Genotyping of TPMT and ITPA and risk of adverse effects among the children with acute lymphoblastic leukemia treated with 6-mercaptopurine in Bangladesh

(バングラデシュにおいて 6-mercaptopurine を投与中の白血病小児に見られる有害作用と薬物代謝酵素遺伝子多型に関する研究)

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SUMMARY

Leukemia is a group of diseases characterized by abnormal and uncontrolled proliferation of haemopoietic cells cause progressively increasing infiltration of the bone marrow. Acute lymphoblastic leukemia (ALL) occurs when there is an acquired damage to the DNA of a single cell in the bone marrow. It results in extensive and uncontrolled growth and accumulation of lymphoblast or leukemic blast, which do not function as normal blood cells. Moreover, it causes the blockage of the production of normal marrow cells and deficiencies in erythrocytes (anemia), platelets (thrombocytopenia) and leukocytes (in particular neutrophil). 6-MP is one of the cornerstones in ALL chemotherapy. 6-MP is converted by TPMT to the inactive metabolite methyl mercaptopurine. TPMT is known to catalyze S-methylation of aromatic and heterocyclic compounds, preferentially thio compounds such as 6-MP and 6-TG. Genetic polymorphism of TPMT have been demonstrated to influence drug metabolism and its effects, constituting one of the most studied and significant example of association between drug clinical effects and a genetic polymorphism. ITPA is a cytosolic enzyme which is involved in the metabolism of 6-MP. It catalyzes the hydrolysis of inosine triphosphate (ITP) to inosine

monophosphate (IMP), thereby recycling purines that might otherwise be trapped in the form of ITP preference, thus protecting cells from the accumulation of harmful nucleotides such as ITP and deoxyinosine triphosphate. Genetic polymorphisms in the ITPA are associated with reduced activity of the ITPA enzyme and increase toxicity to mercaptopurine.

In chapter 1, the current knowledge of leukemia and acute lymphoblastic leukemia and their treatment were summarized. In this chapter, I also summarized about TPMT and ITPA through literature reviews.

In chapter 2, distribution of TPMT and ITPA polymorphism among Bangladeshi children and their association with the adverse effects of 6-MP in acute lymphoblastic leukemia (ALL) patients was identified. In this study, I recruited 75 patients diagnosed with ALL and 75 volunteers with minor illnesses. Genotyping for TPMT (TPMT*3C, *3B, *2) and ITPA (ITPA94C>.A) was done. Relationship between genotypes and adverse effects of 6-MP was

investigated. Minor allele frequency of TPMT*3B, TPMT*3C and ITPA polymorphism among volunteers were 0.006, 0.020 and 0.903 and minor allele frequency of TPMT*3C and ITPA polymorphism among ALL patients were 0.010 and 0.153 respectively. ALL patients with TPMT*3C variant developed leucopenia (P=0.037) neutropenia (P=0.017) and thrombocytopenia (P=0.008). ITPA variant developed fever (P=0.003) neutropenia (P=0.001) and liver toxicity (hyperbillirubinemia, P=0.048, and raised serum ALT, P=0.007). These findings strongly suggest the importance of TPMT and ITPA genotyping in patients with ALL to design more rational and cost effective treatment strategy in children with ALL.

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ABBREVIATIONS

6-MMPRS	6-methyl mercaptopurine ribonucleotides
6-MP	6-mercaptopurine
6-MTIDP	6-methyl thionosine 5'-diphosphate
6-MTIMP	6-methyl thionosine 5-monophosphate
6-MTITP	6-methyl thioinosine 5'-triphosphate
6-TG	6-thioguanine
6-TIMP	6-thioinosine monophosphate
6-TITP	6-thioinosine 5'-triphosphate
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AO	Aldehyde oxidase
AZA	Azathioprine
cDNA	Complementary DNA
DNA	Deoxyribonucleic acid

FAB	French-American-British
EFS	Event free survival
GMPS	Guanosine monophosphate synthetase
HGPRT	Hypoxanthine guanine phosphoribosyl transferase
HPRT	Hypoxanthine phosphoribosyl transferase
IMPDH	Inosine monophosphate dehydrogenase
ITPA	Inosine triphosphate pyrophosphatase
meMP	Methylmercaptopurine
MMR	Mismatch require
MTX	Methotrexate
PAS	P-aminosalicylic acid
PCR	Polymerase chain reaction
PDNS	Purine de novo synthesis
TGDP	Thioguanosine diphosphate
TGMP	Thioguanosine monophosphate

TGNs	Thioguanine nucleotides
TGTP	Thioguanosine triphosphate
TIMP	Thioinosine monophosphate
TPMT	Thiopurine methyltransferase
SNPs	Single nucleotide polymorphisms
TUA	Thiouric acid
TXMP	Thioxanthine monophosphate
WBC	White blood cell
XO	Xanthine oxidase

CHAPTER 1

LITERATURE REVIEWS

1.1 Leukemia

Leukemia was first observed by pathologist Rudolf Virchow in 1845. Virchow called the condition leukemia in German, which he formed from the two Greek words Leuokos, meaning “white” and aima, meaning “blood”. Leukemia is a group of diseases characterized by abnormal and uncontrolled proliferation of haemopoietic cells cause progressively increasing infiltration of the bone marrow (haemopoietic blast cells constitute more than 30% of bone marrow cell), although in certain forms the lymphatic tissues are particularly affected. The process of differentiation in leukaemic cells in often abnormal and this commonly results in an immature morphological appearance (1). The major forms of leukemia are divided on the basis of their morphological characteristics into four categories, i.e., acute and chronic myelogenous and lymphocytic leukemia. Previously, being diagnosed as leukemia was tantamount to receiving a death sentence, but now a day the majority of these patients can be cured by chemotherapy, in combination with intensive supportive care. Originally involving the use of a single drug, but recently such chemotherapy is complex and multidisciplinary, involving combination of chemotherapeutic agents. Despite the availability of effective treatment,

leukemia remains the second major cause of death in children in western world.

1.2 Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia (ALL) occurs when there is a acquired damage to the DNA of a single cell in the bone marrow. It results in extensive and uncontrolled growth and accumulation of lymphoblast or leukemic blast, which do not function as normal blood cells. Moreover, it causes the blockage of the production of normal marrow cells and deficiencies in erythrocytes (anemia), platelets (thrombocytopenia) and leukocytes (in particular neutrophil). Leukemic cells can spread from bone marrow to the blood, lymph nodes, spleen, liver, central nervous system and other organs. ALL remains the most common cancer that occurs in childhood, accounting for approximately 25% of all pediatrics malignancies and 75% of cases of childhood leukemia. ALL can strike children of all ages but most likely to occur before age of 5 years. The 5 year rate of event free survival for children with ALL is nearly 80% (2, 3).

1.2.1 Etiology of acute lymphoblastic leukemia

The etiology of acute leukemia is unknown. The following factors are important in pathogenesis of leukemia:

1) Ionizing radiation
2) Chemicals (benzene)
3) Drugs (alkylating agents, radiation therapy)
4) Genetic consideration
    a. Identical twins
    b. Siblings – 4 times greater than that of general population
    c. Chromosomal abnormalities such as down syndrome, bloom syndrome, fanconi anemia (4)

1.2.2 Presenting manifestation of acute lymphoblastic leukemia

Common:

- Anemia
- Fever, malaise
- Haemorrhage, bruising, petechiae

Less common:

- Infection of mouth and pharynx
- Pain sin bones and joints (childhood especially)
- Upper respiratory tract infection (childhood especially)
- Superficial lymph node enlargement (childhood especially)

Occasional:

- Abdominal pain
- Mediastinal obstruction (childhood especially)
- Nervous system abnormalities
- Skin rash
- Gum hypertrophy (1)

1.2.3 Diagnosis of acute lymphoblastic leukemia (ALL)

a) Laboratories studies

i. Blood count

- Moderate to marked reduction of hemoglobin with normochromic normocytic red cell morphology.
- The white blood cell count may be low, normal or increased in number.
- Blood smear usually shows very few to few blasts (in patient with leucopenia). The WBC count is greater than 10,000/mm blasts are usually abundant. Eosinophilia is occasionally seen in children with ALL.
- 92% of patients have platelet count below normal (thrombocytopenia) (5)

ii. Bone marrow investigation

- The hallmark of the diagnosis of ALL is the blast cells, relatively undifferentiated cell with diffusely distributed nuclear chromatin, one or more nucleolei and basophilic cytoplasm (5). Bone marrow is usually replaced by 80 – 100 % blast cells. Megakaryocytes are usually

absent. Leukemia must be suspected when the bone marrow contains more than 5% blasts.

iii. Immunophenotyping

- Monoclonal antibodies are used to differentiate ALL from AML (acute myelocytic leukemia). A panel of B-and T-lineage markers and lymphocytes maturation markers are used to sub classify ALL.

iv. Morphological classification

- According to the French-American-British (FAB), classification of ALL.
- L1 – a small monomorphic type with small homogeneous blasts, single inconspicuous nucleolus, regular nuclear outline; commonest subtype.
- L2 – a large heterogeneous type, larger blast, pleomorphic and multinucleolate, irregular clefted nuclei with conspicuous nucleoli.
- L3 – Burkitt cell type – large homogenous blasts, abundant basophilic cytoplasm with vacuoles; associated with B – cell phenotype (6).

v. Cytochemistry

- ALL is frequently diagnosed by staining with Sudan black: ALL negative, AML positive, enzymatic peroxides: ALL negative, AML positive, and PAS: ALL positive, AML negative (5).

vi. Cytogenetics

- Specific chromosomal abnormalities are associated with distinct subtypes of acute leukemia. It has major implications for the diagnosis and management of acute leukemia and presence of cytogenetic abnormalities in newly diagnosed patients with acute leukemia provide important prognostic information (7).

1.2.4 Chemotherapeutic treatment of ALL

Chemotherapeutic treatment of ALL involves:

Phase I: Induction of remission with a combination of various drugs like vincristine, prednisolone and L-asperginase often with daunorubicin and cyclophosphamide.

Phase II: This is designed to take care of the small number of

leukemic lymphoblast that remains after clinical remission is achieved. Consolidation phase includes high dose systemic administration of drugs.

Phase III: A maintenance therapy to prevent relapse and to achieve a total cure. Patients receive maintenance chemotherapy for further 2-3 years with daily 6-MP, weekly methotrexate, monthly vincristine and prednisolone (8). Duration of therapy varies among different protocols and centers.

Central nervous system therapy: This starts during the induction phase and continues throughout therapy. This is designed to prevent to relapse with leukemic meningitis.

Following intensive treatment for induction of remission and subsequent consolidation, ALL patients usually receive maintenance therapy for 2-3 years. A patient exhibits continuous and complete remission for a period of 2.5 years. The risk of relapse usually occurs within one year after cessation of therapy, is approximately 20%. So when the chemotherapy can be

terminated most patients have been cured. Relapse usually occurs in the bone marrow. It also may develop alone or even simultaneously in the central nervous system. Even at this situation complete remission can still be obtained in a small proportion of the patients, especially in children, by combination therapy or bone marrow transplantation.

6-MP is one of the cornerstones in ALL chemotherapy. 6-MP, 6-thioguanine, and azathioprine are thiopurine drugs that are usually used to treat acute lymphoblastic leukemia, autoimmune disorder, inflammatory bowel disease and organ transplant recipient (9, 10). Both 6-MP and 6-Thioguanine are pro-drugs.

1.2.5 Survival rate of ALL patients

The children oncology group (COG) recently celebrated the milestone of 50 years of pediatric clinical trials and collaborative research in oncology. This group had its origin in the four legacy pediatric clinical trial groups; the Children's Cancer Group (CCG), the Pediatric Oncology Group

(POG), the National Wilms' Tumor Study Group (NWTS), and the Intergroup Rhabdomyosarcoma Study Group (IRSGG), which merged in 2000 to form the COG. In recent report from COG mentioned that, over the last 50 years, the survival rates for childhood cancer have risen from 10% to 80%. Outcome on ALL has gone from a 6-month median survival to an 80% over all cure rates (11).

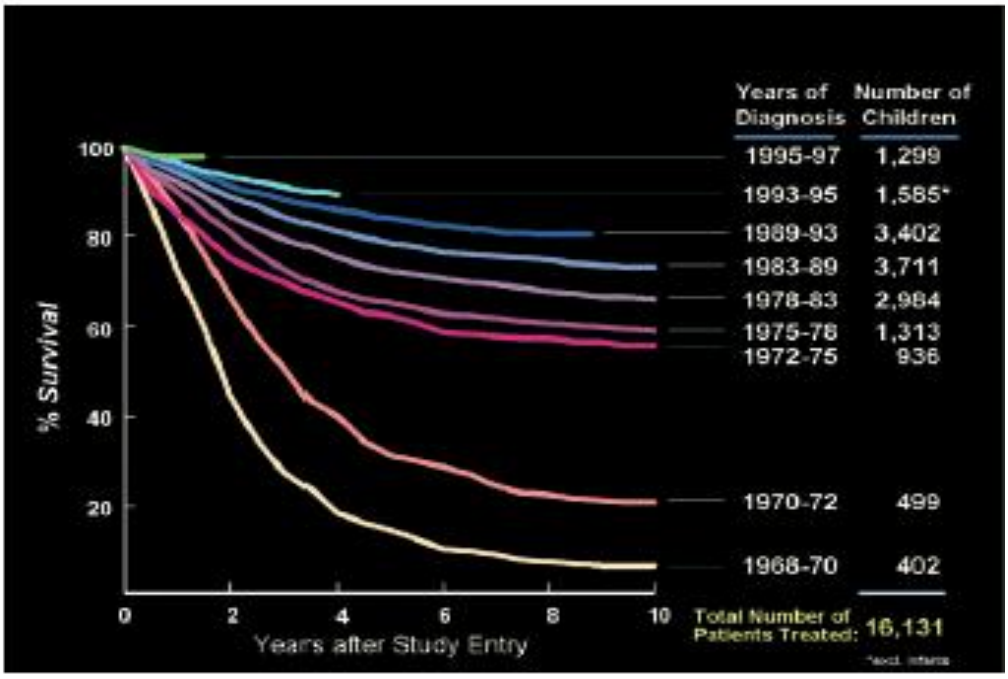

Figure 1.1: Survival of CCG patients with ALL 1968-1997 (11)

1.3 6-Mercaptopurine

a) Discovery of the drug: In 1942, George Hitchings set out to

develop a treatment for cancer. He wanted to create chemicals that were similar enough to normal nucleotides to be used in cells but different enough to interfere with DNA synthesis. Gertrude Elion joined Hitchings in his quest in 1944. 6-MP was originally synthesized by Elion and Hitchings in 1951 (12) by replacement of oxygen atom at carbon 6 of hypoxanthin or guanine, respectively, by sulfur.

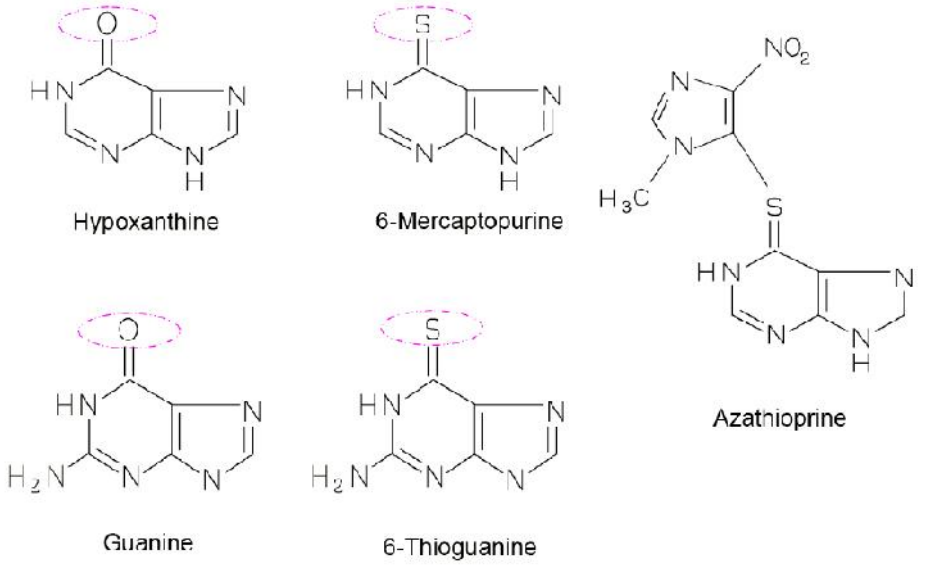

Figure 1.2: Chemical structures of thiopurines and the corresponding endogenous purines.

The first clinical trial with 6-MP involved oral administration and demonstrated a beneficial effects in connection with treatment of acute

leukemia in children (13). Subsequently, extensive experience in the administration of 6-MP was obtained and in the 1960's this drug was established as the backbone of orally administered maintenance therapy of ALL (14).

In 1948, Elion, and Hitchings found that 6-Thioguanine effectively inhibits the growth of both the bacterium *Lactobacillus (L.) casei* and tumors in mice. Later, 6-TG was shown to produce good clinical remission of granulocytic leukemia in 2 adults, although with severe side effects, including nausea, vomiting, and suppression of the bone marrow (15). On the basis of empirical evidence, 6-TG very soon became part of the routine treatment of AML (16). Combination of 6-MP with MTX and steroids (the only drugs available for treatment of acute leukemia at the time of introduction of thiopurines) extended the median survival time from 3-12 months. Only 2 years after its original synthesis and the initial anti-microbiological investigations, 6-MP was approved by authorities for use in the treatment of childhood ALL (17).

In 1958, Schwartz et al. (18) demonstrated the immunosuppressive ability of 6-MP to prevent and antibody response in rabbit injected with an antigen. Shortly thereafter, the Hitchings-Elion laboratory synthesize numerous 6-MP derivatives, including azathioprine, a pro-drug that is reduced non-enzymatically to 6-MP in vivo (10, 19). The first comparison of azathiopurine and 6-MP as immune-suppressor was performed on 10 dogs that had received renal transplant. AZA had better effects in this system (20) and was soon found to be an effective immune-suppressive agent in human as well (21).

Today, thiopurine are widely used to treat ALL of childhood, inflammatory bowel disease and autoimmune diseases, as well as to prevent rejection of organ transplant (22). These drugs, like many cytotoxic agents, have relatively narrow therapeutic index, with potential life-threatening drug-induced toxicity, primarily in the form of myelosuppression (23, 24). The other major toxic effect of thiopurines is hepatotoxicity (25) which has been reported to be related to the amounts of thioguanine nucleotides (TGNs), or methylmercaptopurine (meMP) in erythrocytes and to the accumulation of

6-MP and it's metabolites in the liver (26). 6-MP is now used as a routine component of all modern protocols for maintenance therapy of children with ALL (23) and the combination of high-dose MTX and 6-MP is commonly employed for consolidation therapy of childhood ALL (27).

1.3.1 Metabolism of 6-MP

6-MP is an inactive pro-drug that exerts cytotoxicity only after intracellular metabolism to active metabolite (28). The bioavailability of 6-MP is only 16 percent, because of high first-pass metabolism, after oral administration, 6-MP undergoes extensive intestinal and hepatic metabolism by aldehyde oxidase (AO) and xanthine oxidase (XO), which convert this substance into rather inactive metabolites. Oxidation of 6-MP in the liver via xanthin oxidase (XO), generates thiouric acid (TUA), and inactive metabolite that is eliminated in the urine (29).

Figure 1.3: Scheme of thiopurine drug metabolism. XO = xanthine oxidase, HPRT = hypoxanthine phosphoribosyl transferase, TPMT = thiopurine methyltransferase (30)

6-MP is converted by *TPMT* to the inactive metabolite methyl mercaptopurine (meMP) (31). In a third metabolic route, 6-MP is initially converted by hypoxanthine guanine phosphoribosyl transferase (HGPRT) to 6-thioinosine monophosphate (TIMP) and thioguanosine monophosphate

(TGMP), respectively. Conversion of TIMP to TGMP via thioxanthine monophosphate (TXMP) is catalyzed by two enzymes, IMP dehydrogenase (IMPDH) and GMP synthatase (GMPS) respectively (32). Subsequent reactions by phosphokinases wield 6-thioguanosine diphosphate and triphosphate (TGDP and TGTP). TGMP, TGDP and TGTP are collectively termed as thioguanine nucleotides (TGNS). In corporation of 6-TGDP into DNA triggers cell cycle arrest and apoptosis by a process involving the mismatch require (MMR) pathway (33). The enzyme, thiopurine methyltransferase (TPMT) inactivates 6-MP through the formation of methylmercaptopurine (34). 6-TGNs can be methylated by TPMT. 6-TIMP can be methylated by TPMT to form 6-methyl thionosine 5-monophosphate (6-MTIMP), 6-methyl thionosine 5'-diphosphate (6-MTIDP) and 6-methyl thioinosine 5'-triphosphate (6-MTITP). The above mentioned last three metabolites are called 6-methyl mercaptopurine ribonucleotides (6-MMPRS). 6-TIMP can also be phosphorylated by monophosphate kinase to produce 6-thioinosine 5'-triphosphate (6-TITP) and ultimately back to 6-TIMP by an enzymatic reaction catalyzed by inosine triphosphate pyrophosphatase (ITPase). Besides methylation of 6-MP to the inactive metabolite meMP,

TPMT methylates TIMP to meTIMP, a metabolite that has been shown to inhibit purine de novo synthesis (PDNS) (35).

1.4 Role of pharmacogenetics in drug toxicity and efficacy

Pharmacogenetics aims to use knowledge of genetic polymorphism to 'tailor' therapy for improved response and reduced toxicity. The term pharmacogenetics represents the study of genetic factors that influence response to drugs and chemicals and was first termed in 1959 (36). Most research so far has focused on single polymorphisms. A more comprehensive approach to predict treatment response will be considered genetic variation in entire biological and pharmacological pathways. Genetic variations are the results of multiple mechanisms such as single nucleotide polymorphisms (SNPs) are over 90%, insertion, deletion, tandem repeats, and microsatellites. In an attempt to individualize therapy, pharmacogenetic is used in search for answers to the hereditary basis of individual differences in drug response. Some drugs require metabolism to be activated or inactivated. Some enzymes involve in the biotransformation have polymorphic expression.

Pharmacogenetic causes variability in the metabolism of MP (Fig 1.3), due to polymorphic variation in the activity of TPMT enzyme (37).

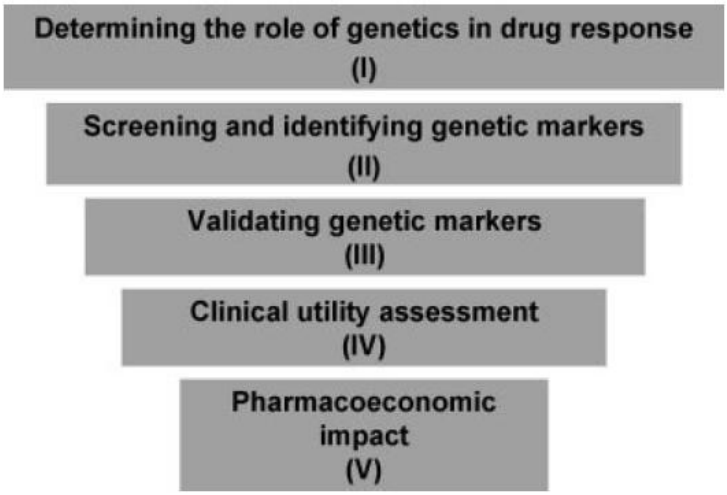

Figure 1.4: Five stages of pharmacogenetics and pharmacogenomics in cancer therapy (38).

1.5 Thiopurine methyltransferase (TPMT)

Thiopurine methyltransferase (TPMT) is a cytosolic enzyme present in prokaryotes and eukaryotes. It has a molecular mass of 26 KDa, comprises 245 amino acids, is not metal dependent and TPMT is encoded by 34 Kb gene consisting of 10 exons and 9 introns and has been localized to chromosome 6p22.3 (Fig 1.4) (39) found in the liver, kidneys, intestine, erythrocytes, leukocytes and a number of other tissues.

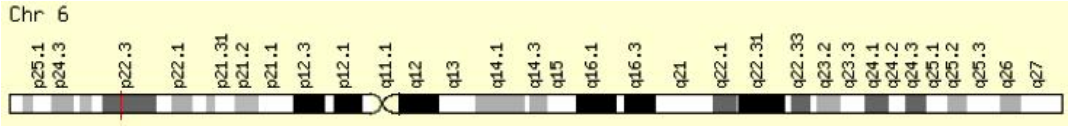

Fig 1.5: Position of TPMT gene on chromosome 6 (40)

TPMT is known to catalyze S-methylation of aromatic and heterocyclic compounds, preferentially thio compounds such as 6-MP and 6-TG (18). The first study of the enzyme that catalyzed this reaction was performed by Remy in the early 1960s using rodent tissue (41). At late 1970s, TPMT activity was first assayed and studied in human tissue (42), with the goal of testing the hypothesis that individual variation in this pathway for thiopurine biotransformation might be related to individual differences in drug toxicity and therapeutic efficacy. The first application of measurement of RBC TPMT activity involved pharmacogenetic experiments performed with large population samples and nuclear families (43). Those studies demonstrated that the "trait" of level of RBC TPMT activity was controlled by a common genetic polymorphism. The level of TPMT activity in erythrocytes reflects the corresponding levels in the kidney, liver and lymphocytes (44, 45).

Characterization and cloning of the human TPMT cDNA and gene expressed that this phenotypic variations were from variation in the sequence of the gene itself (46, 47). To date, at least a total of 23 such genetic polymorphism have been identified which may be associated with decrease level of TPMT enzyme activity or enhanced toxicity of thiopurine methyltransferase have been identified (48).

1.5.1 Molecular basis for altered TPMT activity

The molecular basis of the TPMT polymorphism is largely related to three common non synonymous coding single nucleotide polymorphisms (49), each of which renders the protein unstable (47) and subject to enhanced ubiquitination and degradation (50). TPMT enzymatic activity is controlled by a common genetic polymorphism (49). So, the homozygous deficiency is characterized by almost undetectable levels of TPMT protein; heterozygotes have intermediate protein and activity levels and homozygous wild type individuals have high levels of protein and activity (51, 52).

There is pronounced inherited variations in TPMT activity, ranging from high to virtually undetectable, were first observed in human tissues more than two decades ago. The frequency of distribution of erythrocyte TPMT activity in 298 control subjects was found to be trimodal. Approximately 1 in 300 individuals (0.3%) has low or undetectable level of this activity, which is intermediate in approximately 10% of the general population (43).

1.5.2 Alleles of TPMT and genetic polymorphism

Variant human alleles associated with decreased catalytic activity of TPMT involved point mutations in the open-reading frame or act intron/exon splice sites. TPMT*1 is the wild-type allele, encodes the fully active enzyme; wild TPMT*2 (238G4C), TPMT*3A (460G4A, 719A4G) and TPMT*3C (719A4G) are the most prevalent accounting together for 80-95% of the polymorphic alleles that lead to a significant reduction in enzyme activity (53). TPMT*3B (460G4A) is less common and the remaining TPMT variant can be considered to be family specific ('private') mutations found in individuals belonging to various ethnic groups (54, 55, 56).

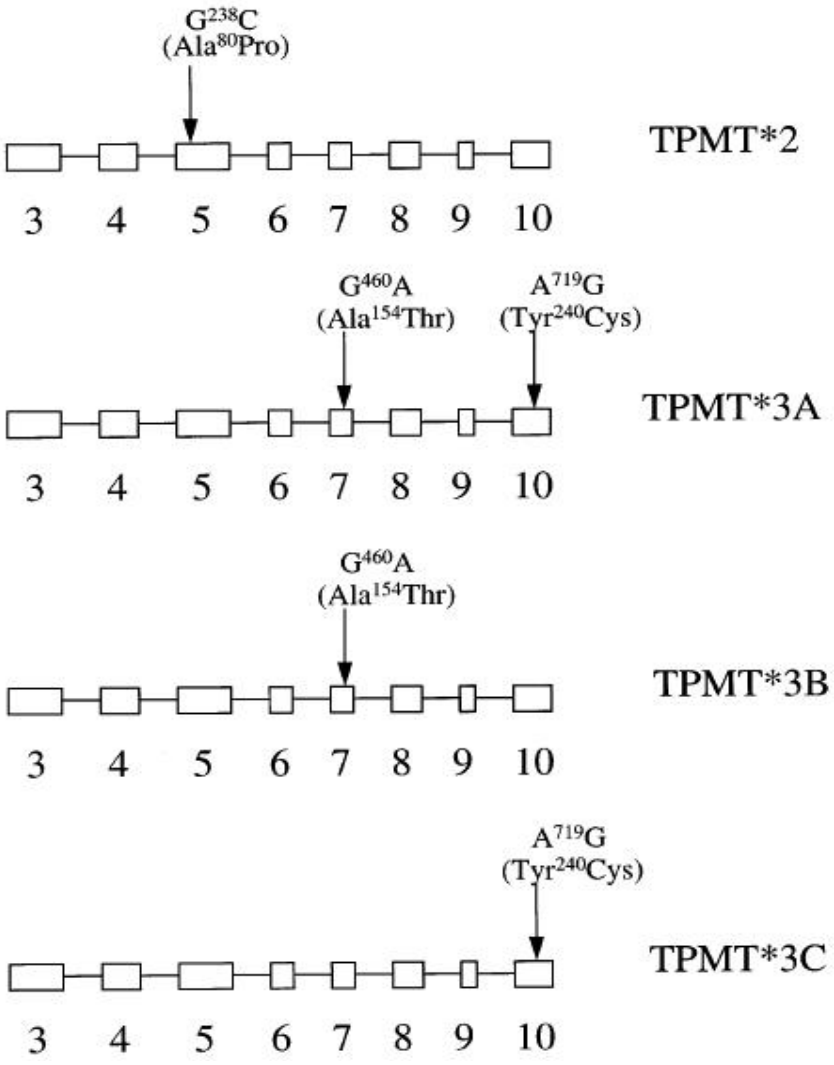

Fig 1.6: Allelic variant of the TPMT gene. Boxes depict exons in the open reading frame of the human TPMT gene. The positions of the three point mutations detected by PCR-based assays and indicated (44).

1.5.3 Relationship between TPMT and 6-MP toxicity

There are many associations between single genetic polymorphisms and drug effects have been clearly demonstrated, showing that inherited

genomic variation causes substantial interindividual differences in drug effects (57). Genetic polymorphism of TPMT have been demonstrated to influence drug metabolism and its effects, constituting one of the most studied and significant example of association between drug clinical effects and a genetic polymorphism (58). TPMT polymorphism is inherited as an autosomal co-dominant genetic trait. The enzymatic activity of TPMT shows a trimodal distribution of high, intermediate and very low activity among the population (43, 59). 89-94% of all individuals studied having a high activity, 6-11% having intermediate activity and 0.3% having a very low or non-detectable activity (47, 60).

Polymorphic variants of the TPMT gene encode enzymes which have significantly reduced activity. Approximately 0.3% are homozygous for non-functional alleles and the consequence absence of TPMT activity is associated with severe thiopurine-induced hematological toxicity and bone marrow suppression (61). Leucopenia, thrombocytopenia or any combination of these may occur in bone marrow suppression

Inactive TPMT alleles screening can be done to identify individuals at risk of bone marrow toxicity. In case of homozygous-TPMT deficient patients (two non-functional alleles), dose reductions are required to avoid the life threatening bone marrow suppression (62). Heterozygous allele with a low activity identifies the patients in whom lower doses of thiopurine can be used safely. If a patient has clinical or laboratory evidence of myelosuppression, TPMT testing should be considered (62). Approximately 90% dose reduction is required for patients with the homogeneous mutations and 40% of dose reduction may be required for patient with heterogeneous mutant.

Table 1.1: TPMT genotype classification and implications to therapy

Genotype	Activity Level	Frequency in the population	Dose Adjustments
Wild type	High TPMT activity	89%	Start at normal dose, may need to increase
Heterogeneous Mutant	Intermediate TPMT activity	11%	Start at 60% of normal dose *
Homogeneous Mutant	TPMT deficiency	0.33%	Start at <10% of normal dose **

* Children's Oncology Group, 2008 (64)

**Weinshiboum and Sladek (43)

1.5.4 Ethnic variation in TPMT mutations

Although the overall prevalence of TPMT deficiency is similar between different ethnic groups, the frequency of variant alleles differs between different populations, for example, there is a higher representation of the TPMT*3A allele in South Asian compared with the high frequency of the TPMT*3C in East and West African (63). The TPMT*1 (wild-type allele, 96%) and TPMT*3A (460G>A and 719A>G, 4%) alleles are most common among Caucasians (65).

1.5.5 Frequency of TPMT allele distribution in different populations

Several studies aimed at determining the allelic variations of the TPMT in different populations. Some of these are summarized in following table.

Table 1.2: Summary of different studies of TPMT allele variation in different populations

Population	No. of alleles	*2	*3A	*3C %	Reference
French	382	_	5.7	0.8	(McLeod, 2002)
British Caucasian	398	0.5	4.5	0.3	(Collie-Duguid, 1999)
Italian	412	0.4	3.9	1	(Rossi, 2001)
Norwegian	132	_	3.4	0.3	(Loennechen, 2001)
African-Americans	496	0.4	0.8	2.4	(Hon, 1999)
Caucasian-American	564	0.2	3.2	0.2	(Hon, 1999)
Japanese	384	0	0	0.8	(Hiratsuka, 2000)
Brazilian	408	2.2	1.5	1	(Boson, 2003)
South-east Asian	698	0	0	1	(Chang, 2002)
Turkish	212	0	0.9	0.9	(Tumer, 2007)
Swedish	_	0	3.7	0.4	(Haglund, 2004)
Mexican	218	0.9	3.2	1.4	(Taja-Chayeb, 2008)
Iranian	254	3.93	0.87	1.57	(Azad M, et al., 2009)

1.6 Inosine triphosphate pyrophosphatase (ITPA)

ITPA is a cytosolic enzyme which is involved in the metabolism of 6-MP. It catalyzes the hydrolysis of inosine triphosphate (ITP) to inosine monophosphate (IMP), thereby recycling purines that might otherwise be trapped in the form of ITP preference, thus protecting cells from the accumulation of harmful nucleotides such as ITP and deoxyinosine triphosphate. Genetic polymorphisms in the ITPA are associated with reduced activity of the ITPA enzyme and increase toxicity to mercaptopurine (66). Deficiency of inosine triphosphate pyrophosphatase (ITPAse) causes accumulation of potentially toxic metabolite 6-thio-ITP (67). ITPase deficiency is polymorphic, 5% of a normal population had decreased ITPase activity which was shown in an early population study (68). High level of endogenous ITP is accumulated in the red cells of completely ITPase deficient individuals, was first demonstrated by Vanderheiden in 1965 (69). ITPase deficiency has not been associated with any disease or clinical condition. It was suggested that ITPase deficient patients would be at risk of suffering from side effects when treated with purine drug analogues such as thiopurines (70).

1.6.1 Structure of ITPA

Gen Bank accession number is A F026816 for ITPA gene. It is located on the short arm of chromosome 20 (20P13) (71). The genomic structure of the ITPA gene was determined by Sumi et al (72). ITPA gene consists of eight exons and is ~ 13Kb long. 2 allelic variances in the ITPA gene were associated with decreased ITPase enzyme activity. ITPA c.94C>A (P32) in exon 2, and ITPA g.IVS2 + 21A>C (73). Zero rate cell enzyme activity was found in homozygotes, whereas 22.5% enzyme activity was found in heterozygotes (74). A C198A transversion (rs1127354) causing a proline to threonine replacement at codon 32 (P32T polymorphism) is the most relevant SNP determining low ITPA enzymatic activity (75, 76). ITPA94C>A transversion causes and amino acid change (P32T), reducing ITPA enzymatic activity to 25% in heterozygotes, and abolishing it in homozygous variants (77). Five single polymorphic sequence variants in the human ITPA gene have been identified, two of which are associated with ITPase loss of activity (94C>A in exon-2, and IVS2+21A>C). These interact and affect branch points resulting in misplacing of exons 2 and 3 leading to shortening of polypeptide stretches in the enzyme (74). The other three coding region ITPA polymorphic sequence variants are

silent mutations (138G>A, 561G>A and 708G>A) (72).

1.6.2 Ethnic variation in ITPA allele

Homozygosity of the ITPA 94A allele, which results in deficiency of ITPase activity in erythrocytes and lymphocytes, occurs in approximately 1 in 1000 Caucasians (73). Carriers represent about 1 in 15 (6.0%) of Caucasian populations, and have an average red cell ITPase activity of about 22% of normal. This allele is more common in Asian populations, with a frequency of 11-15% (78). Homozygosity of the ITPA IVS2+21C allele occurs in approximately 1 in 250 Caucasians, with a carrier frequency of about 1 in 8 (13.0%), resulting in partial reduction of ITPase activity with an average of 60% normal levels in red cells. Its frequency varies greatly in other populations, e.g. the SNP is absent among Japanese. Compound heterozygotes (ITPA 94A/IVS2+21C) have 10% of normal activity (79).

1.7 Survival rate of TPMT and ITPA polymorphism

Genetic variations in the drug metabolisms are known to influence treatment related toxicity and survival rate. TPMT is one of the most well defined genotype of those. TPMT deficient individuals form higher concentration thioguanine nucleotides and are more susceptible to acute thiopurine toxicity such as myelosuppression (80). However, TPMT is more frequent among Caucasians. There is a significant ethnic difference in survival of TPMT and ITPA among Caucasians and Asian. Until now, most of the studies belong to Western countries but a recent report from Korea shown that ITPA variants were the only risk factor for lower event free survival (EFS) which was 95.2% in wild type and 81.9% in AC/AA variant (HR 4.96, 95% CI =1.1-22.7, P=0.039) (81).

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CHAPTER 2

Polymorphism of TPMT and ITPA and adverse effects of chemotherapy for acute lymphoblastic leukemia children in Bangladesh

2.1 Summary

This study was to identify the distribution of TPMT and ITPA polymorphism among Bangladeshi children and their association with the adverse effects of 6-MP in acute lymphoblastic leukemia (ALL) patients. Pharmacogenetics approach reduces the toxicity and increases safety of chemotherapy. 6-mercaptopurin (6-MP) metabolizing enzyme such as thiopurine S-methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) have contribution to variables responses including adverse effects among patients treated with 6-MP in leukemia.

In this study, I recruited 75 patients diagnosed with ALL and 75 volunteers with minor illnesses. Genotyping for TPMT (TPMT*3C, *3B, *2) and ITPA (ITPA94C>.A) was done. Relationship between genotypes and adverse effects of 6-MP was investigated. Minor allele frequency of TPMT*3B, TPMT*3C and ITPA polymorphism among volunteers were 0.006, 0.20 and 0.903 and Monir allele frequency of TPMT*3C and ITPA polymorphism among ALL patients were 0.010 and 0.153 respectively. ALL patients with TPMT*3C

variant developed leucopenia (P=0.037) neutropenia (P=0.017) and thrombocytopenia (P=0.008). ITPA variant developed fever (P=0.003) neutropenia (P=0.001) and liver toxicity (hyperbillirubinemia, P=0.048, and raised serum ALT, P=0.007). In univariate logistic regression analysis, it was observed that ITPA polymorphism had 6 times higher chance of absolute neutropenia (OR = 6.25, 95%CI = 2.01 – 19.42), 3 times higher chance of hyperbillirubinemia (OR = 3.58, 95% CI = 1.21 – 10.61), 4 times higher chance of increase ALT (OR = 4.53, 95% CI = 1.53 – 13.43) and 7 times higher chance of fever (OR = 7.00, 95% CI = 2.05 – 23.84) in compare to non-polymorphic patients. After adjusted with age, sex, height and weight in multivariate logistic regression analysis the association remained similarly significant.

In conclusion, this study strongly suggests the importance of TPMT and ITPA genotyping in patients with ALL to design more rational and cost effective treatment strategy in children with ALL.

2.2 Introduction

The treatment of pediatric acute lymphoblastic leukemia (ALL), the most common malignancy in children, has reached success rates up to 90% in the last 2 decades (1) in the western countries and some industrialized countries. Whereas supportive care plays an important role during chemotherapy for childhood malignancy to avoid/manage complications, pharmacogenetical intervention has been a new approach to get not only a less side effects but also better treatment outcome (2). 6-MP is one of the most widely used and highly effective chemotherapeutic agent for maintenance therapy in childhood ALL (3, 4). In the standard treatment, 6-MP is administered as a daily oral dose, usually 1-2 years of maintenance therapy. It has been found that, in pharmacogenetic studies among childhood ALL have associated toxicity of 6-MP with single nucleotide polymorphisms (SNP) in genes coding for 6-MP metabolizing enzyme such as thiopurine S-methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) (5). Pharmacogenetics provided a molecular approach which guides the individualization of chemotherapy. This approach may reduce the toxicity and increases safety of the therapy (5)

Inosine triphosphatase catalyses the hydrolysis of *ITP* to inosine monophosphate, thereby purines is trapped in the form of ITP (6, 7). Deficiency of Inosine triphosphate pyrophosphohydrolase (*ITPA*se) has been reported to cause accumulation of the potentially toxic metabolite 6-thio-ITP (8). At least 5 variants of ITPA gene have been identified, among them two single nucleotide polymorphisms of *ITPA* c.94C>A and *ITPA* g.IVS2+21A>C are associated with decreased enzyme activity (9). Individual who are homozygous for *ITPA* c.94C>A (P32T) mutation have total deficiency of enzyme activity which accumulate ITP intracellularly whereas ITPA c.94C>A heterozygotes have decreased ITPA activity that is 22.5% of control mean value (7).

6-MP is an inactive pro-drug, which requires to go under metabolism process into thioguanine nucleotides (TGN) in order to perform its cytotoxic effects (10). TPMT is involved in the methylation reactions of 6-MP, it means TPMT is a key enzyme in the metabolism of 6-MP. The activity of TPMT is influenced by genetic polymorphism which can alter the rate of metabolism of 6-MP (11). The cellular accumulation of TGN is inversely related to TPMT

activity; presumably the high TPMT activity shunts more drugs down the methylation pathway, leaving less for activation to TGNs (10). Studies have found that the distribution of TPMT activity in erythrocytes (RBC) to be tri-modal; approximately 90% of persons have high activity, 10% have intermediate activity, and 0.3% have less or not detectable enzyme activity (10, 12). Three of these alleles (*TPMT**2, *3B and *3C) account for 80-95% of intermediate or low enzyme activity cases (13, 14). The cumulative incidence of 6-MP dose reduction due to toxicity was highest among patients who were homozygous mutant for TMPT (*TPMT* *2/*3C/*3B), intermediate among heterozygous patients and lowest among wild type (*TPMT* *2/*3C/*3B) patients (15, 16).

In the treatment of ALL, the knowledge of SNPs in 6-MP metabolizing enzymes and its related drug toxicity has developed more rational approaches to optimize chemotherapy. TPMT and ITPA genetic variant differ from patient to patient and among different ethnic. Determination of the frequency of this genetic variant is necessary to consider the use of pharmacogenetic as a tool to improve treatment outcome. Until now,there is no available report among

Bangladeshi children about the incidence of TPMT and ITPA common allelic varients and the relevance of those alleles on toxicities in patients with ALL. Thus the aim of this study was to determine the frequency of TPMT and ITPA variant alleles among Bangladeshi population and the association of polymorphisms with adverse effects of 6-MP.

2.3 Rational of the study

Bangladesh shows recent advances in relation to manage infectious diseases (17), the chronic diseases in particular cancers are less prioritized. The status of cancer in this country is not clearly known, as there is no population-based cancer registry or national cancer registry of any kind. According to WHO, Bangladesh is experiencing increasing cancer burden with estimated 122,715 new cancer cases in 2012 (18). The number of new cases is projected to be increased by 77% in 2030. These WHO estimate may not reflect the real cancer status, as the estimate was extrapolated based on the incidence and mortality rates from regional data (18). The figures are likely to be underestimated as many cases go unreported due to lack of awareness,

education, misconception, and poverty among populations, in addition due to poor health system and poor governance (19). Moreover, overall cancer care and management system are below international standard due to high treatment cost, lack of oncologist and insufficient infrastructure.

In Bangladesh, pediatric tumors consist of 4.4% of all malignancies during 2005 to 2009. Leukemia constituted about 14.3% of all childhood malignancies; among them ALL were commonly found (20). The relative incidence of malignancies seen at BSMMU in 2012 was acute lymphoblastic leukemia 58%, non-hodgkin lymphoma 11% and acute myeloblastic leukemia 10%, neuroblastoma 5%, hepatoblastoma 3.5%, hodgkins lymphoma 3%, Wilm's tumor 2%, retinoblastoma 2%, germ cell tumor 2%, histocytosis 2%, central nervous system tumor 1%, osteosarcoma 1% (21). Bangladesh has only one state-run specialist cancer hospital, fourteen oncology units of public medical teaching hospitals and few private clinics and hospitals are available to treat cancer patients. While over 70% population live in the rural areas but most of the tertiary care hospitals are situated in the central part of Dhaka, Bangladesh (22). Even though some cancer research has been started now a

days, but most of them are based on the prevalence and risk factors. Chemotherapic treatment is available but there is no scope of dose adjustment. Still there is lack of research in Bangladesh regarding Chemotherapic toxicity and their genetic cause and also how to minimize them. That's why I conducted this study to know the genetic polymorphism and toxicity of Chemotherapic treatment in Bangladesh. Results of this research will guide physician to the importance of prior diagnosis of genetic polymorphism, so that they can adjust dose and minimize toxicity. As a result, continuation of treatment and better prognosis will be expected.

2.4 Methods

This is a retrospective study conducted in two tertiary level hospitals in Dhaka city, central part of Bangladesh. Seventy-five patients diagnosed with acute lymphoblastic leukemia (ALL) recruited from pediatric hematology and oncology department of Bangabandhu Sheikh Mujib Medical University (BSMMU) and 75 children who attended in the outdoor department of Central Hospital Limited who were presented with minor diseases other than cancer

such as cold, diarrhoea or other minor illness have been recruited in this study. All children had been suffering from ALL were under maintenance therapy using 6-mercaptopurine 75mg/m^2/day orally as per UKALL 2003protocol, version 7 (23). All patients and volunteers were recruited over one year period, in between January 2013 to December 2013. Informed written consent was obtained from their legal guardians and/or children prior to the recruitment and data collections. Study was approved by the ethical review committees of BSMMU, Central Hospital Limited and University of Tsukuba Hospital.

2.4.1 DNA extraction and genotyping

Genetic DNA was extracted from 2ml peripheral blood of the patients on maintenance therapy and volunteers using the Genomic DNA Isolation Kit (QiAamp DNA Blood Mini Kit: Qiagen, Vealo, The Netherlands) following the instructions from manufacturer. Polymorphisms of *TPMT**3C(c.719A>G, rs1142345), *TPMT**2(c.238G>C, rs1800462), *TPMT**3B(c.460G>A, rs1800460), and *ITPA*(c.94C>A,rs1127354) were genotyped using the TaqMan Assay-on-Demand SNP Typing System (Applied Bio Systems, Foster City, CA, USA) following the manufacturer's instructions. PCR was performed on a

384-well format with 3ng of DNA each, and automatic allele calling was performed using ABI PRISM 7900HT data collection and analysis software, version 2.2.2 (Applied Biosystems).

2.4.2 Clinical and laboratory data collection

All relevant information such as demography of the participants, sign and symptoms, disease state, toxicity profile and results of blood biochemistries such as complete blood count, serum billirubin, liver transaminases (ALT), and serum creatinine were documented. The worst value of clinical and laboratory data were collected during maintenance therapy. A maintenance therapy is given to prevent relapse and to achieve total cure. Patients receive maintenance chemotherapy for 2 years with 6-MP, methotrexate, vincristine and dexamethasone. 6-MP intolerance was defined as the occurrence of hematopoietic toxicity, hepatotoxicity, and/other toxicities that resulted in delays in subsequent chemotherapy. Hematologic toxicity was defined as neutropenia, an absolute neutrophil count (ANC) $<1.0X10^9/L$. Myelosuppression was defined as leucopenia, a reduction in the number of

white cells (<3.0X10^9/L), and or thrombocytopenia (platelet count <100X10^9/L) Hepatic toxicity was defined as a greater than two fold increase in serum billirubin and ALT. Fever was defined as temperature >38.0^0 C. This toxicity definition was set by my study group for this study purpose and usually used by hematology and oncology department of BSMMU.

2.4.3 Statistical analysis

Statistical analysis was performed by using SPSS statistics version 21.0 (IBM Corporation, NY, USA). Data were expressed as number %, mean $\pm$ SD. Allele frequency was calculated by using Hardy-Weinberg principle. Chi-square test was performed to compare the relationship between polymorphism and development of toxicity. Univariate and multivariate logistic regression analysis was performed to see the effect of ITPA polymorphism on toxicity. Two sided *P* value less than 0.05 were considered to be statistically significant.

2.5 Results

I enrolled 75 patients diagnosed with childhood ALL and 75 healthy volunteer having age of (5 ± 2.5) years and (3.1 ± 1.6) years respectively; 86/150 (57.3%) were male (Table 1). TPMT and ITPA genetic polymorphism were found in 6/150 (4%), and 34/150 (22.7%) respectively. For volunteers, frequency of *TPMT**3B (rs1800460), *TPMT**3C (rs1142345), and *ITPA*94C>A (rs1127354) polymorphism were 0.99, 0.98 and 0.90 respectively. For ALL patients, frequency of *TPMT**3C and *ITPA*94C>A were 0.98, and 0.87. I found 3 homozygous variant for *ITPA*94C>A (rs1127354) genes and no homozygous variant for *TPMT**3C, *TPMT**3B and *TPMT**2 alleles. All 3 homozygous variant were among the ALL patients. I also found 31 heterozygous variant for ITPA gene. *ITPA*94C>A was the most common variant in the subjects screened, no *TPMT**2 variant was detected (Table 2). The frequency of allele *ITPA*94C>A, reported to be higher in the Asian population was 22.7% in people of Bangladesh (in this study). This distribution is similar to those reported in previous publication (24).

It was observed that the ALL patients those who have *TPMT**3C have significantly higher chance of leucopenia (*P*=0.037), neutropenia (*P*=0.017) and thrombocytopenia (*P*=0.008) .It has also been found that the ALL patients those who have *ITPA*94C>A also have higher chance of neutropenia (P=0.001), raised serum billirubin (*P*=0.048), raised ALT (*P*=0.007) and fever (*P*=0.003) (table 3). In univariate logistic regression analysis, it was observed that ITPA polymorphism had 6 times higher chance of absolute neutropenia (OR = 6.25, 95%CI = 2.01 – 19.42), 3 times higher chance of hyperbillirubinemia (OR = 3.58, 95% CI = 1.21 – 10.61), 4 times higher chance of increase ALT (OR = 4.53, 95% CI = 1.53 – 13.43) and 7 times higher chance of fever (OR = 7.00, 95% CI = 2.05 – 23.84) in compare to non-polymorphic patients. After adjusted with age, sex, height and weight in multivariate logistic regression analysis the association remained similar.

2.6 Discussion

The use of genomic sequence information for providing safe and effective medication for developing “personalized/individualized medicine” is

breezing the gap between the basic research and clinical practice (25). There are no molecular analyses on Bangladeshi subjects to identify the pharmacogenomics determinants of 6-MP associated drug toxicity. I conducted this study because of growing interest in employing pharmacogenetics to refine and better individualize treatment for childhood ALL. I determined the laboratory parameters in ALL patients who were under maintenance phase of the ALL treatment to correlate my results with clinical findings. Though my study confirm the previous reports (5, 25), but it was important to demonstrate these findings among Bangladeshi population.

TPMT is a gene, related to the anti-leukemia effects and side effects of 6-MP. It is mentioned as a potential gene selected for polymorphism testing (26). Differences in TPMT polymorphism varies among different ethnic group, it ranges from 2% to 14% prevalence (5). I found that allelic frequency of *TPMT**3C polymorphism among Bangladeshi was 3.3%, *TPMT**3B was 0.7%, although I did not find the *TPMT**2 allele (table 2), most likely due to the small sample size and the known low frequency of TPMT variants in Asian population. The frequency and distribution of TPMT alleles in my study

among Bangladeshi is similar to that of Iranian (27), Turkish (28) and Japanese population (unpublished data).

The ITPA is an enzyme involve in the 6-MP metabolism. Genetic polymorphisms in the ITPA gene are associated with reduced activity of the ITPA enzyme and increased toxicity to 6-MP. There are several polymorphism have been described for ITPA but the P32T (proline to threonine substitution at amino acid 32) variant is the most common SNP (29). *ITPA*94C>A frequency has been investigated in various populations. Studies on Asian population including this study reveal a higher frequency of the variant allele than American Caucasians (0.06) or British Caucasians (0.07) or African (0.05) (29, 30). Among Asian, Chinese was found to be (0.15), East Indian (0.11), Philipino (0.14) (24), which reinforce my findings among Bangladeshi (0.22) and Japanese population (unpublished data).

This is the first ever report from Bangladesh showing the effect of TPMT and ITPA genotypes in optimizing 6-MP therapy based on genetic constitution. Clinical consequence of one particular polymorphism is one of the

major aspects about pharmacogenetics in the treatment outcomes. Several previous studies indicate that TPMT polymorphisms are associated with 6-MP toxicity (31, 32, 33). In my study, patients with predicted deficiency in *ITP*ase, tended to be more likely to develop fever, neutropenia and likely to experience liver toxicity (raised serum billirubin and raised serum ALT) during treatment. It was observed that all homozygous variant (AA) of ITPA allele were found among ALL patients (4.0%). Study found that 6-MP toxicities tended to be higher among homozygous and heterozygous variant allele of ITPA in compare to wild type. I also found that *TPMT**3C and *TPMT**3B deficient patients are more likely to develop myelosuppression such as neutropenia and thrombocytopenia which is supported by previous findings (5, 34).

In my study, toxicity related to ITPA polymorphism was very high. It may occur due to multiple causes. One possible explanation is low nutritional status of the ALL patients in this study (BMI = 16.03 $\pm$ 3.92). It has been well known that undernourished children have higher chance of lower immunity and increase chance of infections and toxicities or chemotherapy itself can cause malnutrition and higher chance of toxicities (35). Other possible

explanations are lack of regular monitoring of this group of children during Chemotherapic treatment and higher dose of Chemotherapy which is 75mg/m^2/day as per UKALL protocol (23) whereas Japanese start with lower dose such as 40-50mg/m^2 /day.

Limitation of the study: As with most genetic studies, my current study was not sufficiently powered to derive definitive conclusion. Results of this analysis showed that 2 cases out of 2 have shown hematological toxicities. It might be due to small sample size and selection bias, and this is a major limitation of this study. A larger sample size and well-designed study might minimize the issue on statistical inference.

2.7 Conclusions

The frequency of *ITPA*94C>A among Bangladeshi children is very high. My results suggest that patients with TPMT and *ITPA*94C>A polymorphism has higher chance of toxicity. My study strongly suggests the importance of TPMT and ITPA genotyping in patients with ALL to design more rational and cost effective treatment strategy in children with ALL. As ITPA is more frequent in compare to TPMT among Bangladeshi population it should be prioritize during screening process. My plan is to recommend stake holders and policy maker to start genetic screening process before initiating the Chemotherapic treatment for ALL children. It will minimize toxicities and continue treatment and finally better outcomes of childhood ALL. I have also plan to identify other associated conditions accompanying ALL such as Gilbert syndrome. Furthermore, I recommend larger study for better findings of TPMT allele frequency.

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Table 2.1: Baseline characteristics of the study participants

Characteristics	ALL patients (mean ± SD)	Volunteers (mean ± SD)
Age (year)	5 ± 2.5	3.1 ± 1.6
Weight (kg)	17.62 ± 7.74	14.10 ± 4.02
Height (cm)	106.51 ± 19.90	97.10 ± 13.11
MUAC (cm)	15. 96 ± 2.78	14.80 ± 2.46
BMI	16.03 ± 3.92	
RBC count ($X10^{12}$/L)	3.27 ± 0.48	
WBC count ($X10^{9}$/L)	3.47 ± 1.67	
Neutrophil count ($X10^{9}$/L)	1.437 ± 571	
Platelet count ($X10^{9}$/L)	187 ± 101	
ALT (U/L)	59.09 ± 54.08	
Serum billirubin (µmol/L)	17.82 ± 6.95	
	(n/%)	(n/%)
Sex (male/female)	43/32 (28.7/21.3)	43/32 (28.7/21.3)

MUAC = Mid upper arm circumference, RBC = Red blood cell, WBC = White blood cell, ALT = Alenine transaminases

SD = Standard deviation

Table 2.2: Frequency of TPMT and ITPA alleles

Variations	rs number	Wild type	Heterozygous	Homozygous	Minor Allele frequency
ALL patients					
*TPMT**2	rs1800462	75	0	0	0.000
*TPMT**3B	rs1800460	75	0	0	0.000
*TPMT**3C	rs1142345	73	2	0	0.010
*ITPA94*C>A	rs1127354	55	17	3	0.153
Volunteers					
*TPMT**2	rs1800462	75	0	0	0.000
*TPMT**3B	rs1800460	74	1	0	0.006
*TPMT**3C	rs1142345	72	3	0	0.020
*ITPA94*C>A	rs1127354	61	14	0	0.093

TPMT = thiopurine S-methyltransferase, ITPA=- inosine triphosphate pyrophosphatase

Allele frequency was calculated by using Hardy-Weinberg principle

Table 2.3: Relationship between clinical adverse event and polymorphism during treatment with 6-MP in total 75 patients

Gene & location	Genotype	Leuco-penia	Neutro-penia	Thrombo-cytopenia	Raised billirubin	Raised ALT	Fever
***TPMT**3Cc.719A>G**							
AA	73	22	18	15	21	28	35
AG/GG	2	2	2	2	1	1	1
P-value		**0.037**	**0.017**	**0.008**	0.515	0.739	0.954
ITPAc.94C>A							
CC	55	6	9	12	12	16	20
CA/AA	20	8	11	5	10	13	16
P-value		0.183	**0.001**	0.167	**0.048**	**0.007**	**0.003**

Table 2.4: Univariate and multivariate logistic regression analysis which showing the relationship between ITPA polymorphism and toxicity

	Crude OR	95% CI	Adjusted OR	95% CI
Leucopenia ($<3\times10^9$/L)	1.62	0.56-4.72	2.18	0.65-7.23
Absolute neutropenia ($<1.0\times10^9$/L)	6.25	2.01-19.42	7.68	2.21-26.61
Thrombocytopenia ($<100\times10^9$/L)	1.19	0.36-3.96	1.11	0.33-3.77
Hyperbillirubinemia (>17.3μmol/L)	3.58	1.21-10.61	4.73	1.39-16.07
Raised serum ALT (>36U/L)	4.53	1.53-13.43	4.73	1.52-14.68
Fever (>38°C)	7.00	2.05-23.84	6.89	1.99-23.91

OR, Odds ratio; CI, Confidence interval. Adjusted by age, sex, height, and weight.

CHAPTER 3

CONCLUSION

This research was conducted to detect the frequency of polymorphism of TPMT and ITPA among children in Bangladesh and also to see the relationship of genetic polymorphism with toxicity. Till to date, there is no research focusing on the polymorphism of drug metabolizing gene and this is the first ever pharmacogenetic study in Bangladeshi, so that, based on this report more extensive study regarding the pharmacogenetic of Asians can be conducted in future.

This research showed several important results. First, this research confirmed that ITPA is more prevalent than TPMT among Bangladeshi population. Second, this research indicated that TPMT*3C is more common than TPMT*3B and there is no TPMT*2 among Bangladeshi. This research also suggested that TPMT and ITPA variant significantly influence the risk of toxicity. Myelosuppression and febrile neutropenia occurred in TPMT and ITPA variant respectively.

Pharmacogenetics provided a molecular approach which guides the individualization of chemotherapy. This approach may reduce the toxicity and increases safety of the therapy. In the treatment of ALL, the knowledge of SNPs in 6-MP metabolizing enzymes and its related drug toxicity has developed more rational approaches to optimize chemotherapy. TPMT and ITPA genetic variant differ from patient to patient and among different ethnic. Determination of the frequency of this genetic variant is necessary to consider the use of pharmacogenetic as a tool to improve treatment outcome. This research strongly suggests the importance of TPMT and ITPA genotyping in patients with ALL to design more rational and cost effective treatment strategy in children with ALL. As ITPA is more frequent in compare to TPMT among Bangladeshi population it should be prioritize during screening process. Furthermore, I recommend larger study for better findings of TPMT allele frequency.

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