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University of Kerbala
College of Pharmacy

Genetic Polymorphism of ATP Binding Cassette G2 (ABCG2) Transporter and the Relationship with Therapeutic Response of Nitrofurantoin in Children with Urinary Tract Infection in Selected Clinics/Hospitals in Kerbala Province.

A Thesis

Submitted to the Council of College of Pharmacy as Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology and Toxicology

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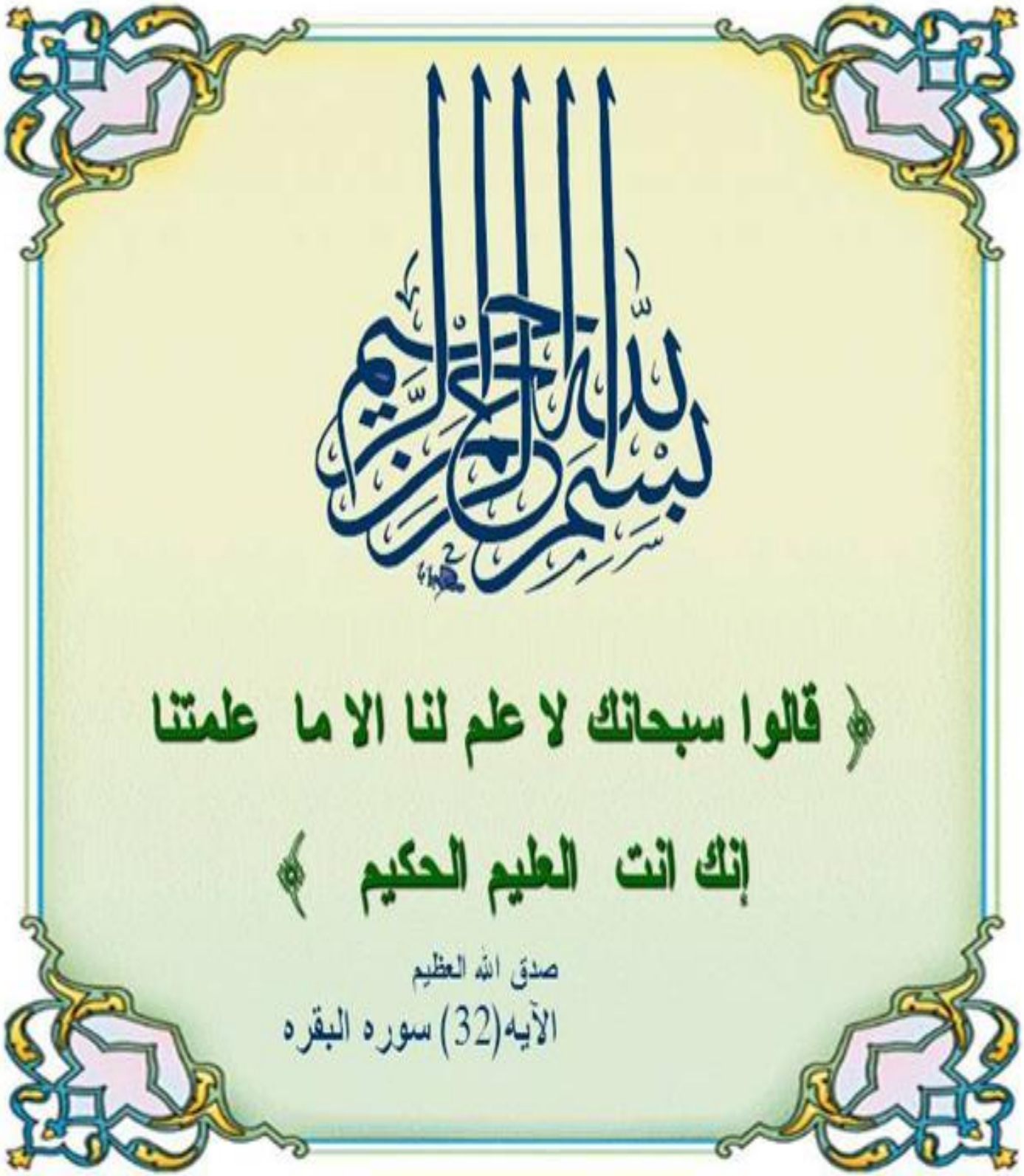
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Dedication

To whom I am proud to bear his name all my life.....and my Idol in life…..the greatest father in world

To who revived her prayers and blessings…..and her satisfaction it from Gods pleasure…..my mother the angle of my life

To my soul mate and companion of my life's journey…..who encouraged me to take this step….. my faithful husband

To my sisters my support in life

Innocent hearts…..precious gifts …..my children

Sisters my way…..my friends

I dedicate this thesis with love…

Thura

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List of Abbreviations

Abbreviation	Description
ABCC1	ATP-binding cassette transporter number 1 of sub-family C
ABCG2	ATP-binding cassette transporter number 2 of sub family G
AS-PCR	Allel specific_ polymerase chain reaction
ATP	Adenosine triphosphate
AUC	Area under the curve
BCRP	Breast cancer resistance protein
CFU	Colony- forming units
DNA	Deoxyribonucleotide triphosphates
dNTps	Deoxynucluotide triphosphates
E.Coli	Escherichia. Coli
EL	Extracellular Loop
GUE	General urine exam
Kg	Kilogram
LE	Leukocyte esterase
MDR	Multidrug resistance
MRP	Multidrug resistance association protein
PCR	Polymerase chain reaction
P-gp	P-glycoprotein
Q141K	glutamine to lysine amino acid substitution at position 141

rpm	Revolution per minutes
Rs	Reference
SD	Standard deviation
SNPs	Single nucleotide polymorphism
SPSS	Statistical package for social sciencess
TBE	Tris Borate EDTA
TM	Trans membrane
UTI	Urinary tract infection

Abstract

Background:

The urinary tract (UTI) is one of the most common area of bacterial infection in humans. The infection of lower urinary tract (UTIs), such as cystitis, are always characterized by symptoms such as frequent urination, urgency, and difficulty urinating. If left untreated, these infections can develop into upper urinary tract infections called acute pyelonephritis or kidney infections.

Nitrofurantoin is a synthetic nitrofuran antibacterial agent that has been used for more than 50 years. It still works and continues to be prescribed, especially in outpatient settings for uncomplicated urological patients, particularly in its microcrystalline formulation, macrodantin.

The ABCG2 gene located on chromosome 4q22 encodes 655 BCRP amino acid breast cancer resistance proteins. Like other G subfamily proteins of the ABC-binding transporter (ABC), BCRP is a semi transporter containing a nucleotide-binding domain and a transmembrane domain fused to a single polypeptide chain. BCRP has a homodimer-like functional form with a molecular weight of 144 kDa. The ABCG2 variant, rs2231142, leads to change of nucleotide cytosine to adenine ABCG2 C421A, a single nucleotide polymorphism associated with decreased protein expression/transport activity in vitro and higher anti-cancer drug concentrations in carriers of the C421A polymorphism.

Objectives:

The present study was designed to evaluate the possible association of ATP- binding cassette G2(ABCG2) genetic polymorphism (rs 2231142) with susceptibility to urinary tract infection. Also to evaluate the possible association of ABCG2 genetic polymorphism (rs 2231142) with the therapeutic response to nitrofurantoin (after 1 week of 5-7mg/kg daily

nitrofurantoin treatment) for pediatric with urinary tract infection in clinics/hospitals in kerbala province.

Patients and Methods:

The study is accomplished by enrolling 110 individuals both male and females aged between 4-14 years, diagnosed with UTI and conducted from outpatient in karbala teaching hospital of pediatrics, Al-hussienia hospital and private clinic of pediatric .A mean dose of nitrofurantoin 6mg/kg/day was administered for 1 week , Blood samples were obtained from eligible patients who had given consent for genetic testing. Also urine collected from the same group and two reading of general urine exam were collected, first reading before treatment, and second reading after 1 week. The genetic variant, and ABCG2C421A genes were carefully selected according to the specialized database, genotype was determined by AS-PCR technique, response of drug measured by clinical, RBC, pus, Leukocyte esterase test , nitrite test.

Results:

This study results was recorded the homozygote wild type (CC allele) 34 cases (30.9%), heterozygote (AC allele) 54 cases (49.1%) and 22 cases homozygote mutant type (AA allele)(20%) on the other hand, the analysis of the effect of the genetic polymorphism on the response to the drug under the study revealed that there was no statistical significant difference of SNPs genotypes (CC, CA and AA) and the response to treatment.

Conclusion:

The current study concludes that nitrofurantoin did not interact with ABCG2 gene polymorphism(rs2231142) in selected clinics/hospitals in kerbala province in urinary tract infection children and regardless of genotype. These results indicate that nitrofurantoin is not a suitable clinical probe substrate for assessing BCRP activity.

Nitrofurantoin is very effective in these subjects.

Chapter one

Introduction

1. Introduction

1.1. Urinary Tract Infection

1.1.1. Definition and Classification.

Urinary tract infection(UTI) is defined as a combination of clinical symptoms, pyuria and positive urine culture with >50,000 CFU/ml of a single uropathogenic organism on an appropriately collected urine culture [1, 2]. UTIs are a common and important clinical problem in childhood and may lead to systemic illness and renal injury in the short term(3), with repeated infections, renal scarring, hypertension and end-stage renal dysfunction may develop[4,5].

Traditionally, UTIs have been classified by the site of infection (ie, pyelonephritis [kidney], cystitis [bladder], urethra [urethritis] [6,7] and by severity or factors that trigger (ie, complicated versus uncomplicated) [8]. A complicated UTI describes infections in urinary tracts with structural or functional abnormalities or the presence of foreign objects, such as an indwelling urethral catheter. This model does not necessarily reflect clinical management [9] and also according to the symptoms to symptomatic and asymptomatic [10], however. In children, a simpler and more practical approach is to categorize UTI as a first infection versus recurrent infection. Recurrent infections can be further subdivided into the following categorize **(fig.1-1)** [11,12].

1.Unresolved bacteriuria.

2. Bacterial persistence.

3. Reinfection.

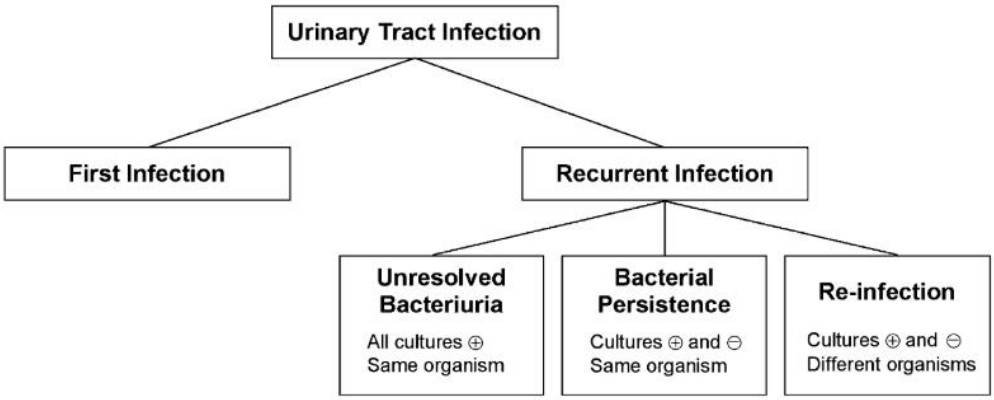

Figure (1-1): Functional classification of UTI [(11)].

1.1.2. Epidemiology

UTIs are one of the most frequent clinical bacterial infections in women[(13)], accounting for nearly 25% of all infections affecting women and man, at a ratio of 8:2[(14)]. It account for more than 8 million visits to physician's offices, 1.5 million emergency doctor visits and 300,000 hospitals admission in the United States per year. These infections cost of more than $3.5 billion[(15)].

Incidence of pediatric UTI varies based on the age, gender and race of the child[(16)]. In the first 3 months of life, the incidence of UTI is higher in males than in females, with the male to female ratio of UTI about 2–5:1. The rate of febrile UTI is higher in white infants than in black infants, with the relative risk being 6-times higher in white infants, according to statistics demonstrating that the rate of febrile UTI is higher in uncircumcised boys compared with circumcised boys [(17)].

1.1.3. Pathophysiology

The urinary tract is sterile [18]. The retrograde ascent of bacteria is the most common mechanism of infection. In girls, bacteria can more easily access and ascend the urinary tract due to the urethral opening closeness to the anus and the shorter length of the urethra. Another proposed route as a uropathogenic bacterial reservoir has been the presence of the intact foreskin in infants, in whom the frequency of UTI is ten fold that of circumcised males[19]. Lower UTIs, also known as cystitis typically start with periurethral contamination by a uropathogen living in the gut[20], followed by colonization of the urethra and, then, migration by the flagella and pili of the pathogen to the bladder or kidney. Bacterial adherence to the uroepithelium is key in the pathogenesis of UTI. Infections happen when bacterial virulence mechanisms overcome efficient host defense mechanisms [21]. Upper UTIs, also called pyelonephritis, develop when uropathogens ascend to the kidneys by the ureters. Infections can arise when bacteria bind to a urinary catheter, a kidney, or a bladder stone or when they are retained in the urinary tract by a physical obstruction[22].

1.1.4. Etiology

UTIs are usually caused by bacteria. The bacteria usually enters the urinary tract from the bowel or back passage (anus), via the urethra (the tube from which urine exits the bladder) [23].

In most cases, the infection is limited to the bladder or urethra. However, especially if left untreated, the bacteria can continue to multiply and movel up the ureters to one or both kidneys [24]. This is a much more serious issue resulting in fever, chills, dehydration and even sepsis [25].

1.1.5. Risk factors

Specific risk factors in children include infrequent voiding, low fluid intake and functional stool retention, which were more frequent in girls with recurrent UTIs than in control girls[26]. If the urinary bladder is not emptied completely, as is the case in neurogenic bladder and other conditions of dysregulated bladder emptying, the residual urine may be colonized with bacteria[27]. In children, congenital hydronephrosis, vesicoureteral reflux, ureteropelvic junction obstruction or ureterovesical junction obstruction are all anatomic abnormalities linked to UTI **(Figure 1-2)**. These structural risk factors highlight the importance of regular emptying of the bladder to avoid bacteria to multiply and entering to the urinary system [28].

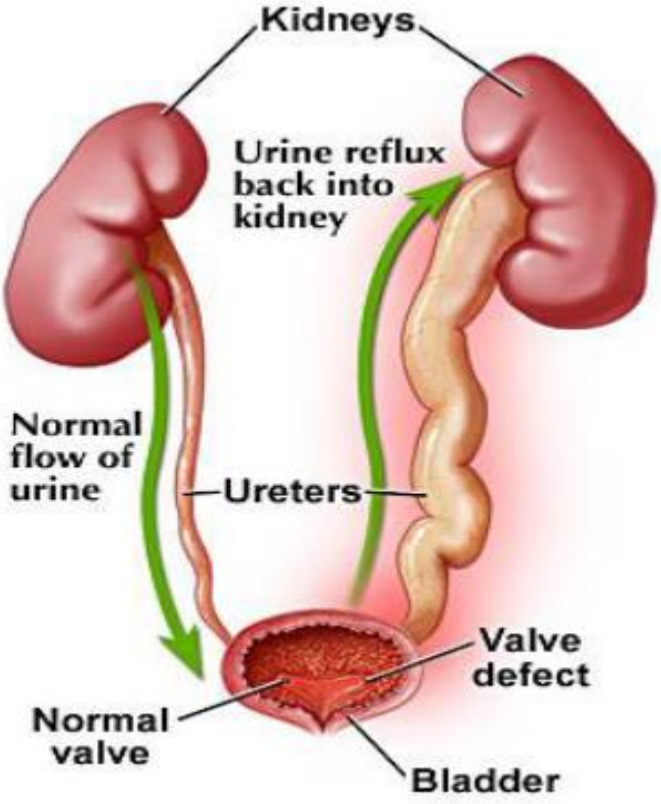

Figure (1-2): Anatomical abnormalities predispose to UTI [29].

1.1.6. Signs and Symptoms

Cystitis may or may not be symptomatic[30]. During an episode of cystitis, there are a variety of symptoms which may be experienced, including:-

- dysuria (pain associated with urinating) [31].
- A pain in the area above the pubic bone[32].
- frequency
- urgency
- urine that is cloudy or odorous
- urinary hematuria (urine with blood) [33].

1.1.7. Prevention and Treatment of UTI

There are a number of ways to relieved and prevent cystitis:

- Plenty of fluids should be drink during the day, especially water. 8-10 cups are aimed to be drink to increase the frequency and amount of voiding and which may lower the risk of recurrent cystitis [34].
- Tea, coffee or fizzy drinks should be avoided as they can irritate the bladder [35].
- Cranberry juice drinking may help to prevent cystitis. It is thought that cranberry juice helps to stop bacteria sticking to the wall of the bladder and helps "flush out the bladder"[36].
- The underwear of the children should be change daily to help keep the area around the bottom as clean as possible. Loose fitting, cotton is preferred to avoid irritation and sweating [32].
- When using the toilet, children should be relaxed, not leaned forward but sitted back fully on the toilet to encourage the bladder to empty completely [37].

- To ensure the bladder emptied completely, counting to 20 slowly while on the toilet can also[38].
- Encouraging about going to the toilet frequently during the day(6-8times).
- Going to the toilet whenever having the urgement without hanging on [39].

Before any antibiotic therapy is started, a urine specimen should be obtained for urinalysis and urine culture[40]. In febrile children with signs of UTI (clinical signs, positive dipstick and/or positive microscopy), antibiotic treatment should be initiated as soon as possible to eradicate the infection, prevent bacteraemia, improve clinical outcome, reduce the risk of renal involvement during the acute phase of infection, and reduce the likelihood of renal scarring[41].

Table (1-1): Treatment of cystitis with antibiotic [42].

Cystitis*/Lower UTI (complicated or uncomplicated)		
	Agent	**Notes**
1st line	Nitrofurantoin	• Most active agent against *E. coli* • Avoid if CrCl < 30 mL/min • **Avoid if systemic signs of infection/suspicion of pyelonephritis or prostatitis** • **Does not cover Proteus**
	TMP-SMX	• Do not use for empiric treatment if resistance >20% • Drug-drug interactions with warfarin • Monitor potassium level if concomitant use of spironolactone, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs) • Renal dose adjustments, avoid if CrCl < 15 mL/min
2nd line	Cephalexin	• Active against *E. coli, Proteus, and Klebsiella*
3rd line	Fosfomycin†	• Active against E. coli, Enterococcus. Is also active against ESBL positive E. coli. **Fosfomycin susceptibility tests recommended.**

1.2. Nitrofurantoin

Nitrofurantoin, a chemotherapeutic agent of the nitrofuran family, was first used into clinical practice in 1952[43]. Nitrofurantoin is a synthetic antimicrobial derived from furan by the addition of a nitro group and a side chain containing hydantoin [44].

Nitrofurantoin is used extensively in the treatment and prevents of uncomplicated lower urinary-tract infections. The usual oral dose for adults is 50-100 mg four times daily, with meals and at bedtime. Treatment is usually continued for 7 days[45]. The daily dose for children is 5-7 mg/kg/24 hr given in 3 to 4 divided doses as nelson book[46]. The dosage is reduced if continued beyond 7 days or if used for prophylaxs. ln long-term treatment, a dose as low as 1 mg/kg may be utilized . A single dose of 50- 100 mg at bedtime may be sufficient to prevent recurrences[47]. Resistance to nitrofurantoin is still uncommon; for example, resistance is ≤ 2% in E. coli from community-acquired UTIs in Europe[48].

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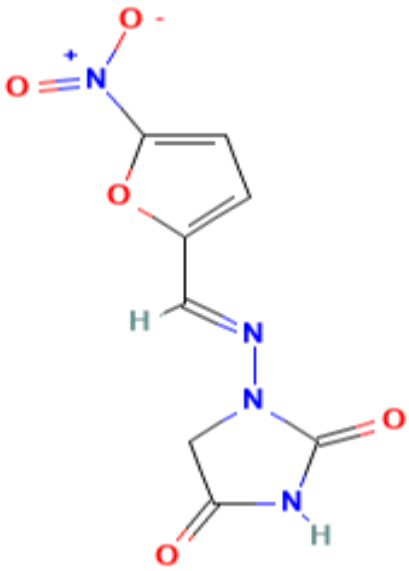


Figure (1-3): Nitrofurantoins chemical structure [49].

1.2.1. Mechanism of Action

The mode of action is reduction of nitrofurans by Bacterial enzymes(nitroredactase) must reduce nitro group in nitrofurantoin to nitrosogroup produce extremely reactive electrophilic metabolites that can interfere with enzymes in DNA, RNA and protein thus impairing energy metabolism, cell wall and carbohydrate synthesis[50].

1.2.2. Antimicrobial Spectrum

Nitrofurantoin has been shown to be effective against the most strains of the following bacteria below.

Gram –positive Aerobes:

Coagulase-negative staphylococci(including staphylococcus epidermidis)

Enterococcus faecalis

Staphylococcus saprophyticus

Staphylococcus aureus

Streptococcus agalactiae

Group D streptococci

Viridans group streptococci [51]

Gram –negative Aerober:

Escherichia coli

Citrobacter amalonaticus

Citrobacter diversus

Citrobacter ferundii

Klebsiella ozaenae [52]

Nitrofurantoin is not active against most strains of Proteus species or Serratia species. It has no activity against Pseudomonas species [53,54].

1.2.3 Pharmacokinetics

Nitrofurantoin is quickly absorbed from the gastrointestinal tract[55]. The macrocrystallne form is dissolved and absorbed more slowly and produces lower serum concentrations than the microcrystallne form, and peak concentrations in the urine are achieved more slowy[56].

The peak blood concentration of nitrofurantoin following an oral dose of 100 mg, is less than 1 µg/mL and may be undetectable; tissue penetration is negligible; the drug is well concentrated in the urine [57]. At the concentrations achieved in urine (>100 µg/mL), nitrofurantoin is

bacteriocidal[58]. Nitrofurantoin is bacteriocidal at the amounts found in urine (>100 µg/ml) [58]. Nitrofurantoin is predominantly removed via biliary and urin excretion (unchanged nitrofurantoin), as well as enzymatic degradation [59]. Around 40% of nitrofurantoin is excreted by the kidneys, with the remainder passing through the gastrointestinal system [60]. The bioavailability of nitrofurantoin is over 90%, however plasma concentrations are relatively low (less than 1 mg/l after a 100 mg oral dose). The half-life of the medication is brief, usually around 1 hours, and 27-50% of it is eliminated unaltered in the urine.although peak urine levels are greater than 100mg/l (range 50-200mg/l) they are only maintained for ashort time [61].

1.2.4. Contraindications

Anuria, oliguria, or significant impairment of renal function (creatinine clearance under 60 mL per minute or clinically significant elevated serum creatinine) are contraindications. Treatment of this type of patient carries an increased risk of toxicity because of impaired excretion of the medicine.Because of the possibility of haemolytic anaemia due to immature erythrocyte enzyme systems (glutathione instability)[62], the medicine is contraindicated in pregnant women during labour and delivery, or when the onset of labour is imminent[63]. For the same reason, the medicine is contraindicated in neonates less than one month of age [64]. Nitrofurantoin is also contraindicated in those patients with history of hypersensitivity to the drug (65).

1.2.5. Dosage and Administraion

Furadantin should be taken with food to increas drug absorption and, in some patients, tolerance[66]

Adult

50-100 mg four times a day, the lower dosage level is recommended for uncomplicated urinary tract infections[67].

Pediatric patients

5-7 mg/kg of body weight per 24 hours, given in four divided doses, contraindicated under one month of age[68].

The **table (1-2)** is based on an average weight in each range receiving 5 to 6 mg/kg of body weight per 24 hours, given in four divided doses. It can be used to calculate an average dose of Furadantin Oral Suspension (25 mg/5mL) for pediatric patients[69].

Therapy should be continued for one week or for at least 3 days after sterility of the urine is obtained. Continued infection indicates the need for reevaluation[70].

For long-term suppressive therapy in adults, a reduction of dosage to 50-100 mg at bedtime may be adequate. For long-term suppressive therapy in pediatric patients, doses as low as 1 mg/kg per 24 hours, given in a single dose or in two divided doses, may be adequate[70].

Table(1-2): Dosing table for children(69).

Weight in killogram (kg)	Pediatric doses and frequency
7 kg to 11 kg	(12.5 mg/2.5 ml) four times daily
12 kg to 21 kg	(25 mg/5 ml) four times daily
22 kg to 30 kg	(37.5mg/7.5 ml) four times daily
31 kg to 41 kg	(50mg/10 ml) foure times daily
42 kg or greater	(50-100mg/10-20ml) foure times daily

1.2.6. Adverse Effects

Common: nausea and vomiting, anorexia, diarrhoea, abdominal pain, allergic skin reactions, headache[71].

Rare: pulmonary toxicity (reversible allergic pneumonitis, sually within the first week or chronic interstitial pulmonary fibrosis generally after approximately 6 months)[72], drowsiness, vertigo, dizziness, peripheral polyneuropathy (usually presents as peripheral paraesthesia and sensory loss in the lower limbs), hepatotoxicity, skin reactions including Stevens-Johnson syndrome, exfoliative dermatitis, lupus-like syndrome, anaphylaxis, drug fever, eosinophilia and arthralgia[73]

1.3. Pharmacogenetic

Pharmacogenetics refers to the variability in response to drug therapies in humans, which is a fast-growing field in molecular biology and clinical medicine[74]. Study of interindividual variation in DNA sequence related to drug absorption and disposition (Pharmacokinetics) and/or drug action (Pharmacodynamics)[75,76] including polymorphic variation in genes that encode the functions of transporters, metabolizing enzymes, receptors and other proteins[77]. The term has been pieced together from the words pharmacology (the study of how drugs work in the body) and genetics (the study of how traits are inherited)[78].

An increasing number of drugs on the market or under development have been identified as substrates of the ATP-binding cassette drug efflux transporter breast cancer resistance protein (BCRP; ABCG2) (79), which can affect the pharmacokinetics of drugs by reducing absorption and/or increasing biliary elimination(80).also genetic variants that cause variability in response to medication(81).

1.3.1. The human ATP- binding cassette(ABCG2)

The human ATP-binding cassette (ABC) proteins belong to a large protein superfamily that now comprises 48 members (82). Many of the human ABC proteins are efflux transporters, and three of them, namely P-glycoprotein (P-gp, gene symbol ABCB1), the multidrug resistance protein 1 (MRP1, gene symbol ABCC1), and the breast cancer resistance protein (BCRP, gene symbol ABCG2), have been implicated to be the major efflux transporters responsible for multidrug resistance in cancer cells(83).

The ATP-binding cassette transporter G2 (ABCG2; also known as breast cancer resistance protein, BCRP) has been suggested to be involved in clinical multidrug resistance (MDR) in cancer like other ABC transporters such as ABCB1 (P-glycoprotein)[84]. As an efflux pump exhibiting a broad substrate specificity localized on cellular plasma membrane[85], ABCG2 excretes a variety of endogenous and exogenous substrates[86], including chemotherapeutic agents, such as mitoxantrone and several tyrosine kinase inhibitors. Moreover, in the normal tissues, ABCG2 is expressed on the apical membranes and plays a pivotal role in tissue protection against various xenobiotics. For this reason, ABCG2 is recognized to be an important determinant of the pharmacokinetic characteristics of its substrate drugs[87]. Human BCRP is encoded by the ABCG2 gene which is located on chromosome 4q22[88]. The gene product ABCG2 has been shown to be a promiscuous transporter of a large number of hydrophobic substrates[89]includes a number of pharmaceutical medications. Several high-throughput assays for ABCG2 have been designed to screen large libraries of compound, and their use has resulted in an explosion in the Identification of novel selective inhibitors of this transporter, some of these substrates are (Nitrofurantoin, ciprofloxacin, doxorubicin, gefitinib, glyburide, methotrexate, norfloxacin and so on…..) while the inhibitors are (abacavir, lopinavir, crysin, 14yclosporine A, nimodipine, tacrolimus, tamoxifen and so on …….)[90].

Structural feature **Figure (1-4)**, unique to ABCG2 among other ABC transporters, is a much longer extracellular loop (EL3) between the TM5 and TM6 [91]. It has three cysteine residues, that produce an one intramolecular (C592-C608) and the other intermolecular (C603) bonding of disulfide [92]. Although the intramolecular disulfide link appears to be important for ABCG2 expression and function, the intermolecular

disulfide bond is probably of secondary importance [93]. Within EL3, there is a functional N- glycosylation site at N596 which most likely is modified by N- acetylglucosamine[94].

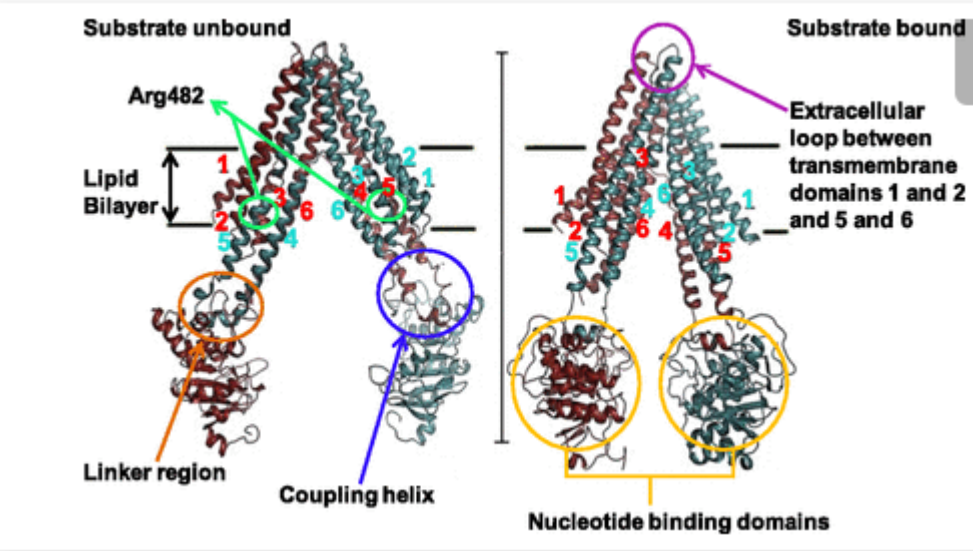


Figure (1-4): Homology modeling of ABCG2

This figure predicts that substrate binding induces significant conformational change, which brings the nucleotide-binding domains closer together and closes the substrate-binding cavity. This structural alteration possibly allows these domains to interact and results in the transport of substrates through the central translocation pathway. The transmembrane domains are numbered 1–6, and possible areas of significance to the transporter's function are highlighted **[95].**

1.3.2. The Association Between Human ATP- Binding Cassette Polymorphism and Nitrofurantoin Response

Polymorphisms affecting the expression or function of ABCG2 may have clinically important roles in drug disposition and efficacy[96]. The most well-studied single nucleotide polymorphism (SNP), Q141K (421C>A), is shown to decrease ABCG2 expression and activity, resulting in increased total drug exposure and decreased resistance to various substrates[97]. The effect of Q141K can be rationalized by

inspection of the ABCG2 structure, and the effects of this SNP on protein processing may make it a target for pharmacological intervention[98].

In vivo pharmacokinetic studies have shown that ABCG2 affects the absorption, biliary excretion and elimination of nitrofurantoin in mice(99). The plasma AUC of oral nitrofurantoin was four fold higher in ABCG2 knockout than wild-type mice. Following intravenous dosing, the nitrofurantoin AUC was twofold higher and the biliary excretion 48-fold lower in ABCG2 knockout mice compared with ABCG2 wild-type mice (100). Furthermore, co-administration of nitrofurantoin with the ABCG2 inhibitor chrysin to rats resulted in a 75% reduction in biliary excretion and significantly increased nitrofurantoin AUC following both oral and intravenous administration(101). Nitrofurantoin is not a substrate for either the P-glycoprotein (MDR1) or multi- drug resistance-associated protein 1 and 2 (MRP1, MRP2) efflux transporters in vitro. The results of the above-mentioned studies suggest that BCG2 activity should influence nitrofurantoin disposition in man(102).

1.4 Aims of The Study

The present study was designed to evaluate:

- The association of ATP- binding cassette G2(ABCG2) genetic polymorphism (rs 2231142) with susceptibility to urinary tract infection in selected clinics/hospitals in kerbala province.

- The possible association of ABCG2 genetic polymorphism (rs 2231142) with the therapeutic response to Nitrofurantoin for children with urinary tract infection in in selected clinics/hospitals in kerbala province.

Chapter Two

Patients, Materials and Methods

2. Patients, Materials and Methods

2.1. Patients

One hundred fifty children newly diagnosed patients with urinary tract infection (their age range from 4-14 years) participated in this study,
Patients had attended in pediatrics teaching hospital, Al-hussienia hospital and private clinic of pediatric from November 2020 to April 2021 seeking for medical treatment. Those patients selected by a consultant pediatrics and diagnosed as UTI. All participant children were enrolled in this study after signing parents on written informed consent and requested to answer a specially designed questionnaire. 40 patients were lost due to infection with bacteria other than E.coli (N=28) and some not continue in this study due to parents infected with coronavirus(N=12).

2.1.1. Patients Criteria

2.1.1.A. Inclusion Criteria

Children aged between (4-14) years with UTI. Received nitrofurantoin for 1 week, present with no other disease.

2.1.1.B. Exclusion Criteria

1- Patient were receiving other drugs (antibiotic, antacid).

2- Patients with other disease (renal failure, G6PD deficiency).

3- Patients with fever.

2.1.2. Ethical Considerations

The research was certified in the "college of pharmacy by the ethic panel", karbala university. All procedures were explained to patients and informed consent was obtained by the principle investigator form participants, as show in (appendix1).

2.1.3. Study Protocol

All precausions have been taken in clinical settings to prevent transfection of covid 19.

This interventional study done on participants (one hundred ten). From each participants blood sample was collected for genetic study and urine samples collected for urine culture, VITEK test, pre-treatment GUE before treated by nitrofurantoin [103]. 40 patients lost due to infection with bacteria other than E.coli (N=28) and due to parents infected with coronavirus [104] (N=12). And only from patients continue the study, urine sample was collected again after 7 days of nitrofurantoin treatment for post- treatment GUE then data collection and statistical analysis as show in **figure (2-1).**

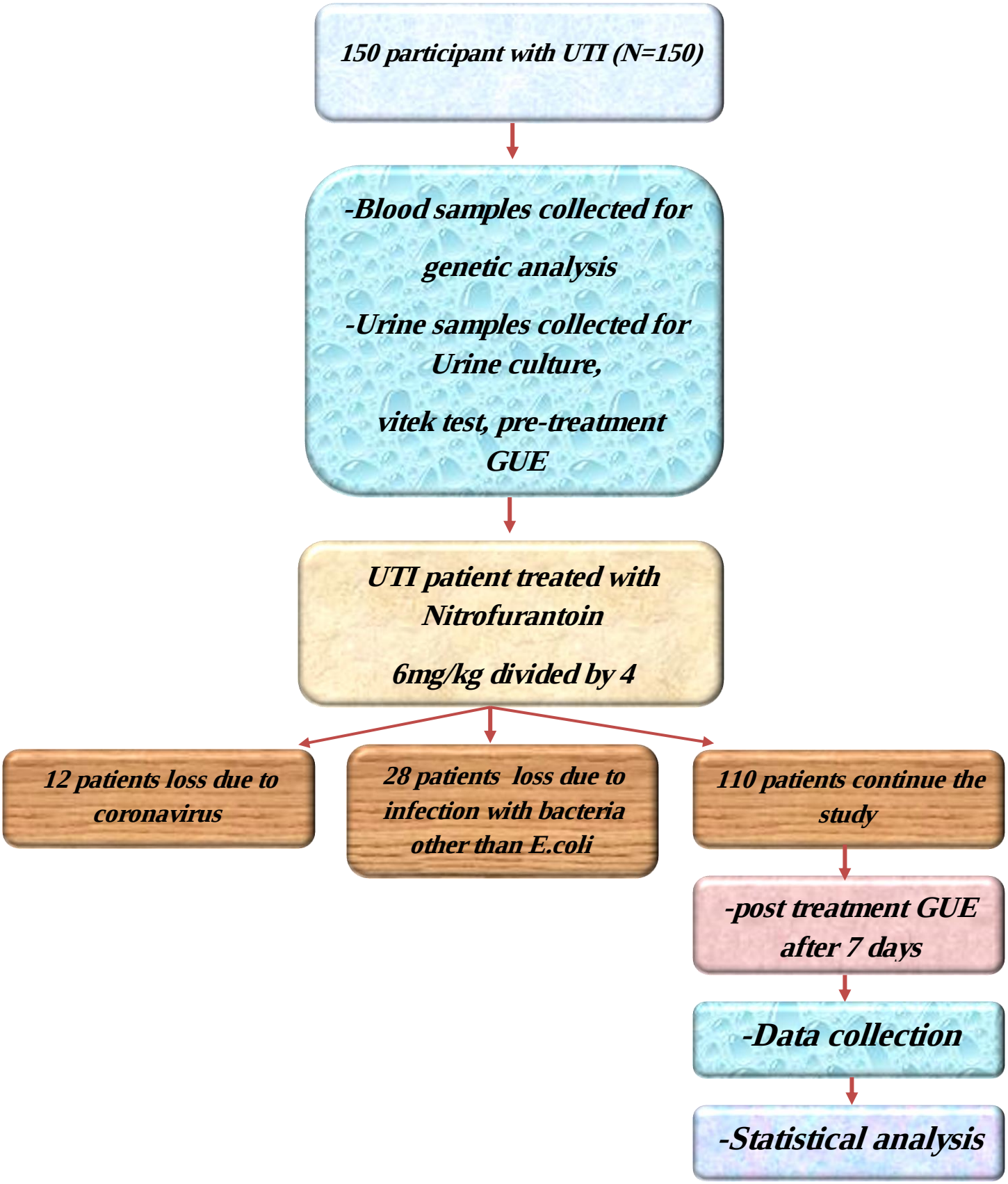


Figure (2-1): Flow chart of study group.

2.2. Materials

2.2.1. Equipment and Instruments

The **table (2-1)** below lists all of the equipment and instruments that were used in this study.

Table (2-1) Instruments and the manufacturing companies

No.	Equipment &instrument	company
1	Centrifuge	SIGMA / Germany
2	Distillatory	Gel / Germany
3	Sensitive balance	NDA / Taiwan
4	Water bath	Lab tech / Korea
5	Vortex	Human twist / Germany
6	Micropipettes 5-50,100-1000µl	SIAMED /Japan
7	Thermocycler PCR	Techne / UK
8	Hood	Lab tech / Korea
9	UV Transilluminator	Syngene / Germany
10	Gel electrophoresis	Techinme / England
11	Digital camera	Canon / England
12	Autoclave	Hiclave-Hirayama / Japan
13	Compound light microscope	Olympus / Japan
14	Incubator	Memmert /Japan
15	Standard loop 0.01 ml	Himedia / India
16	Refrigerator	Ishtar / Iraq
17	Urinalysis reagent strips	Acon / USA
18	VITEK	BioMeriux / France
19	Densichek plus	BioMeriux / France

2.2.2: Culture Media

The chemicals and biological materials used in this study and their origins are given in **table (2-2).**

Table (2-2): Culture media and chemical reagents

chemical	Manufacturer(Origin)
Mueller-Hinton agar	Biolab (Europe)
Blood agar	Liofilchem (Italy)
MacConky agar	OXOID (UK)

2.2.3 Analytical Kits

The kits utilized in this research, along with their manufacturers and countries of origin are given in **table (2-3).**

Table (2-3): Kits used in this study

No.	Kit	Company	Country
1	G-spin DNA extraction Kit	Intron	Korea
2	Accupower^{R} PCR premix	Bioneer	Korea
3	VITEK identification (ID) card	Biomerieux	France

2.2.4 Primers

The PCR primers for ABCG2 gene were designed by Dr. Hayder Ali Mohammed by using primer BLAST software. These primers were provided from (Iraq scientific researcher, Al- diwaniyah), the following **table (2-4)** shows the details sequence for the primer.

Table (2-4): The sequence of ABCG2 (C>A) rs(2231142) genetic polymorphism

Primers	Sequence (5'->3')	Tm
F1- G Allele	ACTCTGACGGTGAGAGAAAACTTAC	57.30
F2- T Allele	ACTCTGACGGTGAGAGAAAACTCAA	57.30
Reverse primer	TTATCCACACAGGGAAAGTCCTAC	57.20

2.2.5. Chemicals and Reagents

All the chemicals and reagents that utilized in this study, as well as the manufacturers name and place of origin showed in **table (2-5).**

Table (2-5): Manufacturer and countries of origin for chemicals and reagents are listed

No.	Chemical	Company and origin
1	Absolute Ethanol	Scarb (Spain)
2	Agaros	Biobasic (Canada)
3	TBE buffer 10X	Bioneer (Korea)
4	Ethidium bromide	Bioneer (Korea)
5	Ladder 100bp	Bioneer (Korea)
6	Free nuclease water	Bioneer (Korea)
7	Nitrofurantoin (tap-susp)	Awa(Erbil)-Bioactive (UK)

2.3. Methods

2.3.1. Diagnostic Work-Up

2.3.1.A. Medical History

The site of pain, episode, complicating factors are identified by taking the patient's history. This includes questions on primary (first) or secondary (recurring) infection, febrile or no febrile UTIs; malformations of the urinary tract (pre- or postnatal ultrasound [US] screening), previous operations, voiding habits; family history; whether there is constipation [105].

2.3.1.B. Clinical Signs and Symptoms

Asked about lower urinary tract symptoms (simple cystitis) include

1- Dysuria (burning when urinating)

2- Frequency (need to urinate frequently often in tiny amounts)

3- Urgency (is the sudden urge to urinate)

4- Enuresis (day or night)

5- Color (cloudy urine) and odor of urine (strong unpleasant smell of urine)

6- Incontinence

7- Hematuria

8- Suprapubic pain

In a girls ask about discharge and menses (if was above the age of ten), for boys (circumcised or not) [106].

2.3.1.C. Physical Examination

To confirm the diagnosis, a physical examination is essential, especially if the signs and symptoms of lower UTI are present.

Physical examination should look for signs of constipation (abdominal distention and abdominal mass), palpate the bladder (to exclude bladder distention), measure temperature (to exclude febrile patient), and perform a genital examination if necessary (to excluded vaginitis, cervicitis, prostatitis, epididymitis, and urethritis) [107].

2.3.2. Urine Sampling, Analysis, Culture and VITEK Test

2.3.2.1. Urine Sampling

The ideal sample for the laboratory diagnosis of UTI is urine. Urine should be collected before the initiation of antibacterial therapy, as a single antibiotic dose can be the cause of sterile urine culture. In toilet trained children, a midstream urine samples is preferable. Urine should be processed as soon as the sample is received in the laboratory. It is best to avoid any delay between the sample collection and processing. Since the generation time of E.coli is 20 minutes, this being the most commonly implicated organism in any UTI any delay will reflect in the culture outcome as a false significant growth, which may not be the case [17].

2.3.2.2. Urine Analysis

Clean catch urine samples were collected in sterile universal containers from patients showing symptoms of urinary tract infection according to the questionnaire form (Appendix2) and they were instructed on how to collect samples, and the samples were labeled with special code for each participant. The urine samples obtained divided into two portions. One portion was for the direct microscopic examination. The urine samples were shaked and 1ml from each sample was transferred to 1.5 ml size

aliquot tube, centrifuged at 1000 rpm for 1 minutes. The supernatant was discarded, and the sediment was re-suspended in 500µl urine. This native urine sediment was dropped on glass slide and covered by a coverslip. The microscopic examination was performed by the bright-field microscopy (x400). The threshold value of least 5 pus cells/HPF, which corresponds to at least 25 leukocytes per ml of non-centrifuged urine, was considered as pyuria [108][109]. The second portion was cultured on the blood agar and macconky agar using standered loop method. After incubation, the culture plates were examined, and bacterial growth of ≥ 103 CFU/ ml was considered a significant cut off for bacteriuria [110]. The pure culture were diagnosed using VITEK system, is a microbiology and automation system, system that uses growth- based technologies. It's used to identify bacteria [111].

2.3.2.3. Urine Sample Testing by Dipstick Screening Technique

1. Just before testing, the urine sample was thoroughly mixed.

2. Without contacting the test pads, the strip was withdrawn from the bottle and the cap was replaced immediately.

3.The test pads of the strip were thoroughly immersed in the specimen before being withdrawn.

4. The strips edge was run against the rim of the urine container to remove surplus pee, and the strip was holed in a horizontal position to prevent chemical mixing with neighboring test pads while removing from the specimen.

5. The color chart on the side of the container was compared to the reagent test strip area, and the read time, which is crucial for optimal result, was taken into account.

6. The strip was held close to the color blocks without touching the bottle, and the colors were carefully matched.

7. The following times were used as a guide for the subsequent test.

For glucose 30 second

For protein and nitrite 60 seconds

For leukocytes 2minutes

A positive nitrite test usually means infection. To render the dipstick positive, it usually needs more than 10,000 germs per ml, making it a specific but not very sensitive test. A negative nitrite test does not rule out the possibility of a urinary tract infection, but a positive one strongly suggests infection. While leukocyte esterase (LE) is a neutrophil produced enzyme, it could indicate pyuria as a result of a UTI [(112)].

2.3.2.4. Cultivation Of Urine Samples and Laboratory Prepared Media

2.3.2.4.A. Blood Agar Base

This culture medium is a nutrient rich foundation that allows all relevant microorganisms to grow optimally. 40 g/liter suspension, autoclave (15 min at 121 °c), cool to 45-50 °c, add 20ml defibrinated blood to each 500ml of medium, mix at 25°c.

The prepared medium is clear and yellowish-brown before blood is added, then blood-colored and not hemolytic [(113)].

2.3.2.4.B. MacConkey Agar Base

MacConkey agar is a slightly selective medium that allows the accurate separation of lactose-fermenting and lactose-non fermenting gram-negative enteric bacilli from feces, urine, foodstuffs, waste water, and other sanitary materials. In 1 Liter of distilled or deionized water, dissolve

51.5g of the powder. Mix thoroughly. Bring to a boil for 1 minute, stirring constantly until the sugar is completely dissolved. Sterilize for 15 minutes in an autoclave at 121°c [114]**.** After being solidified, the produced medium were used for isolation, viable count determination, identification, and susceptibility testing [115].

2.3.2.4.C. Mueller-Hinton Agar Base

Recommended for determining the susceptibility of bacteria isolated from clinical samples to antimicrobial treatments. In 1000 ml purified/distilled water, dissolve 38.0 grams. Bring to a boil to completely dissolve the medium. autoclave at 121°C for 15 minutes to sterilize. Reduce the temperature to 45-50°C. Fill sterile petriplates to about 4 mm thickness of mixture. To allow the extra moisture to evaporate, the solidified plates were incubated at 37°C for 15-30 minutes [116]. Touch four or five isolated colonies of the organism to be tested with a sterile inoculating loop or needle, in 2 ml of sterile saline, suspended the organism. To make a smooth suspension, vortex the saline tube, then used within 15 minutes of preparation [117].The plates were inoculated by dipping a sterile swab into the inoculum, removing excess inoculum by pressing and rotating the swab firmly against the side wall of the tube above the level of fluid, then rubbing the swab all over the medium surface three times at a 60 degree angle after each application, and finally passing the swab around the edge of the agar surface. For a few minutes, the plate was left to dry at room temperature with the lid closed. The antibiotic discs were added after 15 minutes of inoculation, and the plates were inverted for incubation to avoid moisture collection on the agar surface. Each plate had a maximum of 5 antibiotic discs placed on it. The diameter of each zone, including the diameter of zone inhibition, was measured and recorded in mm after an overnight incubation at 37 , and

compared to the standard inhibition zone as resistant, intermediate, sensitive according to clinical laboratory standards institute criteria **table (2-6)**[118].

Table (2-6): According to the clinical laboratory standards institute, standards antimicrobial inhibition zones exist.

Antibiotics	Resistant	Intermediate	Sensitive
Gentamicin (10µg)	≤12	13-14	≥15
Ampicilin (10µg)	≤13	14-16	≥17
Nitrofurantion (10µg)	≤14	15-16	≥17
Tetracycline (30ug)	≤11	12-14	≥15
Nalidixic Acid (30µg)	≤13	14-18	≥19
Cotrimoxazole (30µg)	≤10	11-15	≥16
Streptomycin (10µg)	≤10	12-14	≥15
Sulphamethoxazole (30µg)	≤13	14-18	≥15

2.3.3. Collection and Preparation of Blood Samples

Before starting nitrofurantoin medication, one milliliter of blood was obtained from each subject. After sterilization, blood was sucked from the antecubital vein and stored at 2.5 °C until DNA extraction, which took between 15 and 21 days from the day of collection [119].

2.3.4. Genomic DNA Extraction

Genomic DNA was isolated from blood samples using a DNA G- spin extraction kit (fresh blood) Intron, Korea, and followed the manufacturer's instructions as follows [120].

1. Pipet 200 µl of whole blood or bodily fluids and added into 1.5 Eppendrof tubes.

2. 20 µl of proteinase K and 5 µl of RNase A solution was added to the sample tube then mix by pulse vortex.

3. 200µl of buffer BL was added into the sample tube and mixed thoroughly to yield a lysis solution.

4. The mixture was left at room temperature for 2 minutes.

5. The lysate was incubated for 10 minutes at 56°C with repeated mixing.

6. The sample tube was centrifuged brifly to remove drop from the inside of the lid.

7. 200 µl of absolute ethanol was added into the lysate and mix well by by vortex and briefly centrifuge also.

8. The mixture was applied carfully to the spine column, close the cap, and centerfuged at 13,000 rpm for 1 min, then the filterate was discarded and a new collection tube is replaced.

9. 700µl of WA buffer was added to the spin column and centrifuged for 1 min at 13,000 rpm and discard the filtrate.

10. 700µl of buffer WB was added to spin column and centrifuged for 1 minute at 13,ooorpm and discard the filtrate then the collection tube was replaced with another one for additional centrifuged for 1 min to dry the membrane.

11.The spin column was replaced with a new 1.5 ml tube, then 80 of buffer CE was added directly into to membrane and incubated for 1 min at room temperature the centrifuged for 1 min at 13,000 rpm to elute DNA.

2.3.5. Allele Specific-Polymerase Chain Reaction(AS-PCR) Technique

For genotyping and detection of C421A gen polymorphisms in the ABCG2 gen in UTI patients' blood samples, an allele specific-PCR (AS-

PCR) approach was used. This method was carried out as described as following steps details[121].

2.3.5.A. PCR Master Mix Preparation

The Accupower PCR premix kit was used to make the PCR master mix, which was done according to the company's instructions, as shown in **table (2-7)**

Table (2-7): PCR master mixes preparations in according to the manufacturer's instructions.

PCR master mix	Volume
DNA template 5-50 ng	5 µl
Forward primer (10pmol)	1.5 µl
Reverse primer (10 pmol))	1.5 µl
PCR water	12 µl
Total volume	20 µl

Following that, the PCR master mix component was placed on a standard Accupwer PCR premix kit, which includes all of the other reagents required to start the PCR reaction.

Taq DNA polymerase, dNTPs, Tris-HCl pH: 9;0, KCl, MgCl2, stabilizer, and loading dye are among them. After that, all of the PCR tubes were transferred to an Exispin vortex. After centrifuge for 3 minutes at 3000 rpm, place in PCR thermocycler.

2.3.5.B. PCR Thermocycler Condition

Table (2-8): shows the different PCR thermocycler parameters used for each gene.

Table (2-8): The temperature and timing of PCR in a thermo-cycler

PCR step	Temp.	Time	Cycles Numbers
Initial denaturation	94°C	5min	1
Denaturation	94°C	30 sec	35 cycle
Annealing	57°C	1min.	
Extension	72°C	1min	
Final extension	72°C	5min	1
Hold	4°C	Forever	-

2.3.5.C. PCR Product Analysis

The following steps were used to examine the PCR results using agarose gel electrophoresis:

1. 1% percent agarose gel was made by dissolving 1x TBE in the microwave and then cooling to 50°C.
2. The ethidinum bromide stain was the added to the agarose gel solution in the amount of 3 µl.
3. After placing the comb in the proper position, the agarose gel solution was poured into the tray and left to solidify for 15 minutes at room temperature. The comb was then gently removed from the tray and 5 µl of PCR product added to each comb well, as well as 5µl of (100 bp ladder) in the first well.
4. The gel tray was placed in the electrophoresis chamber and 1X TBE buffer was added. Then , for 1 hour, electric current was applied at 100 volts and 80 Hz.
5. The 197bp PCR products were visualized using a UV transilluminator.

2.4. Statistical Analysis

The data of participants in this study were converted into a computerized database, revised for errors or inconsistencies, and then managed, processed, and analyzed by using the statistical package for social sciences (SPSS) version 26, IBM, US.

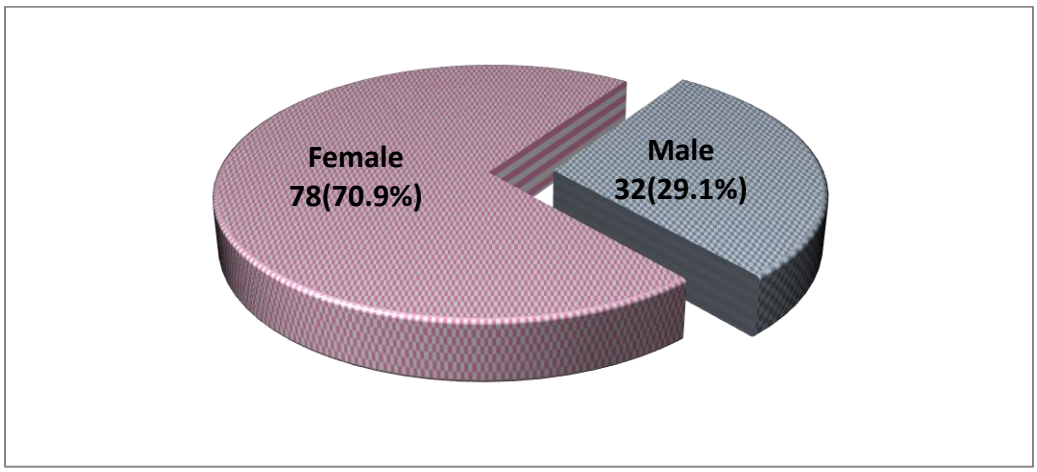
Chapter Three

Results

3. Results

3.1. The Demographic and Biochemical Parameter of Urinary Tract Infection Patients Group

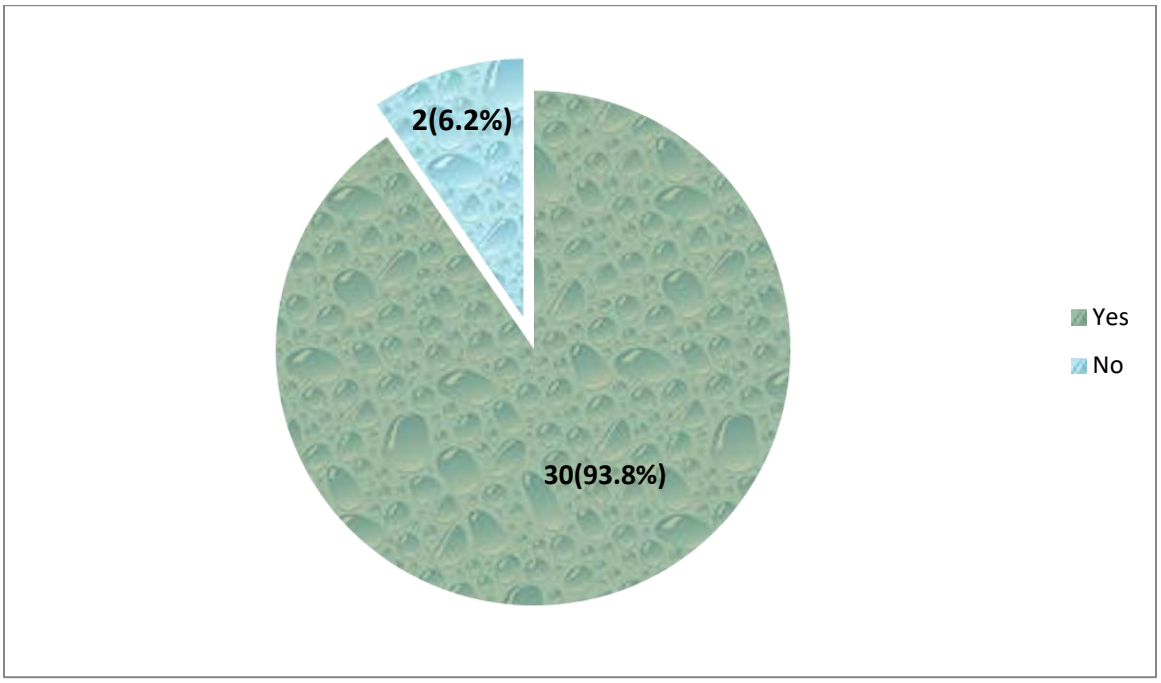
The mean age of the sample of the present study was 7.68±2.86 years, ranged from 4 to 14 years. Male accounted for less than one third of the total study sample (29.1%), most of them were circumcised (93.8%) as showed in **figure(3-1)** and **figure(3-2)** below, also represented according to the age as **tables (3-1)(3-2)**.



Figure(3-1): Numbers and proportions of the gender of the study sample

Table(3-1): Numbers and proportion of gender according to the age in study sample

Variable		Group	
		Male (n=32)	**Female (n=78)**
Age groups	**< than 5 years**	7 (21.9%)	11 (14.1%)
	5-10 years	18 (56.3%)	55 (70.5%)
	< than 10 years	7 (21.9%)	12 (15.4%)

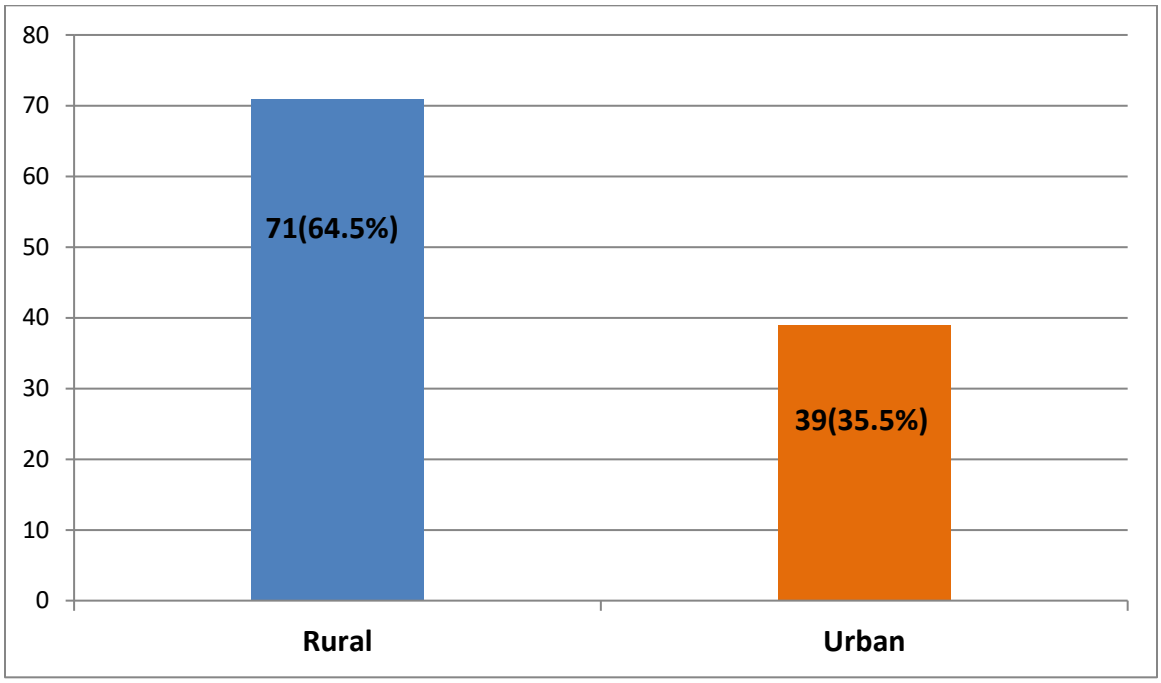


Figure(3-2): Numbers and proportions of the circumcised male of the study sample.

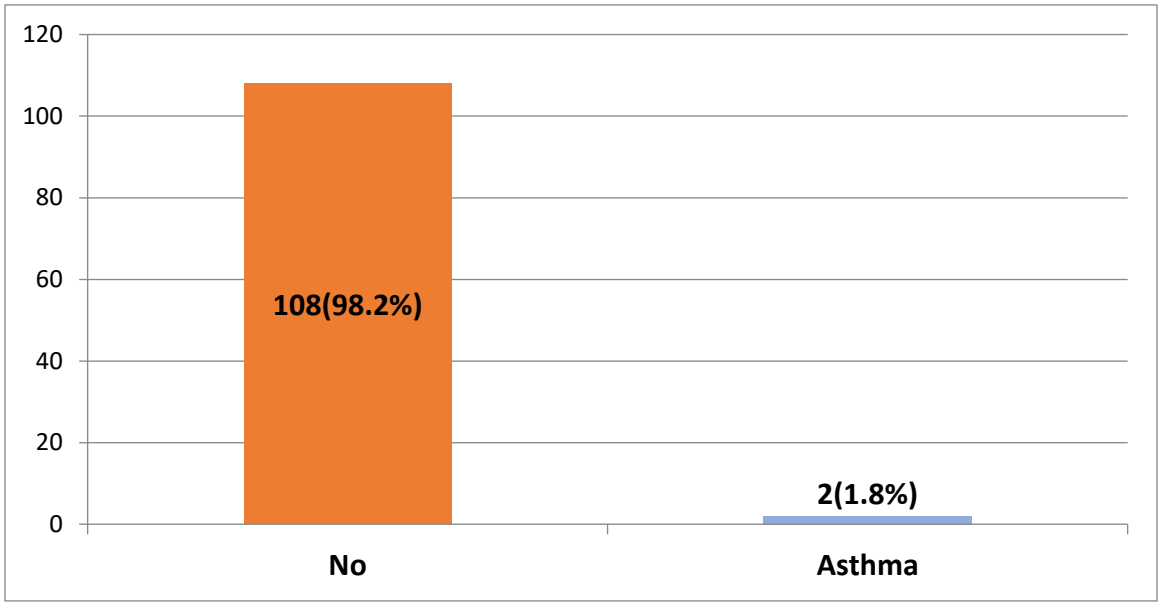
Table(3-2): Numbers and proportion of the circumcised male according to the age in the study sample.

Variable		Group	
		No (n=2)	**Yes (n=30)**
Circumcision Male (32)	**< than 5 years**	2 (100%)	5 (16.7%)
	5-10 years	0 (0%)	18 (60%)
	< than 10 years	0 (0%)	7 (23.3%)

The study revealed that 64.5% of the patients were rural residence **figure(3-3)**. Only 2 patients reported history of other diseases (asthma) as shown in **figure(3-4)**.

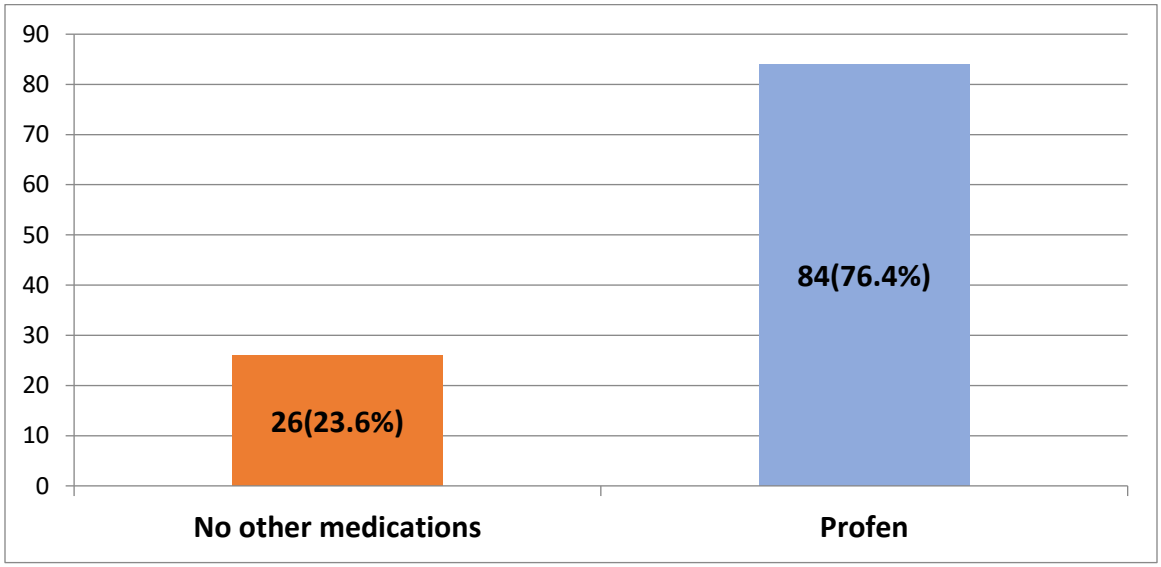


Figure(3-3): Distribution of the patients of the study according to the residence.

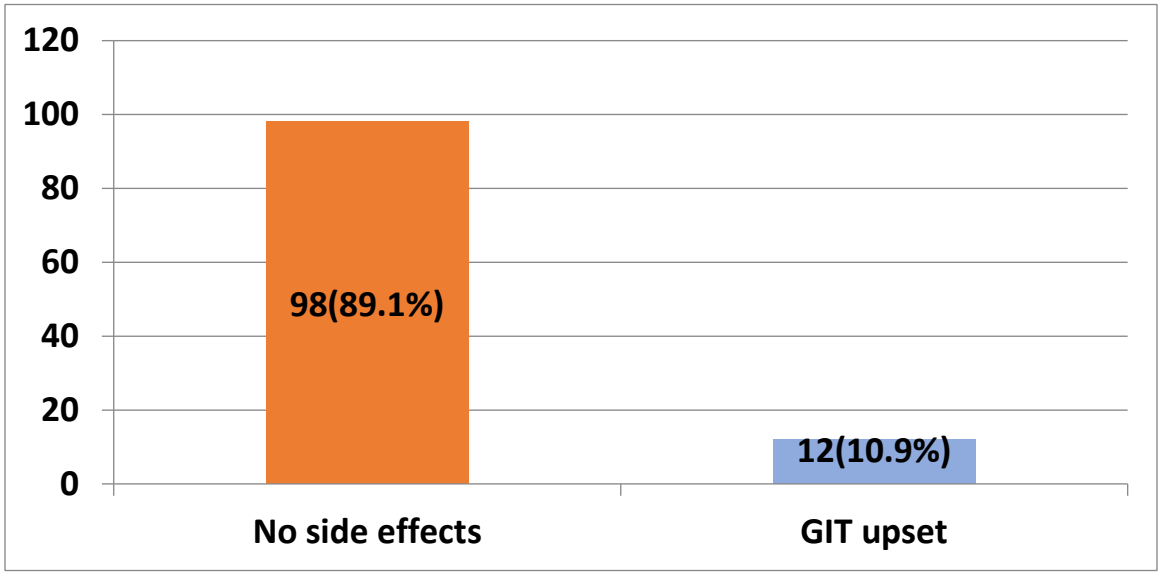


Figure(3-4): Numbers and proportions of the history of other diseases of the study sample.

The study showed that more than 75% of the patients of the current study (76.4%) reported a history of the use of other medication (Ibuprofen) as shown in **figure(3-5)** below. Whearas the history of drug side effects revealed that about one tenth of the patients of the present study (10.9%) had GIT upset figure**(3-6).**

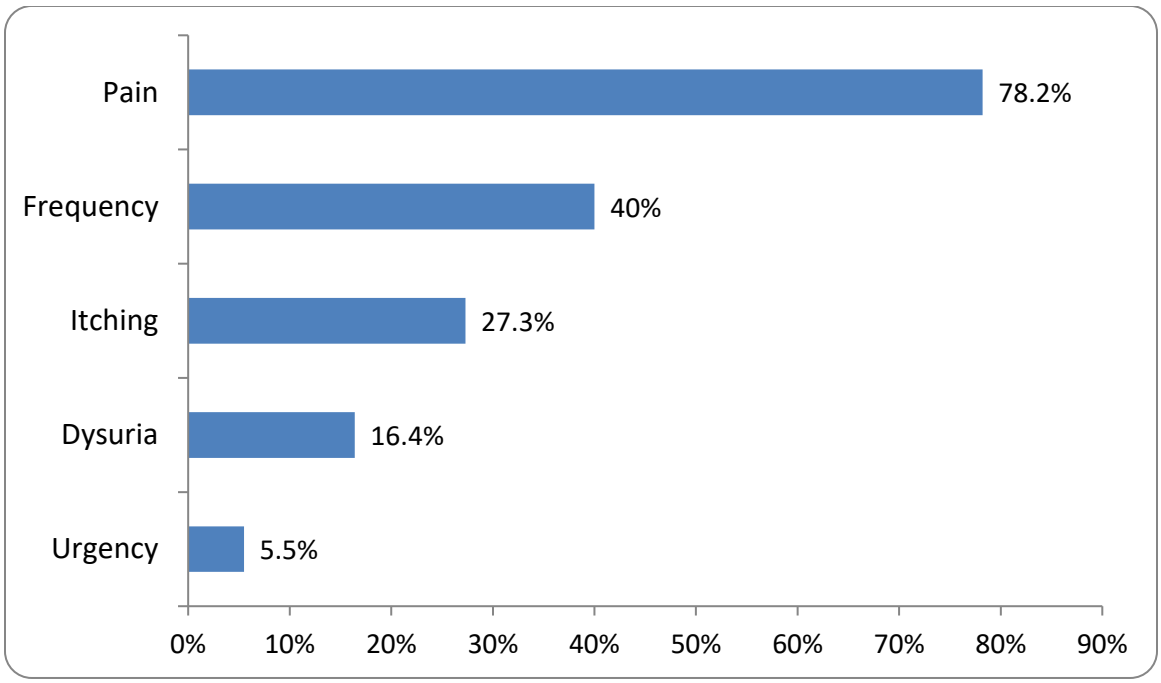


Figure(3-5): Numbers and proportions of the history of use of other medication of the study sample.



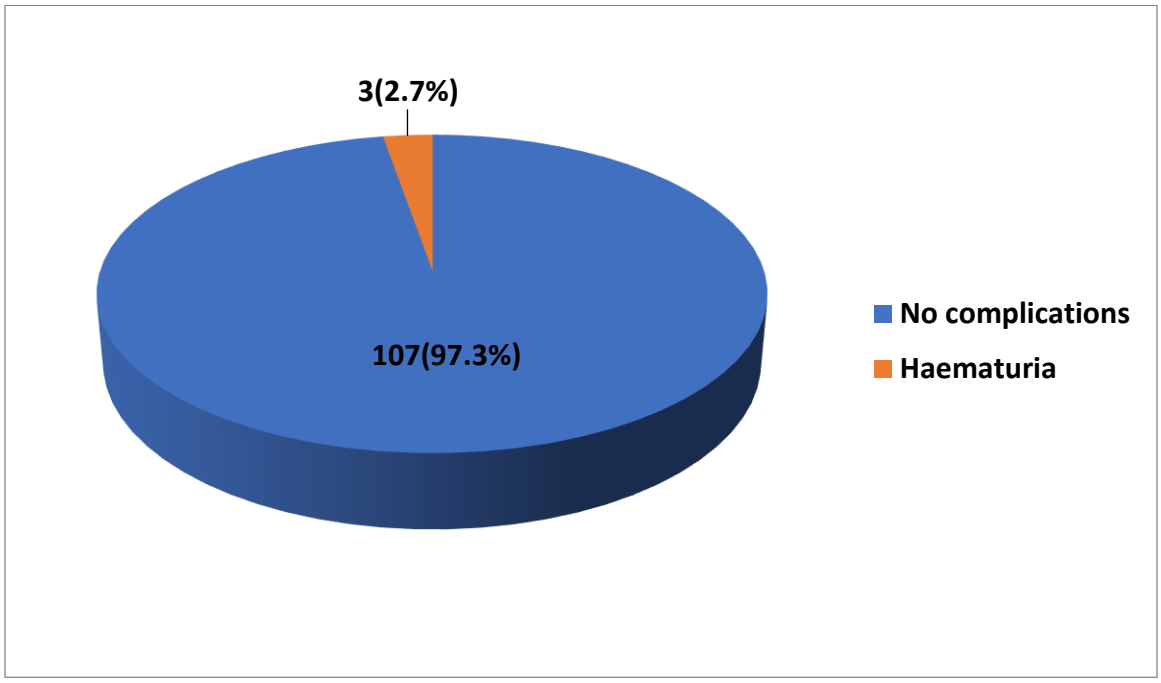
Figure(3-6): Numbers and proportions of the history of drug side effects of the study sample.

The analysis of the present study revealed that, prior to treatment, from the total 110 patients; pain during micturition accounted for the highest proportion of symptoms (78.2% of total), followed by frequency (40%) and the least reported prior to treatment symptoms was urgency (5.5%) as illustrated in **figure(3-7)** below.



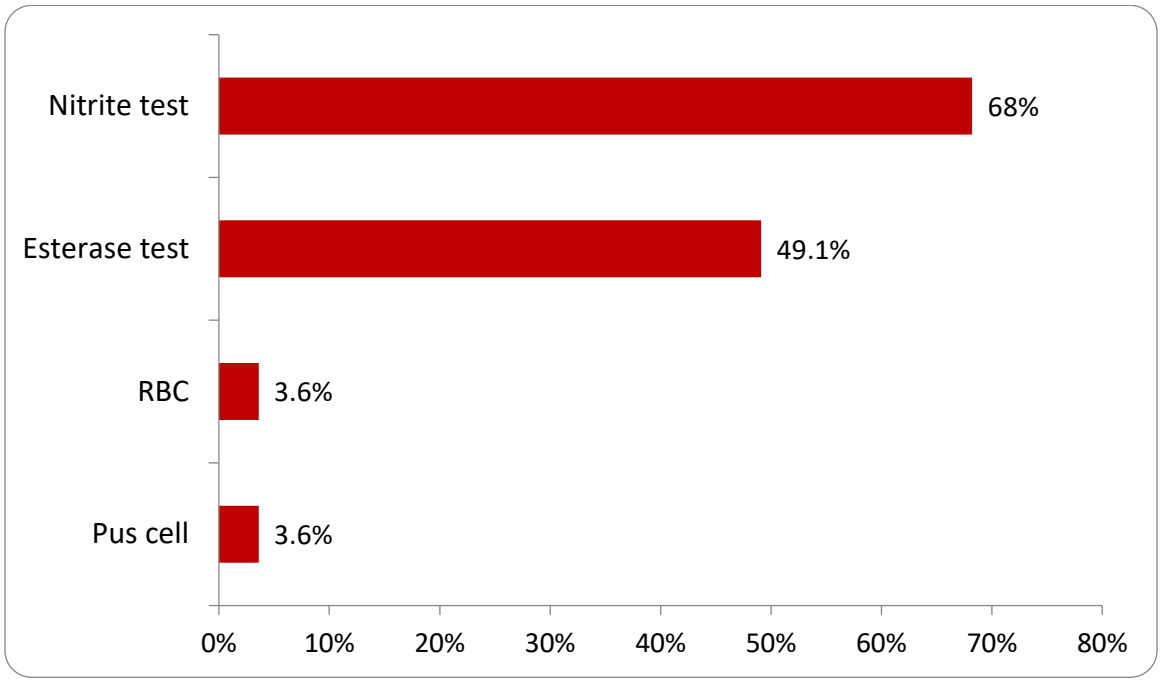
Figure(3-7): Proportions of the symptoms of the study patients prior to treatment.

The analysis of the current study showed that from the total 110 study patients, there were 3 patients (2.7%) experienced disease complication in form of hematuria as shown in **figure(3-8).**



Figure(3-8): Proportions of the complication of the study patients before treatment.

The analysis of the data also showed that, prior to treatment, from the total 110 patients; nitrite test accounted for the highest proportion of signs (68% of total), followed by esterase test (49.1%) and the least reported prior to treatment signs were RBC and pus cells (3.6% for each) as illustrated in **figure(3-9)** below**.**



Figure(3-9): Proportions of the signs of the study patients prior to treatment.

3.2. Molecular Analysis

3.2.1. Genetic Analysis to Assess the Association of ATP Binding Cassette G2 Polymorphism with Urinary Tract Infection Pathogenicity

The analyses were conducted to assess the association between the SNP rs2231142 [CC (wild type), TC (heterozygous type),TT (mutated type)] with the pathogenesis of UTIs according to logistic regression and **figure (3-10).**

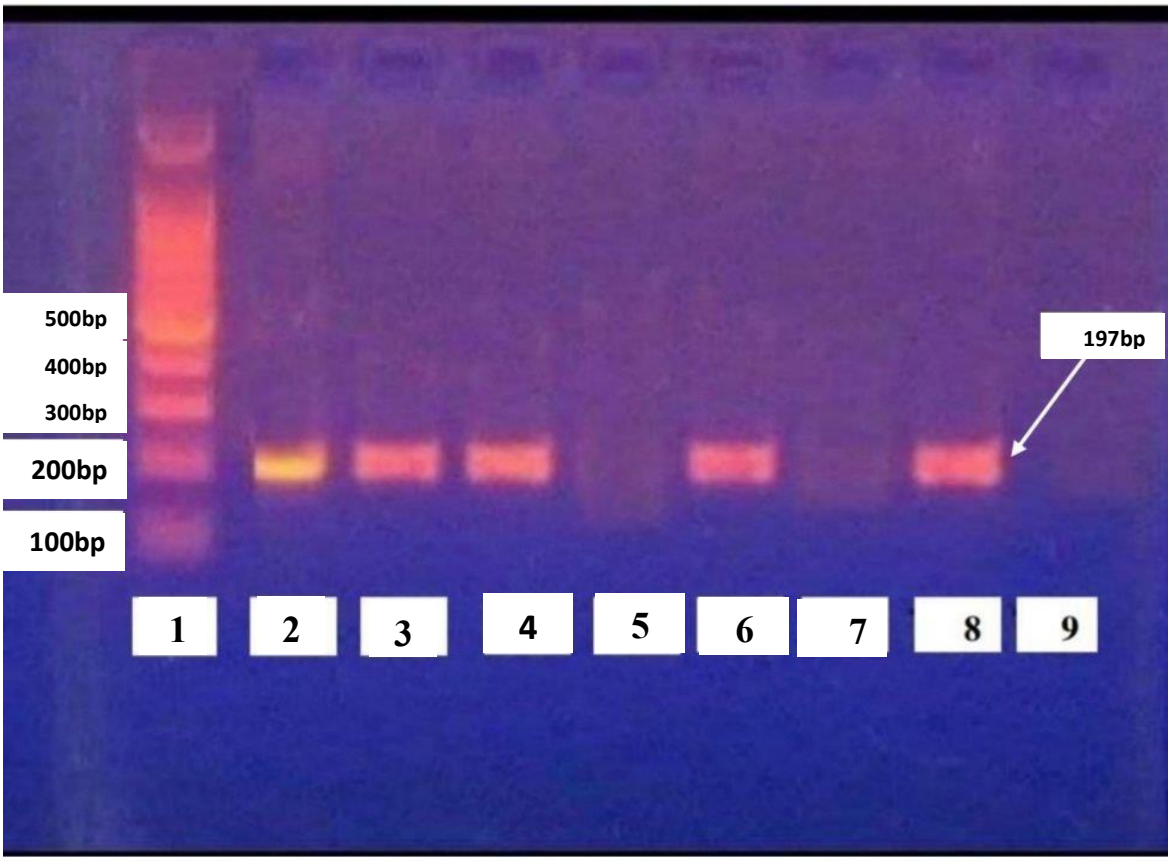
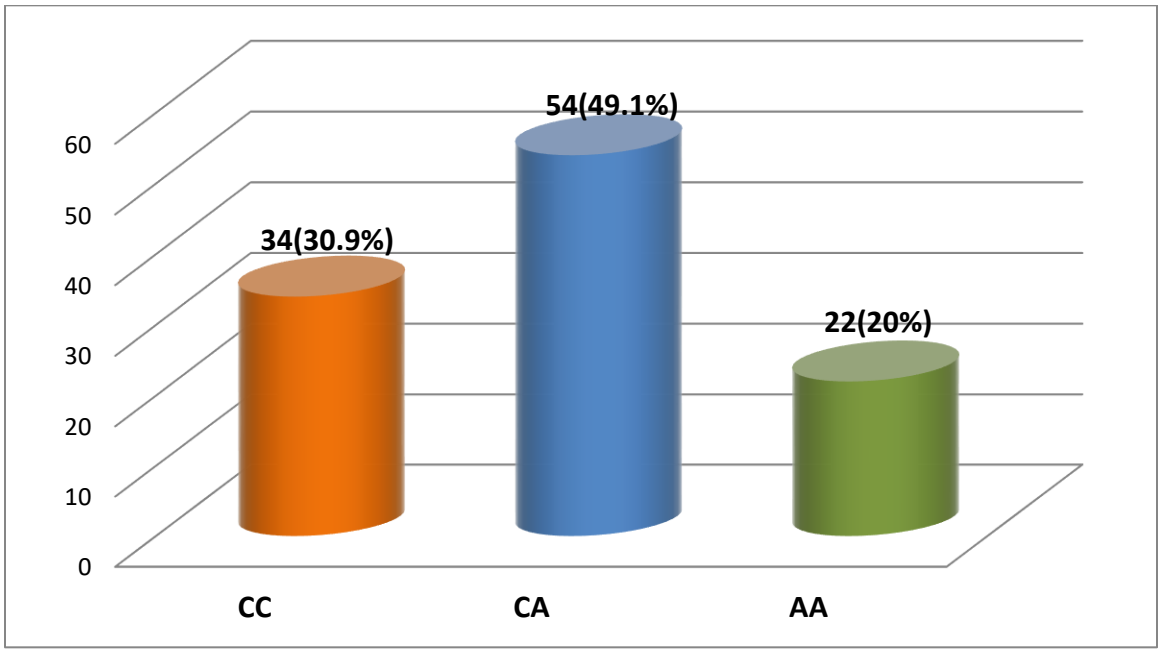


Figure (3-10): Allele specific PCR of ABCG2 (C>A) (rs2231142) genetic polymorphism showed : Lane(1): represented DNA ladder 100-1000 ,Lane (2and3) represented : CA heterozygous genotype ,Lane (4 to 8): represented CC genotype (wild) all were showed in (197bp).

The distribution of the single nucleotide polymorphisms (SNPs) of the study patients illustrated in **figure(3-11)** below



Figure(3-11): Distribution of the single nucleotide polymorphisms (SNPs) genotypes of the study patients.

The analysis of the data showed that there were no significant statistical gender differences in regard to socio-demographic characteristics, SNPs and signs and symptoms of the study patients except for itching (p=0.033) as illustrated in **table(3-3)** below.

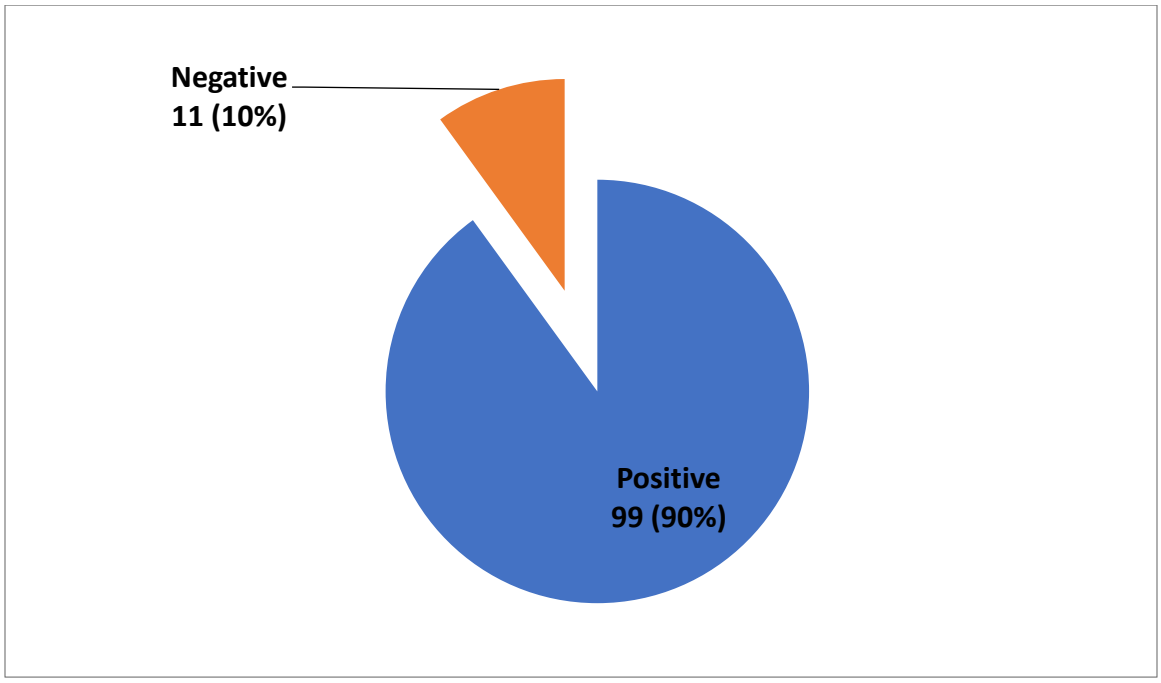
Table(3-3): Gender difference of some socio-demographic characteristics, SNPs and signs and symptoms of the study sample.

Variable		Group		p value
		Male (n=32)	Female (n=78)	
Mean age (y)		7.81 ± 3.22	7.63 ± 2.73	0.761 [NS]
SNP	CC(Wild)	10 (31.3%)	24 (30.8%)	0.747 [NS]
	CA(Hetro)	17 (53.1%)	37 (47.4%)	
	AA(Mutant)	5 (15.6%)	17 (21.8%)	
Itching	Yes	4 (12.5%)	26 (33.3%)	**0.033 [S]**
	No	28 (87.5%)	52 (66.7%)	
Frequency	Yes	10 (31.3%)	34 (43.6%)	0.230 [NS]
	No	22 (68.8%)	44 (56.4%)	
Urgency	Yes	3 (9.4%)	3 (3.8%)	0.354 [NS]
	No	29 (90.6%)	75 (96.2%)	
Dysuria	Yes	7 (21.9%)	11 (14.1%)	0.317 [NS]
	No	25 (78.1%)	67 (85.9%)	
Pain	Yes	22 (68.8%)	64 (82.1%)	0.125 [NS]
	No	10 (31.3%)	14 (17.9%)	
Esterase test	Positive	13 (40.6%)	41 (52.6%)	0.255 [NS]
	Negative	19 (59.4%)	37 (47.4%)	
Nitrite test	Positive	23 (71.9%)	52 (66.7%)	0.594 [NS]
	Negative	9 (28.1%)	26 (33.3%)	
RBC	< 10	29 (90.6%)	73 (93.6%)	0.195 [NS]
	1+	0 (0%)	3 (3.8%)	
	2+	1 (3.1%)	0 (0%)	
	Nill	2 (6.3%)	2 (2.6%)	
Pus cell	<10	30 (93.8%)	76(97.4%)	0.578 [NS]
	1+	2(6.3%)	2(2.6%)	

Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant, , t-test used for numerical data, and chi-square test for categorical data

3.2.2. Assessment of Demographic and Biochemical Parameters in Urinary Tract Infection Pediatric Before and After 1 weeks of Nitrofurantoin Treatment According to SNP rs2231142

The study revealed that ninety nine patients (90% of the study sample) experienced positive response to drug treatment under the study, whereas 11 patients (10% of total) had resistance to study treatment as shown in **figure(3-12)** below:



Figure(3-12): Numbers and proportions of response of the study patients to treatment.

The mean age of patients was (7.68±2.86) years old with a range of (4-14) years old. In current study urinary tract infection reported to be more

prevalent among younger ages (>7) years old with percentage about (69.91 %) compare to (39.09%) in patients with (<7) years old.

More response to treatment occurred at age group (14-13) years old with percentage about (10%). Less percentage of non-response was recorded in age group (12-10) years old, it was (19%), **table(3-4).**

Table (3-4): Distribution the age groups according to percentage of responder and non- responder among the patients.

Age	Total N. Percentage%	Response N. Percentage%	Non response N. Percentage%
(4-6) years	43 (39.09)	40 (40.4)	3 (27.27)
(7-9) years	36 (32.73)	30 (30.3)	6 (54.55)
(10-12) years	21 (19.1)	19 (19.2)	2 (18.18)
(13-14) years	10 (9.09)	10 (10.1)	0 (0)
Total	110(100)	99 (100)	11 (100)

The analysis of post-treatment response in regard to symptoms of study patients revealed that pain significantly dropped from 78.2% prior to treatment to 7.3% post-treatment. Frequency and dysuria were also significantly dropped from 40% to 3.6% and 16.4% to 1.8% respectively (p value < 0.001). Itching and urgency were dropped from 27.3% and 5.5% to zero respectively as illustrated in **table(3-5)** below.

Table(3-5): Comparison of the effect of drug treatment on the symptoms of the study sample.

Symptoms	Categories	Before treatment	After treatment	P value
Pain	**Yes**	86 (78.2%)	8 (7.3)	**< 0.001 [S]**
	No	24 (21.8%)	102 (92.7)	
Frequency	**Yes**	44 (40%)	4 (3.6)	**< 0.001 [S]**
	No	66 (60%)	106 (96.4)	
Itching	**Yes**	30 (27.3%)	0 (0%)	**-**
	No	80 (72.7%)	0 (0%)	
Dysuria	**Yes**	18 (16.4%)	2 (1.8)	**< 0.001 [S]**
	No	92 (83.6%)	108 (98.2)	
Urgency	**Yes**	6 (5.5%)	0 (0%)	**-**
	No	104 (94.5%)	0 (0%)	
Results are presented as n= number of subjects and percentage **p<0.05 considered significantly different** **[S]= Significant** **[NS]= Non significant** **McNemar Test was used**				

The more reduction in urine levels of the following parameters; PH and specific gravity while with marked change in color, turbidity, ketosis, urobilirubin, protein were reported among them than non-responder children after the period of therapy, **table (3-6).**

Table (3-6): Comparison macroscopic parameters between responder and non-responder to the treatment.

Macroscopic Parameters	Normal value	Non-responder	responder
color	Pale yellow to deep amber	45	65
odor	Odorless (vary somewhat)	47	63
volume	4-8 ounce / 24 hour	-	-
PH	4.5-8	80	30
Specific gravity	1.003-1.030	73	37
turbidity	Clear to very slightly cloudy	26	84

The analysis of post-treatment response regarding signs of study patients showed that positive nitrite test proportion significantly dropped from 68.2% prior to treatment to 7.3% post-treatment (p value < 0.001). Also esterase test significantly dropped from 49.1% to 4.5%. Pus cell proportion was significantly elevated from 3.6% to 21.8% (p value < 0.001) as illustrated in **table(3-7)** below.

Table(3-7): Comparison of the effect of drug treatment on the signs of the study sample.

Signs	Categories	Before treatment	After treatment	P value
Nitrite test	**Negative**	35 (31.8%)	102 (92.7)	**< 0.001 [S]**
	Positive	75 (68.2%)	8 (7.3)	
Esterase test	**Negative**	56 (50.9%)	105 (95.5%)	**< 0.001 [S]**
	Positive	54 (49.1%)	5 (4.5%)	
RBC	**Negative**	106 (96.4%)	106 (96.4%)	0.688 **[NS]**
	Positive	4 (3.6%)	4 (3.6%)	
Pus cell	**Negative**	106 (96.4%)	86 (78.2)	**< 0.001[S]**
	Positive	4 (3.6%)	24 (21.8)	

Results are presented as n= number of subjects and percentage

p<0.05 considered significantly different

[S]= Significant

[NS]= Non significant

McNemar Test was used

The analysis of the effect of the genetic polymorphism on the response to the drug under the study revealed that there was no statistical significant difference of SNPs genotypes (CC, CA and AA) and the response to treatment (p value >o.o5) as shown in **table(3-8)** below.

Table(3-8): Effect of genetic polymorphism on the response to the drug under the study of the sample patients.

Variable		Response		p value
		Negative	Positive	
SNPs	CC(Wild)	2 (5.9%)	32 (94.1%)	0.652 [NS]
	CA(Hetro)	7 (13%)	47 (87%)	
	AA(Mutant)	2 (9.1%)	20 (90.9%)	
Results are presented as numbers and percentage p<0.05 considered significantly different [S]= Significant [NS]= Non significant chi-square test used				

The analysis of the effect of the genetic polymorphism on the symptoms prior and after treatment showed no statistical significant difference of the SNPs genotypes (CC, CA and AA) and the symptoms of the patients of the current study prior to and after treatment (p value >o.o5) as illustrated in **table(3-9)** below.

Table(3-9): Effect of genetic polymorphism on the symptoms of the patients sample.

Variable			SNPs			p value
			CC(Wild)	CA(Hetro)	AA(Mutant)	
Itching	Pre	Yes	10 (29.4%)	13 (24.1%)	7 (31.8%)	0.746 [NS]
		No	24 (70.6%)	41 (75.9%)	15(68.2%)	
	Post	Yes	0 (0%)	0 (0%)	0 (0%)	-
		No	34 (100%)	54 (100%)	22 (100%)	
Frequency	Pre	Yes	11 (32.4%)	23 (42.6%)	10 (45.5%)	0.535 [NS]
		No	23 (67.6%)	31 (57.4%)	12 (54.5%)	
	Post	Yes	1 (2.9%)	3 (5.6%)	0 (0%)	0.815 [NS]
		No	33 (97.1%)	51(94.4%)	22 (100%)	
Urgency	Pre	Yes	1 (2.9%)	5 (9.3%)	0 (0%)	0.329 [NS]
		No	33 (97.1%)	49 (90.7%)	22 (100%)	
	Post	Yes	0 (0%)	0 (0%)	0 (0%)	-
		No	34 (100%)	54 (100%)	22 (100%)	
Dysuria	Pre	Yes	8 (23.5%)	7 (13%)	3 (13.6%)	0.431 [NS]
		No	26 (76.5%)	47 (87%)	19 (86.4%)	
	Post	Yes	2 (5.9%)	0 (0%)	0 (0%)	0.132 [NS]
		No	32 (94.1%)	54 (100%)	22 (100%)	
Pain	Pre	Yes	28 (82.4%)	41(75.9%)	17 (77.3%)	0.826 [NS]
		No	6 (17.6%)	13 (24.1%)	5 (22.7%)	
	Post	Yes	0 (0%)	6 (11.1%)	2 (9.1%)	0.102 [NS]
		No	34 (100%)	48 (88.9%)	20 (90.9%)	
Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant, chi-square test used.						

The effect of the genetic polymorphism on the signs of the disease on the study patients prior and after treatment showed no statistical significant difference of the

SNPs genotypes (CC, CA and AA) and signs of the disease on patients of the study (p value >o.o5) as illustrated in **table(3-10)** below.

Table(3-10): Effect of genetic polymorphism on the biochemical parameters of the disease of the study patients.

Variable			Snip			p value
			CC	CA	AA	
Esterase test	Pre	Positive	15 (44.1%)	30 (55.6%)	9 (40.9%)	0.428 [NS]
		Negative	19 (55.9%)	24 (44.4%)	13 (59.1%)	
	Post	Positive	0 (0%)	4 (7.4%)	1 (4.5%)	0.226 [NS]
		Negative	34 (100%)	50 (92.6%)	21 (95.5%)	
Nitrite test	Pre	Positive	21 (61.8%)	37 (68.5%)	17 (77.3%)	0.476 [NS]
		Negative	13 (38.2%)	17 (31.5%)	5 (22.7%)	
	Post	Positive	2 (5.9%)	5 (9.3%)	1 (4.5%)	0.798[NS]
		Negative	32 (94.1%)	49(90.7%)	21 (95.5%)	
RBC	Pre	Positive	1 (2.9%)	2 (3.7%)	1 (4.5%)	0.951 [NS]
		Negative	33 (97.1%)	52 (96.3%)	21 (95.5%)	
	Post	Positive	2 (5.9%)	1 (1.9%)	1 (4.5%)	0.529 [NS]
		Negative	32 (94.1%)	53 (98.1%)	21 (95.5%)	
Pus cell	Pre	Positive	1 (2.9%)	3 (5.6%)	0 (0%)	0.815 [NS]
		Negative	33 (97.1%)	51 (94.4%)	22 (100%)	
	Post	Positive	3 (8.8%)	14 (25.9%)	7 (31.8%)	0.06 [NS]
		Negative	31 (91.2%)	40 (74.1%)	15 (68.2%)	

Results are presented as n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant , chi-square test used.

The data analysis also showed that there were no statistical significant age and gender difference on the response to treatment (p value>0.05) as illustrated in **table(3-1)** and **table(3-12)** below.

Table(3-11): Effect of age on the response to treatment.

Parameters	***Response***		***P value***
	Positive	***Negative***	
Age	7.7 ± 2.94	7.55 ± 2.12	0.869 [NS]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant. t-test was used			

Table(3-12): Effect of gender difference on the response to treatment.

Parameters		***Response***		***P value***
		Positive	***Negative***	
Gender	Male	28 (28.3%)	4 (36.4%)	0.727 [NS]
	Female	71 (71.7%)	7 (63.6%)	
Results are presented as n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant , chi-square test used.				

Chapter Four

Discussion

4. Discussion

Urinary tract infections (UTIs), is a one of the most frequent infectious disorders, they are classified based on the site of infection, the presence or lack of symptoms, anatomical or functional abnormalities, and underlying illnesses [122].

As the development and outcome of UTI is related to nitrofurantoin response and as no study dealing with ABCG2 polymorphism during UTI children ,so this is the first work in kerbala city focusing on determination of ABCG2 polymorphism with nitrofurantoin response in children with UTI.

4.1. The Demographic and Biochemical Parameter of Urinary Tract Infection Patients Group (Before Treatment)

In this study, UTI were confirmed by symptoms, urinalysis result and culture >50,000 colony forming unit/ml results.

The mean age of the sample of the present study was 7.68±2.86 years, ranged from 4 to 14 years. Girls are much more likely than boys to have UTI greater than one year of age [123]. In men, UTIs are less common due to the environment surrounding the male urethra is drier, the urethra is considerably longer, and prostatic fluid contains antibacterial action. Also There is a greater distance between the usual source of microorganisms that cause a UTI (the anus) and the usual entrance point (the opening of the urethra) [124]. This trend has been shown in the present study that showed the Male accounted for less than one third of the total study sample (29.1%), most of them were circumcised (93.8%) and the other non-circumcised (6.2%), this is disagreement to this research that showed The circumcision protects against or prevent recurrence of UTI in male [125]. The circumcision plays an important cultural and religious role in many societies, and some of studies that looked at the number and type of bacteria present found that

the genitals before and after circumcision had a significant increase in bacteria under the foreskin in uncircumcised boys. E.coli are precisely types of germs that can enter the urinary tract and cause infection. The circumcision is based on medical reasons, and its benefits are believed to include improved hygiene, reduced urinary tract infection (UTI), sexually transmitted diseases, penile cancer[126], some of study refers that Circumcision decreased the risk of UTI and given a risk in normal boys of about 1% [127].

The study revealed that 64.5% of the patients were rural residence From the total sample of the current study, while (35.5%) were in urban that was in agreement with this research that indicates People who live in big cities or urban areas are more likely to consult general practices for urinary tract infection and upper respiratory infections. Both living in a big city and living in the country are linked to an increased incidence of UTIs. Living in a rural region is linked to a higher risk of infection [128]. The data suggests that the living environment may influence the rate of consultation under certain circumstances[129], incorrect antibiotic prescriptions are prevalent for uncomplicated UTI. Rural women are more likely to be given antibiotic inappropriately for an extended period of time . Antibiotic management strategies are needed, especially in rural regions, to improve urologists outpatient antibiotic prescriptions and prevent needless antibiotic exposure [130].

The current study included 150 patients diagnosed with UTI but only 110 of them participated in the study, (76.4%) reported a history of use of other medication Ibuprofen (IBU) for pain of UTI as shown in **figure(3-5),** this agreement with this research that indicates that IBU does not have direct antimicrobial properties and nor does it potentiate the activity of trimethoprim or nitrofurantoin in vitro against E. coli but used for symptomatic relieve [131].

The side effects of nitrofurantoin were recorded by some of them only(10.9%) as shown in **figure (3-6),** but none of these children discontinued the medication and these side effects that included gastrointestinal complaints (anorexia, nausea, vomiting), this agreement with this research referred that adverse symptoms related to Nitrofurantoin ranged from 5%-16% [(132)].

The analysis of the present study revealed that, prior to treatment, from the total 110 patients; pain accounted for the highest proportion of symptoms (78.2% of total), followed by frequency (40%), dysuria (16.4%) and the least reported prior to treatment symptoms was urgency (5.5%). which is likely the result of this research that showed Most patients had symptoms associated with UTI and these symptoms might appear suddenly or gradually, and are frequently linked to the urinary process [(133).]

The analysis of the current study showed that from the total 110 study patients, there were 3 patients (2.7%) experienced disease complication in form of hematuria, The detection of even microscopic amounts of blood in a child's urine alarms the patient, parents, and physician, and often prompts the performance of many laboratory studies. Hematuria is one of the most important signs of renal or bladder disease, but proteinuria is more important diagnostic and prognostic finding except in the case of calculi or malignancies[(134).] Hematuria is almost never a cause of anemia, The physician should ensure that serious conditions are not overlooked, avoid unnecessary and often expensive laboratory studies, reassure the family, and provide guidelines for additional studies if there is a change in the child's course [(135).]

This study relied on dipstick test and microscopic examination to know the presence of disease as well as in response to the treatment due to the result of dipstick (nitrite and leukocyte esterase) and microscopic (examination of pyuria)

and urine culture were all the same in detecting UTI. As showed in Iraq, in this research, the authors observed that the nitrite test has a 95.5 % specificity and 67.3 % sensitivity, when compared to the specificity and sensitivity of pyuria, leukocyte esterase has a specificity of 97.7% and a sensitivity of 74.44%, which are not significantly different. Both tests (nitrite and leukocyte esterase) are quick, inexpensive and available for early identification of UTI while waiting for culture and sensitivity results [136].

leukocyte esterase and nitrite test for UTI patients were calculated retrospectively, The analysis of the present data also showed that, prior to treatment, from the total 110 patients; nitrite test accounted for the highest proportion of signs (68% of total), followed by esterase test (49.1%) and the least reported prior to treatment signs were RBC and pus cells (3.6% for each). Physical, chemical and microscopic examinations constitute a complete analysis of urine. In some hospitals, a urine culture is only done when a urine test paper detects abnormalities. Some studies have anegative urine test strip analysis is useful for ruling out a UTI, according to the finding of this research [137].

Leukocyte esterase is a screening test used to detect substances that indicate the presence of white blood cells in the urine. This may mean that child has a urinary tract infection [138]. This result was agreement with this research [139].

The study also found more reduction in urine levels of the following parameters PH, specific gravity and urobilirubin, and protein with marked change in color, turbidity, ketosis and protein was reported among them than non-responder children after the period of therapy. Proteins, glucose, ketones, blood, bilirubin, and blie derivatives are all commonly analyzed chemical constituents in urine. As showed in this reseaech, Plasma proteins, albumin, globulin, and fibrinogen are all proteins present in urine under pathological situations[140]. This

research conducted When a bromophenol-coated strip is dipped in urine, the color of the strip changes, including the presence of protein in the urine. When the color shift is compared to the color chart on the bottle, proteinuria can be graded semi-quantitatively[141]. Proteinuria can be caused by nephrotic syndrome, diabetes, hypertension, UTI, fever, or polycystic kidney disease. This result was agreement with research that found all seven indicators of urine (color, odor, quantity, consistency, sediment, turbidity and froth of urine) come from physical and microscopic examination help in diagnosis or monitor several condition and disease like DM, kidney, UTI and so on [142].

Also this is similar to the rerearch in which authors noted significant persistent proteinuria in the patients associated with refractory or recurrent UTIs [143].

The analysis of the data also showed that, prior to treatment, from the total 110 patients; nitrite test accounted for the highest proportion of signs (68% of total), followed by esterase test (49.1%) and the least reported prior to treatment signs were RBC and pus cells (3.6% for each) as illustrated in **figure (3-9).** When the LE test is used with the urine nitrite test, it creates a powerful screening tool for predicting a UTI. These tests are used to detect bacteria in the urine in an indirect manner [144]. Leukocyte esterase is an enzyme that indicates the presence of white blood cells, which is usually associated with infection. Nitrites, a waste product from the breakdown of some bacteria, are also detected by the dipstick test [145].

The results of this study confirm and expand the previous findings, of this research[146], that bacterial counts in their urine are less than 10^5 CFU/ml for many children with urinary tract symptom. Furthermore, this research suggest that women with bacteriuria with low count (>10^2 to 10^4 CFU/ml) may be experiencing the early stages of UTI that is limited to the urethra[147].

4.2. Molecular Studies

4.2.1. Genetic Analysis to Assess the Association of ATP Binding Cassette G2 Polymorphism with Urinary Tract Infection Pathogenicity

The analyses were conducted to assess the association between the SNP rs2231142 CC (wild type), CA (heterozygous type), AA (mutated type) with the pathogenesis of UTIs according to logistic regression, this study found the distribution of the single nucleotide polymorphisms (SNPs) of the study patients was recorded as 34(30.9%) for CC genotype, 54(49.1%) for CA genotype and 22(20%) for AA genotype.

There were two types (≥1%) of common missense genetic variants in the ABCG2 gen (Q141K; rs2231142 and V12M; rs2231137) and only one common nonsense variants (Q 126X; rs72552713) that is specific to south East Asian groups [148].

No researches present about this SNP with UTI but this SNP studied with other disease such as this research that shown that SNP C421A of the ABCG2 gene predisposed to an increased individual risk of developing multiple myeloma [149].

The results of amplifications were analyzed and three genotypes (wild, mutant and heterozygous) were obtained for each SNP. Showed in **figure (3-10)** Lane (2and3) represented : CA heterozygous genotype , Lane (4 -8) represented :CC genotype (wild) all were showed in (197 bp).

4.2.2. Analysis of ATP Binding Cassette G2 Polymorphism on Nitrofurantoin Efficacy with Comparison of Gender

At the beginning nitrofurantoin should be avoided in patients with moderate-severe renal failure (creatinine clearance <50 mL/min) due to poor excretion of the medicine so increased systemic accumulation lead to sub therapeutic level in urinary tract(150).

The analysis of the data showed that there were no significant statistical gender differences in regard to socio-demographic characteristics, SNPs and signs and symptoms of the study patients except for itching (p=0.033) as illustrated in **table(3-3).**

this result found the most common percentage of itching processes in the female (33.3%) more than male (12.5%) with significant differences (P< 0.05) the most important causes of this mechanism were yeast infections usually cause pain and itching of the genitals and a thick, curd-like discharge. UTI are a type of infection that affects the lower urinary system, which includes the urethra and bladder in some girl this results agreement with this research (151). Another study found that group B streptococcus spp. are other source of the vaginal infection characterized by inflammation and itching, these type of infections are frequently misdiagnosed for yeast since the symptoms are so similar with this research(152).

4.2.3. Assessment of Demographic and Biochemical Parameters in Urinary Tract Infection Children Before and After 1 weeks of Nitrofurantoin Treatment According to SNP rs2231142

The study revealed that 99 patients experienced positive response to drug treatment under the study, whereas 11 patients (10% of total) had resistance to study treatment as shown in **figure(3-12).** Different finding were reported by this

research in which these authors observed 30.1% of Escherichia coli isolates showed resistant against nitrofurantoin[153] .

The multidrug ABCG2 efflux transporter makes it an important determinant of the pharmacokinetics of a variety of substrate drugs. Polymorphisms that change ABCG2 expression or function could have therapeutic implications for medication distribution and efficacy [98], the most well-studied SNP, rs2231142, has been demonstrated to reduce ABCG2 expression and activity, resulting in higher total drug exposure and lower resistance to a variety of substrates [102].

In current study urinary tract infection was reported to be more prevalent among younger ages (>7) years old with percentage about (60.91%) compare to (39.09%) in patients with (<7) years old **table (3-4).**

The clinical signs and symptoms of a UTI vary depending on the childs age, but all febrile children aged two to 24 months with no evident source of infection, with the exception of circumcised boys older than 12 months, should be tested for UTI [154].

The analysis of post-treatment response in regard to symptoms of study patients revealed that pain significantly dropped from 78.2% prior to treatment to 7.3% post-treatment, A number of conditions can cause dysuria. UTIs are a common cause of painful urination . Frequency and dysuria were also significantly dropped from 40% to 3.6% and 16.4% to 1.8% respectively (p value < 0.001). Itching and urgency were dropped from 27.3% and 5.5% to zero respectively as illustrated in **table (3-5).** Therefore, it was noticed that this dropping stage is due to the use of antibiotics nitrofurantoin , it is activated inside bacteria by reduction via the flavoprotein nitrofurantoin reductase to unstable metabolites, which disrupt

ribosomal RNA, DNA and other intracellular components. It is bactericidal, particularly against germs found in acid urine [155].

The analysis of post-treatment response regarding signs of study patients showed that positive nitrite test proportion significantly dropped from 68.2% prior to treatment to 7.3% post-treatment (p value < 0.001). Also esterase test significantly dropped from 49.1% to 4.5%. Pus cell proportion was significantly elevated from 3.6% to 21.8% (p value < 0.001) as illustrated in **table (3-7).** Normal urine contains chemicals called nitrates. If bacteria enter the urinary tract, nitrates can turn into different, similarly named chemicals called nitrites. Nitrites in the urine could indicate a UTI [156], So the quick shift of nitrite to nitrate was due to the antibacterial drug, nitrofurantoin, interfering with vital processes in bacteria, resulting in their death [138]. Nitrofurantoin quickly reaches therapeutic concentration in the urine as well as cleared quickly [157].

The analysis of the effect of the genetic polymorphism on the response to the drug under the study revealed that there was no statistical significant difference of SNPs genotypes (CC, CA and AA) and the response to treatment (p value >o.o5) as shown in **table(3-8),** the positive response was illustrated as 32 (94.1%), 47 (87%) and 20 (90.9%) for CC, CA and AA, respectively, this disagreement with this research In 305 Chinese patients with hypercholesterolemia who were treated with rosuvastatin at a dosage of 10 mg daily, the c.421A variant was found to be significantly associated with greater reduction in low density lipoprotein-c level (LDl-C), in a gene-dose-dependent manner. As compared with subjects with the c.421CC genotype, those with the c.421AA genotype showed a 6.9% greater reduction in LDL-C level, which would be equivalent to the effect obtained by doubling the dose of rosuvastatin [158].

On the other hand the analysis of the effect of the genetic polymorphism on the symptoms prior and after treatment showed no statistical significant difference of the SNPs genotypes (CC, CA and AA) and symptoms of patients of the current study prior to and after treatment (p value >o.o5) as illustrated in **table (3-9)** for the effect of genetic polymorphism on the symptoms of the sample patients, which include the following (Itching, Frequency, Urgency, Dysuria, Pain).

The effect of the genetic polymorphism on the signs of the disease on the study patients before and after treatment showed no statistical significant difference of the SNPs genotypes (CC, CA and AA) and signs of the disease on patients of the study (p value >o.o5) as illustrated in **table(3-10).** It was also found data analysis showed that there were no statistical significant age and gender difference on the response to treatment (p value>0.05) as illustrated in **table (3-11)** and **table (3-12),** this result recorded the effect of age on the response to treatment (7.7 ± 2.94) (7.55 ± 2.12) for positive and negative response to nitrofuraton antimicrobial agent. So that these findings are different from to the findings of this research in which the authors concluded that ABCG2 is an important determinant for the bioavailability of nitrofurantoin and the main mechanism involved in its hepatobilliary excretion [159].

Finally, and from the current study all these parameters which involved in this work summarized by a fact that, the ABCG2 polymorphism had no effect on therapeutic response of nitrofurantoin in urinary tract infection children which firstly referred in our city.

4.3. Conclusions

From the results of the present study it was concluded that the:

-Genetic polymorphism of ABCG2 (rs2231142) may be not associated with the pathogenesis of UTI in selected clinics/hospitals in kerbala children.

-ABCG2(rs2231142) polymorphism not participated therapeutic resistance to nitrofurantoin in selected clinics/hospitals in kerbala children diagnosed with UTI.

4.4. Recommendations

1. Additional SNPs of the ATP binding cassette G2 (ABCG2) gen has to be studied to confirm the role of ATP binding cassette G2 (ABCG2) polymorphism towards UTI and to determine the correlation of different response for nitrofurantoin treatment in UTI children.

2.ATP binding cassette gene polymorphism has to be studied to determine its effects on UTI pathogenicity and variable nitrofurantoin response in children with this disease .

3. Sequencing of this gene is recommended.

4. Using nitrofurantoin antibiotic for treatment UTI of E-coli.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Reference

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Appendixes

Appendix 1: The Consent Form

University of Karbala

Consent to be in Research

Study Title :- Genetic polymorphism of ATP binding cassette G2(*ABCG2)* transporter and relationship with Therapeutic Response of Nitrofurantoin in children with urinary tract infection.

The Researcher Name:- Thura Hassan Al-tayyar

This is a medical research study. The researcher and pediatrician Wisam Khalid Al-samermed F.I.C.Ms will explain this study to you. If you have any questions, you may ask me and/or the doctor.

You are being asked participate in this study because you have urinary tract infection and treated by nitrofurantoin.

In this study, the researcher are collecting blood samples from you to learn more about the association of genetic polymorphism of ABCG2 with therapeutic response of nitrofurantoin.

If you agree to be in this study, you will go to the laboratory and give a blood sample for one time only. The blood will be drawn by putting a needle into a vein in your arm. One small tube of blood will be taken. Also take urine before and after treatment. This will take about five minutes.

The risks?

The needle stick may hurt. There is a small risk of bruising and fainting, and a rare risk of infection.

Will my medical information be kept confidential?

We will do our best to protect the information we collect from you and your medical record. If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used. Information that identifies you will be destroyed when this research is complete.

If you wish to be in this study, please sign below.

Name of participant:

Signature

/ /

Appendix 2: The Questionnaire Form
Questionnaire of Urinary tract infection (UTI) Patients

Name:

Address: mobile number:

parameter		variable	Notes
Age	العمر		
Gender	الجنس		
For males(circumcise or not)	بالنسبة للذكور(مختون او لا)		
Drug side effects	اعراض جانبية للدواء		
Duration of treatment with nitrofurantoin	مده اخذ العلاج		
complications	مضاعفات المرض		
Other diseases	الامراض الاخرى		
Other medication	الأدوية الاخرى		

Demographic characterizations Parameters	Pre_tretment	Post_treatment
Clinical response		
Detection of bacteriuria by nitrite test		
Detection of pyuria by leukocyte esterase tests		
Direct Detection of pyuria by urine microscopy		

الخلاصة

المقدمة: المسالك البولية هي واحده من اكثر مناطق الالتهاب البكتيري شيوعا في البشر. يتميز التهاب المسالك البولية السفلى , مثل المثانة يمتاز دائما بأعراض مثل كثره التبول والالحاح وصعوبة التبول. اذا تركت هذه العدوى دون علاج, يمكن ان تتطور الى التهابات المسالك البولية العلوية تسمى التهاب الحويضة او التهاب الكلى.

نايتروفيورانتوين هو مضاد للبكتيريا من عائله النايتروفيوران المصنعة والذي تم استخدامه لأكثر من 50 عاما. لايزال يعمل ويستمر وصفه, خاصتا في العيادات الخارجية لمرضى المسالك البولية غير المصحوبين بمضاعفات , لاسيما في تركيبته الجزيئية الميكروية , ماكرو دانتين.

الجين ABCG2 الموجود على الكروموسوم 4q22 يشفر 655 بروتينات مقاومه لسرطان الثدي . يشبه بروتينات فصيله Gالاخرى لناقل ربط ABC , فان البروتين المقاوم لسرطان الثدي عباره عن شبه ناقل يحتوي على مكان ربط بالنيوكليوتيدات ومكان عبر غشاء مدمج في سلسله متعددة الببتيدات .يحتوي البروتين المقاوم لسرطان الثدي على شكل وظيفي يشبه الهومودايمر بوزن جزيئي قدره 144 كيلو دالتون.

يؤدي التغيير الجيني, rs 2231142 , الى تغيير اليوكليوتيدات السايتوسين الى الادنين ABCG2 C421A, وهو تعدد اشكال النيوكليوتيدات الفردي المرتبط بانخفاض نشاط العبور /النقل البروتيني في المختبر وتركيزات اعلى من الأدوية المضادة للسرطان في ناقلات تعدد الاشكال C421A .

الهدف من الدراسة: صممت هذه الدراسة لتقييم الارتباط المحتمل بين تعدد الاشكال الوراثية للناقل ABCG2 وعلاقته بالإصابة بعدوى المسالك البولية. وايضا لتقييم العلاقة المحتملة بين تعدد الاشكال الوراثية لنفس الناقل بالاستجابة العلاجية للنايتروفيورانتوين (بعد اسبوع واحد من علاج النايتروفيورانتوين من تناول 6 ملغم / كغم اربع مرات يوميا)للأطفال المصابين بعدوى المسالك البولية في عيادات ومستشفيات مختارة في محافظة كربلاء.

الاشخاص وطرق العمل: تضمنت الدراسة (110 فرد من الذكور والاناث تتراوح اعمارهم بين 4-14سنه) تم تشخيص اصابتهم بالتهاب المسالك البولية التي اجريت في استشارية كل من مستشفى كربلاء التعليمي للأطفال ومستشفى الحسينية وعيادات الاطفال الخاصة.

بمتوسط جرعه 6 ملغم/كغم كل 4 مرات يوميا لمدة اسبوع واحد, وتم الحصول على عينات الدم من المرضى المؤهلين الذين وافقوا على الاختبار الوراثي. كما تم جمع الادرار من نفس المجموعة وتم اخذ قراءتين لفحص البول العام قبل وبعد العلاج. النمط الجيني ABCG2C421A تم تحديده بعنايه بواسطه تقنيه

النتائج: كان النمط الوراثي نوع CC وCA اكثر شيوعا في مجموعة الدراسة هذه من النمط الوراثي المتغير AA. حيث وجد CC في 34(30,9 %) في الاشخاص, بينما كان CA موجود في 54(49,1 %) اما التركيب الوراثي المتغير 22(20 %) فقط من الدراسة. ومن ناحية اخرى اظهرت النتائج التي تم الحصول عليها من هذه الدراسة عدم وجود فروقات معنويه احصائيه في كل انواع الطفرات (AA,CC,CA) واستجابتهم للعلاج.

الاستنتاج: بينت الدراسة الحالية الى ان النايتروفيورانتوين ليس له ارتباط مع تعدد الاشكال الوراثية للناقل ABCG2 في عدوى المسالك البولية لدى الاطفال في عيادات ومستشفيات مختاره في محافظة كربلا وبغض النظر عن التراكيب الوراثية.

تشير هذه النتائج الى ان النايتروفيورانتوين ليس ركيزة مسبار سريري مناسب لتقييم فعالية BCRP.

النايتروفيورانتوين فعال جدا في هذه الدراسة.

جمهورية العراق

وزاره التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الصيدلة

تعدد الاشكال الوراثية لناقل ABCG2 وعلاقته بالاستجابة العلاجية للنيتروفيورانتوين في الاطفال المصابين بالتهاب المسالك البولية في عيادات ومستشفيات مختارة في محافظة كربلاء

رسالة مقدمه الى

كلية الصيدلة في جامعة كربلاء كجزء من متطلبات درجة الماجستير في الادوية والسموم

من قبل

ذرى حسن عبدالجليل الطيار

بكالوريوس صيدلة

بأشراف

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