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Effect of Polymorphisms in Drug Transporters on Cisplatin Efficacy and Nephrotoxicity in Paediatric Osteosarcoma

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: Osteosarcoma (OS) is the most common type of primary bone tumor in children and adolescents. Chemotherapeutic resistance to cisplatin represents such a significant barrier in the successful treatment of Osteosarcoma. The degree of nephrotoxicity and drug resistance (poor tumor necrosis) is associated with cisplatin accumulation in cells which is governed by Copper transporter protein 1 (CTR1) and Organic cation transporter 2 (OCT2). This study **aims** to determine the allelic frequency of CTR1 and OCT2 single nucleotide polymorphisms (SNPs) in osteosarcoma patients. In addition, detect the relation between SNPs in transporters and cisplatin efficacy or nephrotoxicity.
Methods: A group of 120 pediatric osteosarcoma patients was recruited and genotyped for CTR1, rs7851395, and OCT2 rs316019. We detected the allelic frequency of the two gene polymorphisms. We defined good responders versus poor responders depending on tumor necrosis parameters and looked at nephrotoxicity and serum electrolytes according to CTCAEv4 using the Chi-square test ($\chi 2$) and Kruskal-Wallis value, the odds ratio and confidence interval were calculated too.

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Results: We found that the C allele in (rs316019) OCT2 polymorphism was the dominant allele, and patients with (C/C genotype) were the dominant genotype (72%). and the "A" allele is the dominant allele in (rs7851395) CTR1 and patients with (A/A genotype) were (39.5%).
Conclusion: the study had found that the "C" allele is the dominant allele in (rs316019) OCT2 and the "A" allele is the dominant allele in (rs7851395) CTR1, the study didn't find any significant relation between CTR1, OCT2 polymorphisms and cisplatin response or nephrotoxicity and farther multi center studies need to be done.

Keywords: Polymorphisms; osteosarcoma; nephrotoxicity; CTR1; OCT2.

1. INTRODUCTION

1.1 Background

Osteosarcoma is the most common primary malignancy of the bone most commonly diagnosed, especially in children and young people. Incidence rates of Osteosarcoma in females are almost higher than those observed in males who are less than 15 years of age (0–14 years) but it increases in male puberty [1]. Primary osteosarcoma typically occurs during the second and third decades of life and are rare in patients younger than six or older than 60 years [2].

Cisplatin was approved in the United States for cancer treatment after deep research and considered as the first platinum-based compound approved by Food and Drug Administration (FDA) [3].

Cisplatin is an alkylating agent used to treat many human cancers. Its mode of action is related to its ability to interfere with DNA's purine bases, interfere with DNA repair mechanisms, cause DNA damage, and induce apoptosis of cancer cells [4]. Treatment with cisplatin can lead to nephrotoxicity, ototoxicity, neurotoxicity, infections, and secondary gastrointestinal toxicity [5]. The most important complication of cisplatin treatment is the severe and irreversible damage to the kidney, limiting further treatment or even threatening life. About a third of patients treated with a single dose of cisplatin (50–100 mg / m2) will have renal impairment [6].

Pharmacogenomics is applying genetic information in predicting an individual's response to the drug, which plays an essential role in decision-making regarding precision medicine. It has been found to reduce the risk of adverse events and improve patient healthcare outcomes [7]. The extent of variations determined by inherited factors is currently supposed to account for 15–30% of inter-individual differences in drug response [8]. Copies of one specific gene present in a population may not have identical nucleotide sequences, these different gene copies are called single nucleotide polymorphism (SNP) [9].

Cisplatin is transported to kidney proximal tubule cells by copper transporter 1 CTR1 and organic cation transporter 2 OCT2 on the basolateral membrane [10]. cisplatin is excreted by the kidneys through glomerular filtration with the help of OCT2 [11]. CTR1 has a role in cisplatin distribution in cancer cells [12]. Cisplatin resistance is common and represents a significant barrier to successful chemotherapy [13]. Other several transporters contribute to cisplatin accumulation in the cancer cells like AQP2, AQP9, MVP, and LRP. It was found that increasing the expression of these transporters may affect platinum sensitivity [14]. Single nucleotide polymorphisms (SNPs) in cisplatin transporter genes are believed to make a difference in increasing or protecting against nephrotoxicity besides affecting on sensitivity or resistance of cisplatin [11,15]. Genetic polymorphisms of CTR1 at rs 7851395 was associated with platinum resistance in NSCLC patients [16]. SNPs in the OCT2 gene SLC22A2 (rs316019) was associated with reduced cisplatin-induced nephrotoxicity in patients [17].

To date, there is no data about the prevalence of CTR1 and OCT2 polymorphisms in Egyptian osteosarcoma patients who receive platinum-based regimens; therefore, this study assesses the frequency of these SNPs in this population to determine the degree CTR1, OCT2 polymorphisms could affect cisplatin response or nephrotoxicity.

2. MATERIALS AND METHODS

2.1 Patients

This study included 120 newly diagnosed osteosarcoma patients with initial nonmetastatic extremity sites. According to the treatment

protocol for Osteosarcoma, patients received two cycles of cisplatin 120 mg/m2 /course (120 mg/m²) at week 1 and week 6 of the treatment plan.

Patients were examined for CTR1 (rs7851395) (assay1) and for OCT2 (rs316019) (assay2).

Eligibility criteria

Inclusion criteria

- Patients are less than 18 years at the date of diagnostic biopsy.
- Newly diagnosed patients with primary extremity nonmetastatic with Histological evidence of high-grade Osteosarcoma.
- All patients must be planned to receive cisplatin for at least two cycles as part of their treatment protocol.
- Patient must fulfill prerequisites to receive chemotherapy which are:
  Neutrophils > 1.5 x 10^9/L (or WBC > 3 x 10^9/L if neutrophils are not available) and platelet count > 100 x 10^9/L.. Glomerular Filtration Rate > 70 mL/min/1.73 m^2. Serum bilirubin < 1.5 x ULN.
- Parents/guardians signed informed consent.

Exclusion criteria

- Patients with metastatic disease at initial presentation.
- Low-grade central, periosteal and parosteal osteosarcomas.
- Patients with a secondary malignancy.
- Any previous treatment for Osteosarcoma.

2.2 Methods

Whole blood 5 mL was collected in EDTA tubes from each patient. Genomic DNA was extracted from whole human blood using Gene Jet Whole Blood genomic DNA purification kit (ThermoFisher Scientific) according to manufacturer protocol.

Genotyping analysis of (assay 1) CTR1 (rs7851395) and (assay 2) OCT2 (rs316019) were performed using Taqman® assay (ThermoFisher Scientific). Components of genotyping are the following: (3.5 µL purified water, 5 µL master mix, 0.5 µL assay and 1 µL DNA sample). PCR (QuantStudio™ 6 Flex Real-Time PCR System) ran at 95°C for 10 minutes, thermo cycling (40 cycles (90°C for 15 seconds and 60°C for 1 minute). Extension at 60°C for 30 seconds.

2.3 Clinical Assessment

According to the standard clinical protocol, initial assessment:

- Complete blood count and blood chemistry; (creatinine, urea, sodium, potassium, calcium, magnesium, phosphate, alkaline phosphatase, albumin, bicarbonate, liver transaminase, bilirubin).
- Urine phosphate and creatinine
- Measurement of glomerular filtration rate (GFR).

  Before each cycle of chemotherapy, patients were assessed by:

- Blood chemistry (creatinine, urea, sodium, potassium, calcium, magnesium, phosphate, alkaline phosphatase, albumin, bicarbonate, liver transaminase, bilirubin).
- All grades of nephrotoxicity and electrolytes were assessed according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Data regarding renal function tests, serum creatinine, and electrolytes were collected.

2.4 Pathology

2.4.1 Initial diagnosis

The cases were diagnosed as conventional osteosarcoma high grade, telangiectatic Osteosarcoma, or high-grade surface Osteosarcoma according to criteria described in the WHO classification of tumors of soft tissue and bone (2020) [18].

2.4.2 Assessment of cisplatin response

Assessment of chemotherapy response was based upon examination of coronal and sagittal slabs from the resected bone, demonstrating the tumor. Therapy response equal to or more than 90% was considered a good response, while therapy response less than 90% was regarded as a poor response according to CAP guidelines [19].

2.5 Statistics

Data were statistically analyzed using SPSS software (Statistical Package for the Social

Sciences, version 19, SPSS Inc. Chicago, IL, USA). For quantitative data, the range, mean, median, and standard deviation were calculated. For qualitative data, which describes a definite set of data by frequency, percentage, or proportion of each category, a comparison between two groups and more was made using the Chi-square test ($\chi 2$). For comparison between more than two means of parametric data, the F value of the ANOVA test was calculated. For comparison between more than two means of non-parametric data, Kruskal-Wallis ($\chi 2$) value was calculated. The odds ratio and confidence interval were calculated. Significance was adopted at $p<0.05$ [20]. Sample size was calculated using open EPI software (www.openepi.com). The sample size was calculated to be at least 100 patients using effect sizes [21,22].

3. RESULTS

3.1 Patients

The study included 120 patients who presented between 2009 and 2013. All cases were diagnosed with a nonmetastatic Osteosar coma at an extremity site. Most of the cases were at the lower limb as shown in Table (1). The number of lower limb extremities was 114 divided by 69 cases, 38 cases, and 6 cases for femur, tibia, and fibula, respectively.

3.2 Patients Genotyping and Allele Frequency

114 patients were examined for CTR1 polymorphism (rs7851395) and 120 patients were examined for OCT2 polymorphism (rs316019).

We found that in CTR1 (rs7851395) polymorphism, the frequency of (A) allele was (0.60) and (G) allele was (0.39). Genotype frequency for (A/A) (homo1/1) was 39.5%, (G/G) (homo2/2) was 21.2% and heterozygous (A/G) (hetero1/2) was 39.5%as shown in Table (2).

For OCT2 (rs316019) polymorphism, the frequency of (C) allele was 0.85 and (T) allele was 0.15. Genotype frequency for (C/C) (homo1/1) was 72.5%, for (C/T) (hetero1/2) was 24.2% and for (T/T) (homo2/2) was 3.3% as shown in Table 2.

3.3 Correlation of CTR1, OCT2 Polymorphisms with Drug Response

The drug response was assessed by the percentage of tumor necrosis parameter. Good responders and bad responders classification criteria based on examination of coronal and sagittal slabs from the resected bone, demonstrating the tumor, whereas (>90%) considered good responders and (<90%) considered poor responders according to CAP guidelines [19].

Table 1. Patient's characteristics

Variables	n	%
Sex:		
Female	57	47.5
Male	63	52.5
Age:		
Range	4.00-17.86	
Mean ± SD	12.47±3.30	
Median	13.10	
Tumor Site		
Lower limb:	114	95
Femur	69	57.5
Tibia	38	31.6
Fibula	6	5
Upper limb:	7	5.8
Humerus	5	4
Radius	1	0.8
Ulna	1	0.8
Survival status:		
Alive	101	84.1
Dead	17	14.1
Lost follow up	2	1.6

Table 2. Alleles frequency and Genotypes of both CTR1, OCT2 polymorphism of the studied children with osteosarcoma

Type	No.	Allelic frequency	Genotypes	Patients	
				n	%
1-CTR1 (rs7851395)	114	G= 0.39	(G/G)	24	21.1
		A= 0.60	(A/G)	45	39.5
			(A/A)	45	39.5
2-OCT2 (rs316019)	120	C= 0.85	(C/C)	87	72.5
		T= 0.15	(C/T)	29	24.2
			(T/T)	4	3.3

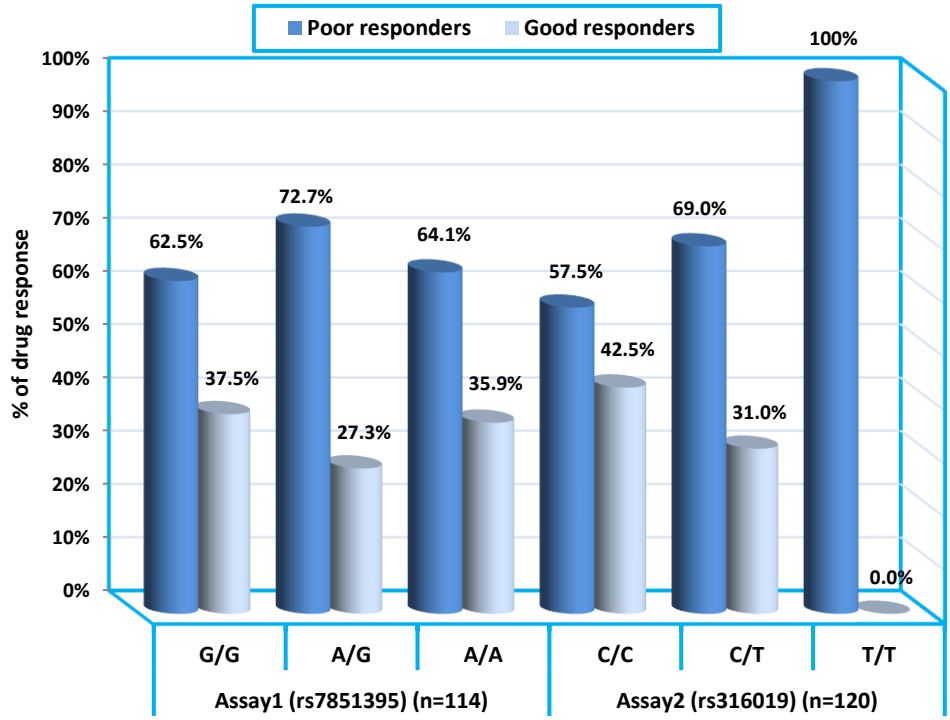

Fig. 1. Drug response percentage in CTR1 (rs7851395) and OCT2 (rs316019) SNPs

Table 3. Drug response and % of tumor necrosis as an outcome of neoadjuvant chemotherapy among the studied osteosarcoma children with Assay1 (rs7851395) and Assay2 (rs316019)

Tumor necrosis	Different Genotypes of CTR1 (rs7851395) and OCT2 (rs316019) among the studied children with osteosarcoma						$\chi 2$	P value
	G/G		A/G		A/A			
	n	%	n	%	n	%		
CTR1(rs7851395):								
•Drug response:								
Poor responders	15	62.5	32	72.7	25	64.1	1.021	0.600
Good responders	9	37.5	12	27.3	14	35.9		
OCT2(rs316019):								
• Drug response	C/C		C/T		T/T			
	n	%	n	%	n	%		
Poor responders	50	57.5	20	69.0	4	100	3.788	0.150
Good responders	37	42.5	9	31.0	0	0		

**Significant (P<0.05)*

Poor responders (< 90%), Good responders (>90%)

We noticed that the group patients with T/T genotype of OCT2 (rs316019) polymorphism showed the worst drug response, but this was not statistically significant. For CTR1 (rs7851395) polymorphism, group patients with A/G genotype showed the weakest drug response but also with no statistical significance, as shown in Fig. 1 and Table 3.

3.4 Correlation of CTR1 and OCT2 Polymorphisms with Nephrotoxicity

There was no significant correlation between CTR1, OCT2 polymorphisms with neither serum creatinine nor creatinine clearance as shown in Fig.1, Table4.

3.5 Correlation of CTR1 and OCT2 Polymorphisms with Magnesium level and other Electrolytes

There was no significant change in serum magnesium, sodium, potassium, and calcium level for CTR1 neither for OCT2 after the first week or after the sixth week of cisplatin treatment in all group patients with different genotypes.

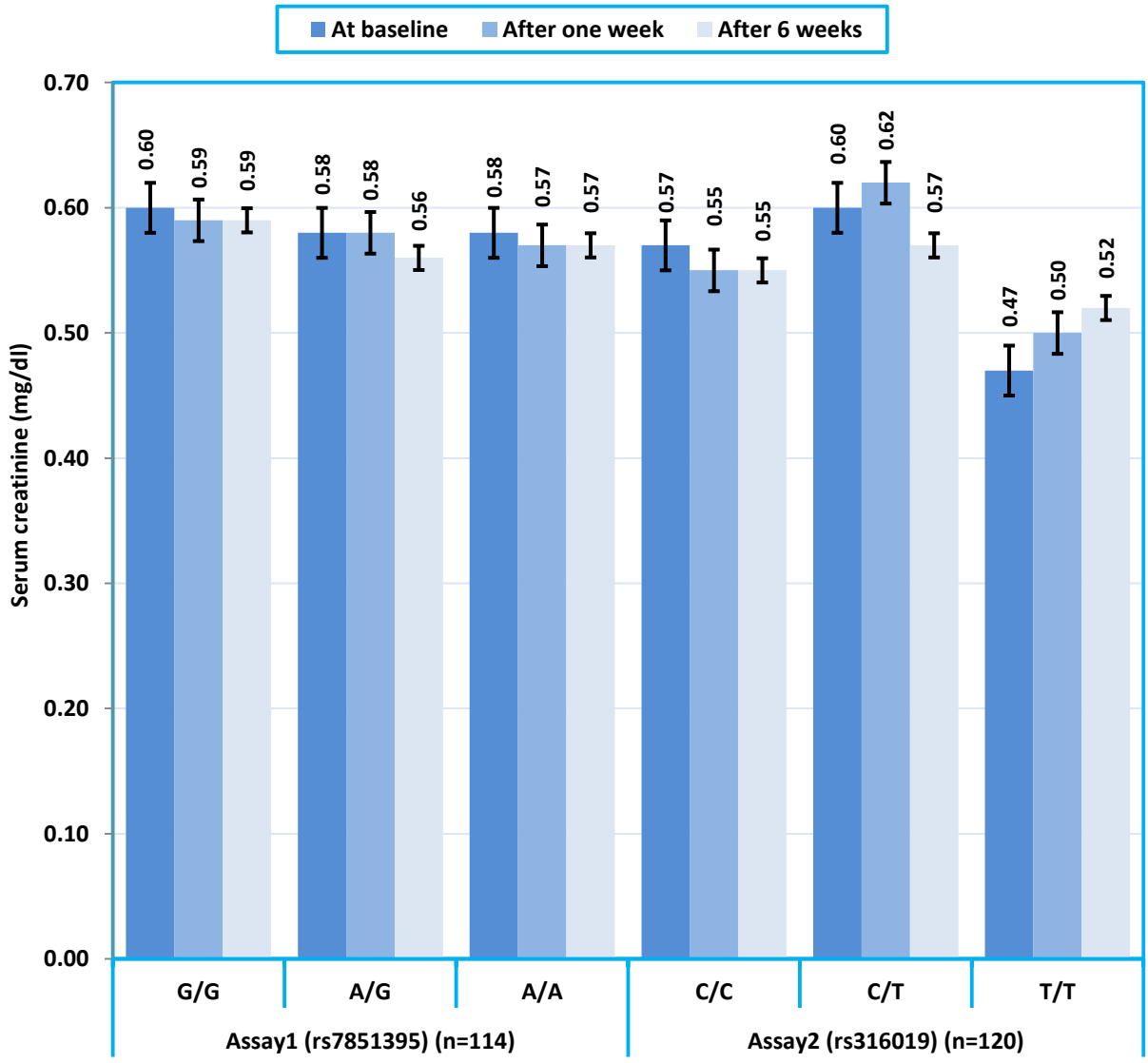

Fig. 2. Mean serum creatinine (nephrotoxicity) among the studied children with Osteosarcoma with CTR1 (rs7851395) and OCT2 (rs316019) SNPs (different alleles) at different times of assessment

Table 4. Creatinine clearance (nephrotoxicity) among the studied children with Osteosarcoma with Assay1 (rs7851395) and Assay2 (rs316019) SNPs (different alleles) at different times of assessment

Creatinine clearance at different times of assessment	Creatinine clearance among osteosarcoma children with (different genotypes)			χ2 value	P value
	G/G	A/G	A/A		
•CTR1 (rs7851395):					
-At baseline:					
Range	44-264	74-248	42-651	0.344	0.711
Mean±SD	151.64±56.77	147.33±52.06	167.60±120.72		
Median	130.00	134.00	131.00		
-After one week:					
Range	85-239	33-275	54-257	1.351	0.267
Mean	152.36±52.82	123.46±52.46	140.24±49.97		
Median	142.00	122.00	141.00		
-After 6 weeks:					
Range	60-188	63-279	58-286	0.475	0.624
Mean	128.81±35.37	146.25±55.92	148.32±65.50		
Median	124.00	131.50	153.00		
#χ2 value	0.727	3.520	0.960		
P value	0.695	0.197	0.619		
EX (B)(OR)	1.006	0.999	1.002		
Confidence interval					
Lower limit	0.993	0.990	0.993		
Upper limit	1.020	1.008	1.012		
•OCT2 (rs316019):					
	C/C	**C/T**	**T/T**		
-At baseline:					
Range	42-651	72-255	125-160	0.271	0.763
Mean±SD	155.00±90.10	139.26±55.96	142.50±24.75		
Median	136.00	127.00	142.50		
-After one week:					
Range	33-356	62-230	142-176	0.212	0.810
Mean	135.80±57.03	132.74±46.09	159.00±24.04		
Median	123.50	133.00	159.00		
-After 6 weeks:					

Creatinine clearance at different times of assessment	**Creatinine clearance among osteosarcoma children with (different genotypes)**			**χ2 value**	**P value**
Range	31-286	58-206	121-211	0.238	0.789
Mean	142.18±61.13	137.42±39.44	166.00±63.63		
Median	129.00	138.00	166.00		
#χ2 value or F value	1.033	0.095	0.167		
P value	0.597	0.910	0.854		
EX (B)(OR)	1.000	1.001	0.993		
Confidence interval					
Lower limit	0.991	0.992	0.972		
Upper limit	1.009	1.011	1.015		

**Significant (P<0.05)*
B=Logistic Regression Coefficient
SE=Standard Error of B
P=Significance level
Exp (B)=Estimated Odds Rat

4. DISCUSSION

Osteosarcoma (OS) is a relatively chemo-sensitive primary bone tumor, with the peak age of onset occurring in late childhood and early adolescence [23]. The treatment paradigm of nonmetastatic OS has typically been multimodality therapy, including neoadjuvant and adjuvant chemotherapy with definitive surgery. However, the majority of recent trials used high-dose methotrexate, doxorubicin, and cisplatin (MAP) chemotherapy [23]. Platinum compounds are used for the treatment of various tumors worldwide [24].

Cisplatin is associated with nephrotoxicity, which is a dose-limiting side effect. Its impact on kidneys depends on the accumulation of cisplatin in renal cells [23]. Platinum resistance is considered a significant obstacle in clinical treatment [25]. As seen in nephrotoxicity, drug resistance to depends on the degree of cisplatin accumulation and cellular uptake [13]. CTR1 and OCT2 contribute to cisplatin uptake; thus, polymorphisms in these transporters are believed to affect nephrotoxicity and drug resistance [13,23].

In the present study, our target was to investigate the prevalence of CTR1 and OCT2 polymorphisms in Egyptian osteosarcoma patients who were treated with the platinum-based regimen and to investigate the relationship between these polymorphisms, drug response, and nephrotoxicity.

To the best of our knowledge, this is the first study to investigate the allelic frequency of CTR1 and OCT2 in the Egyptian population and study their effects on drug response and nephrotoxicity. We found that allelic frequency of polymorphism in CTR1 (rs7851395) was (G/G = 21.1%), (A/A) = 39.5%) and (A/G) = 39.5%). Whereas a previous study demonstrated that allelic frequency was (G/G = 13.5%), (A/A = 33.5%) and (A/G) = 53%) but it was conducted on chinses population [16]. According to the 1000 Genome project, allele frequency was (A=0.57, G=0.43) for Europeans, (A=0.53, G=0.47) for east Asians, and (A=0.44, G=0.56) for Africans but the African population were from south Africa [26]. For (rs316019) we found that allelic frequency was (C/C = 72.5%), (T/C =24.2%) and (T/T =3.3%). whereas Cara Chang et al found that allelic frequency was (C/C =67.9%), (T/C = 18.7%), and (T/T = 1.4%) but it was conducted on European Caucasian population [23]. According to the 1000 Genome project, allele frequency was (T=0.11, C=0.89) for Europeans, (T=0.09, C=0.91) for Americans which makes C allele the dominant allele in different population type [26].

Regarding drug response (CTR1), transporter has been found to play a significant role in cisplatin resistance. Several number of clinical studies found that expression of CTR1 is correlated with cisplatin concentration and, therefore drug resistance [27]. for CTR1 (rs7851395) polymorphism group patients with heterozygotes genotype (A/G) showed the weakest drug response with no statistical significance, which agreed with Xu, X. *et al.* who found that the group patients with AG genotype CTR1 (rs7851395) polymorphism had shown the shortest survival rate [16]. For OCT2 (rs316019) polymorphism, group patients with homozygotes (C/C) showed the best drug response while the group patients with homozygotes (T/T) showed the worst drug response with no statistical significance. The importance of the OCT2 transporter in cisplatin clearance was previously evaluated in a study that found that the presence of the OCT variant was associated with the maintenance of serum creatinine [17]. In addition, a recent study had found that heterozygous patients had associated with higher concentrations and fold-changes in urinary KIM-1 novel biomarker compared to wildtype homozygotes [23]. In this current study, nephrotoxicity was assessed by serum creatinine and creatinine clearance which showed that there was no significant difference between genotypes groups. Regarding magnesium and other electrolytes, there was no significant difference between groups, and to the best of our knowledge; electrolytes weren't estimated before in pharmacogenomics literature.

The limitations of the study were that there are likely many other genes associated with cisplatin efficacy and/or toxicity [28,29]. Another limitation is that cisplatin is not used as monotherapy, so tumor necrosis also depends on anthracyclines and methotrexate and possibly the pharmacogenomics of those two drugs. Also, we defined acute kidney injury using CTCAEv4, which may miss cases of toxicity.

5. CONCLUSIONS

In conclusion, this is the first study detecting the allelic frequency of CTR1, OCT2 polymorphisms in the Egyptian population. The present study came to that the (A) allele was the dominant

allele in CTR1 polymorphism at (rs7851395) while in OCT2 polymorphism at (rs316019) (C) allele was the dominant allele in patient's population. The current study suggesting that polymorphisms of OCT2 at (rs316019) and CTR1 at (rs7851395) may not affect the cisplatin toxicity nor drug efficacy taking in consideration that it needs further studies to illustrate the effects of pharmacogenomics on cisplatin efficacy and nephrotoxicity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL AND CONSENT

This study was approved by the Institutional Reviewing Board of Children's Cancer Hospital-57357. All patients' guardians signed a written document of informed consent approved from the ethical committee before enrolment in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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