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Research Article

The Significance Of Thiopurine Genetic Diversity In The Treatment Of Pediatric Acute Lymphoblastic Leukemia.

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy and 6-mercaptopurine (6-MP) is one of the significant drugs to cure ALL. However, 6-MP toxicity is individualized and influenced by genetic and environmental factors. Further study of genetic variants in Thiopurine Methyltransferase (TPMT) combined with environmental influence constitutes needed research about treatment-related toxicity.
Purpose: The study examines how genetic variants of TPMT affect 6-MP doses required for ALL patients.
Methodology: In this study, the control group consisted of 100 healthy children, while the patient group comprised 100 children with newly diagnosed ALL in the maintenance phase of their treatment. Thiopurine Methyltransferase (TPMT) gene to identify genetic variants. The level of drug toxicity was determined by Complete Blood Count (CBC), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and serum creatinine before and 6 months after switching to 6-MP therapy.
Results: The results show a significant decrease in hemoglobin (HB) and platelet count in patients before treatment and significant improvement after treatment. After treatment, AST and ALT levels were significantly increased, and the White Blood Cells (WBC) count decreased significantly. There were 13% heterozygous patients determined by TPMT genotyping.
Conclusion: TPMT genotype before the treatment will help to determine high-risk patients, and accordingly, the dose of 6-MP could be diminished to reduce the risk of toxicity caused by 6-MP.

Keywords : ALL, TPMT, genetic variants, 6MP, drug toxicity..

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INTRODUCTION

Hematological malignancies known as leukaemia encompass a wide range of cancers in which there is unregulated production of abnormal white blood cells. These cancers originate in the bone marrow but can spread to the blood and other body parts, disrupting normal immune and blood functions. Leukaemia is differentiated by how fast it progresses (acute or chronic) or which type of white blood cell is involved (myeloid or lymphoid). These classifications lead into four major types, these being acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL), and chronic myeloid leukaemia (CML) (Puckett & Chan, 2021). They have unique clinical characteristics, prognosis and treatment options.

These include ALL, which is the most common leukaemia in children and is responsible for the bulk of pediatric leukaemia cases. ALL is a heterogeneous haematological disorder by which immature lymphoid cells proliferate in the bone marrow, peripheral blood and other organs (Hegazy et al.). Symptoms of the disease can vary from fever, fatigue, pallor, weight loss, bone pain, bruising, bleeding, and enlarged lymph nodes (Brown et al., 2020). Generally, diagnosis requires a bone marrow biopsy with findings that guide a professional treatment approach, often including chemotherapy, targeted therapy or stem cell transplantation (Puckett & Chan, 2021). Although ALL can happen in adults and kids, it most commonly develops in the young, with a median age at diagnosis of 15 years. More cases (55.4%) occur in individuals under age 20 and a small percentage (28%) after age 45 (Brown et al., 2020). Specific causes of ALL, while a significant public health issue, are mostly unknown, and genetic and environmental risk factors are associated with its development (Puckett & Chan, 2021).

The treatment of ALL is one of the most complex and often rigorous treatments for any cancer and typically involves phases such as induction, consolidation, and maintenance. 6-Mercaptopurine (6-MP), a purine analogue, is one of the most critical chemotherapy agents in ALL treatment that has improved survival rates drastically in children with ALL. Treatment response and toxicity vary significantly among patients due to genetic factors, namely polymorphisms in the enzyme Thiopurine Methyltransferase (TPMT) responsible for 6-mercaptopurine (6-MP) metabolism. However, genetic variants in TPMT include a risk of severe myelosuppression, limiting the 6-MP dose to a tolerable level and thus increasing the risk of life-threatening complications (Brown et al., 2020). This study evaluates how the generation in leukaemia of both genetic predisposition and oxidative stress affects the development of leukaemia and how thiopurine metabolism, specifically 6-MP, modifies therapeutic outcomes.

METHODOLOGY

The patients were treated according to the St. Jude ALL Total Therapy Study XV treatment protocol (Pui et al., 2019). Treatment comprises three phases: Induction treatment (6 weeks), Consolidation treatment (8 weeks), and Maintenance treatment (120 weeks).

Data collection

This study included 100 children with ALL and 100 healthy children as the control group. Study was done as a collaboration between teams in different private and governmental hospitals according to participant's affiliations from October 2023 to December 2024. Seven months were chosen as the period of completing maintenance therapy to achieve an adequate length of time for monitoring toxicity (Hegazy et al.2023).

Study Design

A six-month prospective observational study was evaluated on the effects of biochemical and hematological parameters due to 6-MP maintenance therapy in pediatric patients. Liver and kidney function markers, such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and creatinine was performed on 5 mL serum collected at baseline. Furthermore, a 4 mL Ethylenediaminetetraacetic acid (EDTA) anticoagulated blood sample was drawn for TPMT genotyping via Polymerase Chain Reaction (PCR) Sequencing and CBC Screen.

After six months of therapy with 6-MP, repeat CBC (for bone marrow toxicity) and liver/kidney function tests (for drug-induced toxicity) were carried out for comparison.

Data Analysis

The present study used R- statistical program version 4.0.4 to analyze the data. This was done to compare a numeric variable between two independent and dependent groups utilizing the Independent T-test (T) and paired T-test (pT). These variables have been counted (percentage) and the correlation between two categorical variables was tested by Fischer's Exact test (F). In all tests, a P-value less than 0.05 is considered significant.

RESULTS

The study included 100 children with ALL in the patient group and 100 healthy children in the control group. The researchers obtained measurements before starting 6-MP treatment and again six months into treatment to determine changes in essential hematological and biochemical parameters.

AS shown in **Table 1**. The baseline hematological and biochemical characteristics of 100 ALL children patients and 100 healthy peers were compared before the introduction

of 6-MP. A significant decrease in hemoglobin levels which was 8.4 ± 1.9 g/dl (P < 0.000), reduced platelet counts, which were 75 ± 30 ×10³/µL (P < 0.000), and leukocyte count, which was 6.3±0.6 ×10³/µL (P < 0.000) indicated the presence of bone marrow suppression. Liver function in this study was evaluated using AST and ALT, which revealed no significant differences between the pretreatment levels and the control (P > 0.05), meaning that hepatic function was relatively healthy before the commencement of therapy. Furthermore, primary data show that 87% of ALL patients had a wild-type TPMT genotype, which means that a low metabolism of 6-MP increases instead of having toxic effects. These results support the initial hypothesis by documenting lower specific hematology components all patients with ALL who have not yet received any form of treatment; however, liver and renal functions are within normal range.

Table 1. Different parameter levels among patients before and after treatment with 6-MP drug and the control group.

Parameter	Control	Before treatment	After treatment	P-Value
HB (g/dl)	12±0.7	8.4 ± 1.9	9.7±1.5	<0.000 (T)
Platelet count(×10^3 µL)	289±68	75±30	105±31	<0.000 (T)
WBC count (×10^3 µL)	6.7[5.7-8.3]	6.3±0.6	5.3±0.4	<0.000 (M)
ALT(U/L)	25±7	31±10	85±23	<0.000 (M)
AST(U/L)	29±7.7	25±9	99±26	<0.000 (M)
Creatinine (mg/dl)	0.7±0.23	0.73±0.2	0.69±0.22	0.356(T)
TMPT genotype (%)	0(0%) Hetero 100(100%) Wild	13 (13 %) Hetero 87(87%) Wild	-	0.117(F)

The impact of 6-MP therapy on pre- and post-treatment hematological and biochemical laboratories in children with ALL.HB level also changed after six months (8.4 ± 1.9 g/dl to 9.7± 1.5 g/dl, P < 0.000): it demonstrated a bone marrow response to Dressler's fibrinogen-supplemented therapy. Likewise, platelet counts were also significantly increased (75 ± 30 ×10³/µL to 105 ± 37 ×10³/µL, P < 0.000), which reflects an improved megakaryocytic activity. Nonetheless, White Blood Cells (WBC) counts remained reduced (6.3±0.6×10³/µL to 5.3±0.4×10³/µL P < 0.000), indicating myelosuppression that often will require modification of the dose. There was a significant rise in liver enzymes specifically in both ALT pretreatment (31±10 U/L) to post-treatment (85±23U/L) P < 0.000) and in AST enzymes pretreatment 25 ± 9 U/L to post-treatment 99 ± 26 U/L P < 0.000 suggesting hepatotoxicity induced by 6-MP therapy. However, creatinine levels were not altered 0.73±0.2mg/dl to 0.69±0.22mg/dl, p = 0.356), so no renal side effects were observed. Hence, these findings indicate that though 6-MP aids in hematologic recovery, it has hepatotoxic impacts, which should be closely monitored to minimize toxicity levels associated with the drug.

DISCUSSION

ALL is a type of cancer that causes too many immature white blood cells to grow and multiply uncontrolled in the bone marrow and the blood. It is due to an increase in the number of leukemia blast cells in the bone marrow and peripheral blood. Malignant disease, specifically ALL, is the most common disease in children (Shafat et al., 2017). The development of this disease may have genetic and environmental factors that cause susceptibility or trigger the condition (Carbone et al., 2020). Many studies indicate that childhood ALL may share certain risk factors with radiation exposure, environmental toxins, and some inherited disorders (Moghaddasi et al., 2018). Many environmental carcinogens, including benzene, polycyclic aromatic hydrocarbons (PAHs), and nitrosamines, can induce DNA damage and affect hematopoietic stem cell function (Birkett et al., 2019). The present report confirms the previously established association between environmental exposures and increased risk of leukaemia, specifically of the AML. However, several studies have shown that certain environmental exposures may induce leukemogenic effects by epigenetic modifications, gene mutations, and dysregulated immune responses (Spatari et al., 2021). Furthermore, exposure to environmental toxins can alter cellular physiology and damage DNA by producing ROS in the hematopoietic cells, thereby promoting malignant transformation (Sackey, 2023).

The analysis shows a strong correlation between environmental factors and leukaemia incidence, which agrees with other epidemiological data. However individual variation in susceptibility to oxidative stress from environmental exposures affects the level of damage cellular damage. Notably, genetic variations in the detoxification enzymes, for example, glutathione S transferase (GST) and the cytochrome P450 (CYP) enzymes, are related to an individual's capacity to metabolize tobacco carcinogens (Silva & Carvalho, 2018). Additionally, a small subset of individuals with impaired detoxification pathways may increase their likelihood of succumbing to leukaemia by a more significant accumulation of carcinogens.

In addition to environmental factors, oxidative stress has a significant role in leukemogenesis as a cause of DNA damage,

chromosomal instability, and apoptosis resistance (Kaweme et al., 2020). Reactive oxygen species (ROS) can promote damage in tumor cells and activate oncogenic pathways, including p53 inactivation and NF-κB activation, which play a central role in tumor transformation and cancer progression. All the above indicates that oxidative damage has to be mitigated with antioxidant defenses. Antioxidant therapy is suspected to affect leukaemia progression via reduced amounts of antioxidants, in particular, superoxide dismutase (SOD) and catalase (Dong et al., 2021).

Biotransformation of 6-MP is an essential part of thiopurine metabolism in leukaemia treatment. The enzyme TPMT regulates 6-MP metabolism, and genetic polymorphism in TPMT affects the efficacy and toxicity of receiving treatment. Patients with low TPMT activity have higher active metabolite levels and a greater chance of myelosuppression while simultaneously having a greater therapeutic response (Franca et al., 2021). However, those with high TPMT activity suffer from rapid drug metabolism that is less than optimal for drug pharmacokinetic measures, too. The genetic variability of thiopurine metabolism is necessary to advance the treatment strategies for leukaemia and minimize toxicity in current treatment plans (Rudin et al., 2017).

This study further supports the role of oxidative stress, and genetic predisposition in the pathogenesis of leukaemia. However, some limitations in interpretation need to be acknowledged (Peluso et al., 2020). Leukaemia is a multifactorial disease; other things may modulate disease risk, such as environmental toxins, viral infections, lifestyle factors, etc. To better illuminate the processes of leukemogenesis, further studies on the causative process of leukemogenesis would be revealed by further studies incorporating a larger spectrum of genetic markers and longitudinal studies to determine the process of leukemogenes (Knisbacher et al., 2022). Additionally, we would better understand the significance of oxidative stress-related biomarkers in the evolution of leukaemia and patient response to therapy.

In conclusion, this study shows that environmental factors are associated with leukaemia. It highlights that inherited genetic polymorphisms and oxidative stress play a role in the susceptibility and response to leukaemia chemotherapy (Carda et al., 2016). Personalized therapeutic approaches, risk reduction of leukaemia, and patient survival all come down to understanding how these factors interact. Therefore, future research must include genetic screening and oxidative stress biomarkers in clinical practice to prevent and treat leukaemia more effectively.

CONCLUSION

It was discovered that TPMT genetic variations significantly increased the risk of hematologic toxicity that affects the pediatric ALL patients receiving 6-MP. However, environmental factors are a known health risk for children. Despite this, the presence of TPMT polymorphisms significantly increased the risk of hematological toxicity, and heterozygous carriers had the most severe effects. These findings show that TPMT genetic screening prior to 6-MP treatment would enable tailoring of the dose according to patient status and diminish toxicity. Genetic testing in clinical practice may improve treatment outcomes and safer ALL therapy.

Future Recommendations

- Expand Genetic Research: Perform large-scale studies with more participants to determine the TPMT genetic anomalies and their part in treatment-related toxicity and reaction in children with ALL.
- Adopt a Multi-Gene Approach: Future prospective research should adopt a multi-gene approach to yielding the genetic factors that can influence the variability in treatment response to enable personalized therapy and increase survival rates.
- Integrate Environmental Factors in Treatment Strategies: Develop and examine the effects on treatment outcomes of environmental factors, including exposure to pollutants, and implement interventions to minimize their impact.
- Promote Public Awareness and Education: Create a well-thought-out education plan to promote awareness of environmental health factors that may affect pediatric leukaemia patients and promote general good health.
- Encourage a Healthy Home Environment: Nurse practitioners and other healthcare providers should actively inform parents about ways through which they can reduce modifiable environmental factors affecting children who are under ALL treatment and adopt protective health measures.

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