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Original Research Article

Study the Relationship between Kidney Stone, Bacterial Infection and Genetic Factors in Kidney Stone Disease Patients

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Abstract: ***Background*:** Urinary stone disease (USD) is among the ancient human known diseases that could be often complicated by pain and severe complications and numerous predisposing factors play a key role in initiation and development of stones. ***Objectives of Research*:** The present research examined several biological and clinical determinants that play a role in urinary stone disease (USD), with identifying the stone chemical composition, determining any corresponding bacterial infections, and molecular evaluating of some genetic predispositions of the (USD) patients. ***Methods and Materials*:** The current study was able to obtain samples of Hundred Stones, Blood, and Urine of (USD) patients in the AL-Hussein Educational Hospital and Safeer AL- Hussein Hospital in Karbala Province between October 2024 and March 2025; where these USD patients were aged between (18-79). Chemical compositions of kidney stones were analyzed using Biolabo- France kit, related bacterial infections were determined using ViteK test kit and Molecular analysis of the corresponding SNPs (rs1042636 of CaSR gene, rs219778 of CLDN14 gene, and rs10917002 of ALPL gene) in the present study was estimated using Allele Specific PCR and further DNA Sequencing was adopted to establish the relationship between stone types, bacterial infection and the genetic predisposing factors in (US). ***Results*:** Evaluating the prevalence of the kidney stones in (USD) patients greater prevalence was reported in the male (60%). And the chemical makeup showed the most Likely type of Stones were Calcium oxalate (40 percent), mixed Sat (31 percent) and last uric (27 percent). Only 33% of patients possessed the history of recurrences in the occurrence of stone formation, whereas diabetes and hypertension were observable in 31% and 23% of cases, respectively. Urine bacterial cultures showed that the majority of the stones (78 percent culture-negative) were of metabolic nature, whereas Klebsiella pneumoniae and Escherichia coli were more commonly noted in culture-positive ones and thus may have a contributory role in culture-related in-vivo urinary tract infection stones. Statistically, significant ($p<0.001$) relationship was determined between the type of bacteria and stone composition. Three (CaSR, CLDN14 and ALPL) of the key genes that were genotyped had a high prevalence of the AG genotype in CaSR (87%) and CLDN14 (97%). It was observed that a strong association ($p<0.001$), between the allele ALPL_C and certain types of stones, in particular calcium phosphate and mixed calcium-uric acid stones, was identified. DNA Sequencing of a small number of the PCR DNA products to analyze the Nitrogen bases of (rs1042636 of CaSR gene and rs219778 of CLDN14 gene) found perfect matches with already published sequences in NCBI Environment based on the Accession Numbers (ACCESSION PV871973) and (ACCESSION PV926278) respectively. This is in line with what the previous literature proposes that dysregulation of the production of ALPL which is a gene that participates in phosphate metabolism can predispose individuals to the occurrence of nephrolithiasis. ***Conclusions*:** the results confirm that the development of urinary stones is multifactorial, and it happens due to genetic variation, microbial effect, metabolic disorder, and demographic outcomes.

Keywords: Kidney Stone, Bacterial Infection, Genetic Factors, Patients.

INTRODUCTION

Urinary stone disease (USD) - is a widespread health issue that affects a considerable amount of people around the world and is one of the oldest medical conditions, it can provoke severe pain and a set of complications at the same

time [1, 2]. Stones cannot be formed without the accumulation of minerals and salts in the kidneys that will later be crystallized to form the solid masses [3].

Bacterial infection is one of risk factors which causes (USD) and classified into third type of stone type and called Struvite or infectious stone; some of bacterial species are known to contribute in forming strokes Proteus mirabilis; Escherichia coli; Klebsiella pneumonia [4, 5], therefore USD might occur directly as a sequel to urinary tract infection, often referred to as infection stones or as a product of underlying metabolic derangements, in which case infection is a secondary event. In infection stones, urinary tract infection becomes a main actor that initiates and stimulates stone formation.

In the case of metabolically derived stones, however, which are complicated by infection, colonization of bacteria can take place within the stone matrix. Whether it belongs to which type does not really matter, but the bacteria inside the urinary calculi pose a serious risk of the disease to recur [6]. Besides the former type, this is calcium stone. The most frequent urinary tract stone in the general population is calcium phosphate and calcium oxalate in either form or combined with each other (calcium stones) [7]. CaOx stones were related with reduced urine volume, increasing urine calcium excretion, increasing urine oxalate excretion and reduced urine citrate excretion, whereas calcium phosphate occurs in alkaline urine (pH 6. 5) [8]. Second Uric acid stones, Two key ways that enhance the uric acid precipitation are high urine uric acid levels and acidic PH of the urine, which causes the next reaction to shift to the rightwards to form the relatively insoluble uric acid [9], and lastly Cystine stones comprise about 1 percent of urinary tract stones, but their frequency seems to be higher in pediatric patients, also, they are present in cystinuria patients, a genetic disorder [10].

The other risk factor that could lead to USD may entail up to 47 risk factors which may include the following exposure to environmental factors such as: Inadequate fluid intake or excessive fluid loss, which may concentrate the urine Dietary factors: Consumption of large quantities of animal protein, sodium, and oxalate-high foods, Medical conditions: obesity, diabetes, and hypertension, medications, lifestyle[11], and genetic polymorphisms (single-nucleotide polymorphisms [SNPs]) as a risk factor of the occurrence of the kidney Genetic differences have also been characterized to elevate the risk of producing kidney stones commonly in cooperation with the environmental and lifestyle variables. Most of the genes associated with nephrolithiasis mainly participate in the cellular signalling pathways or in transferring ions and other intracellular substances across the cellular membranes [13]. There are a wide number of genes linked to the formation of KSD including CASR, FGF23, VDR, CLDN14, ALPL, and SLC26A1 and others [14].

The calcium-sensing receptor (CASR) coded in parasitic and renal tubular cells regulates excretion of calcium [15], Claudin CLDN14 or Wendelin pertains to the Latin version of the word claudere which implies to shut or seal [11]. The biological role of this protein family in creating tight junctions which govern Para cellular transport among epithelial cells is adapted in this naming [16]. Liver Bone and Kidney ALPL produced dysregulated activity of alkaline phosphatase activity could also be a cause of calcium phosphate supersaturation at the urine level and an inductor of KSD [17].

Sometimes the surgery is needed to provide the removal of the stones, especially, in situations when they are not able to pass the urinating tracts or lead to severe complications [18]. Even though surgical methods (e.g., ureteroscopy or percutaneous nephrolithotomy) have proved to be efficient in the treatment of kidney stones, there still exist problems related to postoperative complications.

Study Aims:

1. Kidney stones sample-chemical analysis of USD patients.
2. Identification of the kinds of bacterial infection present within the urine samples of patients USD.
3. Allele Specific Polymerase Chain Reaction (PCR) detection of rs1042636 of CASR gene, rs219778 of CLDN14 gene, and rs10917002 of ALPL gene of blood samples of USD patients and genomic DNA analysis of these genes through DNA sequencing.
4. Examining the links between the type of kidney stones, type of bacterial infections and genetics factors as predisposing factors to form kidney stones in USD patients.

MATERIALS AND METHODS

The current cross-section prospective study was conducted in 2024 between October and March 2025. The readers should know that the current study targeted patients with kidney stones who attended the urology consultant at the Imam AL-Hussein Educational Hospital (peace be upon him) and the Safer of Imam AL-Hussein Hospital (peace be upon him) to the major operating theater with the aim of extracting kidney stones using the Percutaneous Nephrolithotomy (PCNL) process in Karbala city; their age range was (18-75) years.

Sample Taking and Analysis

Kidney stones samples of the patients were obtained using PCNL after a surgical operation in USD patients. The instructions to stone analysis included storage of the stones untreated until the time of analysis, as given by the manufacturer of the diagnostic kit (Biolabo). Besides, (10 ml) of urine specimen was taken in every USD patient and was used in general urine examination (GUE) Note: to prevent false result in the urine culture which occur when performing the test during surgical operation, it was performed prior to the patient undertaking PCNL operation because: the patient takes antibiotics after surgical procedure and they have ureteral catheter which causes infection. In addition to that, (5ml) of venous blood sample was extracted of each USD patient, the blood sample was partitioned in two: the first (3ml) was discarded to collect serum by using.

To know the concentration of Creatinine, centrifuge at 3000 rpm during 5 min.

A second volume (2ml) was pipetted into E.D.T.A. containing tubes to be made use of in molecular analysis in the following manner:

DNA Extraction: genomic DNA of blood samples was extracted following the direction of manufacturing company kit (Blood Genomic Favorgen- Taiwan) with the following steps:

Step 1: RBC Lysis
Step 2: Cell Lysis
Step 3: DNA Binding
Step 4: Column Washing
Step 5: Elution

Primer Design:

The following Oligoprimer sets (Table 1) were locally made as per Snap primer design program and imported to (Macrogen Company (South Korea) were used in the detection of the corresponding SNPs (rs 1042636 of casr gene, rs 219778 of CLDN14 gene, and rs10917002 of ALPL gene) in blood samples of USD patients by use of conventional Polymerase Chain Reaction (PCR).

Table 1: DNA Oligoprimers for PCR Assay in the present study

Gene	Primer sequence (5′ → 3′)	Product Size (bp)
CASR	Forward (A) GAGTTCTGGTGCGTAGAATTCCT Forward (G) GAGTTCTGGTGCGTAGAATTCCC Common Reverse CATCCCGCAACACCATCGAG	400
CLDN14	Forward (A) GGAGCTGTGACCTTGGAAATTCCT Forward (G) GGAGCTGTGACCTTGGAAATTCCC Common Reverse GTTGGTGTGGCAATTAGCATGTTC	488
ALPL	Forward (C) AGGTGCCCAGACAGAGCGCAG Forward (T) AGGTGCCCAGACAGAGCGCAA Common Reverse CCTGCAATTGGTCCTGCCCCTG	200

PCR Amplification Conditions:

Allele Specific PCR also depended on the following PCR amplification conditions in (Table no. 2) in order to determine the capability of the local designed oligoprimers in detecting the target SNPs of the corresponding CAsR, CLDN14 and ALPL genes of the current study.

Table 2: Amplification Conditions of the genes associated with KSD patients

Gene	First denaturation °C (min)	Second denaturation °C (min)	Annealing °C (min)	Extension °C (min)	Cycles	Final extension °C (min)
CaSR:	95 (5)	95 (0.30)	55(0.30)	72 (0.45)	35	72(7)
CLDN14:	95 (5)	95 (0.30)	57(0.30)	72(0.45)	35	72(7)
ALPL:	95 (5)	95 (0. 30)	62(0.45)	72 (0.45)	35	72 (4)

Agarose Gel electrophoresis: All the PCR DNA products underwent agarose gel electrophoresis (using 1.5 % conc. of agarose gel) at (80 volts and 150 mill Ampere over 80 minutes) with the results being recorded.

Subsequently, DNA sequencing of a few (PCR DNA products) were achieved by (Macrogen Company (South Korea) at that time, DNA analysis was made by Bioinformatics programs comparing the nucleotide sequences of the related SNPs of the present study and matching with the Original DNA sequences that have been recorded in NCBI.

Statistical Analysis

Data were analyzed with version 12 of the computer program) SPSS (Information was expressed and presented in line with suitable statistical treatments. Frequency coefficients, Chi-square test, and Fisher exact test were used to estimated the differences between groups, and the p-value was to be computed as one of the indicators of the absence of statistical significance. Remark that the results were deemed to be statistically significant with a level of significance value of (p 1/2 hatapan 1979ab Eva 1996).

RESULTS AND DISCUSSION

Demographics/Clinical Characteristics of Study Participants

It was also found that the percentage of the age groups (The majority aged and kidney stones) (59) calcium oxalate (21), uric acid (19), and mixed stones (18) occurred in patients aged (4059 years) followed by 2039 years (29%) and then age (<20) and 6079 years percent (10%). Concerning the distribution of sexes, most of the sample (60 percent) were male whereas females made 40 percent as indicated in table 1.

Analysis of Kidney Stones Results

Ca Ox stones were the most prevalent (40%) followed by mixed stones (31%), uric acid stones (27%) and the least prevalent were cystine and Ca Ph stones (1% each) in matters relating to the chemical makeup of kidney stones. There was also 33 percent of participants who have had a history of recurrent urinary stone, and the rest of the population 67 percent have not experienced recurrent stones. The percentages of patients with diabetes mellitus and without diabetes were 31 and 69 percent respectively. On the same note, hypertension was reported in 23 percent of the patients who used USD and 77 percent of the non-users as shown in (table 1).

Table 1: Distribution of the study USD Participants and Samples based on variables of demographic features

Variables		No. (%)
Age-group (years)	<20	2(2%)
	20–39	29(29%)
	40–59	59(59%)
	60–79	10(10%)
Sex	Male	60(60%)
	Female	40(40%)
Types of stone	Ca. oxalate	40(40%)
	Mixed	31(31%)
	Uric acid	27 (27%)
	Cystine	1 (1%)
	Ca. phosphate	1 (1%)
Recurrent stone	Positive	33(33%)
	Negative	67 (67%)
Diabetes mellitus (DM)	With DM	31 (31%)
	Without DM	69 (69%)
Hypertension	With	23 (23%)
	Without	77 (77%)

The results, such as in age group, sex, DM and Hypertension, have been addressed and supported by many studies and this has agreed. Recurrence of kidney stones has been established as a common clinical issue with [19], reporting that the recurrence rate depended on the type of stone with Ca Ox being reported with rates of 10 30% yearly and uric acid stones more likely to recur especially in instances where there was inadequate control of the urinary pH. Another cause of the recurrence is dietary in the sense that a low fluid intake, high salt intake, contributed to the ability of this cause to cause recurrence [20], has also observed that about half of the person with a recording in their history book of having nephrolithiasis was at risk of getting another episode of the said condition within 5 - 10 years except during which they employed strategies to prevent the occurrence. Metabolic abnormalities including hypercalciuria and hypocitraturia were the strongest risk factors that related to recurrence among others. Additionally, the guidelines carried out by [21], of the fact that 30-50 percent of the patients experienced a recurrence of stones in a decade and grouped patients, depending on the risk of recurrence, into high and low risk groups with the former patients developing stones at a faster and the latter developing stones at a slower rate due to transient stones, which is attributable to reversible factors such as dehydration.

The correlation between diabetes mellitus and KSD has become more explicit in recent years because of the knowledge on the common pathophysiological pathways. A huge, cohort study that encompassed 1.2 million individuals by [22]. Found that people with diabetes had the 42 percent greater risk of KSD with a significantly higher chance of

developing UA stones. Indeed [23], confirmed that sex, hypertension, obesity, diabetes were significant related factor of KSD, and [24-26], confirmed that sex, hypertension and diabetes were not significant related factors of KSD.

On fig. 1, the horizontal bar chart (A) represented the distribution of variety of kidney stones among a population that was subject of a study. Compared to other stone types, the Ca Ox ones were greatest constituting 40 percent of patients. It was then followed with mixed stones 31% and UA stones 27%. Cystine and Ca Ph stones showed significantly, being 1 percent and 1 percent respectively, and, in second chart (B)the composition of mixed kidney stone was disaggregated and formed 31 percent of the aggregate stones. The most frequent of these was Ca Ox with UA (22%), and subsequent there was Ca Ph with struvite (5%), Ca Ox with struvite (3%), and Ca Ph with UA (1%).

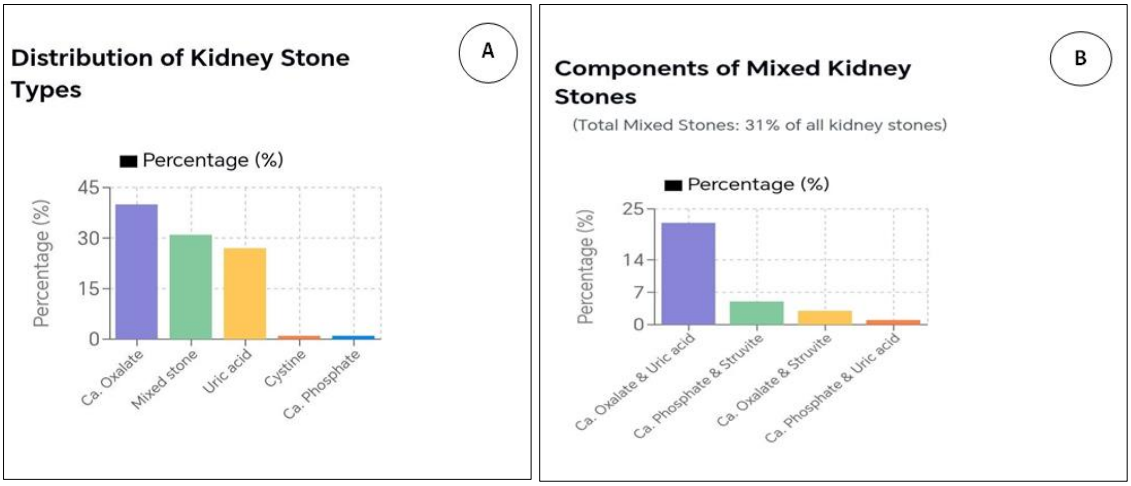

Fig. 1: (A) Distribution the general types of kidney stones. (B) Chemical composition of Mixed kidney stones in KSD patients

In this study only [27], of them were confirmed to be Ca Ox rate 23.3% followed by UA stone was 17.9%, cysteine stones 15.6 %, mixed stone composed of calcium oxalate with uric acid 10 %, and Ca Ox with Ca Ph stone 3% study was in Mosul city, Iraq.

Bacterial Culturing and Identifications Results

Kidney stone in the cohort was shown by most of the samples (78%) to be of metabolic but not infectious origin. Klebsiella pneumonia was the most commonly isolated organism among the stones having positive culture followed by Escherichia coli (6%). Other bacteria like Actinobacteria and Enterobacter among others were few. Fisher-Freeman-Halton Exact test was performed to analyse the connection between various forms of bacteria and the forms of kidney stone. These findings demonstrate a statistically significant correlation ($p < 0.001$), which means that the distribution of species of bacteria was not random but differs significantly depending on the type of stone in (table 3).

Table 3: Correlation between bacterial infection and Kidney Stone Type

		Culture								Total
		Acinobacter	*E.coli*	*Enterobacter*	*enterococcus faecalis*	*klebsiella + pseudomonas*	*Klebsiella*	No Growth	*s. saprophyticus*	
Stone type	Calcium oxalate	1	2**	1	1	0	5	30	0	40
	calcium oxalate+ struvite	0	0	0	0	0	2**	1	0	3
	calcium oxalate+ uric acid	0	1	0	0	0	0	21	0	22
	calcium phosphate	0	1	0	0	0	0	0	0	1
	calcium phosphate+ struvite	0	0	0	0	0	3	0	0	3
	calcium phosphate+ struvite	0	0	0	0	1	1	0	0	2
	calcium phosphate+ uric acid	0	1	0	0	0	0	0	0	1
	Cysteine	0	0	0	0	0	0	1	0	1
	uric acid	0	1	0	0	0	0	25	1	27
Total		1	6	1	1	1	11	78	1	100

** indicates the high significance difference ($p<0.001$)

This finding implied that some bacteria could have played a role towards the development of certain type of kidney stone, especially where the stones were attributable to infection, such as struvite or mixed forms of stone compositions. This finding confirmed the significance of microbiological screening among the kidney stone patients to inform diagnosis and treatment. These results were also in line with the papers of (Flannigan *et al*., 2014 and Mohankumar *et al*., 2024) which focused on the contribution to stone pathogenesis by involvement of bacteria. Contrary, previous works had reported that there were even no major variations in types of bacteria between stone types (Gao *et al*., 2020), even in sterile ones like uric acid stones. In the research, (An L. *et al*., 2021) determined that the Frequency of culture-positive E. coli in the urine and stones of KSD patients was the most prevalent in both the total samples (1,055 patients) culture-positive E. coli rate of culture-positive stones was 32%, and culture-positive urine was 21% For KSD patients, the proportion of E. coli in stones was 43.92% and in urine was 54.30% In addition, In (Daudon *et al*., 2022) the urine culture upper urinary tract urine pathogen was E. coli 19%, pseudomonas 5 %, enterococcal faecalis 4.9, Klebsiella 4, Enterobacter 4 and s. saprophyticus 1.5.

Serum Creatinine

The calculation test Serum Creatinine reported that it was ordered to exhibit the distribution of different Creatinine values, together with important descriptive statistics. The column under the term and title of Value indicates particular concentrations of S.Creatinine in mg/dL, which is (0.5 - 1.2 mg/dL). The respective (Frequency) column denotes the number of people measured at each level of Creatinine. S. Creatinine level in this sample is 0.8 with mean of 0.8 mg/dL. The lowest given level is 0.4 mg/dL, and the highest reading is 1.2mg/dL. The mentioned statistics give the brief description of the central tendency and range of Creatinine values of the tested population. According to the frequency distribution, the Creatinine value which was most frequently reported was 0.9 mg/dL with 24 persons having the value. There was also a fairly high frequency of 18 and 13 in the level of 0.8 mg/dL and 1.0 mg/dL respectively. Table 4: contained the results within the normal reference range of S.Creatinine (0.512 mg/dL) confirming that normal renal functioning was in place during the time of collection. It was done to make sure that every case would be subjected to the inclusion criteria (Shahbaz *et al*., 2024).

Table 4: The results of Serum Creatinine test

Creatinine				
Value	**Frequency**	**Mean**	**Minimum**	**Maximum**
0.5 mg\dl	7	0.8 mg\dl	0.4 mg\dl	1.2 mg\dl
0.6 mg\dl	11			
0.7 mg\dl	13			
0.8 mg\dl	18			
0.9 mg\dl	24			
1.0 mg\dl	13			
1.1 mg\dl	5			
1.2 mg\dl	9			
Total	100			

Findings of Molecular Analysis

The current study used AS-PCR methodology and its positive outcomes confirmed the qualifications of the locally home-designed specific Oligoprimers to detect candidate SNPs (rs1042636 of CaSR gene), (rs219778 of CLDN14 gene), and (rs10917002 of ALPL gene) amongst the blood (DNA) samples of kidney Stone patients. Conversely, AS-PCR was carried out to differentiate the effects of such genes (SNPs) on KSD and kidney stone development. Its outcomes revealed AS-PCR positive detection of the target SNP area of CaSR gene using forward-specific primer of wild SNP (A) allele and a common reverse primer in all (100) blood (DNA) sample, but, upon using forward-specific primer of mutant SNP (G) genotype-ag and a common reverse primer, it produced positive amplification in (87) blood (DNA) samples, whereas in other thirteen (13) blood (DNA) samples displayed negative amplification in KSD patients genotype (AA) as exhibited in table. Besides, with regard to the AS-PCR, amplification of the target SNP region of CLDN14 gene with the forward primer specific to the wild allele (A) and common reverse primer showed positive amplification in all (100) blood (DNA) samples however when the forward primer specific to the mutant allele (G) to the genotype (AG) was used with the common reverse primer revealed positive amplification in (97) blood (DNA) samples and negative amplification in the other (3) blood (DNA) samples of the KSD patients gen.

In fact, according to AS-PCR, the target SNP region of ALPL gene amplification with the forward primer that only allows the amplification of wild allele (T), and the common reverse primer, resulted in positive evidence in (80) blood (DNA) samples amplification, whereas the remaining (20) blood (DNA) samples could not be amplified in KSD patients, however with the mutant allele (C) genotype (CT) as well as the addition of a common reverse primer, there was a positive evidence of (36) blood (DNA) samples.

Table 5: Genotypes Frequencies for the kidney stone Disease patients Associated with the Regarding SNPs.

Genes SNPs	Genotype	Percentage (%)
CaSR rs1042636	AA	13%
	AG	87%
CLDN14 rs219778	AA	3%
	AG	97%
ALPL rs10917002	TT	64%
	CT	36%

In addition, in the subsequent figure (2): the Lanes 3-22: represent amplified DNA samples of KSD patient, a characteristic band was detected in all the lanes, the length was 400bp, which means that the target region SNP of CaSR gene fragment was effectively amplified. The presence of a single sharp band at the target size among all of the DNA samples provides confirmation of specific amplification primers (allele A), whereas no non-specific bands or primer-dimer products were detected indicating specific primers, optimized PCR conditions and a high specificity detection test.

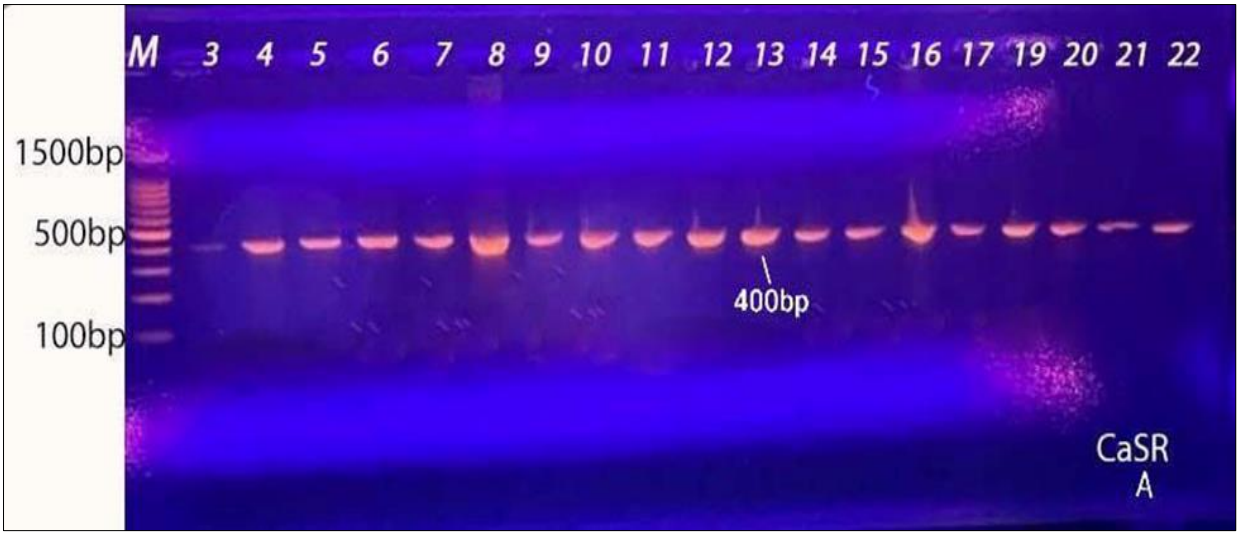

Figure 2: Shows 1.5% Agarose gel electrophoresis of PCR DNA products of the target region of CASR gene (allele A) with positive DNA samples (lanes 3 22) with bands 400bp and lane M= DNA Ladder Marker with molecular weight 100bp. (of KSD patients)

The data on CASR gene indicated that the (G) allele was very common (either AG or GG, but GG was not successfully detected using the primers in a manner suggesting it be in a different percentage than AG, probably indicating that every G-positive sample also contained an (A) allele; thus, they would be (AG heterozygous). Both the 100% amplification at the (A) primer and 87% at the (G) primer indicates that all DNA samples contained at least one copy of the (A) allele and 87 percent had at least one copy of the (G) allele. The 13 per cent who amplified with (A) primer, not with(G) primer, are therefore AA.

These findings provided a notable piece of information on the genetic distribution of particular polymorphism on the CaSR, CLDN14, and ALPL genes in kidney stones patients. These genes demonstrate crucial importance in the metabolism of calcium and phosphate and changes in them are viewed as risk factors of the formation of kidney stones. The result of the CaSR gene expression is an increase of urinary excretion of calcium that may predispose hypercalciuria and form stones. A similar recent finding has been reported on the Indian population, which showed significant correlation between CaSR rs1042636 G allel (Guha *et al*., 2015 A). As it has been shown by yet other related research, the (AA) genotype (RS1042636) has also exhibited serious association with the kidney stone disease. Also, it pointed out that some genotypes and some abnormalities of metabolism, like hyperparathyroidism and hypercalcemia, are substantially associated with the development of kidney stone disease (Patel *et al*., 2022).

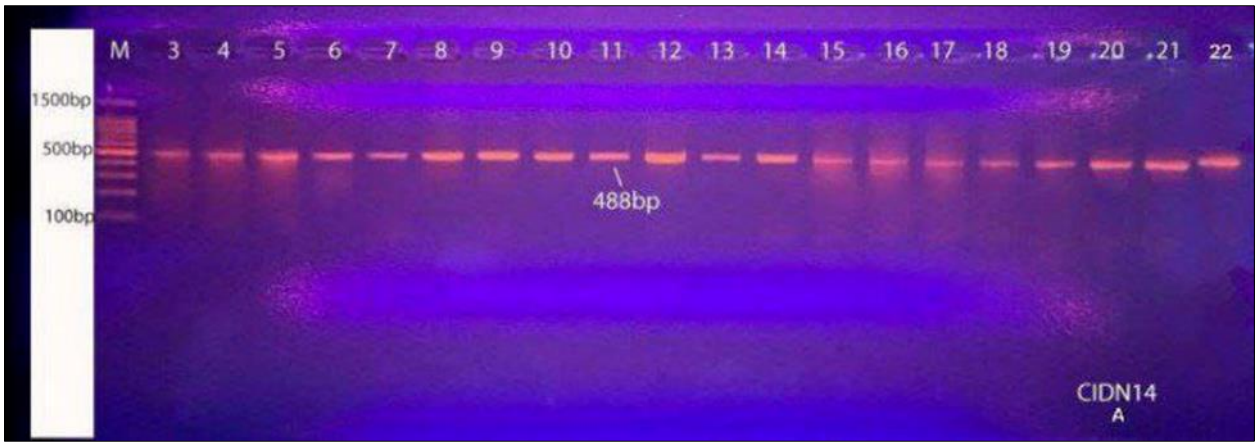

Figure 3: Show 1.5% Agarose gel electrophoresis of PCR DNA products of the target region of CLDN14 gene (allele A) positive DNA samples on lanes 3-22 and bands 488 bp. M Lane = DNA Ladder Marker molecular weight 100 bp. (of KSD patients)

Thers219778 polymorphism of the CLDN14 gene in terms of the patients with nephrolithiasis. All samples were positive in the amplification with a common reverse primer and the forward primer of wild allele (A). An additional primer extension of the forward type-specific allele specific primer (G) genotype (AG) and the universal reverse primer resulted in positive results in (97%) where negative and yielded no detectable products via the G-specific primer). When compared to the similar research by (Ullah *et al*., 2022), it was reported that there was strong correlation of SNP rs219780 and SNP rs219779 with kidney stones, which they are commonly used interchangeably or together since they have high rates of being linked to SNP rs219778. Yet another study explored a variant in CLDN14 gene that is likely to influence or modify functioning in a way biologically relevant, which indicates kidney stone formation (Ure *et al*., 2017). Another similar study dwells on the rs219780 that has been proven to be strongly connected with rs219778, and shows the connection within the framework of Egyptian children (Elshamaa *et al*., 2020). The relationship between the CaSR gene and kidney stone has been mentioned in a same study carried on Guha *et al*., (2015). It also stated the correlation of CLDN14 gene with the development of kidney stone.

Association between the Type and Genotype of Kidney Stones

With regard to the types of kidney stones and genotypes, in the study under discussion, there were no relations of kidney Stone type to any of the analyzed genotypic markers except the ALPL C- variant which revealed statistically significant associations in the study ($P < 0.001$), showing a strong connection between the Presence of this allele and the formation of a specific type of stone, most likely with calcium phosphate-based stones and mixed stones included calcium and uric acid. The result implied a possible involvement of ALPL - C in nephrolithiasis pathogenesis as phosphate metabolism was shown in Table (6).

Table 6: Relationship between Kidney Stone types and Genotypes

		***ALPL* -F ©**		***P*-value**
		available	**Absent**	
Stone type	Calcium oxalate	0	40	
	calcium oxalate+ struvite	1	2	
	calcium oxalate+ uric acid	22**	0	0.0001
	calcium phosphate	1	0	
	calcium phosphate+ struvite	4	1	
	calcium phosphate+ uric acid	1	0	
	Cysteine	1	0	
	uric acid	6	21	
Total		**36**	**64**	

** indicates high significance difference ($p<0.001$)

The close correlation between ALPL - C and the stone type was based on the results indicated by (Li *et al*., 2018) who had revealed that the variants of the ALPL genes, such as rs10917002 were significantly associated with the risk of developing calcium phosphate stones in the Chinese population. Similarly, another study of the Icelandic population established a (21%), risked increase in KSD which was correlated to the T allele of rs1256328 (Oddsson *et al*., 2015). Also, in the related work on the Taiwanese population (Chen *et al*., 2019) reported, TT genotype of the rs1256328 increased the risk of having a calcium stone.

The impact of Alpl gene on kidney stone formation was also confirmed by another study namely: The current analysis of (Li *et al*., 2018) proved the previous study on the effects of Alpl gene on kidney stone formation which are: This gene of ALPL rs1256328 in the region of the Turkish population was associated with the increased possibility of developing KSD (İbrahim *et al*., 2022).

The Correlation of the Type of Kidney Stones, the Bacterial Infections and Genotypes

The results revealed significant links between mixed-type of kidney stones (Calcium Oxalate+ Uric acid) and the ALPL gene (CT) genotype and the bacteria Klebsiella pneumoniae in the present study. A large p- value (< 0.001) associated with this finding indicated that the formation of these kidney stones is multifactorial that is to say that it is caused by genetic, bacterial, environmental and diet factors. Conversely, Calcium Oxalate Stone Type that was the most common in nature (40% chance of occurrence) was observed to have a significant correlation of being connected to E. coli Bacteria (as also determined to be significant in terms of p-value (= 0.001). The latter association means that dietary, bacterial and environmental factors are the major determinants of the etiology of calcium oxalate stones.

Findings of DNA Sequencing and Analysis

In the present work, AS-PCR and LDSOPENOL were able to harness the finding of the candidate SNPs (rs1042636 of CaSR gene) and (rs219778 of CLDN14 gene) and (rs10917002 of ALPL gene) and, DNA sequencing of the three AS-PCR positive DNA products and the specific primers to detect the target parts of the candidate SNPs according to the protocol proposed by Macogen corporation (Macogen Korea) sequencing corporation and the Local analysis of the DNA sequences.

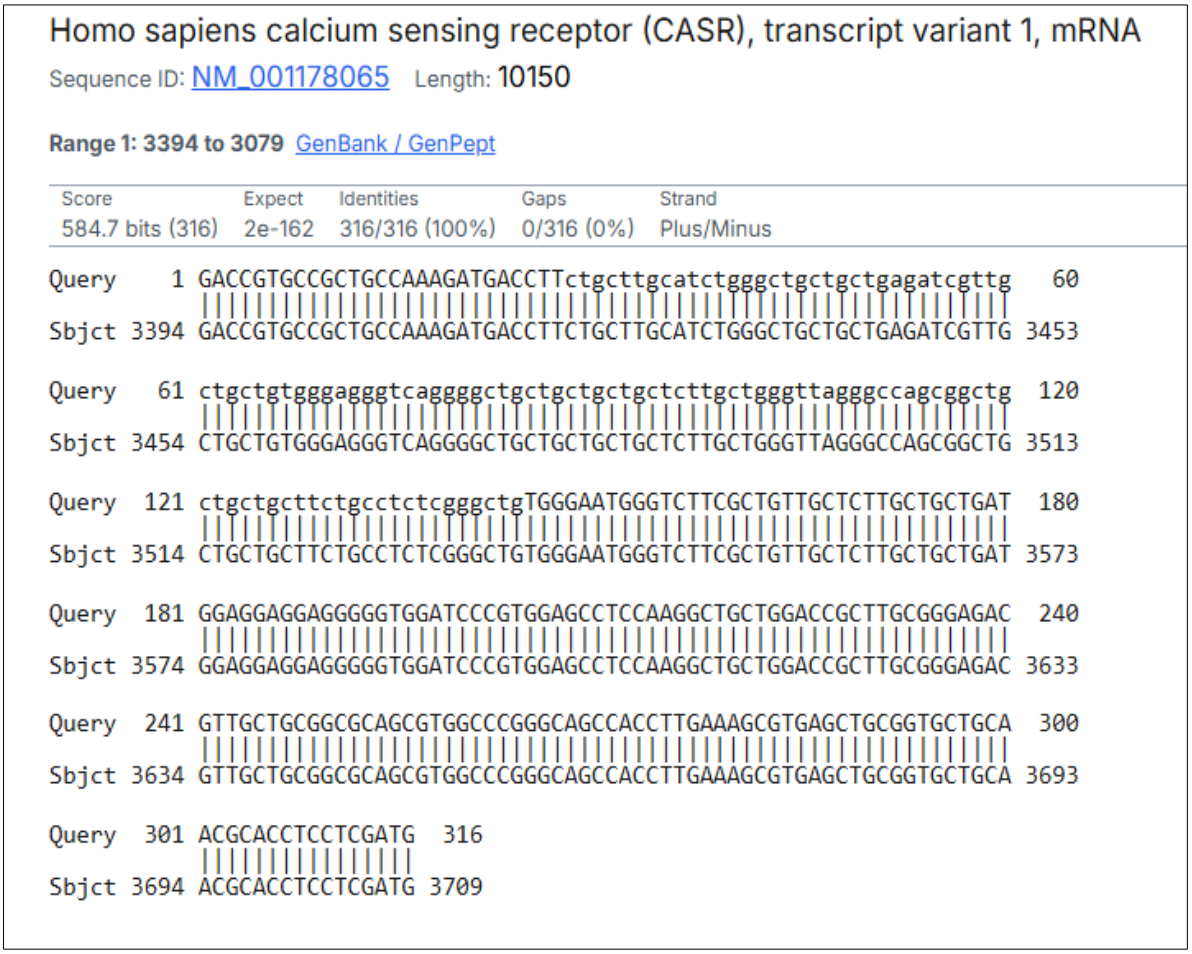

Figure 4: Shows the outputs of DNA sequences analysis of the fragment of interest of SNP (rs1042636 of CaSR gene) and complete matches (100%) of the Nucleotides sequences

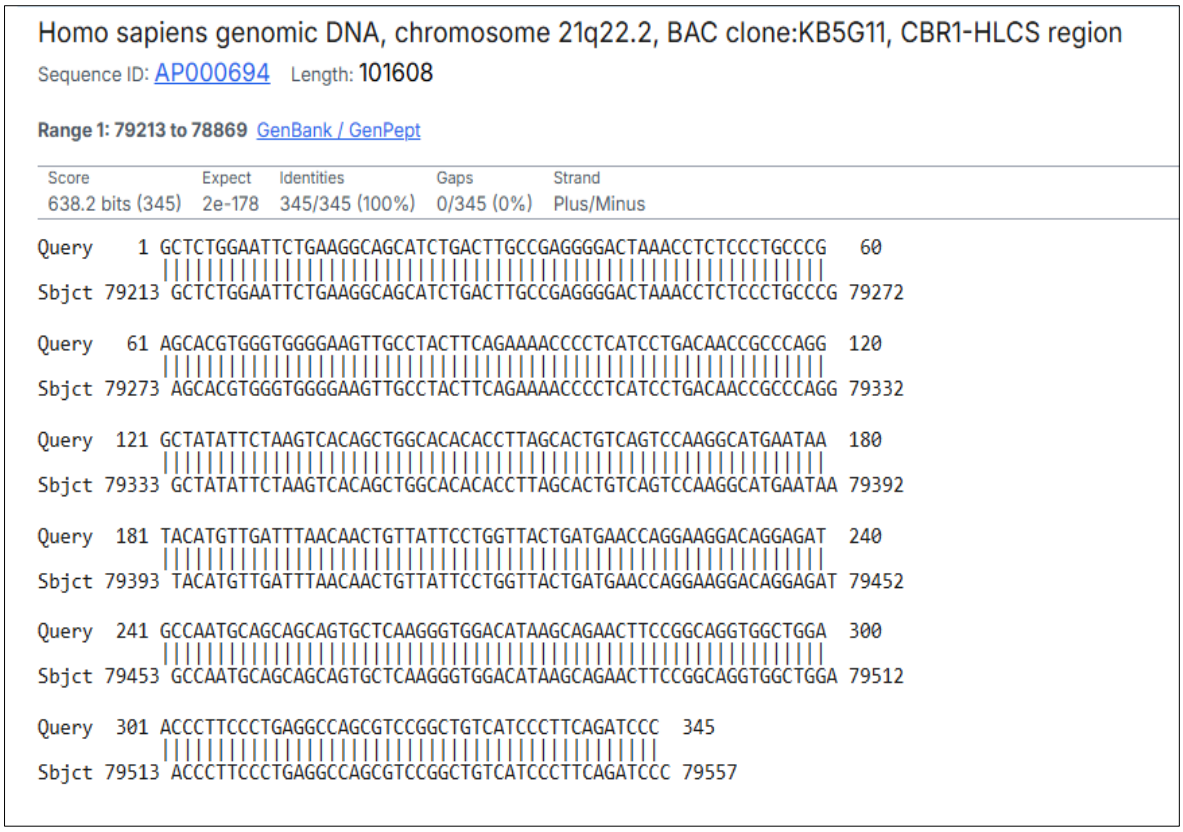

Figure 5: The outcomes of the Analysis of DNA sequences of the sensed fragment of the SNP (rs219778 of CLDN14 gene) with the full congruence (100%) of the Nucleotides sequences are shown

Besides, the above results of (the full identity) of the amino acids (proteins) and DNA nucleotides sequences of the target fragments of the presented SNPs (rs1042636 of CaSR gene) and (rs219778 of CLDN14 gene) are also registered in Gen Bank database of (NCBI) and shall be published based on the received Accession Numbers (ACCESSION PV871973) and (ACCESSION PV926278) respectively.

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