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Meta-Analysis of Methylenetetrahydrofolate Reductase Polymorphism and Methotrexate Related Hematological Toxicities in Pediatric Acute Lymphoblastic Leukemia Patients

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Abstract
Background:*Methotrexate is an antineoplastic drugs which is used to treat various types of neoplasms and autoimmune diseases. In pediatric acute lymphoblastic leukemia methotrexate is widely used. The methotrexate toxicities in acute lymphoblastic leukemia is widely studied with conflicting results due to presence of methylenetetrahydrofolate reductase (MTHFR) polymorphism. Due to this ambiguity we conducted meta-analysis to evaluate the relationship between MTHFR polymorphism e.g. C667T and A1298C, and methotrexate toxicities.*
Method:*we extensively searched scholar.google.com for "MTHFR polymorphism, methotrexate toxicity, and pediatric acute lymphoblastic leukemia", during 2005 to 2016. 675 articles were found, after removal of duplication and irrelevant articles, 23 were selected for meta-analysis.*
Results:*A total of 23 articles were included in this study. Methotrexate toxicities were observed and meta-analysis were performed to check association between MTHFR polymorphism and toxicities. None of any included studies showed statistical significant difference among MTHFR (C667T) CC+CT Vs TT haplotype allele for thrombocytopenia, anemia and leukopenia 95%CI (1.02-3.43). Two studies showed statistically significant difference p<0.01 for neutropenia. None of any study showed any significant difference of MTHFR A1298C haplotypes allele's related toxicities.*
Conclusion:*MTHFR (C667T and A1298C) have little to no relationship with methotrexate toxicities and it can't be used as a marker to predict methotrexate related toxicities.*

I. Introduction

Acute lymphoblastic leukemia (ALL) is a type of malignancy which mainly occur during childhood. About 30% of the pediatric cancers during the age of 2-5 years involves Acute Lymphoblastic leukemia[1, 2]. Due to availability of newer chemotherapy drugs during the past few years, the cure rate of ALL has improved more than 80% dramatically. A folic acid antagonist Methotrexate (MTX), is useful and most important component of ALL Chemotherapy both for maintenance and consolidation therapy. Though, some patients may develop severe toxicities which may require reduction in MTX dose or termination of the therapy[2].Due to the interpatient variation of pharmacokinetic parameters, toxicity and efficacy, it is crucial to monitor MTX serum concentrations in ALL patients [3, 4]. The biological activity of MTX depends on intracellular concentration and intracellular retention time. Reduced folate carrier is responsible for intracellular active transport of MTX [5]. Intracellulary, MTX show its pharmacological effects by inhibiting two enzymes. Inhibition of dihydrofolate reductase during the folic acid cycle also affects other enzymes, e.g. Serine hydromethyltransferase (SHMT1) and methylenetetrahydrofolate reductase or MTHFR. While polyglumated form of MTX inhibit thymidylate synthase. These enzymes are essential for nucleic acid synthesis so inhibition of these enzymes cause cellular death [6].

There are various transporters that decrease the intracellular MTX by pumping it out from the cell. These transporters mainly include breast cancer resistance protein (ABCG2), MDR Protein (ABCB1) [7] And SLCO1B18, Which Is An Organic Ion Transporter[9].

MTHFR is an important intracellular enzyme which regulates the intracellular folate metabolism and homeostasis. This enzyme irreversibly catalyze the conversion of 5, 10-methylentetrahydrofolate (5, 10-CH2-THF), essential for thymidine and purine synthesis, to 5-methyltetrahydrofolate (5-CH-THF), which is essential for nucleic acid methylation and synthesis of protein. Disturbance of folate hemostasis can lead to alteration in MTHFR cellular activity which may significantly effects the pharmacological activity of MTX in both normal and malignant cells. Therefore, it has been suggested that an impaired transformation of ^{5,10}-CH2-THF to ^{5}-CH-THF and the consequent alteration in the intracellular folates level could cause MTX toxicity[10]. In this perspective, many studies has been done on single nucleotide Polymorphism (SNP), including A1298C (Glu429Ala) and C677T (Ala222Val). Reduced enzymatic activity of proteins results when there is a SNP in MTHFR 677T allele as compare to normal MTHFR 677C. People having 677TT genotype exhibit 30% while 677CT genotype exhibit 60% MTHFR activity as compare with normal. [11, 12]. SNP in MTHFR A1298C, the will be milder decrease in activity as compare to normal 1298A allele. The homozygous individual with 1298CC allele have 40% less activity[13]. With some conflicting results, a large number of available studies has explored the possible role of MTHFR polymorphisms in toxicity, susceptibility and response of MTX in pediatric patients with ALL. These conflicting results may be due to ethnic differences, different treatment protocols and inclusion of different criteria for defining toxicity.

In this study, we critically reviewed and analyzed the association between genetic variation of MTHFR and MTX toxicities in pediatric patients suffering from ALL. To evaluate the role of MTHFR A1298C and C677T polymorphism on MTX toxicity in pediatric ALL patients, we performed meta-analysis on eligible studies which were separated according to toxicity criteria.

II. Materials And Methods

We performed in-depth search to classify MTX related toxicities studies that described the relationship between A1298C and C667T polymorphism of MTHFR in pediatric patients with ALL. We used scholar.google.com search engine with subject term and keywords 'MTHFR polymorphism MTX toxicities' and 'ALL pediatric patients' for published research articles between the years 2005 to 2016. Additionally, all the references within published articles were also reviewed.

Data extraction

For each article included in the study, we gathered ethnicity of study population, patient's number, age and diagnosis, MTX dose, MTHFR C677T and A1298C genotype data and toxicity types.

III. Results

We found 254 records with initial search. Duplicate records were eliminated and 167 records remained. From these results, 113 records were excluded because they didn't meet our inclusion criteria.44 remained studies were thoroughly studied and 31studies were discarded. Among 54 remaining studies, 23 studies were selected that investigated Methotrexate toxicity in pediatric patients with ALL and MTHFR polymorphism. 12 studies reported C677T polymorphism (Table 1) while 6 studies investigated A1298C polymorphism (Table 2). These studies could be classified according to the relationship between single nuclear polymorphisms, MTHFR and methotrexate toxicity: those with an association and showing increased toxicity, those with relationship, showing decrease toxicity and those without any association. High dose methotrexate dosage were found in most of the studies (Table 1 and 2). Each study analyzed toxicity with different criteria, we observed each criteria of toxicity (Table 3 and 4). Methotrexate toxicity in pediatric acute lymphoblastic leukemia patients, we didn't observed MTX clearance, therapy interruption, and diarrhea, due to limited data. In our study we mainly focused on neutropenia, thrombocytopenia, leukopenia and anemia.

Neutropenia.

Among four papers that reported neutropenia with both high and low doses of methotrexate[17, 26, 30] (Table 1), only 2 papers were included with patient pool of 200 in meta-analysis [17, 26]. There were no relationship between C667T polymorphism and neutropenia (Fig: 2).

Thrombocytopenia

Ten studies were included to evaluate thrombocytopenia 14,16,17,19,22,23,25,28,30,32. Only one reported toxicity with low dose 28 while rest of studies reported toxicities with high methotrexate doses. Meta-analysis was performed on three studies 17, 19, 23 with 381 patient data. No any relationship between C667T polymorphism and thrombocytopenia was noted. (Fig2)

Anemia

Among 8 studies[14, 16,17,19,22,25,29,32]with high doses of methotrexate, a one study 32 reported increased prevalence of anemia with C667T SNP. Due to lack of significant data we excluded 5 and only 2 studies[17, 19] were included. Meta-analysis was performed but we didn't find any association (Fig 2).

Leukopenia

For leukopenia, ten studies reported leukopenia with both low28 and high[14, 16, 19,22,23,25,29,30,32] methotrexate doses. Only 2 studies reported genotype data in 221 patients. There were no link between leukopenia and C667T polymorphism were found. (Fig2)

MTHFR A1298C SNP

Among the 16 studies, eight studies reported no relationship between A1298C and methotrexate hematologic toxicities (Fig: 3) either with high[15, 22,26,34,35] or low[21, 28, 33] doses. Most of the studies suggested the protective effects of 1298C allele 14, 17,20,23,30. Three studies reported high toxicity with A1298C allele. [16, 19, 25] we performed meta-analysis for hematological toxicities with high doses of methotrexate for, thrombocytopenia, leukopenia and anemia, but only found protective conclusions about A1298C polymorphism.

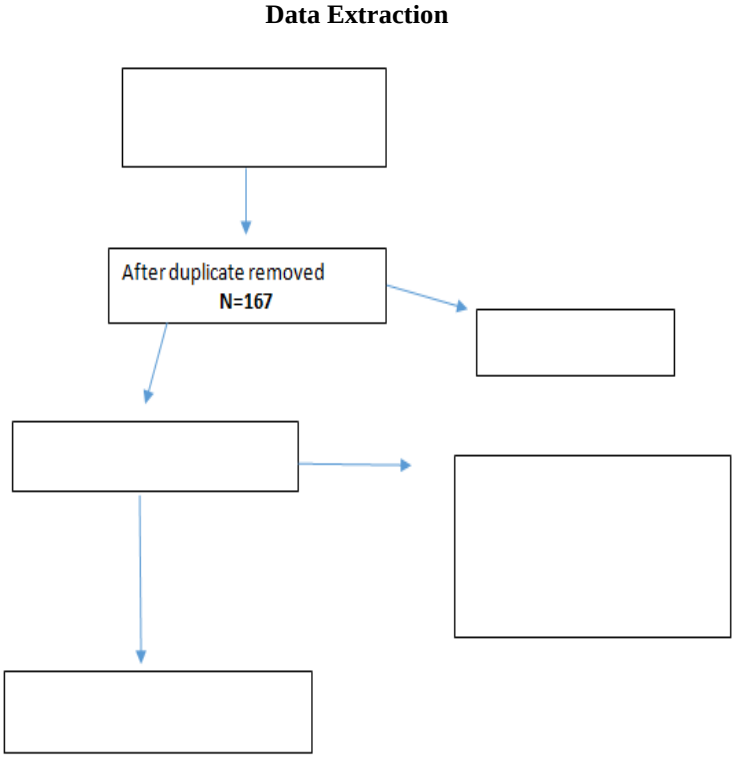

Figure 1: Flow Diagram of Data extraction.

Table: 1 MTHFR C677T Toxicity				
Patient Numbers	**Dose**	**Country**	**Toxicity**	**References**
15	High	Japanese	NA	Shimasaki et al.[18]
24	Low	Japanese	NA	Horinouchi et al.[27]
35	High	Cretan	NA	Karathanasis et al.[19]
46	High	Greek	NA	Hatzidakiset al.[29]
53	High	Various	NA	Kishi et al.[36]
76	High	Thai	NA	Pakakasama et al.[20]
81	High	European	NA	Huang et al.[14]
115	High	Spanish	NA	Lopez-Lopez et al.[15]
16	High	European	NA	Erculj et al.[22]
52	Low	Various	NA	Aplenc et al.[21]
37	High	Turkish	-Y	Kantar et al.[16]
88	High	European	-Y	Van Kooten et al.[30]
186	Low	European	-Y	Costea et al.[28]
20	Low	Japanese	+Y	Shimasaki et al.[37]
26	High	Japanese	+Y	Imanishi et al.[4]
40	High	Egyptian	+Y	Tantawy et al.[26]
40	High	Egyptian	+Y	EL-Khodary et al.[32]
64	High	European	+Y	FaganelKotnik et al.[23]
141	High	Spanish	+Y	Salazar et al.[25]
151	High	European	+Y	D'Angelo et al.[35]
181	High	Chinese	+Y	Liu et al.[17]
557	High	Various	+Y	Sepe et al [31]
29	High	European	+Y	Erculji et al[35]
Y= yes, Y+ = increased toxicity , Y- = decreased toxicity				

Table: 2 MTHFR A1298C Toxicity				
Patient Number	**Doses**	**Country**	**Toxicity**	**References**
40	High	Egyptian	NA	Tantawy et al.[26]
115	High	Spanish	NA	Lopez-Lopez et al.[15]
151	High	European	NA	D'Angelo et al.[35]
167	High	European	NA	Erculj et al.[22]
186	Low	European	NA	Costea et al.[28]
240	High	North American	NA	Kishi et al.[34]
520	Low	Various	NA	Aplenc et al.[21]
64	High	European	-Y	FaganelKotnik et al.[23]
76	High	Thai	-Y	Pakakasama et al.[20]
81	High	European	-Y	Huang et al.[14]
88	High	European	-Y	Van Kooten et al.[30]
181	High	Chinese	-Y	Liu et al.[17]
35	High	Cretan	+Y	Karathanasis et al.[19]
37	High	Turkish	+Y	Kantar et al.[16]
141	High	Spanish	+Y	Salazar et al.[25]
Y= yes, Y+ = increased toxicity , Y- = decreased toxicity				

Table: 3 MTHFR C677T RELATED DIFFERENT TYPES OF TOXICITIES				
Reference	**Anemia**	**Neutropenia**	**Thrombo-cytopenia**	**Leukopenia**
Shimasaki et al.[18]				
Horinouchi et al.[27]				
Karathanasis et al.[19]				
Hatzidakiset al.[29]				
Kishi et al.[36]				
Pakakasama et al.[20]				
Huang et al.[14]				
Lopez-Lopez et al.[15]				
Erculj et al.[22]				
Aplenc et al.[21]				
Kantar et al.[16]			-A	
Van Kooten et al.[30]		-A		
Costea et al.[28]				
Shimasaki et al.[37]				
Imanishi et al.[4]				
Tantawy et al.[26]		+A		

EL-Khodary et al.[32]	+A		+A	+A
FaganelKotnik et al.[23]				
Salazar et al.[25]			+A	
D'Angelo et al.[35]				
Liu et al.[17]				
Sepe et al [31]			+A	
Erculji et al[35]			+A	
-A= decreased toxicity, +A= increased toxicity				

Table: 4 MTHFR A1298C RELATED DIFFERENT TYPES OF TOXICITIES

Reference	Anemia	Neutropenia	Thrombo-Cytopenia	Leukopenia
Tantawy et al.[26]				
Lopez-Lopez et al.[15]				
D'Angelo et al.[35]				
Erculj et al.[22]				
Costea et al.[28]				
Kishi et al.[34]				
Aplenc et al.[21]				
FaganelKotnik et al.[23]				-A
Pakakasama et al.[20]				
Huang et al.[14]				
Van Kooten et al.[30]			-A	
Liu et al.[17]				
Karathanasis et al.[19]				
Kantar et al.[16]	+A		+A	
Salazar et al.[25]			+A	
-A= decreased toxicity, +A= increased toxicity				

Figure: 2 Results of Meta-analysis association between MTHFR C677T and Methotrexate Toxicities.

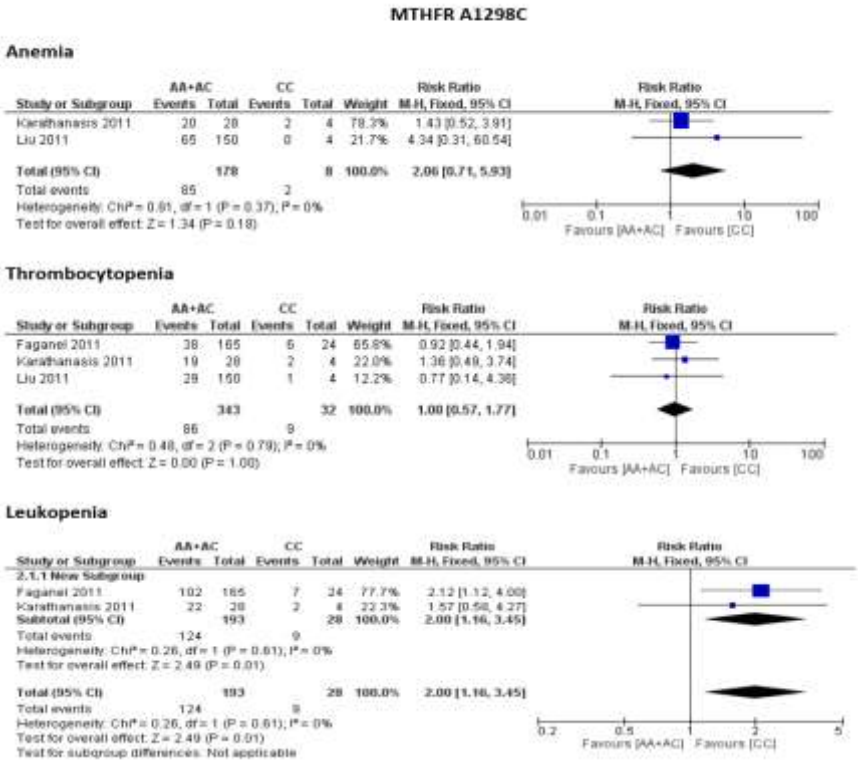

Figure: 3: Results of Meta-analysis, association between MTHFR A1298C polymorphism and Methotrexate Toxicity.

IV. Discussion

MTHFR C677T polymorphism and toxicity in pediatric ALL In the 24 available studies used in this study, 12 did not find a significant relationship between the MTHFR 677T allele and Methotrexate toxicity[14,15,18,22,27,29,33,34,36]. Three studies reportedarelationship between the 677T allele and a reduced toxicity.16,28,30 Nine studies reported relationship between C677T allele and augmented toxicities[4,17,23,25,26,31,32,35,37] (Table 1). Inhabitants studied or MTX doses could not explain anyalterations in results between studies. As different articleexamined toxicity according to different principles, underneath we investigate the results from the 24 studies for each toxicity measure and establish results from meta-analysis if sufficient data was provided to make it conceivable.

Treatment interruption.

3 studies examined MTX treatment disruption. Arelationship between the C677T allele and an increase in stoppage of drug was described by Shimasakiet al.[37]using low doses; though, this study was conducted on a small and heterogeneous inhabitants (20 Patients) and only one patient having TT genotype was described. Two larger studies of 88 and 201 acute lymphoblastic leukemia patients did not find any relationship between C677T and Methotrexatetherapydisruption with low[33] or high doses[30] consequently, the C677TT genotype cannot be reflected a good forecaster of drug therapy interruption. The three studies did not provide sufficientevidence to carry out a meta-analysis to approve it.

Hematologic toxicity.

Some studies have found associations between MTHFR 677T allele and different hematologic toxicity parameters: neutropenia, thrombocytopenia, anemia and leukopenia. From the four papers that analyzed neutropenia, only one reported an association between the 677TT genotype and higher risk of neutropenia with high MTX doses.26 Two larger studies did not find this association with low28 or high MTX doses.17 A fourth study reported the opposite effect, finding an association between the 677TT genotype and a lower risk of neutropenia30 with high MTX doses (Table 1). The controversial reports and the results of our met analysis do not support an association between C677T SNP and neutropenia. Anoverall of 10 studies examined thrombocytopenia with low28 or high methotrexate doses.[14,16,17,19,22,23,25,30,32].Alink between the 677TT and 677CT genotypes and an augmented risk of thrombocytopenia was described in 2 studies,[17,32] but 677CT genotype was only statistically significant. The obvious disadvantage of the heterozygous genotype is hard to elucidate from a practical point of view. Additionally, a new study stated a association between the CT and TT genotypes with declined risk of thrombocytopenia[16].Recent study observedat both C677T and A1298C, arelationship between the collective 677T and 1298C alleles and augmented thrombocytopenia was found.25 Six Other studies didn't find any relationship between thrombocytopenia and C667T polymorphism[14,19,22,23,28,30].The existing data do not support a clear linkamong the C677T allele and a greater risk of thrombocytopenia with methotrexate therapy for ALL, which we found with our meta-analysis.

Among the 8 studies, which reported anemia with high doses of methotrexate[14, 16,17,19,22,25,29,32], only one study reported link between anemia and C667T polymorphism. But we didn't find and association in our meta-analysis. 10 studies reported leukopenia, among them only 1 [32]reported relationship with high doses. Interestingly one study reported decreased link among C667T polymorphism and leukopenia with low methotrexate doses [28]. While 8 studies reported no relationship with high doses [14, 16,19,22,23,25,29,30]. Our meta-analysis and theseoutcomes do not support a clear relationshipamong 677T allele and leukopenia. Therefore, MTHFR 677T allele does not appear to be a noble marker of hematologic toxicity. Lastly, in our evaluation of the literature, we re-analyzed, when probable, the data published in the articles. There was a one case in which author of the article reported relationship among C677T allele and high toxicity [35], we found a statistical error and found opposite results.[38]

According to the published article data and the meta-analysis described, the 1298C allele does not appear to be a good Methotrexate toxicity marker in pediatric acute lymphoblastic leukemia patients. Itlooks more likely a shielding factor relatively than a toxicity marker. In summary, due to lack of significant data, in general, we couldn't find any association with toxicity.in our conclusion, both MTHFR A1298C and C667T polymorphisms,

no significant evidence was found to use either of them as an marker to evaluate toxicity. Recent study suggested that those patients who received high doses of methotrexate had better survival rates.

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Data Extraction

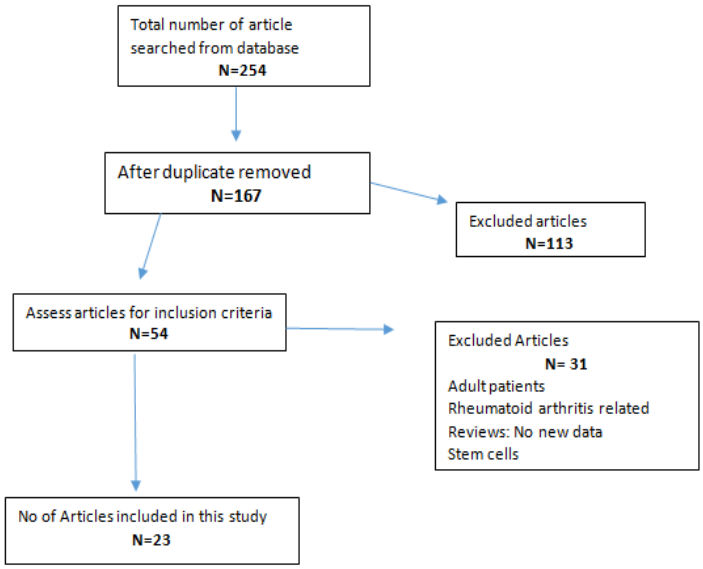

Figure 1: Flow Diagram of Data extraction.

Table: 1 MTHFR C677T Toxicity

Patient Numbers	Dose	Country	Toxicity	References
15	High	Japanese	NA	Shimasaki et al.[18]
24	Low	Japanese	NA	Horinouchi et al.[27]
35	High	Cretan	NA	Karathanasis et al.[19]
46	High	Greek	NA	Hatzidakiset al.[29]
53	High	Various	NA	Kishi et al.[36]
76	High	Thai	NA	Pakakasama et al.[20]
81	High	European	NA	Huang et al.[14]
115	High	Spanish	NA	Lopez-Lopez et al.[15]
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52	Low	Various	NA	Aplenc et al.[21]
37	High	Turkish	-Y	Kantar et al.[16]
88	High	European	-Y	Van Kooten et al.[30]
186	Low	European	-Y	Costea et al.[28]
20	Low	Japanese	+Y	Shimasaki et al.[37]
26	High	Japanese	+Y	Imanishi et al.[4]
40	High	Egyptian	+Y	Tantawy et al.[26]
40	High	Egyptian	+Y	EL-Khodary et al.[32]
64	High	European	+Y	Faganelkotniket al.[23]
141	High	Spanish	+Y	Salazar et al.[25]
151	High	European	+Y	D'Angelo et al.[35]
181	High	Chinese	+Y	Liu et al.[17]
557	High	Various	+Y	Sepe et al [31]
29	High	European	+Y	Erculji et al[35]

Y= yes, Y+ = increased toxicity , Y- = decreased toxicity

Table: 2 MTHFR A1298C Toxicity				
Patient Number	**Doses**	**Country**	**Toxicity**	**References**
40	High	Egyptian	NA	Tantawy et al.[26]
115	High	Spanish	NA	Lopez-Lopez et al.[15]
151	High	European	NA	D'Angelo et al.[35]
167	High	European	NA	Erculj et al.[22]
186	Low	European	NA	Costea et al.[28]
240	High	North American	NA	Kishi et al.[34]
520	Low	Various	NA	Aplenc et al.[21]
64	High	European	-Y	Faganelkotniket al.[23]
76	High	Thai	-Y	Pakakasama et al.[20]
81	High	European	-Y	Huang et al.[14]
88	High	European	-Y	Van Kooten et al.[30]
181	High	Chinese	-Y	Liu et al.[17]
35	High	Cretan	+Y	Karathanasis et al.[19]
37	High	Turkish	+Y	Kantar et al.[16]
141	High	Spanish	+Y	Salazar et al.[25]
Y= yes, Y+ = increased toxicity , Y- = decreased toxicity				

Table: 3 MTHFR C677T RELATED DIFFERENT TYPES OF TOXICITIES				
Reference	**Anemia**	**Neutropenia**	**Thrombo-cytopenia**	**Leukopenia**
Shimasaki et al.[18]				
Horinouchi et al.[27]				
Karathanasis et al.[19]				
Hatzidakiset al.[29]				
Kishi et al.[36]				
Pakakasama et al.[20]				
Huang et al.[14]				
Lopez-Lopez et al.[15]				
Erculj et al.[22]				
Aplenc et al.[21]				
Kantar et al.[16]			-A	
Van Kooten et al.[30]		-A		
Costea et al.[28]				
Shimasaki et al.[37]				
Imanishi et al.[4]				
Tantawy et al.[26]		+A		
EL-Khodary et al.[32]	+A		+A	+A
Faganelkotniket al.[23]				
Salazar et al.[25]			+A	
D'Angelo et al.[35]				
Liu et al.[17]				
Sepe et al [31]			+A	
Erculji et al[35]			+A	
-A= decreased toxicity, +A= increased toxicity				

Table: 4 MTHFR A1298C RELATED DIFFERENT TYPES OF TOXICITIES				
Reference	**Anemia**	**Neutropenia**	**Thrombo-Cytopenia**	**Leukopenia**
Tantawy et al.[26]				
Lopez-Lopez et al.[15]				
D'Angelo et al.[35]				
Erculj et al.[22]				
Costea et al.[28]				
Kishi et al.[34]				
Aplenc et al.[21]				
Faganelkotniket al.[23]				-A
Pakakasama et al.[20]				
Huang et al.[14]				
Van Kooten et al.[30]			-A	
Liu et al.[17]				
Karathanasis et al.[19]				
Kantar et al.[16]	+A		+A	
Salazar et al.[25]			+A	
-A= decreased toxicity, +A= increased toxicity				

Figure: 1 Results of Meta-analysis association between MTHFR C677T and Methotrexate Toxicities.

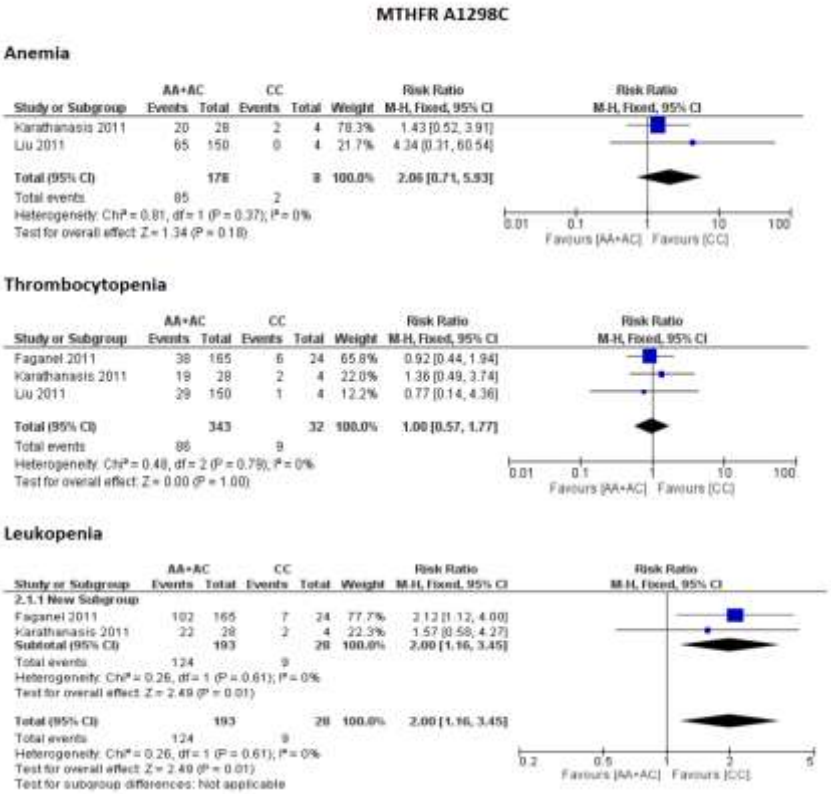

Figure:2 Results of Meta-analysis, association between MTHFR A1298C polymorphism and Methotrexate Toxicity.