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UGT1A1*28 Polymorphism Related Toxicities are more Frequent in Chemotherapy Naïve Patients

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ABSTRACT

Irinotecan(CPT-11) is an important chemotherapy for the treatment of colorectal cancers. The main drawback is its toxicity which includes Neutropenia and Diarrhea. Some studies confirms the correlation between increasing the incidence of Neutropenia and Diarrhea in patients with UGT1A1*28 polymorphism and other studies could not conclude it. So, other factors may incorporate to act with the presence of the polymorphism to increase CPT-11 toxicity. In this study, we try to find these factors. Patients were divided into 2 groups according to the presence or absence of previous chemotherapy treatment. All patients received standard IFL regimen. Primary endpoint was: comparison between the 2 groups as regard to toxicities. Secondary endpoint was assessment of the incidence of UGT1A1*28 polymorphism. UGT1A1*28 polymorphism was present in 20 patients (43%) of them 15% are homozygous. Twenty three patients were chemotherapy naive in group (1) and 20 patients in group (2) had received previous chemotherapy. Grade (II-IV) neutropenia were found in 52 % of group 1 versus 20 % of group 2 (P=0.1). Grade (II-IV) diarrhea was found in 52 % of patients of group 1 and 15% of patients with group 2 (P=0.05).Treatment delay occurred in 29.16 % of group 1 versus 72.4 % of group 2. (P=0.1). The study concluded that UGT1A1*28 polymorphism is present frequently (43%) in a Caucasian population and is associated with more incidence of toxicity in chemotherapy naïve patients.

Keywords: Irinotecan, UGT1A1, Colorectal, Previous Chemotherapy.

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INTRODUCTION

Irinotecan (CPT-11) is currently used in cancer chemotherapy because of its ability to inhibit topoisomerase I.[1] Despite the development of novel anticancer agents, such as bevacizumab, cetuximab or panitumumab, the backbone of metastatic colorectal cancer (CRC) treatment is based on the combination of 5-fluorouracil (5FU) (±leucovorin), oxaliplatin and irinotecan[2]. On the basis of large randomized clinical trials, CPT-11 either alone or in combination with 5FU has been accepted as first- or second-line chemotherapy for the treatment of patients with CRC[3]. Administered as a prodrug, irinotecan undergoes enzymatic conversion by carboxyesterase-2 to yield the clinically active metabolite, SN-38. This active form interferes with tumor cell division by inhibiting the nuclear enzyme topo isomeraseI[4]. SN-38 is eliminated from the body through the biliary system after a process of glucuronidation (conjugation to glucuronic acid) through the uridine diphosphate glucuronosyl transferase (UGT)1A1 enzyme[5]. The UGT1 family, polypeptide A1 gene (*UGT1A1*) **28* polymorphism reduces the enzyme activity, which may lead to severe toxicity in patients treated with irinotecan[6–9]. The activity of UGT1A1 depends on the number of TA repeats in the promoter region of the gene [the wild type has six repeats (TA6) and *UGT1A1*28* has seven repeats (TA7)]. The TA7 allele is associated with decreased expression of the enzyme and less effective glucuronidation of SN 38. Therefore, patients with TA7/TA7 have higher exposure to SN38 and an increased risk of side effects[7–10]. Some studies confirms the correlation between increasing the incidence of Neutropenia and Diarrhea in patients with UGT1A1*28 polymorphism[11-13] and other studies could not conclude it[14, 15]. So, other factors may incorporate to act with the presence of the polymorphism to increase CPT-11 toxicity. In this study, we try to find these factors. One of them, if the patient chemotherapy naïve or not.

MATERIALS AND METHOD

This prospective case control study included all eligible cancer patients who presented to the Medical Oncology clinics of the National Cancer Institute (NCI), Cairo University during the period from October 2010 to March 2012 and were scheduled to receive Irinotecan based chemotherapy. Patients were divided into 2 groups according to the presence or absence of previous chemotherapy treatment. The study was conducted according to the Declaration of Helsinki and the guidelines for Good Clinical Practice. The local ethics committees approved the protocol, and informed consent was obtained from all patients before study entry.

Inclusion Criteria

- Age not less than 18 years.

- Pathological confirmation of cancer.
- Adequate bone marrow function:
  - White Blood Cells (WBC) count ≥3.0 x 10^9 /L.
  - Absolute Neutophilic Count (ANC) ≥1.5 x 10^9 /L.
  - Platelet count ≥100 x10^9 /L.
  - Hemoglobin level ≥9 g/L.
- Adequate renal function:
  - Plasma creatinine (Cr) level <1.5 normal value.
- Adequate hepatic function:
  - Total bilirubin (T.bil) ≤1.5XUNL (Upper Normal Limit).
  - AST and ALT ≤2.5 X UNL.
- Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0-2.

Exclusion criteria

- Pregnant or breastfeeding patients.
- Patients with a currently active second malignancy.

TREATMENT PROTOCOL

To prevent nausea and vomiting:

Patients received pre-medication consisting of intravenous Ondansetron 8mg, dexamethasone 8mg and ranitidine 150mg.

Chemotherapy:

IFL chemotherapy (Irinotecan 125 mg /m^2, Fluorouracil (5-FU) 425 mg /m^2, Calcium Leucovorin 20 mg /m2) were given; 4 weeks on and 2 weeks off. Appropriate dilutions for Irinotecan solution were made in 5% dextrose solution and administered to patients by intravenous (IV) infusion (90 min). Leucovorin and 5-FU are given as IV bolus.

Study assessment

Pretreatment assessment included complete medical history and physical examination. Further assessment conducted within 7 days before treatment included vital signs, performance status (ECOG), complete blood count with differential and full biochemical panel, including liver and renal function tests were performed and repeated before each treatment course. CEA and CA19.9 were done at base line and then every 6 weeks. Radiological evaluation including computerized tomography (CT) scan of the chest, Abdomen & pelvis. Additional radiological imaging such as bone scan were done if indicated.

Post treatment evaluation included

Medical history and physical examination every 3 weeks. CBC and chemistry every 3 weeks. CT chest, abdomen and pelvis every 6 weeks. CEA and CA19.9 every 6 weeks. Other investigations were done if indicated.

Toxicity

Toxicity evaluation was done according to the NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE).[16]

Statistical methods

SPSS package (version 17.0) was used for data analysis. Mean and standard deviation were reported to describe quantitative data. The Chi-square and Fischer exact tests were used to evaluate the differences in the distribution of the variables. The Kaplan–Meier method was used to estimate the overall and progression free survival and the Log rank test to evaluate differences in survival among groups.

UGT1A1 assessment

UGT1A1*28 polymorphism is characterized by the presence of an additional TA repeat in the TATA sequence of the UGT1A1 promoter ((TA) 7TAA, instead of (TA)6TAA). In the current study, UGT1A1*28 polymorphism was assessed in the blood and/or tissues by PCR as previously described by Iyer et al., 2002[17] and Akiyama et al., 2008.[18]

Extraction of DNA from blood Mononuclear cells (MNCs)

Blood samples (7 ml) were collected from each patient on day 1 of the cycle (before starting chemotherapy). Density gradient separation of the MNCs from the collected blood was done using FicollHypaque Solution. DNA was extracted from the MNCs by phenol: chloroform: isoamyl alcohol after proteinase K digestion according to standard protocols. The extracted DNA was used to detect UGT1A1*28 polymorphism by PCR.[17, 18]

DNA Extraction from formalin fixed paraffin embedded tissues (FFPET):

For each tumor sample included in the study, five micron thick sections (5 sections) were obtained in a sterile, eppindorff, plastic tube. DNA was extracted from the homogenized FFPET sections by phenol: chloroform: isoamyl alcohol after proteinase K digestion according to standard protocols. Assessment of the concentration and purity of the extracted DNA was done using the spectrophotometer followed by visualization of an ethidium bromide-stained gel.[17, 18]

PCR amplification and genotyping of UGT1A1*28:

The isolated DNA was amplified by polymerase chain reaction (PCR) using the primer sequences and conditions of Iyer et al., 1999[19]

Primers:

5'-GTC ACG TGA CAC AGT CAA AC-3'

5'-TTT GCT CCT GCC AGA GGT T-3'

PCR conditions

Initial denaturation step at 950C for 5 minutes, 30 cycles of: 950C for 30 seconds, 580C for 40 seconds, 720C for 40 seconds and final extension at 720C for 5 minutes.

Genotypes were assigned as follows

6/6: homozygous for (TA)6TAA

6/7: heterozygous for each (TA)6TAA/(TA)7TAA

7/7: homozygous for (TA)7TAA

5/8: heterozygous for each (TA)5TAA/(TA)8TAA

RESULTS AND DISCUSSION

46 cases of advanced colorectal cancer presenting to National Cancer Institute, Cairo University, aged between 19 and 71 years with a median age of 45 years were included and followed up during the period from September 2010 to January 2013 with a median follow up of 9 months.

Patients' Characteristics

Table 1 summarizes patients' characteristics with regard to age, sex, stage, pathology, side, and smoking history.

Table 1: Patients' Characteristics

Characteristic		**No. of patients (%)**		
		1st line (Group 1)	**2nd line (Group 2)**	**P-Value**
All patients		26 (100)	20 (100)	
Mean Age		45 years	44 years	
Sex				
Male		13 (56)	11 (55)	**0.5**
Female		10 (44)	9 (45)	**0.5**
Stage:				
III		7 (30)	8 (40)	**0.36**
IV		16 (70)	12 (60)	**0.36**
Pathology:				
Adenocarcinoma GII		14 (60)	15 (75)	**0.35**
Adenocarcinoma GIII		4 (18)	2 (10)	**0.35**
Mucinous Adenocarcinoma GII		5 (22)	3 (15)	**0.47**
Side:	Left	8 (35)	4 (20)	**0.35**
	Right	15 (65)	16 (80)	**0.5**
Smoking history		4 (18)	3 (15)	**0.4**

UGT1A1*28 polymorphism were present in 20 patients (43%), homozygous in 7 patients (15%) and heterozygous in 13 patients (28%).figure (1).

Genotypes were:

6/6 in 26 patients (57%).

6/7 in 9 patients (20%).

7/7 in 7 patients (15%).

5/8 in 4 patients (8%).

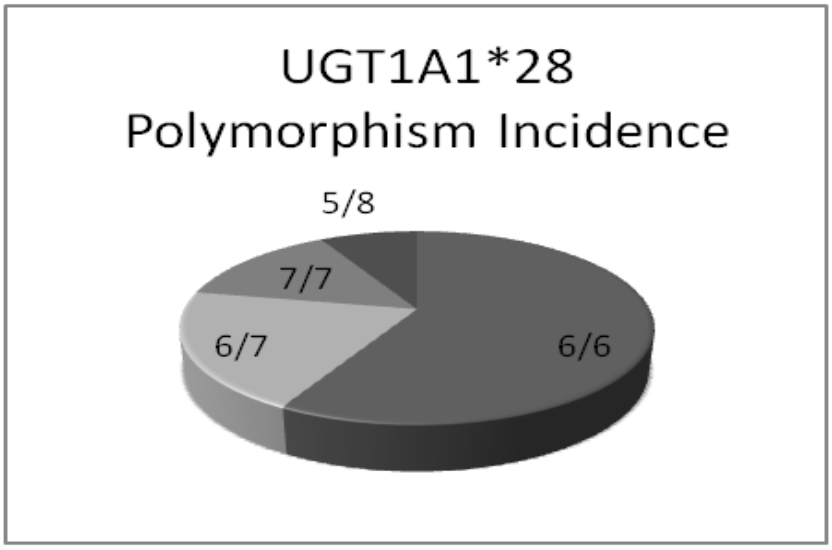

Figure 1: Incidence of UGT1A1 polymorphism

Three patients were excluded from the study due to poor performance status. Forty three patients received IFL chemotherapy regimen, 23 as first line and 20 patients as second line. Of the 23 chemotherapy naïve patients, 4 patients with homozygous polymorphism and 6 patients with heterozygous polymorphism. While 2 patients with homozygous polymorphism and 7 patients with heterozygous polymorphism in the second line group.(P value 0.6 and 0.7 respectively).

TOXICITY

Neutropenia:

There was a lower incidence of neutropenia in patients with 2nd line compared to chemotherapy naïve patients. Grade (II-IV) neutropenia were found in 52 % of group 1 versus 20 % of group2. (P=0.1).).

Diarrhea:

Grade (II-IV) diarrhea was found in 52 % of patients of group 1 and 15% of patients with group 2. (P=0.05).

Vomiting:

Grade (II-IV) vomiting was found in 26 % of group 1 versus 25 % of group2.(P=0.6)

Treatment delay:

There was a lower incidence of treatment delay in patients with 2nd line treatment compared to chemotherapy naïve patients. Treatment delay occurred in 29.16 % of group 1 versus 72.4 % of group 2. (P=0.1).. UGT1A1*28 polymorphism were present in 43 % of patients, 15 % homozygous (7/7) and 28 % heterozygous (6/7). In Christoph Schulz et al. (2009) study, 9.5 % of patients were homozygous and 49.5 % were heterozygous for UGT1A1*28.[20]In other studies, UGT1A1*28 polymorphism was present in a lower percentage such as Y. Akiyama et al. (2008) study, in which there was 16.4% of Japanese patients having this polymorphism, and 10.5% in Xiaoqing Zhang et al. (2012) study, 15.3% in Zhang A et al. (2007) study, 28.6% in Tang et al. (2005) study, 18.7% in Zhou et al. (2009) study, 13% in Saeki et al. (2006) study, 14% in Yea et al. (2008) study and 28.6% in Thomas et al. (2006) study have UGT1A1*28 polymorphism.[18, 21-27]

It is noteworthy that ethnic differences do exist in the UGT1A1*28 polymorphism, that's why there is differences between different studies of different ethnicity. Three patients were excluded from the study due to poor performance status. It is surprising that although UGT1A1*28 polymorphism difference between the two studied groups was not significant, there was an increasing toxicities in the chemotherapy naïve group over the previously treated patients. The difference was significant for diarrhea with P value of 0.05 and there is a trend towarda statistical significant difference for neutropenia and treatment delay. There was a lower incidence of neutropenia in patients with 2nd line compared to chemotherapy naïve patients. Grade (II-IV) neutropenia were found in 52 % of group 1 versus 20 % of group2. (P=0.1).). Grade (II-IV) diarrhea was found in 52 % of patients of group 1 and 15% of patients with group 2. (P=0.05).

There was a lower incidence of treatment delay in patients with 2nd line treatment compared to chemotherapy naïve patients. Treatment delay occurred in 29.16 % of group 1 versus 72.4 % of group 2. (P=0.1).

Other toxicities included 1 case of thrombocytopenia grade III, 1 case of oral mucositis grade II, 1 case of nausea grade II, 2 cases of urinary tract infection, 2 cases of gastritis grade II. All these other toxicities occurred in group 1.

CONCLUSION

Although NCCN guidelines stated that there is no regimen could be preferable over the others as initial therapy for metastatic disease[28], this study may lead to a change if further studies prove that for patients with UGT1A1*28 polymorphism and to decrease chance for toxicities, it is preferable

to use other agent than Irinotecan for chemotherapy naïve patients.

REFERENCES

1. Jaxel et al, 1989; Bissery et al, 1996; Zamboni et al, 1998.
2. O'Neil BH1, Goldberg RM. Innovations in chemotherapy for metastatic colorectal cancer: an update of recent clinical trials. 2008.
3. UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer. E Marcuello, A Altés, A Menoyo, E del Rio, M Gómez-Pardo, and M Baiget*. Copyright 2004, Cancer Research UK.
4. Humerickhouse R, Lohrbach K, Li L, Bosron WF, Dolan ME. Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. Cancer Res. 2000;60:1189-1192.
5. Cost Effectiveness of Pharmacogenetic Testing for Uridine Diphosphate Glucuronosyltransferase 1A1 Before Irinotecan Administration for Metastatic Colorectal Cancer. Heather Taffet Gold, PhD1; Michael J. Hall, MD, MS2; Victoria Blinder, MD3; and Bruce R. Schackman, PhD1
1. 6.Iyer L, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, et al, Gnetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyl transferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest 1998;101:847-54. doi:10.1172/JCI915.MedlineWeb of Science.
6. Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, et alPolymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 2000;60:6921-6. :.Abstract/FREE Full Text.
7. Hazama S, Nagashima A, Kondo H, Yoshida S, Shimizu R, Araki A, et al, Phase I study of irinotecan and doxifluridine for metastatic colorectal cancer focusing on the UGT1A1*28 polymorphism. Cancer Sci 2010;101:722-7. doi:10.1111/j.1349-7006.2009.01428.x.CrossRefMedline.
8. Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, et al, Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J ClinOncol 2004;22:1382-8. doi:10.1200/JCO.2004.07.173.Abstract/FREE Full Text.

9. Marcuello E, Altes A, Menoyo A, Del Rio E, Gómez-Pardo M, Baiget M, et al, UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer. Br J Cancer 2004;91:678-82. CrossRefMedlineWeb of Science.
10. UGT1A1*28 Polymorphism Predicts Irinotecan-induced Severe Toxicities Without Affecting Treatment Outcome and Survival in Patients With Metastatic Colorectal Carcinoma. Chun-Yu Liu, Po-Min Chen, Tzeon-JyeChiou, Jin-Hwang Liu, Jen Kou Lin, Tzu-Chen Lin, Wei-Shone Chen, Jeng-Kae Jiang, Huann-Sheng Wang, Wei-Shu Wang. Received September 4, 2007; revision received October 26, 2007; accepted November 15, 2007. ª2008 American Cancer Society DOI 10.1002/cncr.23370.
11. Pharmacogenetics and Irinotecan Therapy. Kristine K. Hahn; James J. Wolff; Jill M. Kolesar. Authors and Disclosures.Posted: 01/05/2007; American Journal of Health-System Pharmacy. 2006;63(22):2211-2217. © 2006 American Society of Health-System Pharmacists.
12. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. L Iyer, S Das, L Janisch, M Wen, J Ramı´rez, T Karrison, GF Fleming, EE Vokes, RL Schilsky, MJ Ratain. Accepted 7 November 2001.
13. UGT1A1 gene polymorphism: Impact on toxicity and efficacy of irinotecan-based regimens in metastatic colorectal cancer. Christoph Schulz, Volker Heinemann, Andreas Schalhorn, Nikolas Moosmann, Thomas Zwingers, Stefan Boeck, Clemens Giessen, and Hans-Joachim Stemmler. World J Gastroenterol. 2009 October 28; 15(40): 5058–5066.
14. Relevance of different UGT1A1 polymorphisms in irinotecan-induced toxicity: a molecular and clinical study of 75 patients. Rouits E, Boisdron-Celle M, Dumont A, Guérin O, Morel A, Gamelin E. Clin Cancer Res. 2004 Aug 1;10(15):5151-9.
15. Common Terminology Criteria for Adverse Events, Version 4.0, DCTD, NCI, NIH, DHHS. 28 May 2009.
16. L Iyer1, S Das, L Janisch, M Wen, J Ramı´rez, T Karrison, GF Fleming, EE Vokes, RL Schilsky and MJ Ratain: UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. The Pharmacogenomics Journal 2:43–47, 2002.
17. Akiyama Y, et al.: Genetic testing for UGT1A1*28 and *6 in Japanese patients who receive irinotecan chemotherapy. Ann Oncol. 19(12):2089-2090, 2008.
18. Iyer L, Hall D, Das S, Mortell MA, Ramirez J, Kim S et al.: Phenotypegenotype correlation of in vitro SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human

liver tissue with UGT1A1 promoter polymorphism. ClinPharmacolTher 65: 576–582, 1999.

19. Christoph Schulz, Volker Heinemann, Andreas Schalhorn, Nikolas Moosmann, Thomas Zwingers, Stefan Boeck, Clemens Giessen, and Hans-Joachim Stemmler: UGT1A1 gene polymorphism: Impact on toxicity and efficacy of irinotecan-based regimens in metastatic colorectal cancer. World J Gastroenterol. 15(40): 5058–5066, 2009.
20. Xiaoqing Zhang, GuokunAo, Yuewen Wang, Wei Yan, Min Wang, Erfei Chen, Fangfang Yang, and Jin Yang: Genetic variants and haplotypes of the UGT1A9, 1A7 and 1A1 genes in Chinese Han. Genet Mol Biol. 35(2): 428–434, 2012.
21. Zhang A, Xing Q, Qin S, Du J, Wang L, Yu L, Li X, Xu L, Xu M, Feng G, et al.: Intra-ethnic differences in genetic variants of the UGT-glucuronosyltransferase 1A1 gene in Chinese populations. Pharmacogenomics J. 7:333–338, 2007.
22. Tang KS, Chiu HF, Chen HH, Eng HL, Tsai CJ, Teng HC and Huang CS.: Link between colorectal cancer and polymorphisms in the uridine-diphosphoglucuronosyltransferase 1A7 and 1A1 genes. World J Gastroenterol. 11:3250–3254, 2005.
23. YY, Lee LY, Ng SY, Hia CPP, Low KT, Chong YS and Goh DLM: UGT1A1 haplotype mutation among Asians in Singapore. Zhou Neonatology. 96:150–155, 2009.
24. Saeki M, Saito Y, Jinno H, Sai K, Ozawa S, Kurose K, Kaniwa N, Komamura K, Kotake T, Morishita H, et al.: Haplotype structures of the UGT1A gene complex in a Japanese population. Pharmacogenomics J. 6:63–75, 2006.
25. Yea SS, Lee SS, Kim WY, Liu KH, Kim H, Shon JH, Cha IJ and Shin JG.: Genetic variations and haplotypes of UDP-glucuronosyltransferase 1A locus in a Korean population. Ther Drug Monit. 30:23–34, 2008.
26. Thomas SS, Li SS, Lampe JW, Potter JD and Bigler J.: Genetic variability, haplotypes, and htSNPs for exons 1 at the human UGT1A locus. Hum Mutat. 27:e717, 2006.
27. NCCN Guidelines Version 2.2015 Colon Cancer.http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf.

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