**Review Article**

# ROLE OF GLUCARPIDASE FOR INTOXICATION OF HIGH DOSE METHOTREXATE TOXICITY

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## ABSTRACT

Methotrexate is prominently used drug in many disease conditions ranging from rheumatoid arthritis in low-dose and leukemias in high-dose. This high-dose methotrexate (HDMTX) has been widely used for oncology patients, with considerable precautions such as vigorous hydration, urine alkalinization, and leucovorin therapy. However, in spite of taking precautionary measures, many patients are developing HDMTX-induced renal insufficiency, which ultimately ends up with delayed methotrexate (MTX) elimination and persistent elevation in serum methotrexate concentrations. Consequently, it leads to increase in the methotrexate toxicity and deteriorates the patient current condition. To encounter this issue, glucarpidase (an enzyme, carboxypeptidase G2) is an enzyme which instantly inactivates MTX. It hydrolyzes methotrexate into two inactive non-toxic metabolites, and provides an alternative non-renal pathway for methotrexate clearance in patients with renal insufficiency while receiving treatment that contains HDMTX. This article illustrates the evidence from the published articles on HDMTX and glucarpidase to provide effective management strategies for the patients who are receiving chemotherapy containing HDMTX. Glucarpidase is considered to be safe for the patients having intact kidney function if given along with excessive hydration, leucovorin pharmacokinetically guided therapy, and urinary alkalizing agents. Therefore, precise diagnosis of delayed methotrexate clearance and renal insufficiency play a vital role for the management of toxicities induced by MTX. Particularly, the sudden rise in creatinine levels or exponentially decreases in urinary output after high dose MTX administration leads to an emergency like situation. Carboxypeptidase G2 reduces the plasma methotrexate concentration, only if it is administered as soon as possible with concomitantly providing excessive hydration and extended use of pharmacokinetically guided leucovorin therapy to curtail MTX toxicity.

**Keywords:** Glucarpidase, High-dose Methotrexate, Leucovorin, Renal dysfunction

## 1. INTRODUCTION

Methotrexate (MTX) is extensively used anticancer drug for the treatment ranging from childhood to adult cancers. Unlike other anticancer agents, it has an extensive range of dosing requirements depending on the various disease conditions. MTX is used in low dose (e.g. 20 mg/m$^2$) as maintenance chemotherapy and in higher dose ($\geq$500 mg/m$^2$) over continuous intravenous (IV) slow-infusion

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for the management of various malignant conditions. The dose range anything more than 500 to 1000 mg/m$^2$ is referred as high-dose MTX (HDMTX) which requires adequate supportive therapy to minimise or avoid undesirable toxicities.[1]

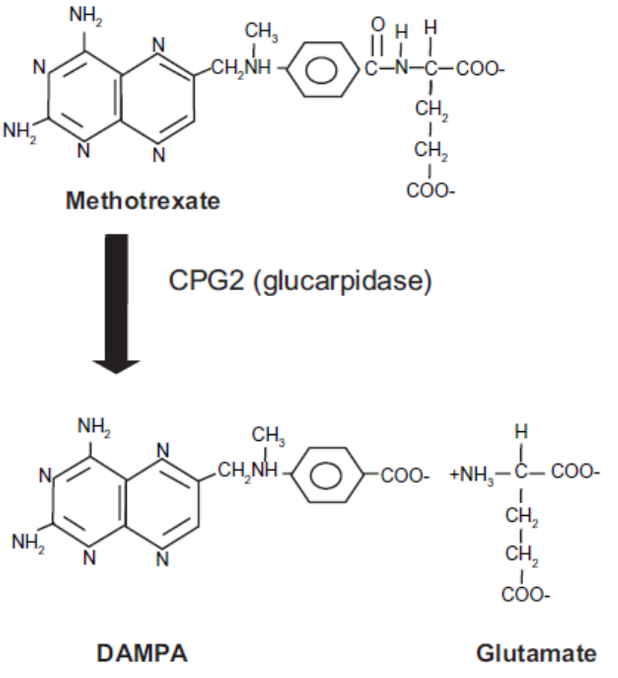
A folate antagonist such as MTX is widely prescribed medicine since 1948 for the management of different malignant conditions and HDMTX is precisely used for the management of haematological malignancies. However, despite of providing vigorous hydration and urinary alkalinizing agents, these treatment regimens could end up with acute kidney failure, proved by a sudden decrease in urine output along with sudden rise in blood urea nitrogen and plasma creatinine concentrations. MTX can considerably potentiate tissue damage because it is mainly excreted by the kidneys.[1]

Pharmacological interventions to encounter this critical condition include administration of high-dose leucovorin,[2, 3] glucarpidase,[4-6] or thymidine.[6, 7] Intravenous administration of glucarpidase can instantly and effectively reduces the serum toxic concentrations of methotrexate. Furthermore, it has been widely used to treat conditions such as MTX toxicities in patients who have been suffering from renal insufficiency due to delayed clearance of methotrexate. [1,4]

## 2. GLUCARPIDASE

Glucarpidase is a carboxypeptidase enzyme approved by US FDA in January 2012 indicated for the treatment of MTX toxicities (having toxic plasma methotrexate concentrations > 1 mcmol/L) for the patients with delayed MTX clearance because of diminished kidney function in spite of using standard leucovorin rescue.[2,3] It was earlier given to manage patients with impaired MTX clearance and methotrexate induced renal insufficiencies.[4] Glucarpidase splits methotrexate into two inactive metabolites namely 2, 4 diamino N-10 methylpteroic acid (DAMPA), and amino acid glutamate which are non-toxic. Hence, it gives an alternative, non-renal route for the elimination of methotrexate in renal failure or impaired patients.[4] This article precisely emphasizes on potential use of glucarpidase in HDMTX toxicities with clinical justification.

**Figure 1. Breakdown of methotrexate into DAMPA and glutamate, the reaction catalyzed by an enzyme glucarpidase**




### 2.1. Mechanism of action of glucarpidase

Glucarpidase is a recombinant bacterial enzyme that inactivates MTX and instantly spilt into the two noncytotoxic metabolites, amino acid glutamate and 4-deoxy-4-amino-N10-methylpteroic acid (DAMPA, see Figure I) which are majorly excreted via liver and not by kidney.[7] Widemann with his colleagues have studied the pharmacokinetic parameters and metabolism of MTX inactive metabolite DAMPA by using Rhesus monkeys.[8] They infused DAMPA over 15 minutes into these Rhesus monkeys and they found that soon after achieving plasma levels of 50 μM, it was rapidly excreted in urine, decreases suddenly to less than 1 μM within 1 to 2 hours. Almost half of the DAMPA eliminated in urine and the remaining half metabolized by the liver into hydroxyl and glucuronide conjugations. These liver metabolites were found significantly in the blood as well as urine of the monkeys and also similarly found in patients samples treated for MTX toxicities along

with glucarpidase. Studying a human leukemic cell line, scientists have reported that DAMPA was safe and did not alter the MTX toxicities. Hence, they concluded that conversion of MTX to its metabolite DAMPA relieves burden on the kidneys for the excretion of MTX and produces non-toxic molecule. A complete breakdown of methotrexate by glucarpidase ends up with rise in DAMPA and amino acid glutamate level in the blood. As levels of MTX are predominantly monitored during the treatment of patients, it should be noted that DAMPA can interfere with in this monitoring process. MTX is commonly quantify by using different methods such as dihydrofolate reductase assays, an antibody-based immunoassay, and a high-pressure liquid chromatography method (HPLC).[9] But, this antibody dependent test usually cross react with the MTX metabolites, and hence interfere in the measurement of MTX concentrations in collected patients samples after receiving glucarpidase treatment. Chromatographic method is the only reliable test to measure MTX levels after 48 hours of carboxypeptidase G2 administrations.[10] The prolonged half-life of DAMPA (9 hours) interferes with other methods for estimation of methotrexate, along with immune-assays; end up with an over-estimation of serum methotrexate level.[10]

### 2.2. Treatment with glucarpidase in HDMTX

Glucarpidase reduces the plasma concentration of MTX by 97% or more within 15 minutes of its administration.[2] However, in spite of the decreasing the intensity as well as duration of MTX systemic concentration after glucarpidase administration, it does not affect the intracellular MTX concentrations.[5,6] Consequently, like dialysis, the concomitant administration of high dose folinic acid is necessary to preserve cells from toxic MTX concentrations till the kidney sufficiently restore its capability to eliminate methotrexate.[2]

As leucovorin is an active metabolite of folic acid which serves as a cofactor supplements to counteract folate antagonist such as methotrexate for dihydrofolate reductase enzyme and inactivated by carboxypeptidase G2. Therefore, carboxypeptidase G2 administration should be avoided within 2 hours of leucovorin administration. The occurrence and intensity of glucarpidase side effects are relatively rare and mild such as pruritus, paraesthesia, vomiting, headache, nausea and flushing.[2, 11-13] The use of glucarpidase is very limited in minimizing the intrathecally administered methotrexate toxicities.

It is generally administered via intravenous injection (50 Units/kg over 5 minutes IV infusion) as a supportive therapy concomitantly with folinic acid.[2] The treatment should be continued with leucovorin unless MTX concentrations persistently retained below the threshold of leucovorin therapy for at least 3 consecutive days. Plus, vigorous hydration and continuous urinary alkalization should be continues unless serum methotrexate concentrations are below its toxic levels.[4]

Buchen and its colleague in 2005 had conducted a prospective, open-label, multicentre nonrandomized trial in patients with a median age around 15 years. They reported a study in which approximately 82 patients were given glucarpidase who were experiencing acute methotrexate overdose toxicities along with kidney failure.[12] Inclusion criteria for the study was serum methotrexate concentrations more than 10 micrometer at 36 hours or more than 5 micrometer at 42 hours after initiation of MTX infusion with renal dysfunction. The patients involved in study were suffering from different malignant conditions, including osteosarcoma, acute leukemias, brain tumors, pleural mesothelioma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma. The therapeutic glucarpidase dose was 50 units per kg. The serum MTX concentration was decreased by 87% soon after the 15 minutes of glucarpidase administration and most of the patients got benefited. However, four patients died because of bone marrow suppression and complications of sepsis. As this study had been done on children, Schwartz and his colleagues had later conducted a similar kind of study in European countries. They have included 43 adults and elderly oncology patients with different malignant conditions namely osteosarcoma, lymphoma, germ cell tumor and acute lymphocytic leukemia[14]. In this study patients had been given

carboxypeptidase G2, followed by leucovorin therapy and appropriate care including urinary alkalization. Amongst 24 patients whose samples were available, they found almost more than 97 % decrease in serum methotrexate concentration in a median of 15 minutes after administration of injection glucarpidase. However, 10 out of the 43 patients were died due to the complications associated with HDMTX. Amongst the 3 patients who treated twice with carboxypeptidase G2 while receiving HDMTX protocol; the carboxypeptidase G2 response was declined in two patients and in third patient the plasma MTX levels was beyond detectable limits. The authors assumed that this must be happened because of either inhibition of carboxypeptidase G2 by DAMPA or due to an immune reaction.

Schwartz and his colleagues assessed effectiveness of carboxypeptidase G2 in a single-arm study by taking adults and elderly patients who were suffering from delayed methotrexate clearance upon receiving high dose MTX regimen.[14] This study included 43 patients having age ranging from 18 to 73 years who received HDMTX (more than 1000 mg/m$^2$) for management of a solid malignant tumor or haematological malignant tumors. Initially, at the time of enrollment, the serum concentration MTX of patients was 1 μM to 1,187 μM. As per the guidance of MTX immunoassay, all patients had been prescribed with adequate supportive care comprised of vigorous hydration, urinary alkalinizing agents, and leucovorin therapy. In addition, patients had been given an infusion of glucarpidase 50 U/kg. However, patients having serum methotrexate levels of 0.1 micrometer or high for them second dose of carboxypeptidase G2 was optional; 1 day later to first glucarpidase dose.[14] At the end, they found that treatment with carboxypeptidase G2 had reduced the level of circulating MTX concentrations by more than 97% (at range, 5.1 micrometer to less than 0.1 micrometer) after 15 minutes (between range, 7-50 minutes) after giving carboxypeptidase G2.[13] Moreover, around 93% patients had shown significant improvisation or normalization of serum creatinine levels followed by glucarpidase rescue. However, In spite of this intervention, the adverse effects of MTX were not subsided. Overall, almost 10 patients died due to high dose MTX related consequences, including neurotoxicity (n =1), infection (n = 7), peritonitis with multiorgan failure (n = 1), and uremia/seizures (n = 1) which amplify importance of timely intervention as well as prevention strategies with vigorous therapeutic care to manage methotrexate toxicities.[14] Widemann and his colleagues had performed a retrospective study in the year 2011 by taking 100 patients from the oncology department who had received leucovorin, carboxypeptidase G2, and in few cases thymidine, for the management of HDMTX induced renal failure (Table 1).[8] Patients whose age was ranging 0.3 to 82 years as well as suffering from various malignant conditions were included in the study. Soon after administration of glucarpidase, about 15 minutes after its administration, the serum concentration of MTX was decreased by 98%. Interestingly, the thymidine rescue did not provided any additional benefits. Amongst 12 died, approximately 6 patients were suffering from irreversible MTX toxicities and mutli-organ failure. The risk factor for methotrexate toxicities (grade 4 & 5) was those patients who were suffering from grade 4 toxicity before providing carboxypeptidase G2, low-dose leucovorin, and glucarpidase treatment more than 96 hours after methotrexate infusion by multiple-regression analysis. Surprisingly, amongst the twenty-eight patients who were given two doses of glucarpidase at 24 hours interval, and 7 patients were given three glucarpidase doses at 4 hours interval, none of the patient had been detected with carboxypeptidase G2 antibodies after receiving therapy at 3, 7 and 14 days interval. Thus, it is obvious that leucovorin and glucarpidase plays pivotal role in the management of high dose MTX induced renal failure, and that it was possible to repeatedly use of glucarpidase. In the study which was conducted by Schwartz, he found an interesting result that the survival was lower for patients having three or more risk factors with deceased methotrexate clearance compares to those who had fewer than 3 risk factors at hazard ration (HR) 3.64; confidence interval (CI) between 1.14 to 17.54 and P value 0.03 which was statistically significant.[14]

**Table 1: Summary of the prominent clinical trials and studies using glucarpidase followed by high-dose methotrexate (HDMTX)**

| Parameters | Buchen and his colleagues[16] | Schwartz his colleagues[17] | Wideman his colleagues[18] |
|---|---|---|---|
| Number of patients (M/F) | 65 (41/24) | 43 (32/11) | 100 (63/37) |
| Age, years (range) | 15.4 (0.9–71.8) | 54 (18–78) | 17 (0.3–82) |
| Underlying pathological conditions (no. of study subjects) | Acute lymphoblastic leukemias (26), Lymphoma (21), Bone cancer (12), Other malignancy (6) | Acute lymphoblastic leukemias(13), Lymphatic malignancy (12), central nervous system involving lymphatic malignancy (16), Other malignancy (2) | Bone cancer (42), Acute or chronic lymphoma or Acute lymphoblastic leukemias (49), Other malignancy (9) |
| MTX dose (range) | 1–12 gm/$M^2$ | 1.2–12.1 gm/$M^2$ within 3 to 6 hours (n=20); 0.9 to 5 gm/$M^2$ within 23 to 26 hours (n=23) | 0.4–12 gm/$M^2$ (median 7.7) |
| Patients inclusion criteria | Plasma MTX concentration more than 10 micrometer at 36 hours or Plasma MTX concentration more than five micrometer at 42 hours or Plasma MTX concentration more than three micrometer at 48 hours; diminished diuresis or serum creatinine more than 1.5 × upper limit of normal | Plasma MTX concentration more than five micrometers at 42 hours or later, or plasma MTX concentration more than one micrometer at 42 hours or later with indications of renal impairement (decreased urine output and/or serum creatinine more than 1.5 × upper limit of normal) or plasma MTX concentration more than 0.4 micrometers at 48 hours or later; indications of kidney impairment | Plasma MTX concentration more than or equal to ten micrometers at 42 hours or later, or Plasma creatinine concentration was more than 1.5 upper limit of normal or creatinine clearance was less than or equal to sixty mL/min/$M^2$ and Plasma MTX level was more than or equal to two standard deviations above the mean ≥ twelve hours followed by methotrexate administration |
| Serum MTX concentration soon before Carboxypeptidase G2 dose, (range) | 11.93 μM (0.52–901) | 10.5 μM (1.0–1187) | 17 μM (0.37–849) |
| Initial Carboxypeptidase G2 therapy Time point, hours, median (range) | 52 (25–178) | 56 (27–176) | 96 (22–294) for forty-four patients along with thymidine; 66 (22–192) for fifty-six patients without thymidine |
| First Carboxypeptidase G2 dose median (range) in U/kg | 50 (33–60) | 50 (10–58) | 50 |
| Carboxypeptidase G2 doses (number of patients) | 1 dose (58); 2 doses (9); 3 doses (1) | 1 dose (40); 2 doses (3) | 1 dose (65); 2 doses (28; interval: 1 day); 3 doses (7; interval: four hours) |
| % decrease in serum MTX (measured by High Performance Liquid Chromatography) Median (range) | 97% (73% to 99%) 15 minutes (after Carboxypeptidase G2 treatment) | About 97% reduction within 15 (7–50) minutes; by taking twenty-four patients | Around 98.7% 15 minutes (after Carboxypeptidase G2 treatment) |
| Patient deaths owing to MTX toxicity | 4/82 (5%) | 10/43 (23%) | 6/100 (6%) |
| Carboxypeptidase G2 anti-bodies (in %) | Not determined | 43 (three out of seven patients) | 00% |

However, the data did not show any established correlation between certain risk factors and resulted toxicity or survival. These factors which did not show correlation to methotrexate toxicity or survival were age of patient, carboxypeptidase G2 dose, serum MTX before glucarpidase administration, the presence of sustained serum MTX reduction, leucovorin doses that were inappropriately low and time of carboxypeptidase G2 intervention depending on time of MTX administration. These factors which did not show any correlation to toxicity or survival were age of patients, dose of carboxypeptidase G2, serum MTX before glucarpidase administration, the presence of sustained serum MTX reduction, leucovorin doses that were inappropriately low and time of glucarpidase intervention relative to the time of MTX administration. However, in this study the authors have not mentioned any significant difference in terms of toxicity or survival amongst monotherapy of high dose methotrexate and methotrexate with other cytotoxic drudg.[14]

Widemann and his colleagues had described their 15 years of experience in the year 2012 with carboxypeptidase G2 for MTX toxicity by taking around 492 cancer patients between the time span of November 1993 to June 2009 (Table 2).[15] In this study, all the patients along with glucarpidase treatment received standardise supportive care with intravenous vigorous hydration, extreme urinary alkalinization and leucovorin. The median methotrexate concentration was 17 micrometers before administering carboxypeptidase G2 rescue, and after the administration of glucarpidase treatment about 15 minutes later the MTX concentration was reduced by a median of 99% and consistently maintained till the last measurement (40 hours, median). While patients initially treated with renal failure shown grade 2 or higher toxicities, after the administration carboxypeptidase G2 rescue, 64% patients amongst them shown grade 0 after a median of 12.5 days. Thus, it had shown that carboxypeptidase G2 was well tolerated by patients, with minor to moderate adverse events including flushing (1.8%), paresthesia (2.0%), and headache (1.0%).[15]

In the year 2008, scientist Phillips and his colleagues have conducted an open-label study in 12 adult subjects who were not suffering from cancer had received glucarpidase.[19] The renal function was normal in 8 study volunteers and abnormal in four study volunteers. All of them had been given a single dose of intravenous carboxypeptidase G2 50 units/kg. From all the study volunteers, patients with normal kidney function had a mean maximum serum carboxypeptidase G2 concentration was found 31 microgram/mL, mean half-life of 9.0 hours and a mean area under the serum concentration-time curve (AUC) of 23.4 microgram × h/mL. Moreover, there was no significant difference for the patients suffering from renal insufficiencies, suggesting that renal damage does not alter the pharmacokinetics parameters of carboxypeptidase G2.

Another study was conducted by scientists on 1,141 pediatric cancer patients at St. Jude Children's Research Hospital. They have reported that all these study patients had received a total of 4,909 doses of high dose MTX ($\geq$ 1 gm/m$^2$) between the time periods 1998 to 2010. Amongst the all patients who received HDMTX, 20 patients (1.8% of study individuals, 0.4% of HDMTX courses) had developed acute kidney injuries as well as delayed methotrexate elimination and inclusively needed 22 doses of glucarpidase.[19] The median plasma methotrexate level was 29.1 μM (range, 1.3-590.6 μM) at the time of glucarpidase administration. All the eligible patients were given with a median carboxypeptidase G2 dose of 51.6 unit/kg (range, 13-65.6 unit/kg).[20] Out of 20 almost 13 patients were able to recommence high dose MTX therapy, and had received a total of 39 doses of high dose MTX followed by administration of glucaridase.[20] High dose MTX rechallenge was effectively tolerate by 11 out of 13 patients. The median time taken for complete removal of MTX was 355 hours with plasma creatinine concentration2.2 mg/dL during the initial course of HDMTX for which the carboxypeptidase G2 administration was required. For the second HDMTX course the elimination time was 90 hours and 72 hours for the third HDMTX courses with plasma creatinine concentration 0.8 mg/dL and 0.6

mg/dL.[20] It suggested that methotrexate clearance recovers to the normal range once kidney function improves. It had been noted in all the study patients that renal function was normalise to its baseline levels and none of the patients died due to methotrexate toxicities.[20]

**Table 2: Effects of carboxypeptidase on serum MTX concentrations in 492 cancer patients.**

| | |
|---|---|
| **Indications for High dose methotrexate (Patients; N=492)** | |
| Non-Hodgkin lymphoma (NHL) | 201 |
| Bone cancer | 148 |
| Acute leukemias (ALL, CLL) | 113 |
| Other malignant conditions | 30 |
| **Pre-treatment methotrexate level (intermediate)** | 17 μM |
| **Decreasing plasma methotrexate concentration followed by administration of the first carboxypeptidase G2 dose by High Performance Liquid Chromatography (N = 156)** | |
| After fifteen minutes of glucarpidase administration | 97% |
| After forty hours of glucarpidase administration | About 99 % |
| **Common adverse drug reactions (N=492)** | |
| Burning sensations in the lower extremities | 10 |
| Skin blushing | 9 |
| Headache | 5 |

### 2.3. Drug interactions of glucarpidase with other moieties

Carboxypeptidase G2 has shown a significant drug interaction with leucovorin and hence, leucovorin should be avoided with glucarpidase. This is mainly because leucovorin competes with MTX to bind with dihydro-folate reductase enzyme (DHFR).[21, 22] Although the effectiveness of glucarpidase is more prominent in reducing the serum MTX concentrations, it does not show any significant effect on intracellular MTX concentration; mainly because leucovorin enters into the cells, and then combats with intracellular MTX. Consequently, the effects of these drugs will be reduced if leucovorin and glucarpidase are given altogether.

Leucovorin is the widely used in cancer protocols especially with the anti-folate agents. It is generally administered after some period of delay to anti-folate drug administration to preserve the effectiveness of anti-cancer drugs.[23-25] The prime objective is to destroy malignant cells, which grow fast and their survival will be dependent on folate concentration in the blood. Therefore, these cells take up more folate along with anti-folate which is necessary to kill the cancerous cells, and this leucovorin preserves the normal cells. This phenomenon was demonstrated in 1954 for the first time in a mouse model of mouse suffering from leukemias. In this model, the scientists Goldin and his colleagues had shown that effectiveness of amino-pterin along with leucovorin rescue was more than the use of aminopterin alone.[26] Thus, MTX along with leucovorin rescue has become most prominent therapy for a different malignant conditions.[27] Surprisingly, the studies conducted earlier about treatment of MTX overdose had investigated about carboxypeptidase, not to prevent MTX toxicities but to decrease the blood folate level in order to increase the effectiveness of anti-folate agents.[28] Furthermore, the therapy consisting of leucovorin, glucarpidase, and thymidine is equally effective for the management of methotrexate induced kidney failure.[29] As thymidine in patients with normal kidney function prevents methotrexate toxicity and it does not compete with methotrexate, hence it is a good complement to leucovorin and glucarpidase.[30,31]

In 1997, Widemann and his colleagues had mentioned for the first time about the leucovorin/thymidine/glucarpidase combination protocol. They had taken 20 patients who developed nephrotoxicity after receiving high dose MTX.[29] The study result had shown that patient had increased serum creatinine concentrations (3.2 mg/dL, median) and elevated serum methotrexate concentrations (201 micrometers at hour 46, median). All the patients were administered with following protocols: (1) thymidine (8 gm/$m^2$/day), (2) 1-3 doses of glucarpidase (50 U/kg), and (3) leucovorin rescue. This protocol had a reduced serum methotrexate level (95.6% to 99.6%) and return to the normal renal function (glomerular filtration rate > 80 mL/min/1.73 $m^2$) at a median of 22 days. Two patients were died due to methotrexate toxicity in spite of providing rational treatment. Amongst them one had developed per-

sistent pancytopenia along with pulmonary complications mainly because of sub-therapeutic leucovorin rescue, and the other had developed acute gastrointestinal haemorrhage with declining of kidney function, pleural effusion, and febrile neutropenia.[29]

Another study was conducted by Widemann and his colleagues in 2010 to report their experience with carboxypeptidase G2, leucovorin, and thymidine in 100 patients.[30] In that study all the patients were managed with standard supportive-care measures, including leucovorin rescue and to 3 doses of carboxypeptidase G2. Initially around 35 patients had been given continuous infusion of thymidine and then thymidine was restricted to some patients receiving methotrexate for more than 96 hours or suffering from methotrexate toxicity. Those patients who had received the first dose glucarpidase along with thymidine (n = 44) had shown reduction in serum MTX levels within 15 minutes by 98.7% and patients who had not received thymidine (n = 56) had shown 98.8% reduction in serum MTX level. However, almost 12 patients died during the study period, from which 6 patients died due to methotrexate toxicities despite of receiving thymidine.[30] These patients suffered from the range of toxicities such as grade 3 (n = 1) or grade 4 (n = 3) mucositis, grade 4 myelosuppression (n = 5), sepsis (n = 5), and toxic epidermal necrolysis (n = 2). The main cause of grade 4 and 5 toxicity comprises of the presence of grade 4 toxicity before administration of carboxypeptidase G2, low-dose leucovorin, and carboxypeptidase G2 administration more than 96 hours after the initiation of the methotrexate infusion. However, the deaths of other patients were associated mainly due to rapidly progressing cancerous condition. The main risk factors for sever toxicity such as pre-existing toxicity, inappropriate leucovorin increase, and delayed glucarpidase indicated by light blue, dark blue, and black boxes, respectively. This had shown statistically significant associations between the development of grade 4 or higher toxicity and: the presence of grade 4 toxicity before the administration of carboxypeptidase G2, low-dose leucovorin rescue within the first 3 days after HDMTX treatment (P = 0.001), the use of diuretics (P = 0.022), and the delayed therapy of carboxypeptidase G2 (more than 96 hours after high dose MTX exposure) (P = 0.033). However, it is very crucial to consider that this was a retrospective study containing diversified patients from numerous study sites with the wide variation in the collecting different data points.[30]

## 3. CONCLUSION

High dose methotrexate is safe when administered in the patients having intact kidney function concomitantly with vigorous excessive hydration, folinic acid rescue and urine alkalinization. However, the leucovorin rescues with other concomitant agents are not effective for patients who are suffering from renal insufficiency, which has been seen in the majority of cancer patients. Thus, to reduce HDMTX toxicity in patients who are suffering from kidney disorders, carboxypeptidase G2 along with leucovorin rescue is considered as highly effective and safe. Therefore, in order to manage methotrexate toxicity it needs the early detection of delayed methotrexate clearance and renal insufficiencies. Particularly, sudden rise in creatinine concentrations or conditions like oliguria or anuria followed by HDMTX administration is an emergency condition. However, plasma methotrexate levels can effectively reduced by timely administration of glucarpidase, but it should be given promptly along with vigorous excessive hydration and extended use of folinic acid rescue to reduce MTX toxicity induced suffering. Therefore, treatment regimen consisting of folinic acid and glucarpidase effectively reduces and avoids MTX further toxicities, restore the renal function, and hence patients is eligible for subsequent chemotherapy comprises of high dose methotrexate.

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