العلاقة بين التعدد الشكلي لجينة NUDT15 ذات الرقم (rs116855232) والسمية النقوية المحرضة بمركبات البورين Mercaptopurine عند المرضى السوريين المشخص لديهم ابيضاض لمفاوي حاد

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الملخص

خلفية وهدف الدراسة: في دراسات سابقة وجد علاقة مهمة بين السميات النقوية المحرضة بمركبات البورين وبين التعدد الشكلي لجينة NUDT15 عند مرضى الابيضاض اللمفاوي الحاد ، هدف هذه الدراسة هو اختبار هذه العلاقة عند مجموعة من المرضى السوريين المصابين بالمرض السابق .

مرضى وطرائق الدراسة: ضمت هذه الدراسة الراجعة كل المرضى المشخص لديهم ابيضاض لمفاوي حاد والذين وصلو لمرحلة الصيانة من المعالجة (على الأقل 6 شهور) ،بدأت الدراسة من كانون الأول عام 2018 حتى آيار 2020 في ثلاث مواقع في دمشق، سوريا . لقد تم إجراء تحاليل خاصة لكشف جينة NUDT15 ذات الرقم rs116855232 ، كما تم ربط التعدد الشكلي للجينة السابقة مع العوامل التالية: شدة جرعة المركابتوبورين ، نقص الكريات الباكر، السمية الكبدية، الانقطاع عن المعالجة.

نتائج الدراسة: لقد ضمت الدراسة 107 مرضى (54.21 % منخفضي الخطورة، 33.64 % متوسطي الخطورة، 12.15% عاليي الخطورة)، إن شدة جرعة المركابتوبورين كانت على الشكل التالي: 82.61%، 56.90%، 4.99% للنماذج الجينية CC، TC، TT على التوالي (P = 0.0071) . لقد وجد علاقة مهمة بين نقص المعتدلات الباكر والتعدد الشكلي لجينة NUDT15 (TT أو TC) OR= 8.92 (95% CI = 1.79 – 44.48) (P= 0.012).

لايوجد أحد من المرضى الذي يمتلك تعدد شكلي طافر لديه ارتفاع في خمائر الكبد .

الاستنتاج: لقد أكدت دراستنا علاقة التعدد الشكلي لجينة NUDT15 ذات الرقم rs 116855232 مع السمية الدموية المحرضة بالمركابتوبورين، كما أكدت عدم وجود علاقة مع السمية الكبدية في عينة من مرضى المجتمع السوري المصابين بالابيضاض اللمفاوي الحاد.

الكلمات المفتاحية: التعدد الشكلي لجينة NUDT15، الابيضاض اللمفاوي الحاد ، سمية المركابتوبورين، السمية النقوية .

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Association between NUDT15 (rs 116855232) Genetic Polymorphism and Mercaptopurine Myelo-Toxicity in Syrian Patients with Acute Lymphoblastic Leukemia

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Abstract

Background and Aims: NUDT15 genetic polymorphism is known to be associated with frequent hematopoietic toxicities during acute lymphoblastic leukemia (ALL) mercaptopurine therapy. The aim of this study is to test this association in a group of Syrian ALL patients.

Patients and Methods: This is a retrospective study that included all patients with acute lymphoblastic leukemia reaching at least 6 months of maintenance therapy between January 2018 and May 2020 at three recruitement sites in Damascus, Syria. The NUDT15 rs116855232 genetic polymorphism was performed and linked to the current clinical factors: 6-mercaptopurine dose intensity, early onset leukopenia, hepatotoxicity and therapy interruption.

Results: A total of 107 patients were enrolled (54.21% low-risk, 33.64% intermediate-risk and 12.15% high-risk). The mercaptopurine dose intensity was 82.61%, 56.90% and 4.99% for the genotypes CC, TC and TT respectively (*P*: 0.0071). A significant association was found between early onset neutropenia and NUDT15 polymorphism (TC or TT), OR: 8.92 (95% CI: 1.79-44.48, *P*: 0.012). None of the patients with NUDT15 polymorphism had significant liver transaminases elevation.

Conclusion: Our study confirms that NUDT15 rs116855232 polymorphism is associated with mercaptopurine hematopoietic toxicity but not with hepatotoxicity in a population of Syrian patients with ALL.

Keywords: NUDT15 polymorphism, Acute lymphoblastic leukemia, Mercaptopurine toxicity, Myelotoxicity.

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Introduction:

Acute lymphoblastic leukemia (ALL) is responsible for almost a third of all cancers in children (Hunger, Stephen P. Mignon L.Loh 2011) (Pui et al. 2011) (Lopez-Lopez et al. 2014) and can be cured with a combination of chemotherapy alone (Narayanan and Shami 2012).

One chemotherapeutic drug agent, 6-mercaptopurine (6MP), is an antimetabolite pro-drug that is widely used in different stages of ALL treatment in children (Yi et al. 2018). 6MP needs to be converted to thioguanosine triphosphate (TGTP) which is incorporated into DNA to form DNA-TG compound triggering futile DNA damage, and subsequently cell apoptosis (Moriyama et al. 2017) (Dean 2020).

Genetic polymorphism in specific genes, that play a role in metabolizing thiopurine, can directly influence drug toxicity and anti-leukemic efficacy. In fact, several single nucleotide polymorphism (SNPs) have been reported to diminish thiopurine metabolism enzymatic activity and lead to the accumulation of thioguanine nucleotides, inducing hematologic toxicity. NUDT15 is a negative regulator nucleotide di-phosphatase that converts TGTP to thioguanosine monophosphate (TGMP) and is thus responsible for the inactivation of thiopurine metabolites (Yang et al. 2014) (Genet 2016) (Zgheib et al. 2017) (Yang et al. 2015).

NUDT15 was previously sequenced in 6MP treated patients, trying to identify the cause of the significant variability in tolerated dose in patients with similar clinical characteristic treated by the same therapy plan. Polymorphism in NUDT15 rs116855232 (c415c>T) leads to changing arginine 139 to cysteine. This change causes protein instability and decreases enzymatic activity, resulting in accumulation of thioguanine and excessive 6MP-related toxicity (Genet 2016) (Yin et al. 2017a).

In two previous reports, the presence of heterozygous and homozygous $NUDT15^{R139C}$ allele increased the chance of severe leukopenia related to 6MP treatment 13.4 and 807 times, respectively (Tatsumi et al. 2020) (Kakuta et al. 2018). Therefore, NUDT15 genotype can guide 6MP dose for ALL therapy. This is an example of pharmacogenetics driven precision medicine in cancer.

The primary objective of this observational study was to confirm the association between the deleterous variant NUDT15 (c415c>T) and 6MP tolerated dose and toxicity.

Methods:

Patients and treatment protocol:

A total of 107 subjects with ALL were enrolled in study between January 2018 and May 2020. Patients below 17 years of age were treated using one of two protocol either ECOG or St Jude protocol total XV. And patients above 17 years were treated by Hyper CVAD or GMALL 84 protocols according to attendant desire. The standard 6MP dosage during the maintenance phase of most the previous protocols was 50 mg per m^2 except in St Jude protocol was 75 mg per m^2 and 60 mg per m^2 for adult protocols. But in our study the final aim was 6MP dose intensity % which was computed as the ratio of the final 6MP dose to that of the prescribed 6MP maintenance dose as per protocol. The 6MP dose are adjusted so as to maintain the WBC above 2000 per UL and the platelet count more than 50.000 per UL. Interruption was defined as the cessation of the administration of medicine resulting from infections and or hepatotoxicity. Hepatotoxicity was defined as an ALT or AST level > 500 U/L at any time point during maintenance therapy. Early-onset leukopenia was defined as leukopenia occurrence during the first 60 days of the maintenance therapy. The study was conducted with the approval of research committee in Damascus University. Informed consent was obtained from the parents or guardians of the patients or from the patients themselves depending on the age and conceptual ability of the patients.

Genetic Analyses:

Total genomic DNA was extracted from peripheral blood using the blood DNA preparation kit (Jena Bioscience, Germany) according to the manufacture instructions and stored at -20 degree C until analysis. The total genomic DNA concentration was determined

using a spectrophotometer (MAESTROGEN, MaestroNano,ProMN913A,Taiwan).Genotypes of rs116855232 were determined by PCR and Sanger sequencing. The sequences of the forward and reverse primers were AAGCAAATGCAAAGCATCAC and GGCTGAAAGAGTGGGGGATA. Each 20 UL PCR amplification reaction contained 1 UL of each primer (10 UM), 2 UL DNA specimen (concentration different between specimen nm) ,6 UL free nuclease water and 10 UL direct PCR Master Mix (KAPABIOSYSTEMS, KAPA2G, KR0374-v11.23) . The reactions were amplified using a DNA Engine (Labcycler SensoQuest, Germany). The PCR conditions were as follows: initial holding at 95 degree C for 5 minutes, 35 cycles of denaturing at 95 degree C for 30 seconds, annealing at 52 degree C for 30 seconds and extension at 72 degree C for 1 second and post extension at 72 degree C for 10 minute. At completion of the PCR reactions, 450 bp long amplicon containing the rs116855232 site were amplified. We stored the specimens at -20 degree c until sequencing them in Macrogen company (Seoul, Republic of Korea).

Statistical analysis:

All statistical analyses were performed using STATA version 6, statistical package and statistical significance was defined as differences with two-sided $P< 0.05$. The collected data were summarized using descriptive statistics and presented as means, standard deviation (SD), and medians (range) for continuous variables. . The mann-whitney U-test was performed to compare continuous variables. Patients were grouped based on NUDT15 genotypes. Multivariate logistic regression analysis was assessed to identify the predictive genetic and clinical factors independently associated with frequency of myelotoxicity (WBC < 2000/mm^3). Odd ratio and corresponding 95% CI were also calculated. Kruskal-wallis test was used to evaluate the difference among patient groups for WBC and 6MP dose intensity.

Results:

Patient characteristics and genetic polymorphisms:

The demographics and clinical characteristics of recruited patients (n=107) are summarized in (table 1). Therapy induced leucopenia was observed in patients during the weeks 1-8(early myelotoxicity). While therapy –induced leucopenia was observed in 32 patients during the weeks 9-24 (late myelotoxicity).

Association between NUDT15 variants with 6MP toxicity:

6MP -induced leukopenia was noticed in 49 patients (45.79%) of total patients, 17 patients was diagnosed during the first 8 weeks of maintenance therapy. Early onset - leukopenia was more frequent in NUDT15 T allele carriers (CT+TT) and was associated with a 8.92 fold increased risk compared with that in patients with wild type (P=0.012, OR =8.92; 95% CI =1.79-44.48) (Table 2).

In contrast 6MP-related hepatotoxicity was just found in 5 patients with NUDT15 CC genotype (4.67% of total patients), and no patient with NUDT15 T allele experienced hepatotoxicity during maintenance therapy (table2) .

32 patients (29.9% of total patient) required 6MP interruption due to severe infection but the odds ratio was not statistically significant (P=0.65, OR=0.93; 95%CI=0.17-5.08).

The relationship between genetics variants and 6MP sensitivity:

Regardless of the used protocols, 6MP dose was modulated according to toxicities during maintenance therapy .therefore, 6MP dose intensity defined as the ratio of prescribed 6MP dose over the protocol dose directly reflected drug tolerance.

Only one patient was diagnosed with homozygous for NUDT15 c415 c >T and this individual was highly sensitive to 6MP with a dose intensity of 4.99 % compared with these with the heterozygous genotype (n=6) or wild type (n=100) who tolerated an average dose intensity of 56.9 and 82.61% respectively (Table3) Figure (1).

Table1: Characteristics of patients with ALL according to NUDT15 genotype

		NUDT15 (rs116855232, C.415c> T)		
	Total patients	CC	CT	TT
		100	6	1
Gender				
Female	43 (40.19%)	39	3	1
Male	64 (59.81%)	61	3	0
Age(Year)	9.6 (0.9-56.2)	9.7 (0.9-56.2)	7.5 (3.1-24.5)	5
BSA.m^2	1 (0.52-2)	1 (0.52-2)	0.91 (0.6-1.75)	0.71
Immunologic subtype				
B-lymphoblastic lymphoma	9 (8.41%)	8	1	0
Bi-phenotype	1 (0.93%)	1	0	0
T-ALL	12 (11.21%)	12	0	0
T-lymphoblastic lymphoma	7 (6.54%)	7	0	0
Precursor B	78 (27.9%)	72	5	1
Protocols				
EORTC AR1	57 (53.27%)	52	5	0
EORTC AR2	23 (21.5%)	23	0	0
GR-All	3 (2.8%)	3	0	0
Hyper CVAD	11 (10.28%)	10	1	0
St Jude LR	1 (0.93%)	0	0	1
St Jude VHR	12 (11.21%)	12	0	0
Risk group				
High risk	13(12.15%)	13	0	0
Intermediate risk	36(33.64%)	35	1	0
Low risk	58(54.21%)	52	5	1
Leucopenia				
No	58(54.21%)	58	0	0
Yes	49(45.79%)	42	6	1
Days to first WBC toxicity				
Mean (min-max)	118 (20-457)	129 (20-457)	57(42-69)	42
Thrombocytopenia				
No	100 (93.46%)	93	6	1
Yes	7 (6.54%)	7	0	0
Days to first PLT toxicity				
Mean (min-max)	137(21-376)	137(21-376)	-	-
AST/ALT >500				
No	102(95.33%)	95	6	0
Yes	5 (4.67%)	5	0	1
Alopecia				
No	101(94.39%)	95	6	0
YES	6(5.61%)	5	0	1
Fever				
No	81(75.7%)	76	4	1
Yes	26(24.3%)	24	2	0
Rash				
No	106(99.07%)	99	6	1
Yes	1(0.93%)	1	0	0
Therapy interruption				
No	75(70.09%)	70	4	1
Yes	32(29.91%)	30	2	0
6MP dose intensity<60%				
No	80(74.77%)	29	1	0
Yes	27(25.23%)	21	5	1

Table 2: Associations between NUDT15 and risk of leucopenia , hepatotoxicity and therapy interruption:

		NUDT15 genotype				
		CC(n=100)	TC,TT(n=7)	P-value	OR	95%CI
Leucopenia	No	58	0	0.003		
	Yes	42	7			
Early Onset Leucopenia	No	87	3	0.012	8.92	(1.79-44.48)
	Yes	13	4			
AST/ALT>500	No	95	7	0.708		
	Yes	5	0			
Therapy interruption	No	70	5	0.652	0.93	(0.17-5.08)
	Yes	30	2			

Table3: Comparison of 6MP dose intensity % by NUDT15 genotype

		6MP dose intensity%		
		Mean	Std. Dev.	P-value
NUDT15	CC	82.61	22.35	0.0071
	TC	56.90	9.56	
	TT	4.99	0	
		CC	TC	TT
	CC		25.71	77.62
	P-value		0.019	0.002
	TC			51.91
	P-value			0.091

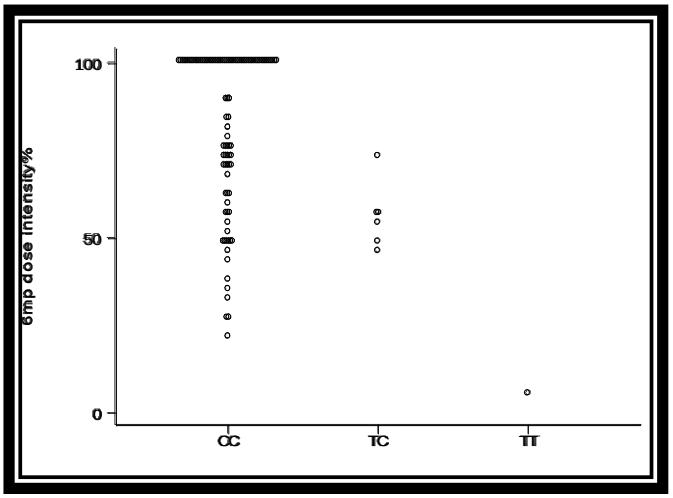

Figure 1**: illustrated the relationship between genetics variants of NUDT15 rs 116855232 and 6MP dose intensity**

Discussion:

Over the past two decades, emerging clinical trials have focused on predicting chemotherapy tolerance according to specific genotypes to guide dose and duration of therapy. Confirmed differences of genetic variants prevalence were observed in multiple ethnic groups in these studies. 6MP is the backbone of maintenance chemotherapy in all acute lymphoblastic leukemia protocols in children. NUDT15 genetic polymorphism was confirmed to be associated with 6MP toxicity in multiple populations (Yin et al. 2017b) . Only two previous studies (Zgheib et al. 2017) (Moradveisi et al. 2019) have investigated this association in Middle Eastern or Arab children with ALL.

More importantly, TC genotype was associated with a tolerable MP dose of 56.9% of the planned MP dose during maintenance therapy for ALL. This percentage is almost equal to that reported from the SJCRH cohort which was median MP dose intensity being 63% for TC (J. J. Yang et al. 2015). But it is higher than that reported from the Lebanese cohort; The 6MP dose intensity was just 33.33% (Zgheib et al. 2017). In addition, TT genotype was associated with a little value of MP dose intensity 4.99% and P-value was significant 0.0071. The same applies to East Asian patients treated on different protocols for ALL but the TT genotype was correlated with a tolerable MP dose of 8.3% of the planned MP dose as Yang et al reported (J. J. Yang et al. 2015). This finding was confirmed recently in Chinese pediatric ALL patients with NUDT15 homogenous genotype (TT) sensitive to 6MP but the dose intensity was 60.27% (Zhou et al. 2018).

Consistent with previous reports, we further confirmed the findings that NUDT15 C415c>T is strongly associated with 6MP induced leukopenia in patients with ALL, Notably the previous polymorphism was also a strong indicator of early –onset leukopenia (Leukopenia that developed within the initial 60 days of the maintenance therapy). The odd ratio was 8.92 (p-value =0.012) which is compatible with another study that found a significant association between intermediate and low NUDT15 activity groups and increased risks of leucopenia (OR=15 , P= 0.000253) and neutropenia (OR=9 , P=0.002)(Sutiman et al. 2018).

Yang et al (S.-K. Yang et al. 2014), Asada et al and Kakuta et al reported that the relationship between NUDt15 R139C and early onset leucopenia in different Asian population. A recent systematic review with meta-analysis by Van Gennep et al revealed that carrying NUDT15c415 C> T (OR=6.9; 95% CI = 5.2-9.1) significantly increased the risk for thiopurine induced leukopenia.

NUDT15, also known as MTH2, is a 164 amino acid protein that belongs to the nudix hydrolase enzyme family , whose members can hydrolyze compounds with the general structure of a nucleoside di-phosphate such as converting 8-oxo-dGDP to 8-oxo-dGMP (Loscalzo 2011)(Takagi et al. 2012)(Valerie et al. 2016). NUDT15 rs 116855232 is located in exon 3 causing an arginine to cysteine(p.Arg139cys) mutation that consequently leads to changes in the amino acid sequence of the NUDT15 protein (S.-K. Yang et al. 2014). The impacts of NUDT15 rs 116855232 on thiopurines induced myelo-toxicity and on thiopurines intolerance are well established. The mechanism underlying the NUDT15 related leukopenia remains unknown as reported by Moriyama T. el al. (Moriyama et al. 2016) .NUDT15 inactivates thiopurine metabolites and decreases thiopurine cytotoxicity in vitro and patients with NUDT15 risk alleles have excessive levels of active thiopurine metabolites leading to an increase in mercaptopurine induced toxicity . In contrary, no significant differences in the 6-TGN levels of patients with wild-type genotype and those who were heterozygous at the C415c> T (rs 116855232) locus (Sutiman et al. 2018).

Intriguingly, a new study revealed that NUDT15 P-Arg 139 cys mutation failed to affect enzymatic activity but negatively influenced protein stability, possibly due to a loss of supportive intra-molecular bonds that caused a rapid proteasomal degradation in cells (Valerie et al. 2016). In addition, the findings of Carter et al suggest that the structural change resulting to variation which would occur at the base of the

substrate –binding pocket of the NUDT15 monomer (Sutiman et al. 2018).

Based on the previous result, we believe that, it is worthwhile to preemptively genotype for the NUDT15 C415c>T .since the effect on 6MP dose tolerability is quite significant and we use 6MP in all phases of treatment induction, consolidation and most importantly continuation phase (Zgheib et al. 2017)

Our study has some limitations, first of all, this study has a small size of population (n=107) resulting in a low power to detect differences between small subsets .Second, we use a plenty of protocol to treat patients which may affect the results. Third we performed a population based retrospective analysis of NUDT15 variants in Syrian patients with ALL and we do it after the 6MP is administered. Fourth the present study restricted to the most common allele of NUDT15 and did not include other rare variants and other SNPs that have been reported previously in other studies.

In conclusion: This is the first report on the association of NUDT15 polymorphisms with MP dose intolerance in Syrian patients with ALL. . Further studies are needed in Arabs especially Iraqis to evaluate the frequency of NUDT15 c415 c>T polymorphism and its role in thiopurine induced myelo-toxicit . Also we need to do more genotype for additional SNPs in our population to know their role in myelo-toxicity during treating patients with acute lymphoblastic leukemia.

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