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

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Abstract
Irinotocan (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 irinotocan 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 pharmacogenetics 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 irinotocan-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 irinotocan therapy.

Keywords: Irinotocan, UGT1A1*93, ABCC5, polymorphism.

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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Prevalence of UGT1A1*93 and ABCC5 Polymorphisms in Cancer Patients

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Irinotecan (CPT-11) is a semisynthetic derivative of the antineoplastic agent camptothecin. It is water-soluble and derives its name from the Camptotheca tree where it was first isolated. 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. 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. 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. 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.

Patient selection and study design

Twenty-five cancer patients at Middle Euphrates Cancer Center who received irinotecan as a preliminary study to demonstrate their relation with irinotecan toxicity in future work. 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

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% |
| | Ninawa | 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’-AGGTGGGGCTGGTCACTGCTGTCATAAG-3’ | |
|                   | rs562-outer-F | 5’-CCCTTGCAACCAACCAGCCTTGTACAC-3’ | |
|                   | rs562-outer-R | 5’-CCGCAGTCTGTCGACAGTCCTCTCTCTCT-3’ | |
| UGT1A1*93         | rs569189-inner-F | 5’-GACATTTTCTGACACACCCCTGGGAAT-3’ | |
|                   | rs569189-inner-R | 5’-CCAGTACTGGCCTTTTTCATCCAGGGAAG-3’ | |
|                   | rs569189-outer-F | 5’-CCGTCCCATAAACCTCCTGCAAGTT-3’ | |
|                   | rs569189-outer-R | 5’-CCACCACAGCTGGAATGTCAGTCT-3’ | |

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 patients have positive family history for the occurrence of cancer distributed as 16% for their father and 16% for their siblings. Non-pharmacological treatment for both radiotherapy plus surgery constitute (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 (23). 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 diarrhea, vomiting, neutropenia, and alopecia (Table 3). These adverse effects reported in the study could be attributed to the presence of polymorphism in genes sequences that are
responsible for the metabolism of irinotecan. 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 neutrophil 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). 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. 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. 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. 

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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References 
1. Osanlou O, Pirmohamed M, Daly AK. Pharmacogenetics of adverse drug reactions. Advances in Pharmacology. 2018; 1st ed. (83), 155-190 p. 
2. Weinshilboum RM, Wang L. Pharmacogenomics: Precision medicine and drug response. Mayo Clinic Proceedings. 2017;92(11):1711–22. 
3. Pirmohamed M. Pharmacogenetics: Past, present and future. Drug Discovery Today.

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1. 2011;16(19–20):852–61. 
2. Mroziewicz M, Tyndale RF. Pharmacogenetics: A tool for identifying genetic factors in drug dependence and response to treatment. Addiction science and clinical practice 2010;17–29. 
3. Haddy CA, Ward HM, Angley MT, et al. Consumers’ views of pharmacogenetics-A qualitative study. Research in Social and Administrative Pharmacy. 2010;6(3):221–31. 
4. Shin J, Kayser SR, Langae TY. Pharmacogenetics: From discovery to patient care. American Journal of Health-System Pharmacy. 2009;66(7):625–37. 
5. Palmirota R, Carella C, Silvestris E, et al. SNPs in predicting clinical efficacy and toxicity of chemotherapy: walking through the quicksand. Recent Results in Cancer Research. 2018;9(38):25355–82. 
6. Sanghani SP, Quinney SF, Fredenburg TB, et al. Hydrolysis of irinotecan and its oxidative metabolites, 7-ethyl-10-[4-n-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin and 7-carboxylesterases ces1a1, ces2, and a newly expressed carboxylesterase isoenzyme, CES3. Drug Metabolism and Disposition. 2004;32(5):505–11. 
7. Santos A, Zanetta S, Cresteil T, et al. Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. Clinical Cancer Research. 2012; 
8. Marsh S, Hoskins JM. Irinotecan pharmacogenomics review. Pharmacogenomics 2010;11:1003–10. 
9. Li M, Seiser EL, Baldwin RM, et al. Original Article ABC transporter polymorphisms are associated with irinotecan pharmacokinetics and neutropenia. Pharmacogenomics Journal. 2018;(May 2016):35–42. 
10. Ahn KRKH, Olff JAJW, Olesar JILLMK. Pharmacogenetics and irinotecan therapy. Am J Health-Syst Pharm. 2006;63:2211–7. 
11. Hoskins JM, Marcello E, Altes A, et al. Cancer therapy: clinical irinotecan pharmacogenetics: Influence of pharmacodynamic Genes. 2008;14(6):1788–97. 
12. Fujita K, Sparreboom A. Pharmacogenetics of irinotecan disposition and toxicity: A Review. Current Clinical Pharmacology 2010; 5, 209-217. 
13. Campbell JM, Bateman E, Peters MDJ, et al. Irinotecan-induced toxicity pharmacogenetics: An umbrella review of systematic reviews and meta-analyses. Pharmacogenomics Journal 2016;17(1):21–8. 
14. Chen S, Laverdiere I, Tourancheau A, et al. A novel UGT1 marker associated with better tolerance against irinotecan-induced severe neutropenia in metastatic colorectal cancer.
patients. Pharmacogenomics Journal 2015;(January):513–20.
17. Fujiwara Y, Minami H. An overview of the recent progress in irinotecan pharmacogenetics R. eview. Pharmacogenomics 2010;11:391–406.
18. Chen S, Villeneuve L, Jonker D, et al. ABCC5 and ABCG1 polymorphisms predict irinotecan-induced severe toxicity in metastatic colorectal cancer patients. 2015;573–83.
19. Adam Paulík JN. Irinotecan toxicity during treatment of metastatic colorectal cancer: focus on pharmacogenomics and personalized medicine. Journal, Tumori. 2018;
20. Man FM De, Goey AKL, Schaik RHN Van. Individualization of irinotecan treatment: A review of pharmacokinetics, pharmacodynamics, and pharmacogenetics. Clinical Pharmacokinetics. 2018;57(10):1229–54.
21. Innocenti F, Kroetz DL, Schuetz E, et al. Comprehensive pharmacogenetic analysis of irinotecan neutropenia and pharmacokinetics. Journal of Clinical Oncology 2009;27(16).
22. Côté J, Kirzin S, Kramar A. UGT1A1 polymorphism can predict hematologic toxicity in patients treated with irinotecan reated with irinotecan. Clinical Cancer Research 2007;3269–75.
23. Teresa M, Martino D, Arbitrio M, et al. ABCG1 transporter genes correlate to irinotecan-associated gastrointestinal toxicity in colorectal cancer patients: A DMET microarray profiling study Single nucleotide polymorphisms of ABCC5 and ABCG1 transporter genes correlate to irinotecan-associated. Cancer Biology and Therapy 2011;4047.
24. Aliaa Abdul sattar, Sarmed Katnem. The protective effect of ethanolic extract of mentha spicata against irinotecan-induced mucositis in mice. Iraqi J Pharm Sci 2019;28(1):37–44
25. National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). National Institutes of Health Publication. 2009;Versão 4.0:194.
26. Horsfall LJ, Zeitlyn D, Tarekken A, et al. Prevalence of clinically relevant UGT1A alleles and haplotypes in African populations. Annals of Human Genetics 2011;236–46.
27. Ferraldeschi R, Minchell LJ, Roberts SA, et al. UGT1A1*28 genotype predicts gastrointestinal toxicity in patients treated with intermediate-dose irinotecan Aims: Future Medicine. 2009;10:733–9.
28. Premawardhena A, Fisher CA, Liu YT, et al. The global distribution of length polymorphisms of the promoters of the glucuronosyltransferase 1 gene (UGT1A1): Hematologic and evolutionary implications. Blood Cells, Molecules, and Diseases. 2003;31(1):98–101.
29. Crona DJ, Ramirez J, Qiao W, et al. Clinical validity of new genetic biomarkers of irinotecan neutropenia: An independent replication study. Pharmacogenomics Journal. 2016;16(1):54–9.
30. Lal S, Sutiman N, Ooi LL, et al. Pharmacogenetics of ABCB5, ABCC5 and RLIP76 and doxorubicin pharmacokinetics in Asian breast cancer patients. Pharmacogenomics Journal 2016;17(4):337–43.

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