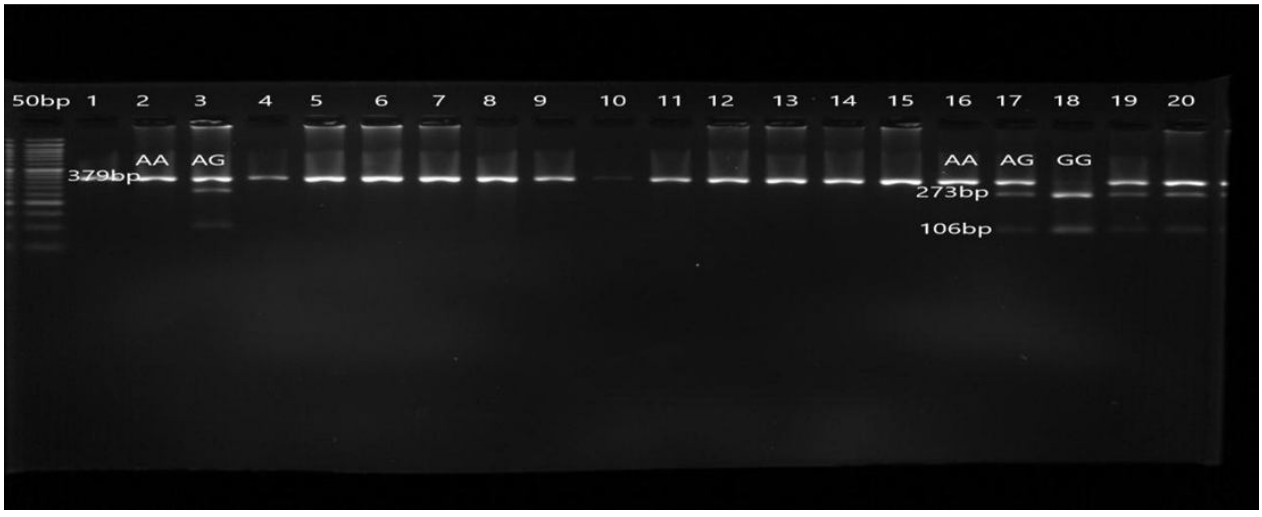


Figure 2: PCR-RFLP of rs3740051.Lane 1-16 shows wild type (A/A genotype) ; Lane 3,17,19,20 shows carrier (A/G genotype) ; Lane 18 shows mutant type (G/G genotype). 50 bp ladder was used.

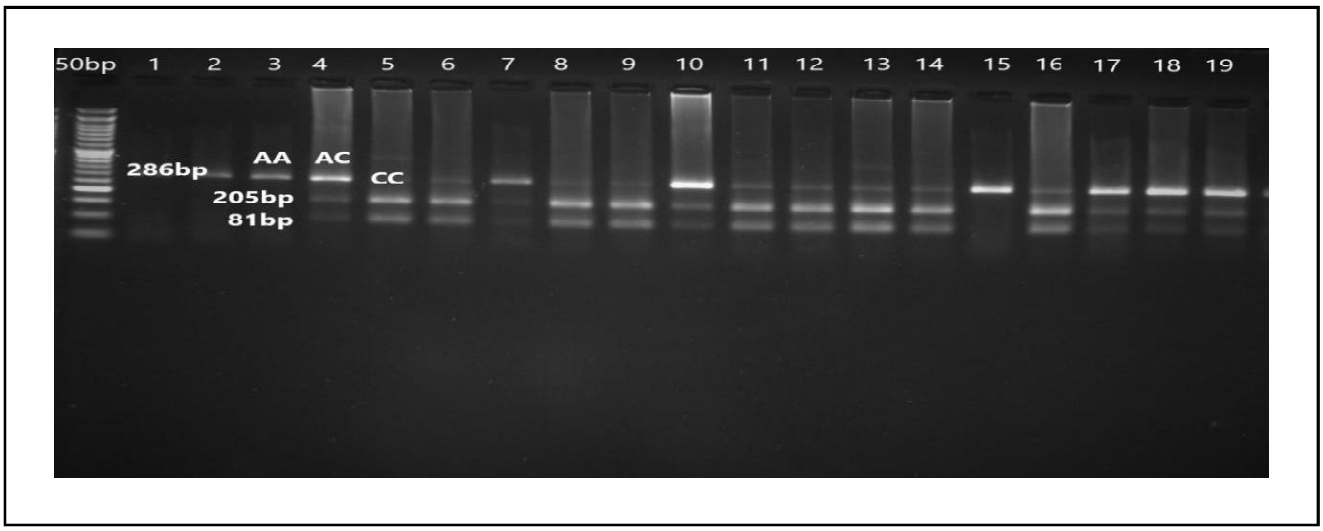


Figure 3: PCR-RFLP of rs932658.Lane1-4,7,15 shows wild type(A/A genotype) ; Lane 4,6,8-19 shows carrier type(A/C genotype); Lane 5 shows carrier type (C/C genotype).

Haplotype analysis using SNPstatonline is depicted in Table 2. Haplotypes with frequency <0.03 were ignored. Haplotype association with kidney stone disease was evaluated using the SNP Stat online tool in a pairwise manner between the SNPs. The associations were significant effects for (T A C, T G A, A G A and T G C) haplotypes in SIRT1 SNPs (rs3740051, rs10509291 and rs932658) (p=0.028, p=0.0052, p<0.0001 and p=0.028) when compared the distribution frequencies of the patients and control (Table 2) .

The study investigated the association between different combinations of single nucleotide polymorphisms (SNPs) and their occurrence in individuals with kidney stones (cases) compared to healthy controls. The results revealed a mixed pattern of associations. Notably, the SNP combination TAC showed a significant but relatively weak association with kidney stones, as indicated by its odds ratio (OR) of 0.74 and a statistically significant p-value of 0.028. On the other hand, the SNP combination TGA demonstrated an inverse association with the disease, supported by an OR of 0.12 and a significant p-value of 0.0052. Interestingly, the SNP combination AGC exhibited a substantial OR of 18.87, suggesting a strong association with kidney stones; however, this association was not statistically significant, possibly due to the limited number of controls with this combination. Conversely, other combinations like AAA showed no significant association. Additionally, the SNP combination AAC, though having an exceptionally high OR, lacked statistical significance, possibly owing to its absence in cases. The presence of the SNP combination TGC in cases but not in controls pointed to a potential association, though it did not reach statistical significance. In conclusion, this study underscores the complexity of genetic factors in kidney stone development, with certain SNP combinations showing varying degrees of association and significance, which may require further exploration with larger sample sizes and additional research.

Table 2: Haplotype analysis

Sl. No.	SNP1	SNP2	SNP3	Case (freq)	Control (freq)	OR(95% of CI)	p value
1	T	A	A	0.4878	0.4204	1.00	0.43
2	T	A	C	0.3016	0.2932	0.74 (0.35 - 1.57)	0.028
3	T	G	A	-	0.1363	0.12 (0.02 - 0.78)	0.0052
4	A	G	C	0.1128	0.015	18.87 (2.46 - 144.65)	0.54
5	A	A	A	0.0456	0.0695	1.41 (0.47 - 4.25)	0.73
6	A	G	A	0.0416	0.0237	1.46 (0.17 - 12.71)	<0.0001
7	A	A	C	0	0.0418	746564566.08 (746564564.29 - 746564567.86)	0.43
8	T	G	C	0.0106	0	1.00	0.028

In the global analysis, the study included a total of 100 control subjects and 100 cases with kidney stones. The chi-squared test yielded a value of 33.858, and Pearson's p-value was calculated to be 7.97, indicating a significant association between the genetic factors investigated and kidney stones. The global haplotype association p-value was found to be less than 0.0001, further supporting the genetic association with kidney stones.

To assess the linkage disequilibrium (LD) between different combinations of SNPs in the SIRT1 gene, D' and R^2 values were computed using the SHEsisplus platform. The LD R^2 values were

represented in a linkage disequilibrium map, where an R^2 of 1 indicated a high level of LD (darkest colored square), values between 0 and 1 suggested varying degrees of LD (light-colored squares), and R^2 equal to 0 indicated low LD (lightest colored squares). Both D' and R^2 values were taken into consideration when predicting the co-inheritance of alleles.

The analysis of combined genotype data for the three SNPs in SIRT1 revealed a strong LD among them, as indicated by high D' values of 0.53, 0.55, and 0.73. The R^2 values of 0.27, 0.1, and 0.06 further supported the co-inheritance of these alleles. Higher D' values were observed for the SNPs of fetuin A, suggesting a strong co-inheritance among these genetic factors. These findings emphasize the potential interplay between specific genetic variations in the SIRT1 gene and their association with kidney stone susceptibility.

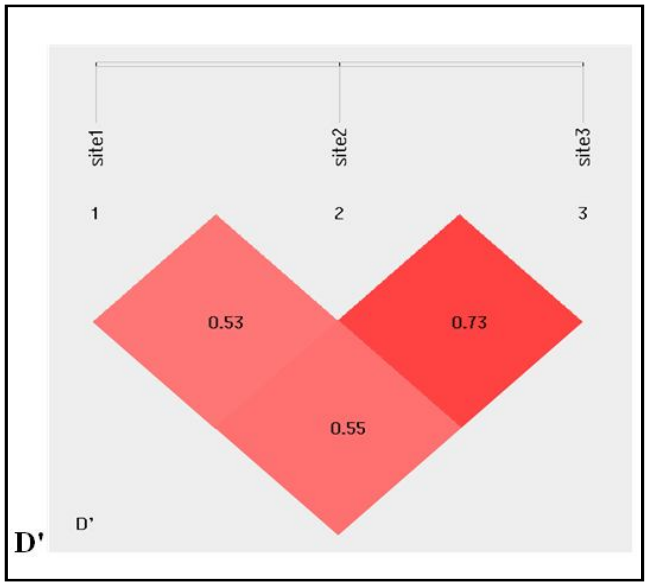


Figure 4a: Linkage disequilibrium analysis.

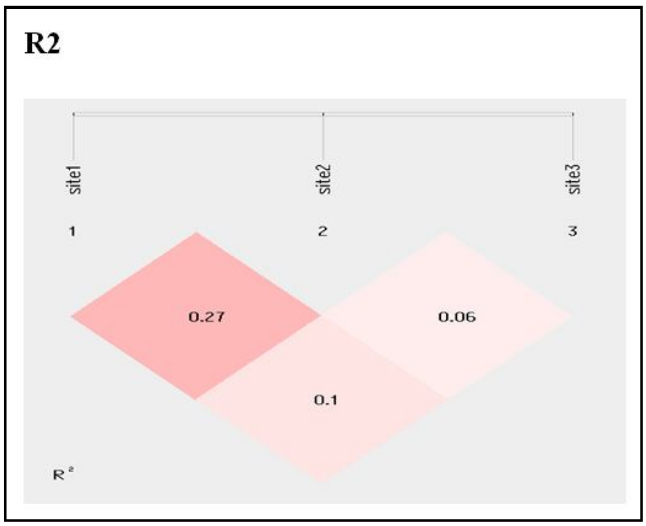


Figure 4b: Haplotype block (R^2) of three sites, site1 is rs10905251, site3 rs3740051 site3 is rs932658 SNPs.

The serum level of sirtuin1 levels,were insignificantly reduced in the patients. Data were expressed as median (IQR) (Table 3).

When biochemical parameters were compared, uric acid and phosphorous levels were significantly low in cases with values [4.23(2.15-5.77) and 5.35(4.7-6.2)] versus controls [6.11(4.8-7.3) and 5.13(4.3-6.14)] respectively (Table 3). Creatinine levels were significantly high in cases [1(0.83-1.27)] compared to controls [0.8(0.56-0.96)]. No significance differences were found in the distribution of calcium and albumin in both groups.

The receiver operating characteristic (ROC) analysis is a statistical method used to evaluate the diagnostic accuracy of a biomarker or test. In this study, ROC analysis was employed to assess the utility of Sirtuin-1 (SIRT1) levels as a potential marker for kidney stone disease. The results of the ROC analysis are presented in Figure 4.

Table 3: Comparison of biochemical parameters

Parameters	Cases (n=100)	Controls (n=100)	*p* value
Sirtuin 1(ng/ml)	16 (14.12-24.64)	21.16 (14.25-29.20)	0.166
Uric acid (mg/dl)	4.23 (2.15-5.77)	5.13 (4.3-6.14)	<0.0001***
Creatinine (mg/dl)	1 (0.83-1.27)	0.8 (0.56-0.96)	<0.0001***
Phosphorous (mg/dl)	5.35 (4.7-6.2)	6.11 (4.8-7.3)	0.0071**
Calcium (mg/dl)	10 (8.95-11.35)	9.97 (8.83-12.39)	0.739
Albumin (g/dl)	4.31 (2.76-4.9)	4.02 (3.13-4.64)	0.226

The ROC curve is a graphical representation that plots the trade-off between sensitivity and specificity for different threshold values of the biomarker (in this case, SIRT1 levels). Sensitivity measures the ability of the test to correctly identify individuals with the disease (true positive rate), while specificity measures the ability of the test to correctly identify individuals without the disease (true negative rate).

In a ROC curve, the diagonal line represents the scenario where the test has no diagnostic accuracy, essentially equivalent to making random guesses. The closer the ROC curve is to the upper-left corner of the graph, the better the test's diagnostic accuracy.

In this study, the ROC curve for SIRT1 levels fell below the diagonal line, indicating that SIRT1 levels alone had limited diagnostic utility for kidney stone disease. The Area Under the Curve (AUC) is a quantitative measure of the overall diagnostic performance of the test. An AUC value of 0.5 represents a test with no diagnostic accuracy (similar to random guessing), while an AUC value of 1.0 represents a perfect test.

In this case, the AUC for SIRT1 levels was 0.439, which is significantly below 0.5. This suggests that SIRT1 levels are not effective in accurately distinguishing between individuals with kidney stones and those without. The 95% confidence interval (CI) of the AUC ranged from 0.343 to 0.536, reinforcing the uncertainty regarding the diagnostic accuracy of SIRT1 levels.

Additionally, the optimal cut-off value for SIRT1 levels was determined to be 16.82 ng/ml. At this threshold, the test demonstrated a sensitivity of 45.5%, meaning it correctly identified 45.5% of individuals with kidney stones. However, it also had a relatively low specificity of 54.7%, indicating that it correctly identified only 54.7% of individuals without kidney stones.

In summary, the ROC analysis suggests that SIRT1 levels, when used as a standalone marker, do not provide strong diagnostic accuracy for kidney stone disease. The relatively low AUC and the trade-off between sensitivity and specificity indicate that SIRT1 levels alone may not be a reliable diagnostic tool for this condition.

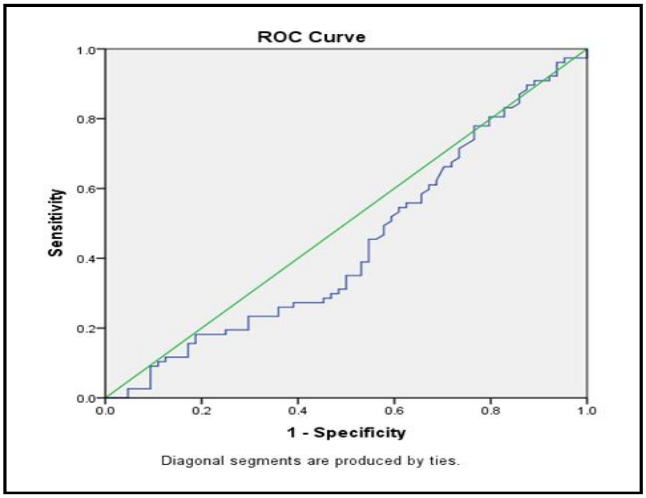


Figure 5: ROC curve for Sirtuin-1.

Table 4: Details of ROC for Sirtuin-1

Area under curve	Sensitivity	1-Specificity	Std error	*p* value	95% CI	
					Lower Bound	Upper Bound
0.439	0.455	0.547	0.049	0.214	0.343	0.536

The analysis revealed that there was no statistically significant association between serum Sirtuin-1 levels and the various SIRT1 gene polymorphisms. The results presented in Table 5 indicate that the established cutoff value for Sirtuin-1 levels was set at 16.82 ng/ml. Despite examining different single nucleotide polymorphisms (SNPs) in the SIRT1 gene, the study found no significant correlation between Sirtuin-1 levels and these genetic variations.

However, it is interesting to note that among the three investigated SNPs, specifically rs932658, individuals with Sirtuin-1 levels greater than 16.82 ng/ml exhibited a higher frequency of mutations. This suggests that there might be a potential association between elevated Sirtuin-1 levels and the rs932658 SNP. The odds ratio of 1.930 for this particular SNP and Sirtuin-1 levels implies that individuals with the mutation at this SNP are 1.93 times more likely to have Sirtuin-1 levels exceeding 16.82 ng/ml.

In summary, the study did not find a general significant relationship between Sirtuin-1 levels and SIRT1 gene polymorphisms. However, the data suggests that one specific SNP, rs932658, may be associated with higher Sirtuin-1 levels, indicating a possible genetic influence on Sirtuin-1 concentration in the presence of this particular mutation. Further research may be needed to confirm and explore this potential link.

Table 5: To assess the association between SIRT1 gene polymorphism and serum levels of Sirtuin-1 in patients with urinary oxalate stones

rs10509291 Sirtuin 1 Level	Wild type (TT)	Mutant (TA+AA)	Chi-square, df	p value	OR (95% CI)
>16.82	40	18	X^2=0.2413 df=1	0.6233	1.235(0.5264 to 2.834)
<16.82	27	15			
rs3740051					
>16.82	40	18	X^2=0.2776df=1	0.5983	0.7885(0.3274 to 1.918)
<16.82	31	11			
rs932658					
>16.82	12	46	X^2=1.332 df=1	0.2484	1.930(0.6794 to 5.276)
<16.82	05	37			

4. Discussion

SIRT1 is currently recognized as a pivotal target for mitigating renal injury and combating mitochondrial oxidative stress by virtue of its role in nuclear acetylation regulation, metabolic modulation, and anti-inflammatory and antioxidative effects (Hou *et al.,* 2019). Previous studies have underlined the significance of uric acid, calcium, and phosphorus among the predisposing factors for kidney stone disease (KSD) (Nasiri *et al*., 2018). The present research identified notable hypercalcemia, considerable hypophosphatemia, and elevated creatinine levels in the cases, which aligns with findings reported by by Shen *et al. (*2022).

Among the sirtuin protein family (SIRT1-7), SIRT1 has emerged as a prominent subject of study. Activation of SIRT1 offers potential in mitigating the metabolic syndrome by exerting protective effects against oxidative stress and genotoxic factors through its deacetylation impact on substrates such as p53 and forkhead transcription factors (Nasiri *et al.,* 2018). Previous investigations have linked gene polymorphisms to various health conditions, including type 2 diabetes, cardiovascular diseases, breast cancer, and neurodegenerative disorders (Han *et al.*, 2015; Kilic *et al.*, 2014; Rizk *et al*., 2016; Siwak *et al*., 2022).

In our study, we focused on three specific SIRT1 tag SNPs (rs10509291, rs3740051, and rs932658) located in the 5'-flanking region, primarily involved in gene transcription regulation. These SNPs have been widely explored in association with conditions like obesity, breast cancer, diabetes, and hypertension, but their relationship with kidney stone patients remained uncharted. Building on the work by Hou *et al.* (2019), we aimed to examine the interplay of SIRT1 gene polymorphisms and their expression in patients afflicted by calcium oxalate kidney stones.

Our investigation into the genotypic distribution and its association with kidney stone patients did not reveal significant findings (Table 2). Furthermore, our findings indicated reduced Sirtuin-1 expression in cases compared to controls, consistent with the report by Hou *et al*. (2019). Specifically, the rs3740051 SNP, an upstream transcript variant of the SIRT1 gene involving an A>G substitution, displayed a higher frequency of the minor (G) allele in the control group, implying a protective role in the development of the disease. This observation harmonizes with studies conducted in the Japanese population, where the G allele was linked to increased energy expenditure and a heightened risk of diabetic nephropathy (Maszlag-Török *et al*., 2021; Maeda *et al*., 2011). In our study, no significant differences in genotypes and sirtuin 1 levels were observed in the cases.

Emerging evidence underscores that tissue injury induced by oxidative stress amplifies the retention of causative crystals, particularly calcium oxalate (CaOx), within renal tubules. Several reports have identified an overproduction of reactive oxygen species (ROS) in renal tubular epithelial cells, culminating in cellular damage. To counteract this, numerous antioxidant compounds have been introduced to mitigate oxidative stress, diminish cellular and tissue injury, and curtail kidney stone formation (Chaiyarit *et al*., 2020).

The SIRT1 gene plays a role in stimulating antioxidant expression, facilitating cellular repair in the wake of oxidative stress (OS), and averting cellular dysfunction. Reduced SIRT1 expression leads to mitochondrial dysfunction by elevating the levels of reactive oxygen species (ROS), lipid peroxidation, and DNA damage (Alam *et al.*, 2021). Of particular interest, the rs10509291 SNP is associated with the regulation of mitochondrial genes in the nucleus. This SNP has previously been nominally linked to type 2 diabetes, with the major (T) allele being connected to an increased risk of diabetes (Dong *et al.*, 2011).

Our current study did not unveil significant differences in gene polymorphisms between cases and controls, but it did confirm lower Sirtuin-1 expression in the serum samples of cases. The UCSC Genome Browser revealed that the rs932658 SNP resides within a potential regulatory region. Further in silico analyses identified rs932658 as a potential functional SNP within the SIRT1 promoter region (Yang *et al.,* 1992). This SNP variant has been shown to alter the transcriptional activity of the SIRT1 gene promoter, subsequently influencing SIRT1 levels. This observation is attributed to the regulatory roles of the SIRT1 gene in the differentiation of human cells (Hou *et al.,* 2019). Notably, the present study indicated that the heterozygous recessive (AC genotype) appears to play a protective role in kidney stone patients, while the homozygous dominant (AA genotype) poses a risk for renal stone development (Yang *et al*., 1992). Prior research has linked the A allele of rs932658 to higher SIRT1 expression.

Hypercalcemia was evident in our study; however, a comprehensive urinary metabolic evaluation was not conducted. Hypercalciuria, characterized by the excessive excretion of urinary calcium, is a common anomaly in calcium stone formers, affecting 35-65% of individuals and contributing to the supersaturation of urinary calcium salts. The etiology of hypercalciuria can be multifaceted, involving complex interactions between the intestines, kidneys, and bones in regulating calcium homeostasis. Idiopathic hypercalciuria, which remains unexplained by this classification, represents at least 50% of stone formers, and these patients likely exhibit a more generalized systemic abnormality impacting calcium homeostasis in the intestine, kidney, and bone.

5. Conclusion

In conclusion, our study did not identify significant associations between the selected SIRT1 gene polymorphisms and kidney stone disease. Heterozygous dominant alleles were prevalent among the control group. Additionally, we observed lower serum Sirtuin-1 levels in cases. In summary, our findings suggest that genetic mutations in the SIRT1 gene may not be advantageous, as they result in decreased serum Sirtuin-1 expression.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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