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Prevalence of UGT1A1*93 and ABCC5 Polymorphisms in Cancer Patients Receiving Irinotecan-Based Chemotherapy at Al-Najaf Al-Ashraf

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Abstract

Irinotecan (CPT-11) is a semisynthetic derivative of the antineoplastic agent camptothecin used in a wide range as an anti-cancer agent in many solid tumors because of its cytotoxic effect through the interaction with the topoisomerase I enzyme. The major limiting factors for irinotecan treatment are its association with potentially life-threatening toxicities including neutropenia and acute or delayed-type diarrhea, results from distinct interindividual and interethnic variability due to gene polymorphism.

This is a cross sectional pharmacogentics study was conducted on 25 cancer patients to estimate the prevalence of UGT1A1*93 and ABCC5 allele single nucleotide polymorphism (SNP) in Iraqi cancer patients treated with irinotecan-based therapy at Middle Euphrates Cancer Center. Four drops of venous blood was drawn for each patient and was applied onto the FTA classic card to perform a genotyping assay for the 2 SNPs. After DNA isolation and purification, real time PCR was performed to detect the SNPs of each gene.

Results of this study showed the prevalence of one allele variant (heterozygous mutation) of UGT1A1*93 was 64% compared to 36% of patients were wild type to this SNP. No patient (0%) could be detected with homozygous polymorphism of the UGT1A1*93. For the ABCC5 polymorphism, results revealed that 32% of patients have one polymorphic allele (heterozygous), while 28% of them have two polymorphic alleles (homozygous mutation). Wild type ABCC5 gene constitutes 40% of patients. As a conclusion**,** high prevalence of UGT1A1*93 and ABCC5 polymorphic alleles were detected in patients at Middle Euphrates Cancer Center which may explain the high toxicity features associated with irinotecan therapy.

Keywords: Irinotecan, UGT1A1*93, ABCC5, Polymorphism.

تقيم نسبة انتشار الطفرة الوراثية في الموروثتين UGT1A1*93 و ABCC5 لدى مرضى السرطان الذين يتعاطون علاج الايرينوتكان في النجف الاشرف

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الخلاصة

علاج الايرينوتيكان هو مشتق من الكامبتوثيشين الشبة صناعي و الذي يستخدم في علاج العديد من الاورام الصلبة و ذلك لقدرته على منع انزيم ال توبو-ايزوميريز ١. من اعظم الاسباب المعرقلة لاستخدامه هو ضهور اعراض جانبية مصاحبة له مثل نقصات عدد العدلات الدموية بالاضافة الى الاسهال بنوعية (الحاد و المؤجل) التي يتعتقد ان ضهوروها المتباين لدى المرضى يعتمد على اختلافات في الموروثات الجينية بين المرضى او بين المجتمعات. سحب ٤ قطرات دم من الاوردة الطرفية و وضعها في بطاقات ال FTA و من ثم استخدامها لاستخلاص ال DNA و بعدها بدء الدورات الحرارية في ال PCR لاكتشاف وجود الطفرات في الموروثات الجينية.

النتائج اظهرت الطفرة الوراثية المتغايرة (الضاهره في الاليل الواحد) هي السائدة مقارنتة بال الطفرة الوراثية المتماثلة (الضاهره في ايلي الموروثة) فيما يخص الموروثة UGT1A1*93. بالمقابل ان ٣٢% من المرضى يحملون طفرات وراثية متغايرة و ٢٨% من المرضى يحملون طفرات وراثية متماثلة فيما يخص ال موروثة ABCC5.الاستنتاج ان النسبة العالية لاتشار الطفرات الموراثية في الموروثتين UGT1A1*93 و ABCC5 لدى المرضى قد تفسر النسبة العالية لضهور الاعراض الجانبية المصاحبة لعلاج الايرينوتيكان.

الكلمات المفتاحية : اختلاف في الموروثات الجينية، UGT1A1*9 ، ABCC5 .

Introduction

Toxicity and efficacy as a result of the administered drugs is the field of interest for any health care providers (HCP) during the patients follow up.

There is an evidence suggesting that the effectiveness and toxicity of any drug may be affected, at least in part, by a genetic polymorphism

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(changes in the genetic code that found in more than 1% of the human population) responsible for drug metabolism or drug targets[1]. Pharmacogenetics is the filed that interest in the study of how genetic variation affects individuals' response to drugs[1,2]. This inter-individual variation can range from inadequate therapeutic efficacy to serious, potentially life-threatening adverse drug reactions.[2,3] The clinical use of this field aims to reduce the potential for adverse drug reactions and maximize drug response by matching the best available drug or dose to an individual's genomic profile[4–6].

Irinotecan (CPT-11) is a semisynthetic derivative of the antineoplastic agent camptothecin. Is water-soluble and derives its name from the Camptotheca tree where it was first isolated. [7] Irinotecan is a pro-drug, and it activated through a hydrolysis reaction catalyzed by two isoforms of carboxylesterases enzyme (CES1 and 2) in vivo to active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) through cleavage of the ester-bond at C10[8,9]. Its active metabolite SN-38 is 100–1000 times more potent than its parent drug. SN-38 is actively transported into the hepatic cells by the organic anion transporting polypeptide (OATP) 1B1 transporter (i.e., SLCO1B1). Then it is further conjugated and detoxified in the hepatic cells by UDP-glucuronosyltransferase (UGT) 1A1 and 1A9 enzyme and extrahepatic by UGT1A7 enzyme to yield its h-glucuronide, SN-38 G.[10] SN-38 and SN-38G are substrates for protein pumps, responsible for a unidirectional compound efflux from hepatocytes into bile and urine [ATP binding cassette (ABC) transporters] to be eliminated. [11]

Irinotecan used in a wide range as an anti-cancer agent in many solid tumors because of its cytotoxic effect through the interaction with the topoisomerase I enzyme.[12] It is approved worldwide for first-line therapy in combination with 5-fluorouracil and leucovorin for patients with metastatic colorectal cancer and as a single agent for second-line therapy of fluorouracil refractory metastatic colorectal cancer[13]. In addition, irinotecan-based therapy is effective in the lung, pancreatic, esophageal and ovarian cancers. The major limiting factors for irinotecan treatment are its association with potentially life-threatening toxicities include neutropenia and acute or delayed-type diarrhea, with distinct interindividual and interethnic variability as a result of the unique pharmacogenetic profile of each patient and/or ethnicity.[14] screen patients for DNA variations prior to selecting irinotecan therapy or dose can guide the decision-making process in terms of individually tailored irinotecan dose adjustments with subsequent toxicity risk reduction while maintaining therapeutic benefits.[15].

In June 2005, the FDA changed the irinotecan package insert for UGT1A1*28 pharmacogenetics testing in patients, recommending reduced irinotecan doses in homozygous UGT1A1*28 carriers, without specifying the extent of reduction[16]. This irinotecan label revision based on genetic studies have been established that patients who are UGT1A1*28/*28 allele carrier are at the highest risk of developing severe toxicity of irinotecan[14]. Recently, and after identifying the irinotecan pharmacology in many studies, a series of genes has investigated for their possible contribution to the variability in irinotecan disposition and adverse effects.[17,18] Other polymorphisms in metabolizing enzymes (UGT1A1, UGT1A7, UGT1A9, and CES) and transporters [ATP-binding cassette (ABC) and SLCO1B1] genes extensively studied in relation to pharmacodynamics and pharmacokinetics of irinotecan[11,19–21]. UGT1A1*93 (rs569189,-3156C>T) and ABCC5 (rs562, 1243T>C) show a significant relation with irinotecan-induced neutropenia and diarrhea respectively in many recent studies[22-24]. In this study, we will determine the prevalence of these polymorphisms in Middle Euphrates Cancer Center patient who received irinotecan as a preliminary study to demonstrate their relation with irinotecan toxicity in future work.

Patients and Methods

Patient selection and study design

Twenty-five cancer patients at Middle Euphrates Cancer Center were selected to participate in the current cross-sectional study and were all on irinotecan monotherapy or irinotecan-based regimen therapy. Four drops of venous blood were drawn for each patient and applied onto FTA classic card to perform a genotyping assay for the 2 genes. Toxicity features were graded according to the National Cancer Institute (MD, USA) – Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0[25]. Ethical approval was obtained from the department of clinical pharmacy at the college of the pharmacy and from the center management. Singed informed consent from all the patients were also obtained.

A total of 25 patients were included in the study. The age distribution of the patients was 16% with an age of (20-39 years), 68% with an age of (40 -59 years) and the remaining 16% with an age of ≥60 years. Males included in the study were (72%) and females (28%). The higher percentage of the population in this study were from Al-Najaf governorates (44%), while the rest distributed from the neighboring governorates. The majority of the patient were Arab (96%). The demographic data and the patient characteristics are illustrated in table 1.

Table 1. Demographic data and patient characteristics

Variable	Category	Number	Percent
Age Group	20-39 y	4	16.0%
	40-59 y	17	68.0%
	≥ 60 y	4	16.0%
Sex	Male	18	72.0%
	Female	7	28.0%
Province	Babil	3	12.0%
	Baghdad	1	4.0%
	Diwania	3	12.0%
	Muthanna	3	12.0%
	Najaf	11	44.0%
	Ninava	1	4.0%
	Thiqar	1	4.0%
	Wasit	2	8.0%
Ethnicity	Arab	24	96.0%
	Kurd/ Turkmen	1	4.0%

Genotyping

Genomic DNA was isolated from venous blood according to the protocol of ReliaPrep™ Blood gDNA Miniprep System (Promega, USA). From National Center of Biotechnology Information (NCBI) database rs562 and rs569189 information was obtained, and specific primers designed in order to make specific assay for allelic detection using amplification refractory mutation system–polymerase chain (ARM system –PCR). Primer sequence listed in table 2. Real time

PCR was performed in a 10µl total reaction mixture containing 1 µl (10-30 ng) of DNA template, 0.5µl (10 µM) of each primer, 5µl of GoTaq qPCR master mix (Promega, USA). After several trails for optimization, PCR conditions consisted of an initial melting step of 10 minutes at 95°C (initial denaturation); followed by 40 cycles of 10 sec. at 95°C (denaturation), 10 sec. at 60°C (annealing) and 72 C for 20 sec. (extension). Melting curves were constructed by increasing the temperature from 70°C to 95°C (0.3 C/sec).

Table 2. Primers sequences and annealing temperature

Gene polymorphism	Primer Name	Sequences	Annealing temperature
ABCC5	rs562-inner-F	5`-CACGACATGCAACGCTGACCATTCCAT-3`	**60°C**
	rs562-inner-R	5`-AGGTGGGCGTGGTCACTGCTGTCATAAG-3`	
	rs562-outer-F	5`-CCCTTGCAACCAACCAGCTTTGCTACCA-3`	
	rs562-outer-R	5`-CCGCAGTCGTCGCACAGTCTCTCTCTCT-3`	
UGT1A1*93	rs569189-inner-F	5`-GACATTTCTGGACACACCCTGGGCAAT-3`	
	rs569189-inner-R	5`-CCAGTACTGGGCCTTTTCATCCAAGGAAG-3`	
	rs569189-outer-F	5`-CCGTCCCATAACCCTTGTCTGCACAGTT-3`	
	rs569189-outer-R	5`-CCACCACAGCTGGAAATGTGCTGAGTCT3`	

Statistical Analysis

Statistical package for social sciences version 24 (SPSS v24) was used to analyze data. Continuous variables presented as means with standard deviation and discrete variables presented as numbers and percentages.

Results

Clinical characteristics of patients

Thirty two percent of pateints have positive family history for the occurance of cancer distributed as 16% for their father and 16% for their siblings. Non-pharmacological treatment for both radiotherapy plus surgery constitue (36%).

Irinotecan dose modification (dose reduction) was done for 12%. The most common toxicity associated with irinotecan included diarrhea (grade 3 and 4) (54.2%), vomiting (16.7%), severe neutropenia toxicity (grade 3 and 4) 20.8%, and toxic alopecia (25%). The clinical characteristics of the patients are shown in table 3.

Table 3. Clinical characteristics of patients.

Variable	Category	Number	Percent
Positive family history of malignancy	• Father	4	16.0%
	• Mother	0	0.0%
	• Sibling	4	16.0%
Adjuvant non-pharmacological treatment	• None	8	32.0%
	• Surgery	8	32.0%
	• Radiotherapy	0	0.0%
	• Both	9	36.0%
Dose modified [a]	• Yes	3	12.0%
	• No	22	88.0%
Sever toxicity features [b]	• Alopecia	6	25.0%
	• Neutropenia	5	20.8%
	• Diarrhea	13	54.2%
	• Vomiting	4	16.7%

a: irinotecan dose reduction (25% -35%) **b: grade 3 and 4 toxicity**

Prevalence of UGT1A1*93 and ABCC5 single nucleotide polymorphism:

Genotyping assay of UGT1A1*93 SNP revealed that patient have one allele polymorphism (heterozygous mutation) constitute 64% compared to 36% of patient were wild type for this SNP. No patient has homozygous mutation could be detected in the studied population (Table 4).

Concerning ABCC5 genotyping, results showed that the one allele variant (heterozygous mutation) prevalence calculated as 32%, while the homozygous mutation 28%. Patients carried wild type ABCC5 gene comprised 40% (Table 4).

Table 4. prevalence of studied mutation in patients

Gene	Allele	Number	Percent
UGT1A1*93	T/T	9	36.0%
	T/C	16	64.0%
	C/C	0	0.0%
ABCC5	C/C	10	40.0%
	C/T	8	32.0%
	T/T	7	28.0%

Discussion

Up to our knowledge, this study is considered the first study that estimate the prevalence of the UGT1A1*93 and ABCC5 polymorphisms in Iraq. There are no other studies in the neighboring countries that could be compared to the results reported in this study; however we compared the findings of this study to studies conducted on American, European and African populations.

It was found that the heterozygous variant of the UGT1A1*93 detected in (64%) of the study population, while there was no homozygous variant could be detected. Innocenti *et al.* study which was conducted in the USA on African American patients in which no homozygous polymorphism was detected [(21)]. Other studies reported the prevalence of homozygous variant in American Whites (13%), France (7%) and United kingdom (7%)[(21,22,26)].

Another study measured variant alleles frequency in European, Asians, and Africans reported a prevalence of 26%, 8% and 36% respectively [(17)]. The reasons for the recorded variation between the present study and other studies are not clear, but could be attributed to ethnicity-related variations, and/or due to low sample size of the studied population[(26,28)].

Many patients participated in this study were complaining from several severe toxicity features including diarrhrea, vomiting, neutropenia, and alopecia (Table 3). These adverse effected reported in the study could be attributed to the presence of polymorphism in genes sequences that are

responsible for the metabolism of irinotecan [27]. This finding was consistent with Li *et al.* and Crona *et al* studies, who postulated that a polymorphism of UGT1A1*93 was a strong predictor of irinotecan induced neutropenia, that is associated with higher SN-38 AUC and lower absolute neutrophils3 counts (ANC) nadirs. [11,29]

The ABCC5 transporter function was unclear until recently, in which it was found that ABCC5 transporter plays a role in the transport of cyclic nucleotides and platinum-based and nucleoside-based analog used in anticancer treatment (e.g. irinotecan and its active metabolite SN-38).[18] Among ABCC5 gene was detect that (40%) of studied patients carried a wild type allele, the homozygous mutation represent (32%) and (28%) of them with a homozygous mutation. Di Martino *et al.* study which was conducted in Italy metastatic colorectal cancer patients showed that the polymorphic allele (T) frequency was (51%) while the (C) allele detected in (48%) of the patient, with homozygous mutation (5/26) and (14/26) of the patient a heterozygous carrier[23]. Another study measured variant alleles frequency in Asians breast cancer patient reported that (T) allele carried by (60%) of the patient and only (40%) of them was carried (C) allele. The genotyping assay through Lal *et al.* was showed that (17%) of the patient with homozygous mutation and (43%) with heterozygous mutation.[30] This high polymorphic allele frequency incidence in Iraqi patient could be attribute the high sever GI toxicity among them (Table 3). This finding was consistent with Chen *et al.*, who assume that a polymorphism of ABCC5 was a strong predictor of irinotecan induced Diarrhea. [18]

Conclusion

A high prevalence of UGT1A1*93 and ABCC5 polymorphic alleles were detected in patients at Middle Euphrates Cancer Center. Further studies should be conducted in multicenter all around Iraq to evaluate the effects of such gene variants on irinotecan associated toxicity features and to maximize the treatment efficacy.

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