GENETIC POLYMORPHISM OF ATP BINDING CASSETTE G2( ABCG2) TRANSPORTER AND RELATIONSHIP WITH THERAPEUTIC RESPONSE OF NITROFURANTOIN IN FEMALE IN KERBALA CITY.

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ABSTRACT
Recent genome-wide association and functional studies have shown that the ABCG2 gene encodes for a drugs transporter, and a common causal 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, cross-sectional observational study, 78 patients (male) with urinary tract infection was identified. with an age 4-14 years. The subjects were newly diagnosis and give nitrofurantoin treatment, dipstick tests are effectively for screening, definitive confirmation requires urine culture, The patient with UTI. received Nitrofurantoin for 1 weak, with no other disease, mean dose was 6mg/kg divided by 4 time per day, genotyping and detection of C421A gen polymorphisms in the ABCG2 gen in urinary tract infection patients' blood samples, an allele specific-PCR (AS- PCR) approach was used in the current study, The study showed that more than three quarters of the patients of the current study (76/97.43%) reported a history of use of other medication (Ibuprofen) and (2/2.57%) have no any medication, the history of drug side effects revealed that about nine out 78 of the female patients of the present study (11.53%) had GIT upset, our results was recorded the wild type (CC allele) 24 cases (30.77%), heterozygosity (AC allele) 38 cases (48.72%) and 16 cases homozyoite mutant (20.51%) 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.

Key words: ABCG2 polymorphism, allele specific PCR and urinary tract infection

I. INTRODUCTION
The urinary tract (UTI) is one of the most common area of bacterial infection in humans.(1) The infection of lower urinary tract (UTIs), such as cystitis, are always characterized by symptoms such as frequent urination, urgency, and difficulty urinating.(2)If left untreated, these infections can develop into upper urinary tract infections called acute pyelonephritis or kidney infections, which may be associated with other symptoms such as fever, nausea, vomiting, and back pain. This infection may also increase the risk of developing bacteremia. It is estimated that 40% of women and 12% of men will have symptomatic UTIs during their lifetime, and about a quarter of affected women will have recurrent UTIs within 6-12 months.(3) Infants and children are also susceptible to UTIs. UTIs in children are often associated with the possibility of vesicoureteral reflux and kidney scarring. Urinary tract infection in pediatric patients may predispose to infection in adults.(4).

Urinary tract infection can be classified as simple or complex. Minor infections occur in patients who were originally considered healthy. On the other hand, complicated urinary tract infections occur in patients who have been damaged in some way, for example, if the urinary tracts have anatomical or functional abnormalities, have another disease, have a weak immune system, or are undergoing long-term catheterization.(5)

Most minor urinary tract infections are caused by E. coli. In contrast, complex UTIs, especially those associated with long-term catheters, may be a variety of microorganisms. These infections are usually caused by Proteus mirabilis, Proteus stewarti, morganella, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa. (6)
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 mechanism of action of nitrofurantoin is unclear, but the activity appears to require reduction of the enzyme in bacterial cells (7).

The ABCG2 gene located on chromosome 4q22 encodes 655 BCRP amino acid breast cancer resistance proteins (8). 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(9).

Genetic background largely determines the dynamics of BCRP, ranging from single nucleotide polymorphisms (SNPs) to large chromosomal aberrations that alter protein properties and functions, leading to increased cancer risk (10).

The aim of study is to determine the association of genetic polymorphism of ABCG2 with therapeutic response of Nitrofurantoin in children with UTI.

II. MATERIALS AND METHODS

Place and Blood sampling:
This is a prospective study conducted on 78 patients (female) with an age 4-14 years. with urinary tract infection was identified. This case were newly diagnosis and treatment with nitrofurantoin. The study was conducted at outpatient in Karbala teaching hospital of pediatrics, Al-Hussenia hospital and private clinic of pediatric, in this time all samples collection were sent to laboratory in the department of pharmacology and toxicology, college of pharmacy, Karbala university at Karbala province, between March 2020 to April 2021. although to dipstick tests are effectively for screening, definitive confirmation requires urine culture (11), samples of urine were taken using a fresh midstream urine sample (12).

The Nitrofurantoin received from patient suffer from UTI for 1 weak, with did not infected with other disease and the mean dose was 6mg/kg divided by 4time per day.

Exclusion criteria which include patient were receiving other drugs (antibiotic, antacid), patients infected with other disease such as renal failure, G6DP deficiency and patients with fever. For genotyping and detection of C421A gen polymorphisms in the ABCG2 gen in Urinary tract infection patients' blood samples, urine samples were collected from patients showing symptoms of urinary tract infection in sterile universal containers as described by (13), these samples were labeled with special code for each participant, These urine samples collected were divided into two portions. The first portion was for the direct microscopic examination. The urine samples were shacked 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(8). By using standard loop method the second portion was cultured on the blood agar and maccuny agar. After incubation, the culture plates were examined every 12hr, and bacterial growth of ≥ 10^3 CFU/ ml was considered a significant cut off for bacteriuria (14). All urine samples were cultivation by using laboratory prepared media blood agar, MacConkey agar and muller hinton agar were used for detection the type of bacterium.

Molecular analysis:
An allele specific-PCR (AS- PCR) approach was used. The PCR primers oligonucleotides for ABCG2 gene were designed by present study. These primers were provided from Iraq scientific researcher, Al- Diwaniyah shows the sequence of the as PCR primer. These primers were designed from an IE of Salm. enteritidis (accession number NC_000004.12) by computerized sequence analysis.

Table (1) primer nucleotides for ABCG2 polymorphism
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. The time and temperature of PCR in a thermocycler were obtained as the following steps: The PCR product was denatured at 95°C for 5 min, followed by amplification through 35 cycles of denaturing, annealing and extension. The denaturation was performed at 95°C for 30 s, annealing 57°C for 55 s, and final extension was performed at 72°C for 5 min.

**Statistical analysis**

Statistical analysis was done using Sas version 23.0 with the mean +/- SD will be used for continuous variables; frequency and percentages for categorical variables; and Chi-square test for categorical variables.

### III. RESULT AND DISCUSSION

The mean age of the sample of the present study was 7.68±2.86 years, ranged from 4 to 14 years. Most of them have abdominal pain (27/34.62%) and (22/28.2%) have voiding frequency, (18/23%) have urinary incontinence and finally (11/14.1%) have dysurea as showed in figure (1).

**Figure(1): Numbers and proportions of the circumcised female of the study sample.**

Young girls may also complain of painful urination when they have associated symptoms, such as the itchiness seen on pinworms (pinworms). The difficult urinating can be caused by a variety of infectious and non-infectious causes, but it usually results from one of the following common illnesses in children and adolescents. Most children who complain of dysuria have primary urogenital diseases (Demetriou et al., 1982), the most common causes of urinary incontinence is constipation, infections, diet, emotional stress, and sexual abuse (Schmidt, & Copp, 2015).

The study revealed that (51/ 65.83%) of the patients were rural residence and (27/34.62%) have urban region figure (2).
Figure (2): Distribution of the patients of the study according to the residence

One of the most girls have uncomplicated urinary tract infections was improper antibiotic prescription. Rural girls are more likely to take antibiotics inappropriately for a long time. Antibiotic management interventions are needed to improve outpatient antibiotic prescriptions for urologists and reduce unnecessary antibiotic exposure, especially in rural area (Clark et al., 2021).

The study showed that more than three quarters of the patients of the current study (76/97.43%) reported a history of use of other medication (Ibuprofen) and (2/2.57%) no have any medication as shown in figure (3)

Ibuprofen is one of the most successful drugs used worldwide for the treatment of mild to moderate pain and various inflammatory condition, The drug is remarkably free of side-effects and no serious toxicity has been demonstrated in extensive clinical trials, apart from one case of gastrointestinal bleeding and Minor toxic effects which have been reported e.g. Anorexia; nausea and vomiting; dyspepsia; upper abdominal pain; constipation

Figure (4): Numbers and proportions of the history of use of other medication of the study sample

The history of drug side effects revealed that about nine out 78 of the female patients of the present study (11.53%) had GIT upset figure(5).
There are various anatomic, physiological, and emotional factors peculiar to young children age group that present significant difficulties and challenges for getting drugs. Most Childhood poisoning is a major cause of morbidity in both developing and developed countries. In spite of the success of some interventions to prevent accidental poisoning in the pediatric population, toxic ingestions continue to be a common occurrence (20).

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 (6), our results was recorded the wild type (CC allele) 16 cases (20.51%), heterozygosity (AC allele) 38 cases (48.72%) and (AA Allele) 24 cases homozyoite mutant (30.77%).

A loss-of-function mutation (Q141K, rs2231142) in the ATP-binding cassette, subfamily G, member 2 gene (ABCG2) has been shown to be associated with female have urinary tract infection. Previous studies have shown...
that the association between rs2231142 and uric acid and gout is stronger in men than in women, suggesting that sex modifies this association (Kolz et al., 2009).

The allelic frequency of C allele was 0.551 while A allele is 0.449, P values for Hardy-Weinberg equilibrium were ≥0.05 in all study population.

![Figure(7): Distribution of the single nucleotide polymorphisms (SNPs) genotypes of the study patients.](image)

The study revealed that eighty nine point seven percentage patients (70 cases of the study sample) experienced positive response to drug treatment under the study, whereas 8 patients (10.3% of total) had resistance to study treatment nitrofurantoin antimicrobial agents as shown in figure (8)

![Figure (8): Numbers and proportions of response of the study patients to treatment](image)

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
The association of the causal ABCG2 rs2231142 variant with

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 >0.05) as shown in table(4)

Table (4): Effect of genetic polymorphism on the response to the drug under the study of the sample patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>Response</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>SNPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>24</td>
<td>2 (8.3%)</td>
<td>20 (91.7%)</td>
</tr>
<tr>
<td>CA</td>
<td>38</td>
<td>5 (13.4%)</td>
<td>33 (86.6%)</td>
</tr>
<tr>
<td>AA</td>
<td>16</td>
<td>3 (18.75%)</td>
<td>13 (81.25%)</td>
</tr>
</tbody>
</table>

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

The association of the causal ABCG2 rs2231142 variant with Nitrofurantoin antimicrobial agents levels was confirmed in a sample of Iraqi children. Our study emphasizes the importance of this common causal variant in a population with a low risk allele frequency, especially as more young children adopt a Urinary tract infections.

REFERENCES


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