WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 22-34. Research Article ISSN 2277– 7105

ASSOCIATION OF DIHYDROPYRIMIDINE DEHYDROGENASE GENE POLYMORPHISM WITH TOXICITY OF 5-FLUROURACIL IN COLORECTAL CANCER IN KHARTOUM STATE

Rayan Ahmed Yousif Mohamed Ali[1]*, Hind Mohamed Abushama[2]

[1]School of Pharmacy, Department of Pharmaceutical Biotechnology, Ahfad University for Women, Khartoum, Sudan.

[2]Department of Zoology, Faculty of Science, University of Khartoum.

Article Received on
17 Sep 2015,

Revised on 11 Oct 2015,
Accepted on 03 Nov 2015

***Correspondence for Author**
Rayan Ahmed Yousif Mohamed Ali
School of Pharmacy, Department of Pharmaceutical Biotechnology, Ahfad University for Women, Khartoum, Sudan.

ABSTRACT

5-Fluorouracil (5-FU) remains one of the most frequently prescribed chemotherapeutic drugs for the treatment of colorectal cancer. The 5-fluorouracil pathway is affected by a number of genes that are known to be polymorphic, one of them is dihydropyrimidine dehydrogenase (*DPYD*) gene. The present study is one of the leading study to document the genotype and allele frequency of *DPYD* IVS14+1G>A polymorphism among Sudanese individuals diagnosed with colorectal cancer and receiving 5-FU chemotherapy compared to healthy controls. The wild and mutant types of *DPYD* gene were detected and a possible association with 5-FU toxicity and ABO blood groups has been evaluated. A PCR-RFLP designed to detect *DPYD* IVS14+1G>A polymorphism was used. Results have shown the frequency of cases to controls with *DPYD* IVS14+1G>A polymorphism was (37%:25% respectively), while cases to controls with wild type was (63%:75% respectively). There is a significant association between male and female cases with heterozygous G/A genotype and carriers of blood group O and A respectively. There was no significant association between *DPYD* IVS14+1G>A polymorphism and neutropenia toxicity. Significant association were found between *DPYD* IVS14+1G>A polymorphism and 5-FU GI toxicity. The experienced 5-FU toxicities by cases in the present study emphasize the importance to incorporate *DPYD* gene polymorphism assessment test to all colorectal cancer patients who candidate to use 5-FU to enhance the prediction of 5-FU toxicity. This could be

added to *DPYD* IVS14+1G>A polymorphism screening with the PCR-RFLP technique is simple and inexpensive (Approximately 8-10$).

KEYWORDS: *DPYD* IVS14+1G>A, 5-flurouracil, Toxicity, Colorectal cancer, Khartoum state.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer- related death worldwide.[1,2] The 5-flurouracil (5-FU) chemotherapy belongs to a group of substances called anti metabolites which are very similar to normal substances within the cell. Anti metabolite drugs work by inhibiting essential biosynthetic processes, or by being incorporated into macromolecules, such as DNA and RNA, and inhibiting their normal function so the cell become unable to divide. 5-FU does both inhibitions.[3] 5-Fluorouracil (5-FU) remains one of the most frequently prescribed chemotherapeutic drugs for the treatment of cancer including colorectal cancer. 5-FU is metabolized via two routes. The anabolic route gives rise to active metabolites which disrupt RNA synthesis and the action of thymidylate synthase (TS). The catabolic route which inactivates 5-FU and leads to the elimination of the drug from the system.[4]

The rate-limiting enzyme in 5-FU catabolism is dihydropyrimidine dehydrogenase (DPD), which converts 5-FU to dihydrofluorouracil (DHFU) in normal and tumor cells. More than 80% of administered 5-FU is normally catabolized primarily in the liver, where DPD is abundantly expressed.[3] In two large prospective cohorts no significant association between ABO blood group and risk of colorectal cancer was observed.[5]

The 5-fluorouracil pathway is affected by a number of genes that are known to be polymorphic, one of them is dihydropyrimidine dehydrogenase (*DPYD*) gene.[4] The *DPYD* gene is located on the short arm of chromosome 1, 1p22, and has been found to harbor several mutations.[6] The most prominent mutation of the *DPYD* gene resulting in severe DPD deficiency is IVS14+1G>A, resulting in the replacement of guanine 1986 (G) with an adenine (A) at the end of exon 14. This mutation does not allow the recognition of the splice site in the sequence GT at the 5´ of the intron 14, causing the deletion of the entire exon 14, the loss of 165bp and the generation of a protein with reduced or absent enzymatic activity.[6, 7, 8&9] The mutation of the *DPYD* gene which codes for DPD enzyme may occur both in

homozygosity and in heterozygosity and results in the synthesis of a functionally inactive enzyme transmitted in an autosomal recessive inheritance.[6]

The most convenient and accurate platform useful clinically for the prediction of DPD enzyme activity and 5-FU efficacy is genetic analysis.[8] As example the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method requires only PCR and one or two enzyme (s), and is technically less demanding than most other molecular biological approaches. It has been found that homozygous DPYD*2A genotype results in complete deficiency while the heterozygous DPYD*2A genotype results in partial deficiency of DPD.[7] DPD activity is completely or partially deficient in 0.1% and 3%–5% of individuals in the general population, respectively and DPD deficiency have been associated with severe toxicity occurred early during treatment due to drug accumulation in tissues.[6, 7 &11]

The toxicity resulting from treatment is routinely assessed following each cycle of chemotherapy, and may result in therapy being modified on subsequent cycles, for example, a dose reduction, a delay in treatment or, in some cases, an alternative treatment. A number of international rating scales are available for rating predictable acute reactions arising from chemotherapy, including that of the National Cancer Institute Common Toxicity Criteria.[12] Many studies were performed around the world concluded that the mutations in exon 14 of *DPYD* gene are responsible for a significant proportion of life-threatening toxicity to 5-Fluorouracil.[7 & 13]

Earlier studies had reported genotype and allelic frequencies of the *DPYD* IVS14+1G>A mutation in different ethnic populations. For example, 3% of the Caucasian population carry DPYD*2A in exon 14 of *DPYD* and 6.5% in south Indian populations while the allelic frequency of 0.75%, 0.98%, 0.91% and 0.94% in Turkish, French, Dutch and German, respectively. However, no IVS14+1G>A mutation was found among 121 Korean, 300 Taiwanese, 239 Egyptians, 190 African Caucasian-African, 105 African –Americans and Japanese populations in previous studies.[8, 14 & 15]

Previous study concluded that carriers of the DPD exon 14-skipping mutation are at significantly increased risk to experience life-threatening myelosuppression upon 5-FU treatment, even when the allelic status is heterozygous. These data lead us to suggest routine testing for the exon 14-skipping mutation before 5-FU treatment.[16] The data reported in

previous study suggest that greater dose reductions or alternative therapies are needed for patients with *DPYD* IVS14+1G>A mutations.[6]

The activity of dihydropyrimidine dehydrogenase enzyme (DPD) varies widely, with most of the variability arising from genetic polymorphisms in the *DPYD* gene. Patients who are partially or totally deficient in DPD activity due to mutation in *DPYD* gene cannot adequate degrade 5-flurouracil (5-FU). 5FU has a relatively narrow therapeutic index, therefore toxicity increases as the dose is increased, resulting in escalated plasma levels of the drug leading to an increased risk to severe toxicity.

Pharmacogenetic testing for *DPYD* polymorphisms can help to predict the risk of toxicity with 5-FU based chemotherapy. PCR-RFLP test can be used to screen patients for DPD deficiency before their first treatment with 5-FU in a rapid and low invasive way, allowing oncologists and their patient make more informed treatment decision. This would greatly improve cancer patients' quality of life.

The aim of this study is to investigate the allele and genotype frequency of *DPYD* IVS14+1G>A polymorphism among Sudanese individuals diagnosed with colorectal cancer and receiving 5-FU chemotherapy compared to healthy controls. The wild and mutant types of *DPYD* gene were detected and a possible association with 5-FU toxicity and ABO blood groups has been evaluated.

MATERIALS AND METHODS

Ethical consideration

Approval and ethical consideration were obtained from Ahfad ethical committee review board and all study participants were signed written consent.

Study participants

One hundred and one cases were enrolled after being diagnosed with colorectal cancer by clinicians at Radio Isotope Center Khartoum (RICK) and received 5-Flurouracil chemotherapy as part of FOlFOX4 protocol. Individuals who refused to participate in the study, did not have 5-flurouracil in their treatment protocols or individuals with blood transfusion within three days from sample collection had been excluded from this study. Blood Samples of hundred healthy controls were taken from central blood bank had agree to participate in this study.

Sample collection

Three milliliters of venous blood were obtained from each participant in EDTA container. Blood Samples were taken in a period from March 2014 to August 2014.

The hematological parameters (ABO blood groups and neutropenia) were examined. The ABO blood typing was tested by the classical method and neutropenia was assessed routinely before each cycle as a measurement of toxicity. The non hematological parameters (nausea, diarrhea and vomiting) had also been evaluated. They were the most prevalent toxicities recorded according to RICK center and to previous studies.[10, 17& 18] All of the cases who had received at least one course of chemotherapy were evaluated for toxicity which was measured according to Common Toxicity Criteria (Version 0.2).

The samples were stored at -20˚C for genotype analysis. Genomic DNA was extracted from nuclear cells by standard protocol with chelex 6% (Instagene) method. *DPYD* IVS14+1G>A polymorphism was evaluated by PCR-RFLP for 5-FU toxicity.

The PCR temperature profile was as follow: An initial denaturation step at 94˚C for 3 minutes was followed by 35 cycles of denaturation at 94 ˚C for 30 seconds, annealing at 60˚C for 30 seconds, and extension at 72˚C for 60 seconds, with a final extension step of 72˚C for 5 minutes. Primers sequences (*DPYD*-R 5´-CTT GTT TTA GAT GTT AAA TCA CAC ATA-3´, *DPYD*-F 5´-ATC AGG ACA TTG TGA CAT ATG TTT C-3´) were selected to allow the amplification of polymorphic *DPYD* gene.[13]

The PCR product sizes were estimated from their distance of migration relative to the DNA marker size. Samples were scored as positive when a PCR product of a band specific to the amplified sequence was detected (198 bp).

The allele and genotype frequencies of the *DPYD* IVS14+1G>A mutation were tested by Hardy- Weinberg equilibrium and the chi-square test in SPSS statistic program. Statistical significance was defined as *P value* <0.05.

RESULTS AND DISCUSSION

Results

One hundred and one cases that diagnosed with colorectal cancer were received 5-Flurouracil chemotherapy at different stages of treatment, 59.4% were males and 40.6% were females

with median age 46 years. In addition hundred healthy controls, 64% were males and 36% were females with median age 40 years were investigated in this study.

A diagnostic band has been obtained by RFLP using *Nde1* enzyme. The digested mutant alleles IVS14+1G>A yielded fragments of 154 bp, 27 bp and 17 bp and the wild type allele yielded 181 bp and 17 bp fragments as shown in figure.1.

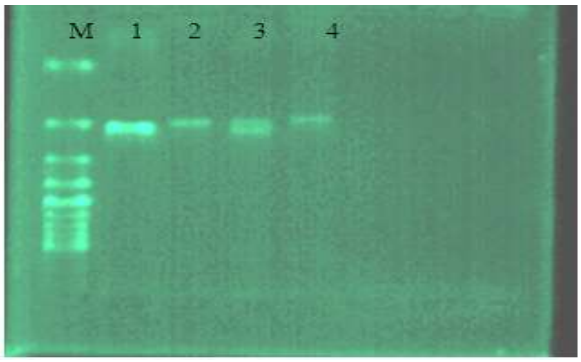

Figure.1 The *Nde*1 restriction profiles of *DPYD* gene IVS14+1G>A polymorphism

lane M shows DNA ladder 100-1000bp, Vivantis; lane 1 shows undigested PCR product 198 bp; lane 2&4 shows homozygous A/A genotype 154, 27 and 17 bp; lane3 shows heterozygous G/A genotype 181, 154, 27,17 bp. The 27 and 17 bp fragments are not visible on the gel.

Among all cases there were 11.9% of cases detected as heterozygous for the *DPYD* IVS14+1G>A polymorphism compared to 16% healthy controls *(P value* = 0.39).

The homozygous for the *DPYD* IVS14+1G>A polymorphism were 24.8% cases compared to 9% controls (*P value* 0.003). There were 63.4% cases detected as wild type for the *DPYD* IVS14+1G>A polymorphism compared to 75% healthy controls (*P value* = 0.07).

The frequency of allele A among cases was found to be 20.5% compared to 11.3% among controls (*P value* 0.002). Whereas the frequency of allele G among cases was 46.2% compared to 55.3% among controls (*P value* 0.025).

Among all cases, twelve cases were detected as heterozygous genotype for *DPYD* IVS14+1G>A polymorphism as shown in figure.2.

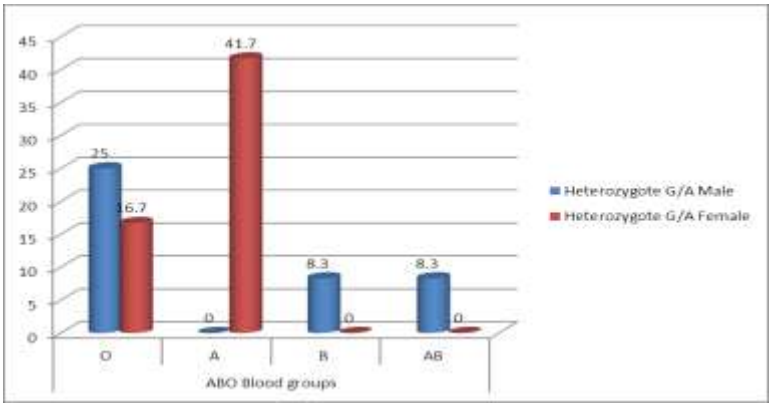

Figure.2 Association of heterozygote G/A genotype with ABO blood groups among cases

There was no significant association detected with neutropenia toxicity.

Among twelve cases who were detected as heterozygous genotype for *DPYD* IVS14+1G>A polymorphism, figure.3 shows the correlation of heterozygote G/A genotype with nausea grades.

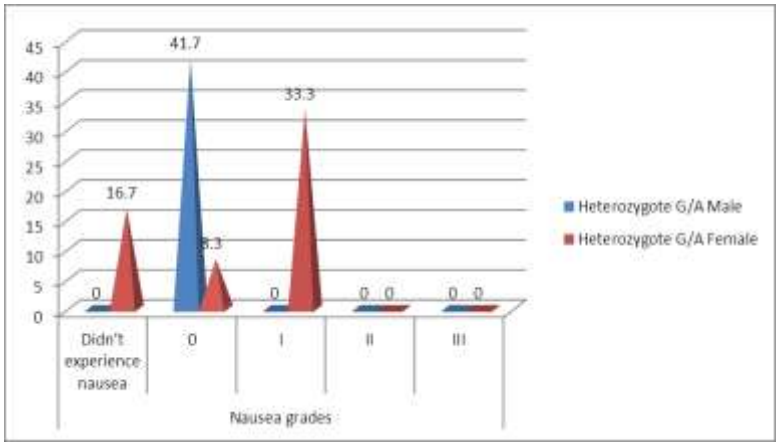

Figure.3 Association of heterozygote G/A genotype with nausea grades among cases

Twenty five cases were detected as homozygous genotype for *DPYD* IVS14+1G>A polymorphism, the correlation with diarrhea grades were shown in figure.4.

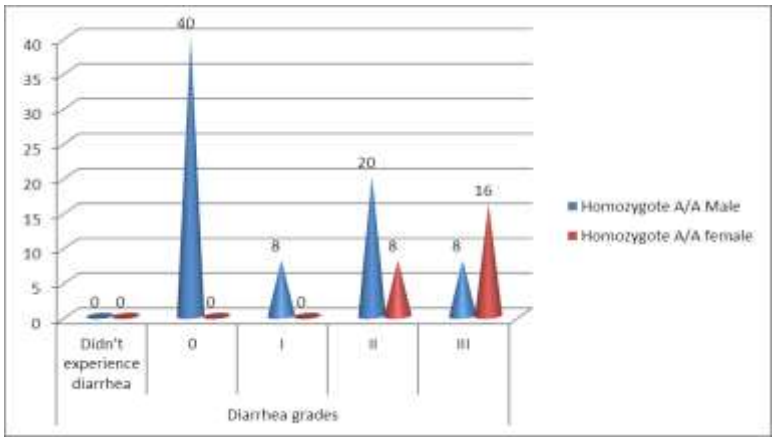

Figure.4 Association of homozygote A/A genotypes of *DPYD* with diarrhea grades among cases

Twenty five cases were detected as homozygous genotype for *DPYD* IVS14+1G>A polymorphism, figure.5 shows the correlation with vomiting grades.

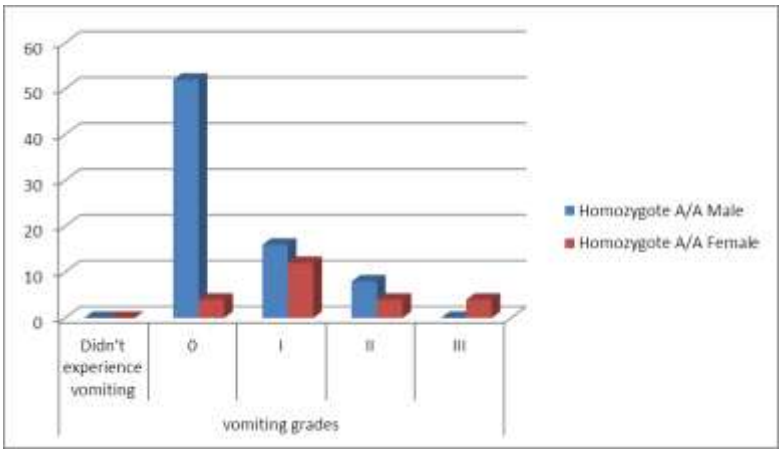

Figure.5 Association of homozygote A/A genotype with vomiting grades among cases

DISCUSSION

Regarding the genotype analysis there was a wide interethnic and intergeographical difference in the allele types and frequency of *DPYD* gene.

The present study is one of the leading study to document the allele and genotype frequency of *DPYD* IVS14+1G>A polymorphism among Sudanese population. It has been found that the frequency of cases to controls with *DPYD* IVS14+1G>A polymorphism was 37%:25% respectively, while cases to controls with wild type was 63%:75% respectively.

The individuals with homozygote A/A genotype were 25% among cases compared with 9% among healthy controls (*P value* =0.003). While the individuals with heterozygote G/A genotype were 12% among cases compared with 16% among healthy controls. On the contrary, no *DPYD* IVS14+1G>A mutation was found among the 239 Egyptians.[17]

These findings might indicate that a possible association between homozygote A/A genotype of *DPYD* and the toxicities of 5-FU due to DPD enzyme deficiency in cases with colorectal cancer as demonstrated in previous study.[7] The present study have also found allele A frequency was 20.5% among cases and 11.3% among healthy controls (*P value*=0.002), whereas allele G frequency was found to be 46.2% among cases and 55.3% among controls (*P value*=0.025). Several studies had reported allelic frequencies of the IVS14+1G>A mutation in different ethnic populations.[17] However, the sample size in some of these studies was too small to yield reliable and consistent data, thus leading to conflicting conclusions. In

one Turkish study which was done to investigate the allele frequency of *DPYD* IVS14+1G>A mutation, it has been found that, there were no significant frequency difference of allele A and G in cases compared to controls. This is more supported by the Egyptian study.[14]

The present study findings may suggest that a possible association between allele A and susceptibility to 5-FU toxicity in cases with colorectal cancer, while allele G is highly expressed in normal individuals.

Regarding the relation between genotype and ABO blood groups as hematological parameters, it has been found that there is significant association between males and females cases with heterozygous G/A genotype and carriers of blood groups O and A (*P value* 0.070 and 0.023 respectively). From the present study, it is likely to suggest that males carriers of blood group O are heterozygous G/A of *DPYD* gene and females carriers of blood group A are heterozygous G/A of *DPYD* gene.

Regardless male to female ratio, the cases were detected as homozygous A/A genotype were clearly observed with blood group A carriers while, cases detected as wild type G/G and heterozygous G/A genotype were highly observed with both blood group O and A carriers.

Regarding the relation between genotype and neutropenia as hematological parameter, there was no significant association between *DPYD* IVS14+1G>A polymorphism and neutropenia toxicity which is contradicted with a study which reported that IVS14+1G>A mutation was most frequently associated with toxicity including neutropenia.[19]

Regarding the relation between genotypes and non-hematological parameter, nausea toxicity, significant associations were found between males and females cases with heterozygous G/A genotype and nausea grades with (*P value* 0.014 and 0.004 respectively). It has been found that males cases with heterozygous G/A genotype mainly had nausea within normal level while female cases with heterozygous G/A genotype mainly had grade I nausea toxicity.

These finding agrees with earlier study suggest that profound DPD deficiency and death secondary to 5-FU do not always involve simple homozygote genotypes.[16]

Regarding the relation between genotype and non-hematological parameter, diarrhea toxicity, significant associations were found between males and females cases (*P value* 0.02 and 0.009 respectively) with homozygous A/A genotype and diarrhea grades toxicity. The males with

homozygous A/A genotype mainly had diarrhea within normal level while females with homozygous A/A genotype had grade III diarrhea toxicity.

These findings in line with many studies were performed around the world concluded that the polymorphism in IVS14+1G>A of *DPYD* gene are responsible for a significant proportion of life-threatening toxicity to 5-Fluorouracil.[9, 10, 11&20]

Regarding the relation between genotype and non-hematological parameter, vomiting toxicity, relevant associations were found between males and females cases (*P value* 0.07 for both) with homozygous A/A genotype and vomiting grades toxicity. Males with homozygous A/A genotype had vomiting within normal level while females with homozygous A/A genotype mainly had grade I vomiting toxicity.

Importantly to mention that the majority of females had experienced nausea, diarrhea and vomiting with variation in grades I, II and III toxicity while the majority of males had been within normal level.

A significant relation between vomiting grades with diarrhea grades (*P value* 0.01) has been noted. These findings indicate the cases that had diarrhea toxicity may be susceptible to have vomiting toxicity too.

CONCLUSION

The high number of cases receiving 5FU-based therapies per year and the experienced 5-FU toxicities by cases in the present study emphasize the importance to incorporate *DPYD* gene polymorphism assessment test to all colorectal cancer patients who candidate to use 5-FU in their treatment protocol to enhance the prediction of 5-FU toxicity. This could be added to *DPYD* IVS14+1G>A polymorphism screening with the PCR-RFLP technique is simple and inexpensive.

The important gains in terms of health care costs will be the reduction in hospitalization or death for severe complications (e.g: extensive and expensive medical care is often required to manage toxicities induced by anticancer agents) and the possibility for the patients to maintain their treatment regimens within a simple and economic screening test. Further studies are recommended to detect the other polymorphisms of *DPYD* gene, drug metabolizing genes and explore in detail the impacts of it on drug toxicity, with regarding the

factors considered to be promoters of carcinogenesis as age, gender, diet, growth factors, and chronic irritation.

Conflicts of interest: No conflict of interest between authors.

ACKNOWLEDGEMENTS

I would like to thank all study participants for kind donation of the samples and the team of Radio Isotope Center Khartoum. Thanks are extended to Mr. Salaheldin Jumaa (Ahfad University of Women) for his assistance and guidance with PCR. I also like to thank Dr. Hisham Mohamed Hassan (University of Khartoum) and Ms. Amanda Gamal (institute of tropical medicine) for their help in statistical analysis. I would like to express my deep appreciation to Prof. El.Sheikh A. El.obeid (Ahfad University of Women) for providing laboratory facilities.

REFERENCES

1. Xu L, Li M, Wang M, Yan D, Feng G, An G. The expression of microRNA-375 in plasma and tissue is matched in human colorectal cancer. BMC Cancer, 2014; 14:714.
2. Gado A, Ebeid B, Abdelmohsen A, Axon A. Colorectal cancer in Egypt is commoner in young people: Is this cause for alarm. Alexandria Journal of Medicine, 2014; 50(2): 99-196**.**
3. Longley DB, Harkin D, Johnston PG. 5-Flurouracil: Mechanisms of action and clinical strategies. Nature Reviews, 2013; 3: 330-331.
4. Dhawan D, Panchal H, Shukla S, Padh H. Genetic variability & chemotoxicity of 5-fluorouracil & cisplatin in head & neck cancer patients: a preliminary study. Indian J Med Res, 2013; 137:125-129.
5. Khalili H, Wolpin B, Huang E, Giovannucci E, Kraft P, Fuchs C, Chan A. ABO blood group and risk of colorectal cancer. Cancer Epidemiology Biomarkers and Prevention, 2011; 20(5): 1017-20.
6. Magnani E, Farnetti E, Nicoli D, Casali B, Savoldi L, Focaccetti C, Boni C, Albini A, Banzi M, Fluoropyrimidine toxicity in patients with dihydropyrimidine dehydrogenase splice site variant: the need for further revision of dose and schedule. Intern Emerg Med , 2013; 8: 417–423.
7. Lee W, Lockhart C, Kim R, Rothenberg M. Cancer Pharmacogenomics: Powerful Tools in Cancer Chemotherapy and Drug Development. The Oncologist, 2005; 10: 104-111.

8. Teh L, Hamzah S, Hashim H, Bannur Z, Zakaria Z, Hasbullani Z, Shia J, Fijeraid H, Nor A, Zailani M, Ramasamy P, Ngow H, Sood S, Salleh M. Potential of Dihydropyrimidine Dehydrogenase Genotypes in Personalizing 5-Fluorouracil Therapy Among Colorectal Cancer Patients. Ther Drug Monit, 2013; 35(5): 624–630.
9. Kristensen MH, Pedersen P, Mejer J. The Value of Dihydrouracil/Uracil Plasma Ratios in Predicting 5-Fluorouracilrelated Toxicity in Colorectal Cancer Patients. Journal of International Medical Research, 2010; 38: 1313-1323.
10. Ezzeldin H, Diasio R. Dihydropyrimidine Dehydrogenase Deficiency, A pharmacogenetic Syndrome Associated with Potentially Life threatening toxicity following 5-Flurouracil Administration. Clinical Colorectal Cancer, 2004; 4(3): 181-190.
11. Katzung BG, Masters SB, Trevor AJ. Basic & Clinical Pharmacology, international edition (Chicago), McGraw-Hill companies., 2012.
12. Walker R, Whittlesea C. Clinical pharmacy and therapeutics, London (New York). Elsevier: 2012.
13. Kleibl Z, Fidlerova J, Kleiblova P et al. Influence of dihydropyrimidine dehydrogenase gene (DPYD) coding sequence variants on the development of fluoropyrimidine–related toxicity in patients with high-grade toxicity and patients with excellent tolerance of fluoropyrimidine–based chemotherapy. Neoplasma, 2009; 56: 303–316.
14. Uzunkoy A, Dilmec F, Ozgonul A, Van kuilenburg A, Akkafa F. Investigation of IVS14+1G>A polymorphism of DPYD gene in a group of Turkish patients with colorectal cancer. Anticancer Research, 2007; 27: 3899-3902.
15. Kalyana C, Sudha M, Kaiser J. Possible Associations of Splice Site Mutation of Dihydropyrimidine Dehydrogenase (IVS14+1G>A) in Adverse Drug Reactions in Some Invasive Ductal Carcinoma Patients. International Journal of Pharmacology, 2007; 3.
16. Raida M, Schwabe W, Hausler P, Vankuilenburg A, Vangennip A, Behnke D, Hoffken K. Prevalence of a common Point Mutation in the Dihydropyrimidine Dehydrogenase (DPD) Gene within the 5-Splice donor site of Intron 14 in Patients with Severe 5-Flurouracil (5-Fu)-related toxicity Compared with controls. Clinical Cancer Research, 2001; 7(9): 2832-2841.
17. Garg M, Lincz L, Adler K, Scorgie F, Ackland S, Sakoff J. Predicting 5-fluorouracil toxicity in colorectal cancer patients from peripheral blood cell telomere length: a multivariate analysis. British Journal of Cancer, 2012; 107: 1525–1533.

18. Saif M, Syrigos K, Mehra R, Mattison L, Diasio R. Dihydropyrimidine dehydrogenase deficiency (DPD) in GI malignancies: Experience of 4-years. Pak J med Sci Q, 2007; 23(6): 832-839.
19. Staveren M, Guchelaar H, Kuilenburg A, Gelderblom H, Maring J. Evaluation of predictive tests for screening for dihydropyrimidine dehydrogenase deficiency. The Pharmacogenomics Journal, 2013; 13: 389–395.
20. Saif M. Dihydropyrimidine dehydrogenase gene (DPYD) polymorphism among Caucasian and non Caucasian patients with 5-Fu and capecitabine-related toxicity using full sequencing of DPYD. Cancer genomic proteomics, 2013; 10(2): 89-92.