EFFECT OF THROMBOPOIETIN RECEPTOR AGONIST ELTROMBOPAG ON CHEMOTHERAPY INDUCED THROMBOCYTOPENIA

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ISLAMABAD, PAKISTAN
JULY 2018

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A thesis submitted in partial fulfilment of the requirements of the degree of MS Biomedical Engineering and Sciences

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Acknowledgements

In the name of Allah Almighty, the most Merciful, the most Beneficent and with countless salutation upon our beloved Holy Prophet Muhammad (peace be upon him). It is a pleasure for me to thank the people whose guidance, motivation and support has made this work possible.

First of all, I am grateful to Allah Almighty, who gave me the strength and ability to accomplish this work successfully. Secondly, with utmost gratification, I would like to express my profound and intense sense of indebtedness to my ever affectionate and worthy supervisor, **Dr. Adeeb Shehzad**. His proficient counselling, valuable suggestions, boundless forbearance, unwearyingly help, invaluable advice, and giving me enough independence to decide the things throughout the study, helped me to grow in both my competence and in confidence as a researcher. I feel highly indebted to **Dr. Muhammad Ayaz Mir**, HOD Shifa Cancer Centre, for his guidance and supervision without which my research would not have been possible.

I would like to acknowledge **Amna Arshad** and **Momina Masud** at Shifa clinical research center for their invaluable support and cooperation. I am grateful to all my M.S. colleagues who have been extending their cooperation to me in a number of ways while carrying out this piece of work.

Lastly, I do not have words to express my gratitude and profound admiration to my loving parents and family members. They provided me with their continuous support and love during my entire life.

Kholood Abid Janjua

Dedicated to my beloved parents, to whom I owe everything.

Abstract

Thrombocytopenia occurs when platelet formation is insufficient to balance the physiological or pathological platelet consumption. It is a result of rapid destruction of the platelets in blood or an impaired/failure in production of platelets from precursor cells. Chemotherapy-induced thrombocytopenia (CIT) is a common problem faced by cancer patients undergoing chemotherapy. Eltrombopag is a non-peptidic oral thrombopoietin receptor (TPOr) agonist that interacts with the transmembrane domain of human TPOr by inducing the proliferation and differentiation of megakaryocytes from the bone marrow, thus increasing platelet count but has not been approved for use in CIT. The purpose of this research was to investigate the effectiveness of Eltrombopag in the management of CIT. Patients experiencing CIT were divided into two groups; patients taking Eltrombopag and matching controls not taking the drug. Clinical outcomes including days to platelet recovery, bleeding episodes, length of hospital stay, delay in next chemo cycle and reduction in chemo dose were compared in both the groups. The study showed a positive effect on the platelet recovery status of the patients receiving Eltrombopag.

Key words: *thrombocytopenia, chemotherapy, thrombopoietin agonist, eltrombopag*

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List of Abbreviations

CIT	Chemotherapy induced thrombocytopenia
TPO	Thrombopoietin
TPOr	Thrombopoietin receptor
rh-TPO	Recombinant human thrombopoietin
PEG	Polyethylene glycol
MGDF	Megakaryocyte growth and development factor
DIC	Disseminated intravascular coagulation
JAK-STAT	Janus kinase/signal transducers and activators of transcription

CHAPTER 1: INTRODUCTION

1.1 Chemotherapy Induced Thrombocytopenia

In patients suffering from cancer, radiation therapy and chemotherapy are considered to be the main factors contributing towards thrombocytopenia. However, it is recommended to consider other possible causes as well. The conventional approach is to evaluate the following criteria whenever the platelet number falls below 100,000/µL [1].

- Contribution of the malignant disease – In patients with neoplasm of breast and lungs, there is high chance of spread to the bone marrow. Additionally, patients with primary cancers of blood and lymphatic system are also prone to involvement of the marrow. All of these conditions lead to the suppression of the bone marrow which results in a decreased production of the blood cells.
- Related conditions like Idiopathic Thrombocytopenic Purpura (ITP) – In certain hematologic malignancies like Hodgkin's lymphoma, about 1% of patients [2], 2 – 10% suffering from chroming leukemia [3] whereas 0.7% of patients experiencing non-Hodgkin's lymphoma [4] develop ancillary ITP. In these patients, the treatment approach is the same as with those having primary ITP along with treatment of the malignant disease [4].
- Current history of infectious disease – It is known that infectious agents lead to an abnormal escalation of the coagulation pathways. However, some pathogens produce a substance called "neuraminidase" that ultimately leads to the reduction of platelet count. They do so by the detaching the sialic acid layering over the platelet. This results in an enhanced metabolism of platelets in the liver [5]. Similarly, infections caused by viruses also suppress the production of platelets in the bone marrow..
- Use of concurrent medications – Anticoagulation medicines like heparin are noted to induce thrombocytopenia. As well as anti-biotic agents including vancomycin [6] and

anti-viral medicine ganciclovir [7] directly reduce the platelet count through suppression of bone marrow activity [8].

- Link to blood transfusion – An infrequent adverse reaction to blood transfusion is a condition known as “post-transfusion purpura” (PTP). This condition leads to the drop in platelet number [9]. This rare condition occurs in the patients in which the platelet agent “PLA1” is absent. This phenomenon is also observable in pregnant women. During PTP, the transfusion of platelets containing the antigen into the recipient who lacks this antigen, the antibody attacks the transfused cells. Furthermore, the platelets of the recipient are also attacked. To counter this condition, immunoglobulins “IVIGs” are injected to the patient and the condition can be stabilized.
- Patients suffering from bleeding disorders – The malignant neoplasms in stomach and pancreatic cancers not only give rise to infections but also contribute to the development of coagulopathy [10]. In these cases, the low platelet count is accompanied by an increase in the “D-dimer” level as well as a decrease in fibrinogen. These patients also exhibit a delayed prothrombin time and “partial thromboplastin time” PTT [11]. It becomes quite challenging to treat this DIC. This coagulopathy can be managed by the use of heparin; however, an adequate improvement can only be achieved by the treatment of the primary malignancy.
- Small vessel disease or “micro-angiopathy” caused by cytotoxic drugs or transplant – Cytotoxic drugs like gemcitabine and docetaxel damage the endothelial lining of the blood vessels. This leads to small vessel disease which in turn causes kidney injury and thrombocytopenia. The resulting phenomenon is described as “chemotherapy related hemolytic uremic syndrome [12]. These cases are diagnosed by the presence of numerous schistocytes despite normal functioning of the protein. There is also an escalated level of lactate dehydrogenase. This condition can be mitigated by symptomatic management stopping the cytotoxic drug therapy. The use of monoclonal antibodies and exchanging plasma is not found to be beneficial [13].
- Time elapsed since receiving the most recent radiation or chemotherapy – The platelets on an average live in the body for a maximum of 10 days. Post radiation or chemotherapy administration, the platelet count in the body starts to decrease after a week. The platelet count continues to drop for 2 weeks after which it starts increase, going back to normal. If

left untreated, generally the thrombocytopenia resolves after 14 days and reaches normal levels near day 28. The number of days required for the platelet level to return to normal depends on multiple factors like the dose of radiation therapy received as well as the duration of the radiation. In some cases, the platelet count can take up to a month or two months to reach the normal levels [14].

- Type of cytotoxic agent – The type of chemotherapeutic drug administered affects the thrombocytopenic episode. The lowest platelet count reached after receiving chemo as well as the number of days to platelet recovery are both dependent on the drug given. The dose and combinations of a majority of chemotherapeutic regimens have been tailored over a number of years to account for thrombocytopenia. Presently, most of the regimens are designed as such to induce minimal incidence of thrombocytopenia. As a result, majority of conventional chemo combinations result in a minimal rate of dose-limiting thrombocytopenia. In cases where thrombocytopenia does occur, it often resolves in a few days. The common management approach is platelet transfusion that has been proven to be successful for a majority of patients.

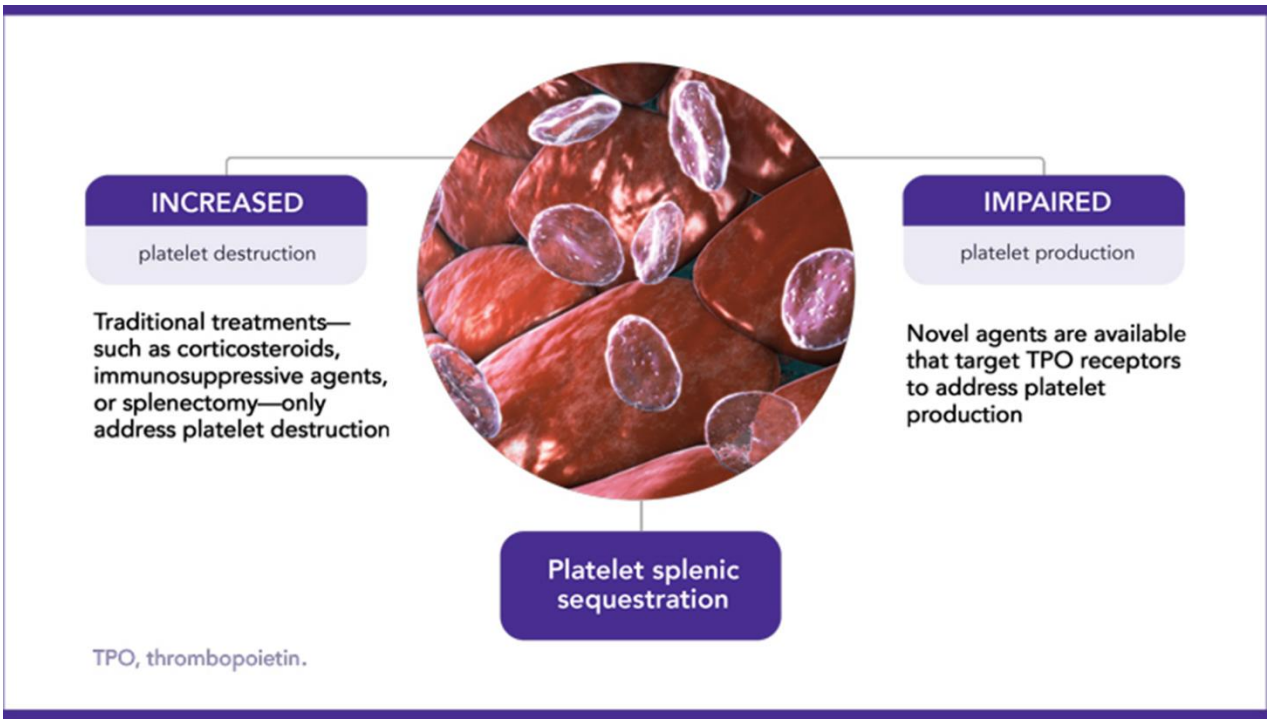


Figure 1: Chemotherapy induced thrombocytopenia

CHAPTER 2: LITERATURE REVIEW

2.1 Role of Platelets

Platelets are the blood cells that, together with the clotting factors present in the blood, serve the function of clotting the blood whenever there is bleeding. Also known as thrombocytes, platelets originate from the precursor cells called megakaryocytes in the bone marrow. They lack a nucleus and are considered to be “fragments of cytoplasm’. Platelets possess a small size and a light mass that allows them to get squeezed out of blood vessels.

Platelets play the vital role of maintaining hemostasis i.e. they prevent bleeding from a damaged vessel. The process of hemostasis takes place in several steps. In the beginning, the platelets bind to the exterior surface of the endothelial lining. Then the platelets modify their configuration and release chemicals. Thirdly, they clump to one another, forming bridges with their receeptors. The development of this platelet plug activates the chain steps of coagulation, the end result of which is the “clot”.

Thrombocytopenia is the decrease in the number of platelets below the normal range. The normal range of platelets in the blood may vary from population to population. A generally acceptable normal range for platelets is between 150,000 to 450,000/µL [15].

2.2 Pathophysiology of Chemotherapy Induced Thrombocytopenia

Each cytotoxic agent induces thrombocytopenia in a unique way. The process of platelet production is important to understand the mechanism of thrombocytopenia. The hematopoietic stem cells develop into “megakaryocyte colony-forming cells” Mk-CFCs. When these cells halt their division by mitosis, they start the process called “endomitosis”. In this process, the DNA of the cells continue to replicate but the nucleus and the cell as a whole remain undivided. This process results in the production of precursor cells that contain up to 32 times the actual amount of DNA matter. In the next step, these precursor cells undergo the inhibition of DNA synthesis. Once there is no more DNA synthesis, the precursor cells develop into bigger cells called megakaryocytes.

The fine details of the process of platelet production by these full grown megakaryocytes are still poorly explained. One possible explanation is that these mature megakaryocytes release extended structures made up of cytoplasm protruding into the cells of endothelium. These cytoplasmic structures undergo a final step of division inside the lung [16]. The life span of a mature platelet is determined by a "platelet-clock" that leads it to apoptosis [17]. Bax and Bak are "pro-apoptotic proteins" that are antagonized by the "anti-apoptotic protein" Bcl-x (L) [17]. The levels of this anti-apoptotic protein control the platelet clock by regulating the signals for platelet death through alteration of Bax and Bak levels [18]. The platelets marked to undergo programmed cell death are destroyed by the mononuclear phagocyte system. Whereas the spleen performs a minimal function in the platelet clearance process [19].

Depending on the nature of cytotoxic drug, the platelet generation process can be interrupted at various stages. Alkylating antineoplastic drugs target the pluripotent "master cells" [20]. The nitrogen mustard drug, cyclophosphamide, does not attack the master cells instead its targets are the megakaryocyte progenitors [21]. On the other hand, the proteasome inhibitor drug bortezomib disrupts the platelet clearance process by hindering the protein NF kappa B [22]. It can be linked to the considerably brief period of thrombocytopenia after the use of the drug [22].

The thrombocytopenia mechanisms described above are all related to a decrease in production of platelets. Some cytotoxic drugs exert their effects be speeding up the process of platelet death. Several chemotherapeutic drugs can change the course of platelets' life span. One such novel chemotherapeutic drug decreases the action of the "platelet clock" thereby enhancing the process of platelet death [23]. It was found in controlled experiments that even after the initial administration, the platelet counts decreased up to one-third of the initial number within just two hours. This continued to decrease to 5% of the starting number after which the platelet count started recovering, increasing till the starting number in just 72 hours [23]. Contrary to the generalized mechanism, this process did not involve platelet activation. Instead, a cascade of steps led to the programmed platelet death by the reticuloendothelial system that destroyed the platelets and cleared from circulation. One such example of drug that employs this mechanism is etoposide [23].

Lastly, some chemotherapy agents are thought to put the body's immune system to work for the process of platelet clearance. A commonly used drug for the treatment of lymphoma, fludarabine, has been proven to induce immune system driven thrombocytopenia in about 5% of patients [24]. This can be treated by use of the chimeric monoclonal antibody rituximab [25]. A relatively rare mechanism is immune mediated thrombocytopenia that is drug-dependent, resulting in an increase in platelet destruction [24].

2.3 Role of Thrombopoietin in Platelet Production

Thrombopoietin is a peptide molecule of low molecular weight that plays a salient role in the production of platelets. It has been shown in both animal and human studies that subjects that lack the normal level of the protein thrombopoietin, or the target receptor it binds to, have low platelet counts [26]. The platelet count in these subjects is as low as 10% of the acceptable standard [27]. It was also proven that the thrombopoietin protein does not affect the production of the leukocytes or the erythrocytes [28]. However, the levels of megakaryocytes are significantly decreased. The liver is primarily responsible for the production of thrombopoietin which is not stored as an intermediate product, but delivered directly into the blood circulation. The rate of production of thrombopoietin by the liver varies with the change in the number of platelets with sialylate [29]. After its release into the circulation from the liver, the major amount of thrombopoietin is removed by the binding receptors on the surface of platelets. There are also similar receptors present on the megakaryocytes found in the bone marrow. The mechanism of thrombopoietin removal is by the attachment of the protein to these receptors which in turn degrade the peptide molecule. The remaining uncleared thrombopoietin in the blood circulation is responsible for maintaining the normal amount of platelet production. It is important to note that the drop in platelet counts in the blood circulation does not induce the production of thrombopoietin inside the liver. Similarly, other biological signals have also shown not to change the rate at which thrombopoietin is produced. As a result of abnormally low platelet count, there is found to be a twenty times elevation in the concentration of thrombopoietin even though the mRNA concentration remains the same

[30]. A significant damage to the liver has been shown to elicit a comparative reduction in the rate of thrombopoietin production [31].

The concentration of thrombopoietin protein in the blood circulation results in a reciprocal decrease in platelet production rates [32]. In the case of chemotherapy induced thrombocytopenia, the rate of thrombopoietin removal from the circulation is deceased as a result of which its levels increase. The elevation in the concentration of thrombopoietin and the subsequent drop in platelet count following chemotherapy administration has been found to follow a "log-linear" relationship [33]. Interestingly the mechanism involved in ITP is considerably different as it does not involve any decrease in the production of platelets or an alteration in the clearance of thrombopoietin protein [34]. The basic process involved in the regulation of a hematopoietic growth factor is believed to be analogous to that of granulocyte or macrophage colony-stimulating factor. This regulation is directed by the number of neutrophils and monocytes in the blood circulation. The regulation of red blood cells production in contrast depends on the concentration of erythropoietin which in turn is altered by a change in hemoglobin level causing release of hypoxia induced factor by sensors in the kidney [35]. It has been established that there is an absence of a similar sensor mechanism in the regulation of platelets.

The thrombopoiesis stimulating protein attaches to the receptors present on the surface of pluripotent cells. The outcome of this attachment results in stimulation at multiple levels of megakaryocyte development. The protein thrombopoietin is crucial for the normal functioning of human physiological process. In humans lacking the target receptor for the protein, there is a severe deficiency of not only platelets but also white and red blood cells [36]. This protein also induces the mitotic stage of megakaryocyte progenitor cells. The dominant function of this peptide is to speed up the mitosis thereby doubling the chromosome number. This results in an expansion of the megakaryocyte colony. The thrombopoietin further matures the precursor megakaryocyte [37]. A pivotal characteristic of the thrombopoietin is that it inhibits the programmed death of megakaryocytes at the initial and advanced stages of development [38]. This phenomenon renders a safeguarding effect on patients undergoing cytotoxic and radiation therapy.

Apart from the thrombopoiesis inducing peptide, there are other chemical secretions like interleukins that induce the production of platelets [39].

2.4 Clinical Development of Theombopoietin Molecules

The protein thrombopoietin was discovered in 1994 [40]. Ever since its discovery, researchers around the world started looking for its analogue molecules to be used in the treatment of thrombocytopenia. There was an early development with the invention of recombinant molecules. This ultimately led to the invention of the present day thrombopoietin receptor agonists [41].

Soon after the recognition of thrombopoietin as the platelet production stimulator, scientists were successful in creating two recombinant molecules. The recombinant thrombopoietin molecule was made from the cells of Chinese hamster ovary. This molecule was glycosylated. The second molecule was made by attachment of polyethylene glycol molecules to the initial 163 amino acid molecules to form megakaryocyte growth and development factor (PEG-rhMGDF). These two molecules demonstrated a highly effective induction of platelet production. They also exhibited a long half-life of up to 40 hours. These two agents were tested on non-diseased volunteers and the onset of action of the drugs was found to be on day 3. It was observed that the platelet counts continued to increase and reached a maximum number by day 14.

During the time period after their discovery till early 2000, these recombinant agents went through considerable upgradation to be used in clinical settings. The commercial development of these agents was halted due to concerns regarding emergence of neutralizing antibodies against PER-rhMGDF [42]. It was later suspected to be linked with to the subcutaneous route of drug administration which led to the development of intravenous rhTPO with no further antibody formation.

In one study involving 525 healthy participants who received PEG-rhMDGF for three months, 2.5% experienced thrombocytopenia due to the emergence of antibodies [43]. The antibodies react with the body’s own produced thrombopoietin leading to decreased level and eventually thrombocytopenia. The 13 thrombocytopenic subjects were treated with immunosuppressants and all recovered [44].

These results from these experiments highlighted the need for the development of new thrombopoietin receptor agonists with better safety profile and decreased chances of antibody formation. A 14-amino acid long peptide was discovered in 1997 that displayed affinity to bind to the thrombopoietin receptor. Even though this molecule did not have any homology to the actual thrombopoietin molecule, it displayed the same level of activity as rhTPO [45]. This new molecule however, had a considerably short half-life. To resolve this problem, the peptide molecule was attached to an immunoglobulin heavy chain, resulting in the drug called romiplostim which has a 120-hour half-life [46]. The onset of action of romiplostim in healthy individuals is 5 days and reaches its maximum at around day 14 [47]. The highest platelet count reached was 1,600,000/μL when given to non-diseased subjects but there was no change in the white blood cell or red blood cell counts.

2.5 Effect of rh-TPO and peg-rhMGDF in Cancer Patients

To fully comprehend the effect of thrombopoietin drugs in patients suffering from cancer, it is imperative to consider that functional receptors of thrombopoietin are not present in solid tumors [48]. In a cell culture study conducted on 39 cell lines of human normal and cancerous tissues it was found that transcripts for thrombopoietin receptors were present in the cell lines of megakaryocytes as well as the cell lines of leukemia and hepatic carcinoma [49].

Another study showed that the mRNA for thrombopoietin receptor was absent in breast cancer tissue and at a low level in lung cancer tissue [50]. Even though the receptor was not identified in the ovarian, breast and lung tumor when tested through immunohistochemistry, real-time quantitative PCR showed the presence of receptor mRNA in the malignant tissues. All of the studies demonstrated that the use of recombinant thrombopoietin agents did not promote tumor growth in any of the subjects. There is a limited amount of data available regarding the use of these recombinant thrombopoietin agents in patients receiving non-myeloablative chemotherapy. Yet the available results all indicate a favorable trend in terms of platelet count restoration. Results from these clinical studies indicate that the use of these drugs results in a

relatively higher platelet count at nadir as well as a reduction in the thrombocytopenic episode and need for platelet transfusions or chemotherapy delays.

A randomized placebo-controlled trial conducted in patients with lung cancer undergoing treatment with carboplatin and taxane showed that administration of PEG-rhMGDF resulted in a significant increase in the mean platelet count at nadir (188,000/μL vs 111,000/μL). These patients also showed a shorter duration to reach platelet nadir, 7 days as compared to 14 days in the placebo group. It was also important to that the days required for platelet count to return to normal were also significantly less in the treatment group; 14 days vs greater than 21 days in the placebo arm [51]. However, no difference was observed in the need for platelet transfusions or bleeding events in both the groups. Similarly, number of thrombotic episodes was also same.

Another similar study was conducted in women with ovarian cancers. Paired comparison was performed in this group. The patients' data was collected in one cycle where no treatment for thrombocytopenia was given. It was compared with the next cycle where rhTPO was administered. Upon analysis it was found that the chemo cycle when rhTPO was administered, the women experienced a higher platelet count at nadir (44,000/μL vs 20,000/μL) as well as decrease in thrombocytopenic episode (1day vs 4days). Also, number of patients requiring platelet transfusion was one third in the cycle when treatment was given. The days till recovery were also reduced from a mean of 23 days to 20 days with the use of rhTPO [52].

In a third similar study on patients receiving combination therapy of cyclophosphamide and carboplatin, the first chemo cycle was give without any support therapy [53]. On the next cycles, the patients were given thrombopoietin factor along with the same dose of chemo as the previous cycle. The lowest platelet count (nadir) was found to be 47,500/μL in the cycle accompanied by thrombopoietin factor compared with 35,500/μL in the initial cycle. Number of days required for platelet count to recover was same in both groups. However, the time span of grade 3 and 4 thrombocytopenia was 0 days in treatment cycle and 3 days without treatment. It was also established that the PEGylated thrombopoietin does not offer any advantage if given before chemotherapy.

Another study has shown that there is a possible improvement in overall survival of cancer patient with the use of this drug [54]. In patient with non-Hodgkin's lymphoma

whose disease has relapsed, the chemo regimen ICE is proven to be beneficial if given without dose reduction. The need for reducing chemo dose arises due to chemotherapy related adverse effects. One of the common side effects with this regimen is thrombocytopenia. A placebo controlled randomized controlled trial with 38 patients, PEGylated thrombopoietin was given to 22 patients. Patients receiving thrombopoietin agent were able to receive chemotherapy at full dose and only 25% required chemotherapy delay compared with 58% of placebo group. In the treatment group, 59% patients were able to complete 8.5 years survival vs 31% in placebo group.

2.6 Use of "thrombopoietin Receptor Agonist" in Cancer Patients Receiving Chemotherapy

Even though the thrombopoietin receptor agonist was approved for treatment of the condition immune thrombocytopenia ITP in 2009, the usefulness of this drug has not been studied with detail in chemotherapy induced thrombocytopenia. There have been very few trials conducted in patients with similar condition. To the best of our knowledge, few case reports [55] and case series have been published studying the effect of the drug in chemotherapy induced thrombocytopenia [56].

One of the published studies included a cohort of cancer patients with platelet count less than 100,000/µL who were studied retrospectively for decrease in chemotherapy dose or a delay in the subsequent cycle [57]. All of the 20 patients experienced increase in their platelet counts. In 75% of the patients, it was possible to resume the scheduled chemotherapy cycle.

A single blind randomized placebo-controlled trial was conducted in patients with solid cancer who were either receiving gemcitabine or a combination of gemcitabine and a platinum agent (cisplatin or carboplatin) [58]. In the cohort receiving gemcitabine alone, the patients in the treatment group had a significantly higher platelet count at nadir compared with the placebo group (143,000/µl vs 103,000/µL). As for the delay in chemotherapy or need for chemo dose reduction, only 14% of the patients were from eltrombopag group whereas 50% from the placebo group. There was no reported case of deep vein thrombosis in either group but a majority of patients receiving eltrombopag exceeded platelet count of 400,000/µL.

2.7 Challenges of Therapy with Eltrombopag

Despite the evidence from a few studies suggesting beneficial effects of eltrombopag in chemotherapy induced thrombocytopenia, some challenges are anticipated in confirmatory studies. Following shortcomings are identified in studies reporting use of eltrombopag in CIT:

- The dose of conventional chemotherapy regimens used these days are adjusted to minimize the risk of thrombocytopenic episode. In rare cases when it does occur, it resolves quickly.
- The widely accepted management strategy is platelet transfusion and in most cases, it is required for a few days.
- Most of the chemotherapy regimens reported to induce thrombocytopenia are still in experimental phases. It is still unclear if the use of thrombopoietin receptor antagonist will be effective in these drugs.
- The limited amount of data available is unable to demonstrate a confirmatory dose and schedule for the use of eltrombopag. The studies conducted used eltrombopag both concurrently and post chemotherapy. The possibility of interaction between eltromopag and chemotherapeutic drugs resulting in an inadvertent dose increase or decrease cannot be fully ruled out.
- An alternate way of gathering evidence regarding the drug's efficacy is through animal models. Apart from the selective human studies, the animal studies on eltrombopag are also very few. This is because the only other animal in which the drug is active is chimpanzees. This limits the options for the drug to be tested in animal models.
- High quality clinical studies are needed to confirm the therapeutic dose and schedule for eltrombopag in chemotherapy induced thrombocytopenia. Accurate results can be obtained by testing it on patients who have history of thrombocytopenia in previous chemotherapy cycles [59]. Experts in this area have developed a consensus that trials to confirm eltrombopag's efficacy should include following endpoints:
    - Prevention of platelet nadir below 50,000/µL
    - Reduced or no need for platelet transfusions
    - Reduction in bleeding episodes
    - Maintaining full chemotherapy dose in the following cycle

- Prevention of delay in next chemotherapy cycle.

2.8 Treatment of CIT

There is a lack of clear guidelines for the proper approach to manage or treat thrombocytopenia in cancer patients receiving chemotherapy. Majority of the approaches depend on the underlying treatment objectives of the particular cancer patient. The individual patients also need to be assessed for their risk level. Management strategy is also dependent on whether the patient is receiving chemotherapy for curative or palliative intent. As a general rule, the first step is to assess the patient's need for chemotherapy and the intent of the therapy. Next it is important to take into account the patient's risk for bleeding specially those taking concurrent medication for anti-coagulation. After review of the existing literature, the following approaches to the management of chemotherapy induced thrombocytopenia have been identified:

- Whenever possible, it is recommended to treat the underlying causes e.g. use of suspected antibiotics, treatment of infection and control of any bleeding disorder.
- Decrease the dose, frequency or both, of the chemotherapy drug used. Changing the combination of chemotherapy drugs can also be a helpful option specially when chemo is given as palliative therapy.
- When the intent of chemotherapy is curative, reduction in dose or frequency of drugs is not recommended. To cope with the thrombocytopenia, platelet units are transfused to maintain the normal blood count before the next scheduled cycle. The platelet transfusions are usually recommended if there is a bleeding episode or the platelet count drops below 10,000/μL [60]. Transfusion can be recommended at a higher platelet count if the patient is experiencing fever or in an outpatient setting.
- If the patient has a history or an increased risk of bleeding, anti-fibrinolytic drugs can be used.

2.9 Development of Eltrombopag

Eltrombopag olamine is a peptide molecule of small molecular weight that has been developed for treatment and management of conditions that lead to thrombocytopenia. It is an agonist of the thrombopoietin receptor (TpoR) which in normal conditions is bound by the thrombopoietin

hormone. Eltrombopag was developed by the collaboration of pharmaceutical industries Lingand pharma and GlaxoSmithKline. In the early stages of its clinical trials, eltrombopag was classified as an “orphan drug”. It is a label used to classify drugs developed for use in rare conditions, like thrombocytopenia. It was approved by the US food and drug administration in 2008 to be used for treating thrombocytopenia in patients suffering from ITP in whom treatment by steroids, immunoglobulins or surgical resection of spleen was considered ineffective.

After further clinical trials, the US food and drug administration in 2014 granted eltrombopag the approval for use in patients suffering from aplastic anemia in whom the immunosuppressive therapy had failed. In last year, the National Institute of Health NIH-USA designated eltrombopag as standard of care in the treatment of aplastic anemia. It is commercially available under the brand names Promacta and Revolade. In Pakistan, the drug became available in 2011 as Revolade.

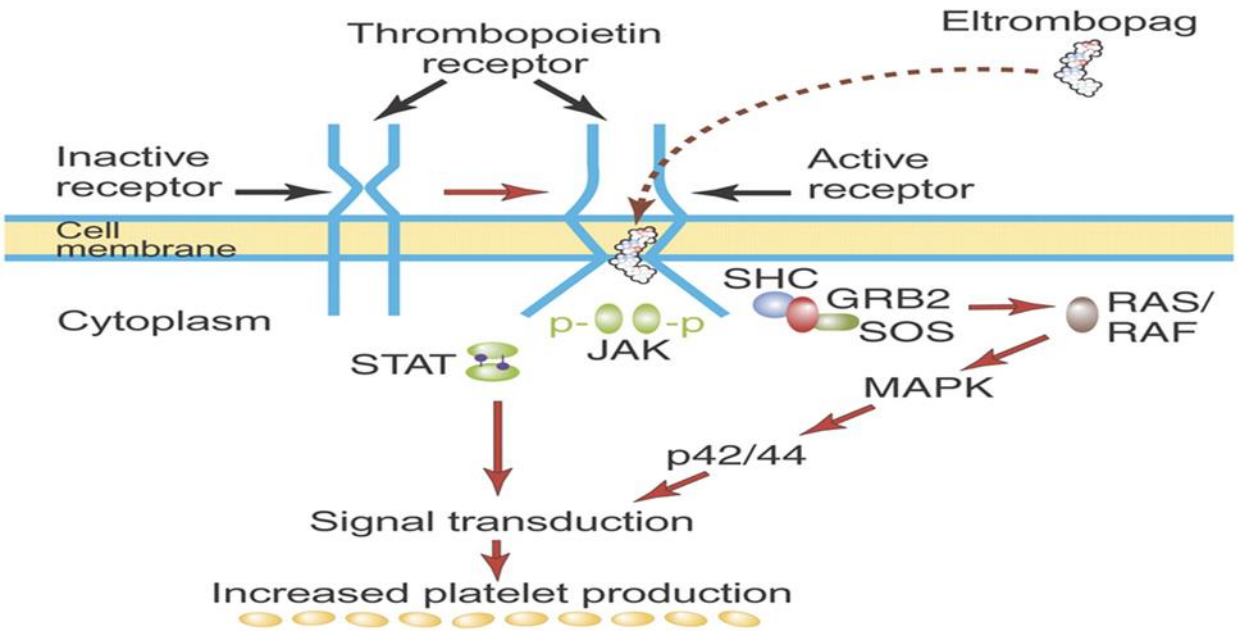


Figure 2: Action of Eltrombopag

2.10 Eltrombopag: Mechanism of Action

The eltrombopag molecule selectively interacts with the thrombopoietin receptor. This leads to the activation of the signaling pathway known as the JAK-STAT pathway. The cascade of reactions causes increase in the proliferation as well as differentiation of platelet precursor cells called megakaryocytes. Eltrombopag is a non-peptide molecule. It works by interacting with the transmembrane domain of the thrombopoietin receptors found on the megakaryocytes. This initiates intracellular signaling pathways. The bone marrow progenitor cells are activated to mature and differentiate into megakaryocytes. Another vital characteristic of the eltrombopag molecule is that it binds to a different site on the TPO receptor than that where the body's own thrombopoietin binds. Therefore, both the thrombopoietin produced by the body and the drug molecule can attach to the receptor at different sites at the same time. This elicits a twofold activity for the production of platelets.

CHAPTER 3: METHODOLOGY

3.1 Rationale of the Study

Eltrombopag can be considered as the first oral platelet growth factor. Presently eltrombopag is approved for three uses:

1. Primary Immune Thrombocytopenia with failed response to other treatments in patients who have undergone surgical resection of the spleen.
2. As a second line treatment in patients where because of increased risk or health reasons, splenectomy is contraindicated and not a feasible option.
3. Patients suffering from chronic Hepatitis C virus infection.

The only approved treatment for chemotherapy induced thrombocytopenia is Oprelvekin. It is a recombinant form of Interleukin-11 that was granted approval in 1999. Randomized controlled trials had shown that the use of oprelvekin decreases the platelet transfusions in patients from 96% to 70% [40]. The patients underwent same dose and regimen of chemotherapy but in first cycle received platelet transfusions only and in the subsequent cycles their thrombocytopenia was managed with oprelvekin in addition to platelet transfusions. But the use of this drug in clinical settings demonstrated an alarming increase in adverse effects. The safety issues led to a constant decline in the use of oprelvekin in the treatment of CIT.

Because of lack of approved agents for CIT, eltrombopag has been used “off-label” to achieve optimal chemotherapy dose and schedule in cancer patients with thrombocytopenia. Off-label use indicates that the drug is being used in patients with conditions that have not been approved or proven to be clinically beneficial as deemed by the drug regulatory authorities or high quality clinical trials.

The need for the off-label use of eltrombopag in patients with chemotherapy induced thrombocytopenia can be better understood by analyzing the various burdens on cancer patients suffering from such condition. Data published by Partners Healthcare Center for Drug Policy in the us shows that a single platelet transfusion can cost around 3000 US dollars whereas the transfusion of packed red blood cells on an average costs around 2000 US dollars. The cost of the FDA approved drug Oprelvekin can be up to $2000 per week. Apart from these direct costs,

there are some indirect costs as well. Indirect cost includes patient's hospital charges in case of prolonged hospitalization due to thrombocytopenia or its management. If the thrombocytopenic episode results in delay in the next chemo cycle, there will be additional cost to the patients. A patient with low platelet count needs to be monitored on regular intervals increasing the cost of laboratory tests.

A single dose of eltrombopag 50 mg costs PKR 4000. This by no means is a highly affordable option. But the availability of eltrombopag in oral form cuts down the need for patient hospitalization. The tablet can be safely administered in an outpatient setting without the need of strict monitoring. This makes eltrombopag a favorable option for both patients and health care providers alike.

3.2 Objectives of the Study

3.2.1 Primary Objectives

The primary objective of this prospective cohort study was to assess the efficacy of thrombopoietin receptor agonist Eltrombopag in reducing days to platelet recovery in chemotherapy induced thrombocytopenia CIT.

3.2.2 Secondary Objectives

The secondary objectives of this study were to assess the efficacy of thrombopoietin receptor agonist Eltrombopag in reducing frequency of chemo delay, chemo dose reduction and length of hospital stay recovery in chemotherapy induced thrombocytopenia.

3.3 Hypothesis of the Study

3.3.1 Null Hypothesis

There is no significant difference in days to platelet recovery in CIT patients receiving Eltrombopag compared with control group

3.3.2 Alternate Hypothesis

There is a significant reduction is days to platelet recovery in CIT patients receiving Eltrombopag compared with control group

3.4 Study design

This study was a prospective comparative cohort study.

Cohort A: CIT Patient receiving Eltrombopag with platelet transfusion, also called treatment group.

Cohort B: CIT Patient receiving standard management, also called control.

Patients were further divided according to type of cancer (solid tumor, hematological malignancy) and type of chemotherapy regimen received.

3.5 Study Settings

This study was conducted at the Oncology Clinic, Shifa International Hospital Islamabad. The patients were recruited from the outpatient clinic as well as the inpatient cancer ward.

Patients were enrolled in this study from February 2017 till April 2018.

3.6 Study Groups

GROUP 1: Treatment group - Patients receiving Eltrombopag with standard management (platelet transfusions) for the treatment of chemotherapy induced thrombocytopenia.

GROUP 2: Control group - Patients receiving standard management (platelet transfusions) for the treatment of chemotherapy induced thrombocytopenia.

3.7 Patient Selection Criteria

3.7.1 Inclusion Criteria:

Patients who experienced chemotherapy induced thrombocytopenia and scheduled to receive platelets transfusion only or Eltrombopag with platelets transfusion for the management.

Patient with platelet count less than $50x10^{\wedge 9}$/ L.

Both male and female patients.

Patients who gave consent for their data to be used in this study.

3.7.2 Exclusion Criteria

Patients with platelet count less than $50x10^9$/L before the start of chemotherapy

Thrombocytopenia due to other causes e.g. ITP, non-anticancer drugs, aplastic anemia, hepatitis C, radiation sickness, MDS and leukemia.

3.8 Sample Size Determination

The reference values for the sample size were taken from a phase I randomized study. The sample size was calculated using WHO Sample Size Calculator and taking the following parameters:

Level of significance	5%
Power of the test	90%
Population proportion 1	0.24 [57]
(chemo delay/reduction in control group)	
Population proportion 2	0.55 [57]
(chemo delay/reduction in Eltrombopag group)	
Sample size	52 (per group)

3.9 Ethical Considerations

This study was approved by Institutional Review Board and Ethics Committee IRB & EC at Shifa International Hospital with reference number: 868-143-2017. Consent was obtained from the participants to use the data for research and publication purpose. The research study was conducted according to the good clinical practice guidelines adapted from Declaration of Helsinki. All personal identifiers of the participants like name and medical record number are removed.

3.10 Study Parameters

After considerable review of the published evidence in previously conducted studies, both observational and interventional, the study parameters were decided. The local practices and the feasibility of measuring the outcomes was also deliberated. It was decided that the following outcomes or end-points will be used in our study:

- Mean days to platelet recovery till 50,000 x 10^9/L
- Patient outcome i.e. thrombocytopenia recovered or not recovered, at discharge or 30 days or whichever comes first
- Number of bleeding episodes experienced after chemo administration till the patient is discharged or at 30 days
- Number of platelet unit transfusions required after chemo administration till the patient is discharged or at 30 days
- Length of hospital stay after chemo administration till the patient is discharged or at 30 days

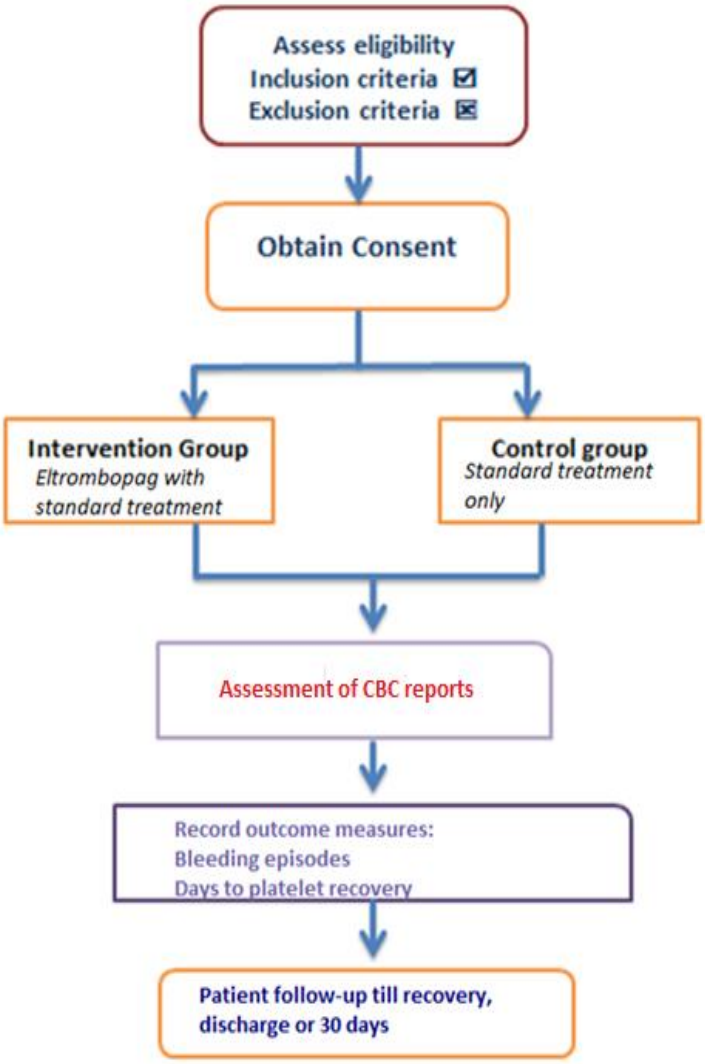
3.10.1 Outcome Measures

The study participants were assessed every day during their hospital stay. Platelet counts were recorded and number of bleeding episodes were monitored. The participant's recovery status was assessed at the time of discharge or at 30 days, whichever occurred first.

3.10.2 Determination of Platelet Counts

The platelet counts of the study participant were measured by collecting blood sample of study participants daily. The samples were sent for CBC analysis to the centralized laboratory. Complete blood count analysis was performed one the standardized CBC analyzer machine.

STUDY FLOW CHART:



3.11 Statistical Methods

3.11.1 Descriptive Statistics

All data was entered in SPSS version 21. Quantitative variables like age, BSA, no of chemo cycles received, platelet count at start, are presented as Mean± Standard deviation. Qualitative variables such as gender, tumor type, co-morbidities, 30-days outcome, are presented as frequency and percentages.

3.11.2 Tests of Association

Independent student's T-test is performed to compare quantitative variables. Chi-square is used to determine association between categorical variables. Pearson's correlation is used for relationship between two continuous variables. Binary logistic regression is applied to determine relationship of outcome variable with the predictor variables.

CHAPTER 4: RESULTS

4.1 Baseline Characteristics

The baseline characteristics of the participants in both the groups are mentioned in the table below. Mean age of the participants was 48.04±17.02 years. The mean body surface area was 1.76±0.21m^2. The patients had received 3.30±2.178 chemotherapy cycles before experiencing the thrombocytopenic episode. The days required for the participants for platelet recovery till 50,000/µL were 6.86±4.55 days. Mean length of stay was 6.70±5.42 days.

Table 1: Baseline characteristics of study participants

BASELINE CHARACTERISTICS					
		Mean	Std. Deviation	Minimum	Maximum
1	Age (year)	48.04	17.02	13.30	81.52
2	Body Surface Area (m^2)	1.76	0.21	1.23	2.22
3	No of chemo Cycles before Thrombocytopenia episode	3.30	2.78	1	14
4	Platelet count at start of Eltrombopag (x10^9/L)	14524.75	11153.111	3000	44000
5	No of days from last chemo administration	12.07	7.24	1	43
6	Days of therapy	6.86	4.55	1	27
7	Days to platelet recovery till 50*10^9/L	6.62	3.41	1	19
8	No of platelet units transfused	10.46	11.78	0	67
9	No of PRBC units transfused	2.17	2.91	0	18
10	Length of Hospital Stay	6.70	5.42	1	31

There was a similar number of participants from both genders, 51.92% of the participants were female whereas 48.08% were male. The mean age and body surface area of the participants is also depicted in the figures.

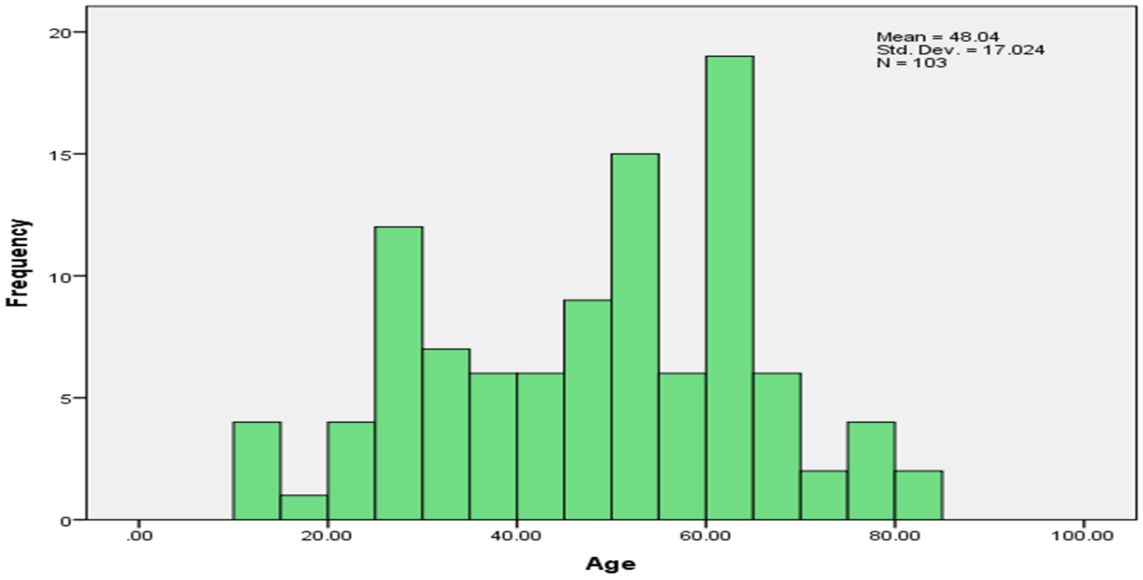


Figure 3: Mean age of study participants

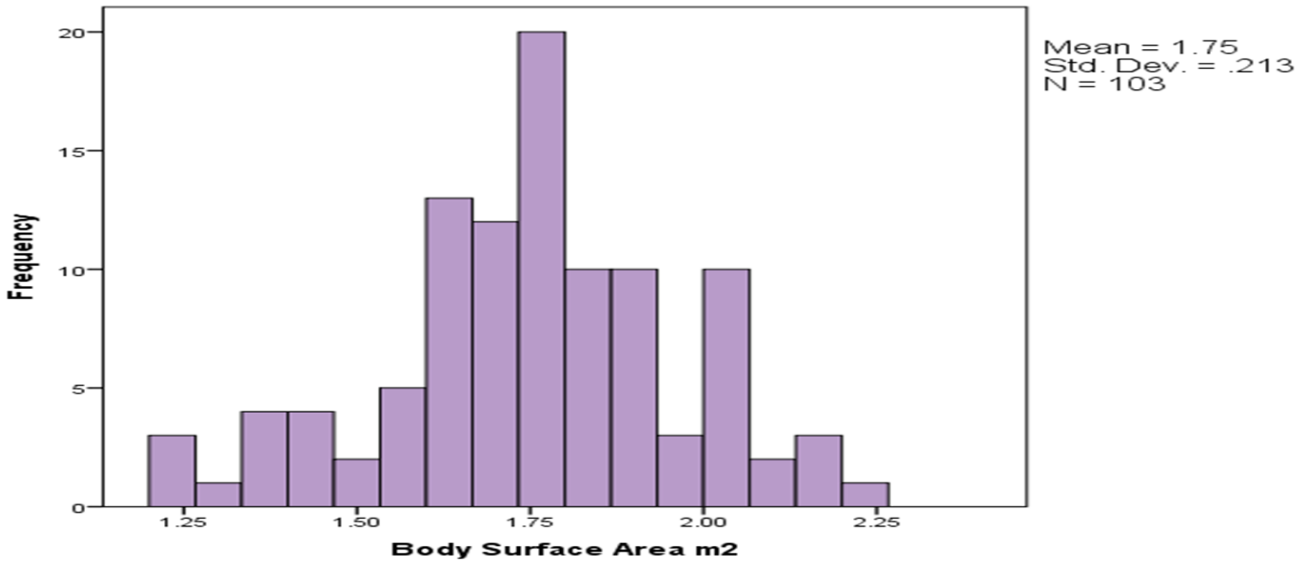


Figure 4: Body surface area of study participants

The most prevalent diagnosis was lymphoma, 26.9%. Second most recurring cancer was acute leukemia which include both acute lymoophocytic leukemia and acute myelocytic leukemia. Among solid cancers, the most frequent was genitourinary and breast cancer, both were 8.7%. Solid tumors also included sarcoma 6.7%, gastric 4.8% and lung cancer 5.8%. 5.8% tumors were classified as others including pancreatic and thyroid cancer

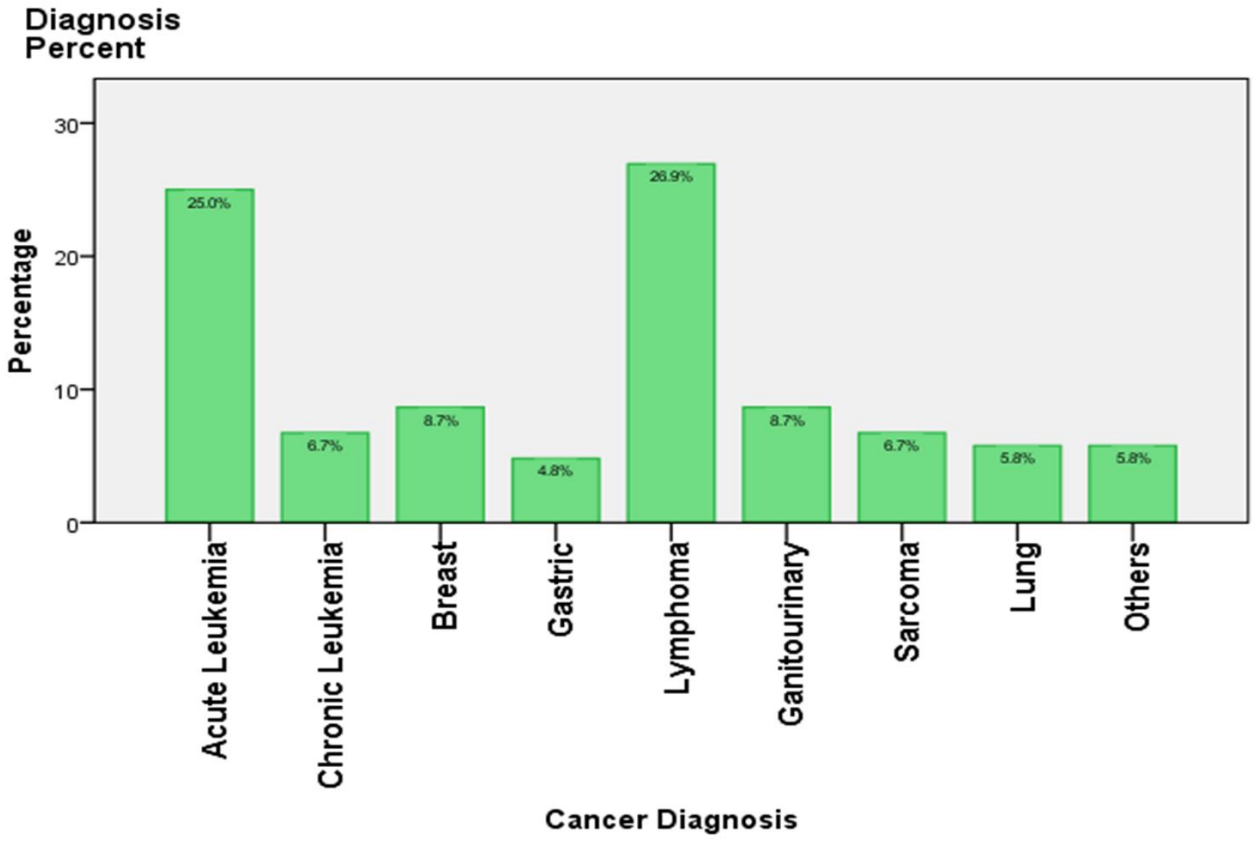


Figure 5: Percentages of various cancer diagnosis.

The study participants were also assessed for the presence of cancer metastasis and previous exposure to metastasis. 41.8% of the participants had presence of metastasis, most frequent of which was bone metastasis. 25.49% of the participants had previously been exposed to radiation therapy as part of their cancer treatment.

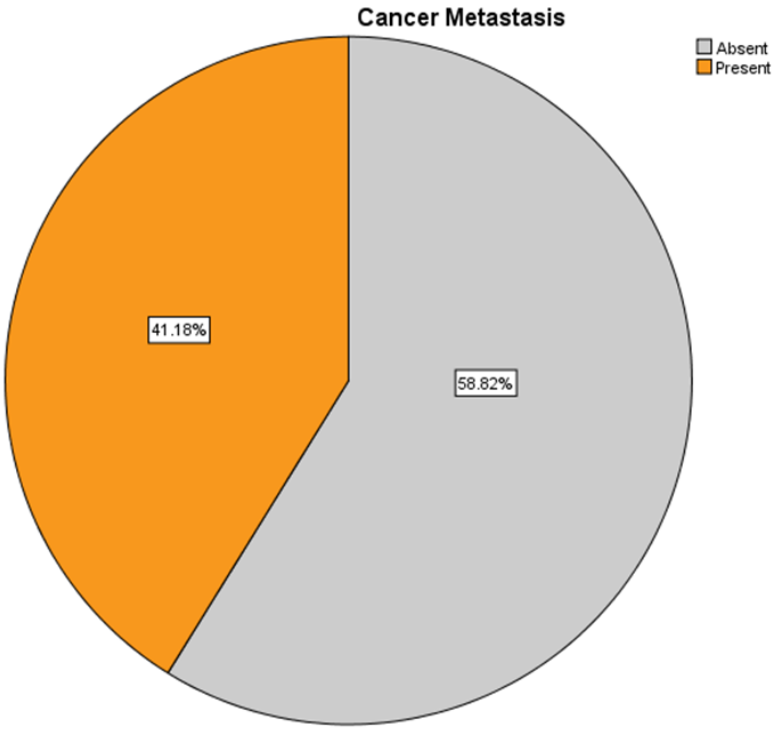


Figure 6: Presence of metastasis

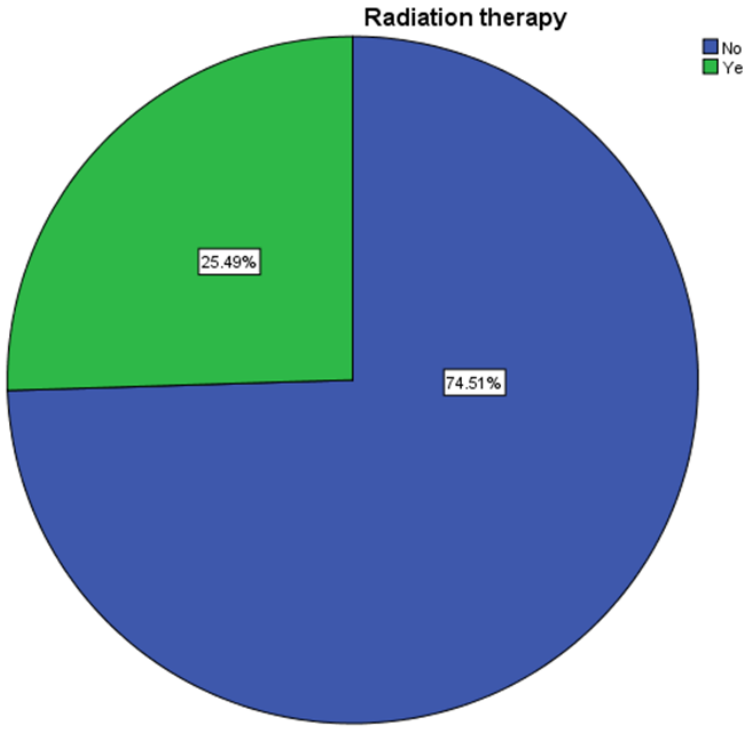


Figure 7: Previous exposure to radiotherapy

The co-morbidities of the study participants in both the groups were assessed. The most common concurrent diseases were diabetes and hypertension, 21% and 21%. Whereas 13.5% of participants were suffering from both diabetes and hypertension. A small number of study participants, 6.7% had history of cardiac diseases including ischemic heart disease and coronary artery disease. Other minor frequency included thyroid, hepatic and renal disease.

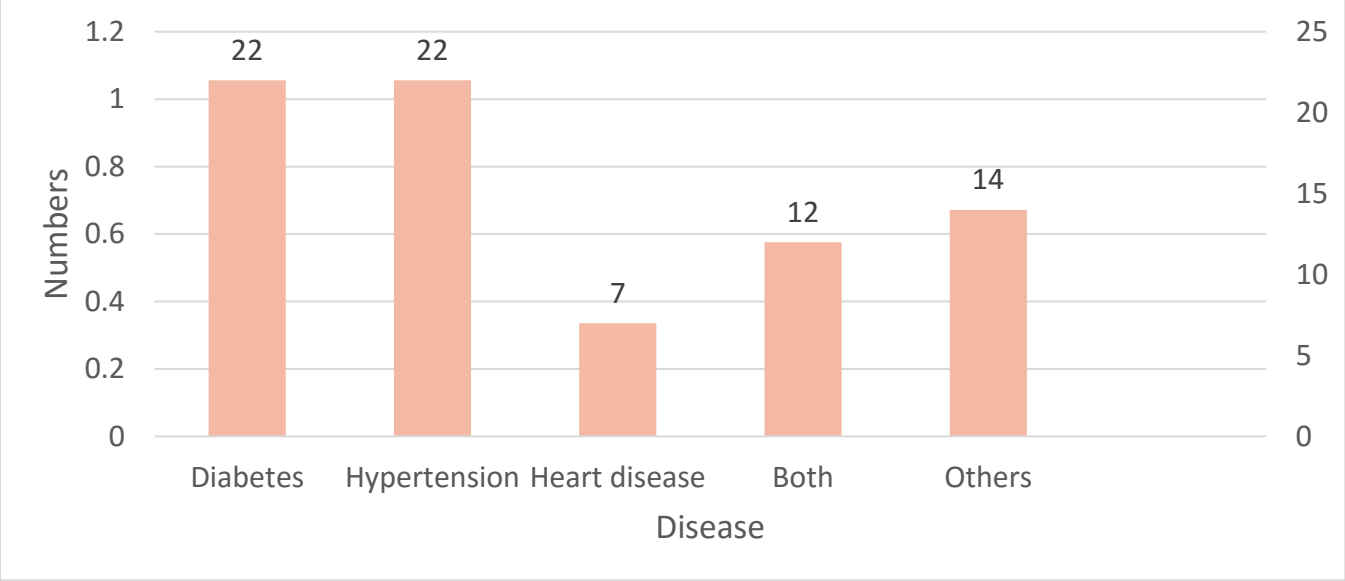


Figure 8: Presence of co-morbidities

4.2 Comparison of characteristics

The basline characteristics of participants in both treatment and control groups were compared. The mean age, body surfae area, number of chemo cycles before thrombocytopenic episode and number of days from the last chemo administration were similar in both the groups. No statistical difference was found in the two groups. The platelet count at the start of eltrombopag therapy or platelet transfusion was higher in the treatment group, 18274±12721 vs 11000±7720 (p=0.01).

Table 2: Comparison of eltrombopag group and standard group

		ELTROMBOPAG GROUP		STANDARD GROUP		
		Mean	Std. Deviation	Mean	Std. Deviation	P-Value
1	Age (year)	48.79	16.01	47.29	18.08	0.66
2	Body Surface Area (m^2)	1.76	0.17	1.75	0.25	0.69
3	No of chemo Cycles before Thrombocytopenia episode	3.29	2.84	3.31	2.68	0.97
4	Platelet count at start of Eltrombopag (x10^9/L)	18274.51	12721.76	11000	7720.6	0.01*
5	No of days from last chemo administration	13.16	7.56	10.46	6.21	0.05
6	Days of therapy	6.80	4.47	6.12	4.22	0.54
7	Days to platelet recovery till 50*10^9/L	6.83	3.822	6.31	2.70	0.54
8	No of platelet units transfused	10.02	8.9	10.94	7.48	0.69
9	No of PRBC units transfused	2.02	2.44	2.29	2.25	0.64
10	Length of Hospital Stay	5.43	3.34	7.9	6.71	0.02*

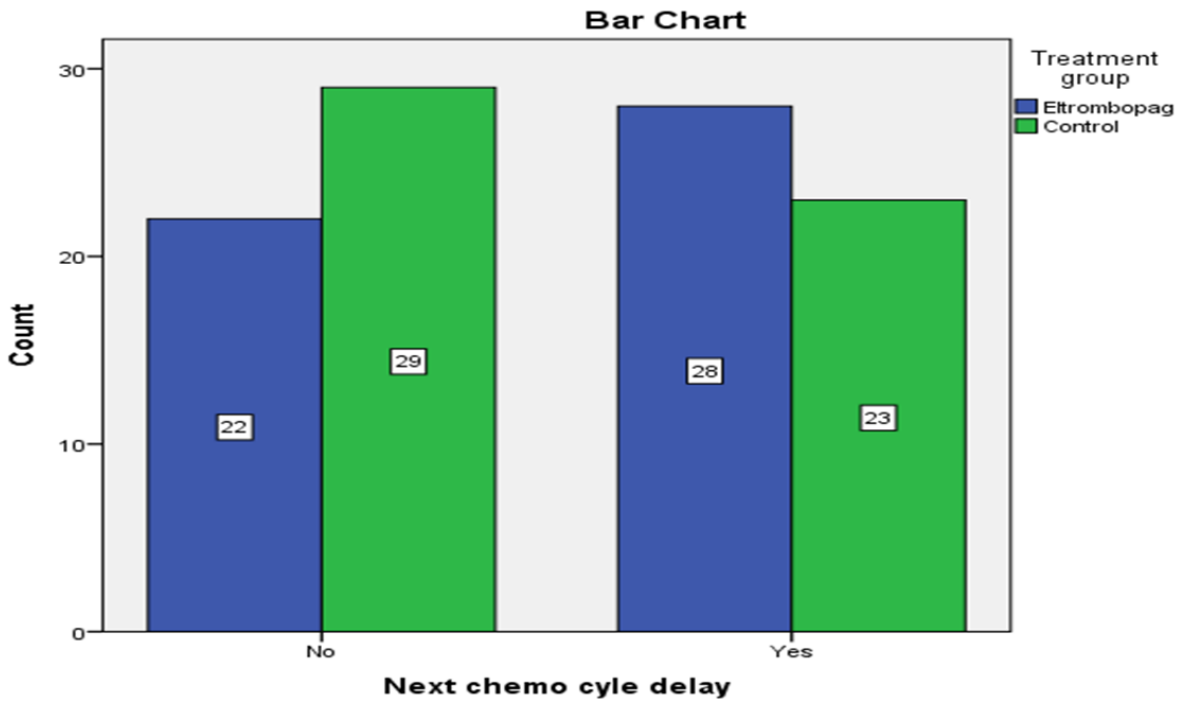


Figure 9: comparison of chemo delay

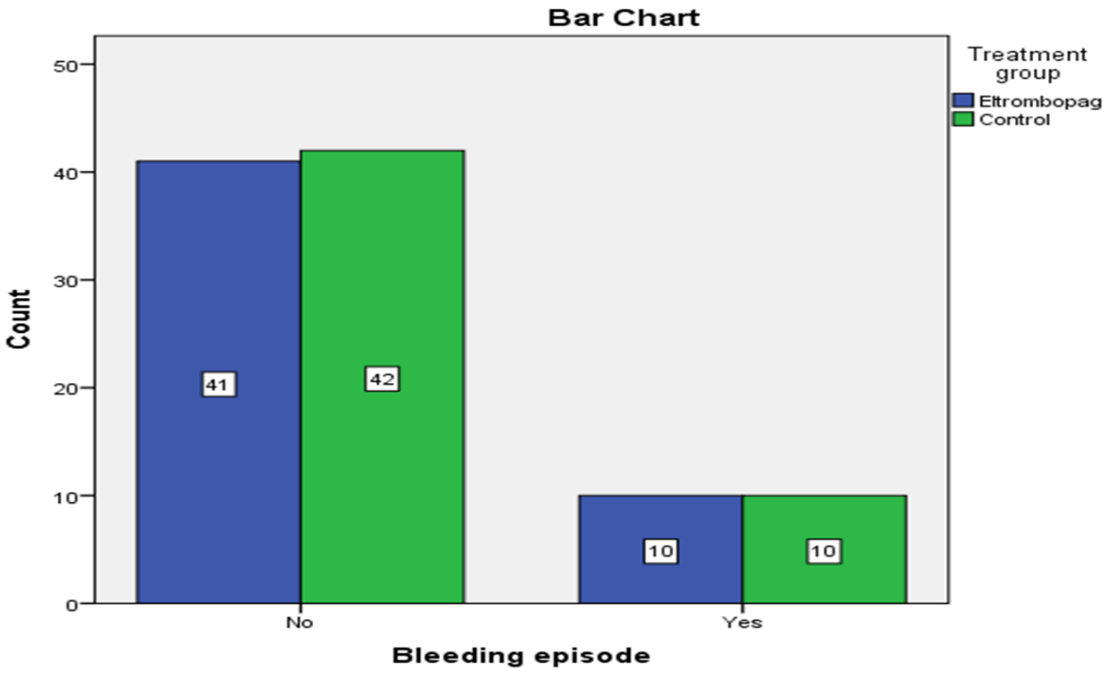


Figure 10: comparison of bleeding episodes

The need for reduction or discontinuation of the subsequent chemotherapy was recorded. In the eltrombopag group, 25 participants received their next chemo cycle without any reduction compared with 27 participants in the control group. 12 patients in treatment group required reduction in chemotherapy dose compared with 9 patients without treatment. However, the number of participants that required discontinuation of the next chemotherapy cycle were higher in control group, 16 vs 13 in the eltrombopag group.

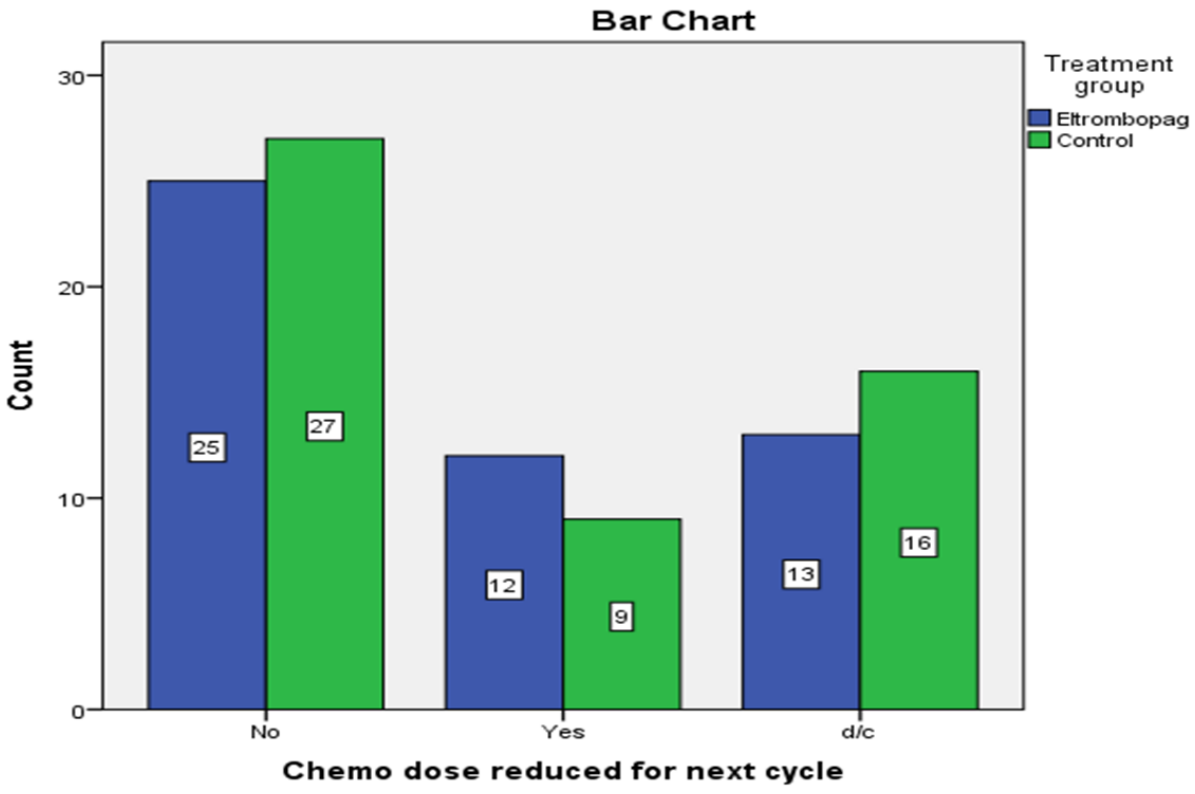


Figure 11: Comparison of chemo dose reduction

Another clinically important end-point was whether the patient's platelet count had recovered at 30 days post-chemotherapy. 39 participants in the eltrombopag had fully recovered compared to only 29 participants in the control group. The number of patients not recovered was significantly higher in the control group, 8 vs 21 ($p<0.05$). The number of deaths in eltrombopag group was 4 and control group was 2.

The figures 13 and 14 show the comparison of need of platelets transfusions and length of hospital stay in both eltrombopag and control group. The treatment group required 10.02 ± 8.9 units compared with control group: 10.94 ± 7.48 units ($p= 0.69$). As for the comparison of length of hospital stay, the eltrombopag group required hospitalization for 7.9 ± 6.71 days compared to 5.4 ± 3.34 days in the control group ($p = 0.02$).

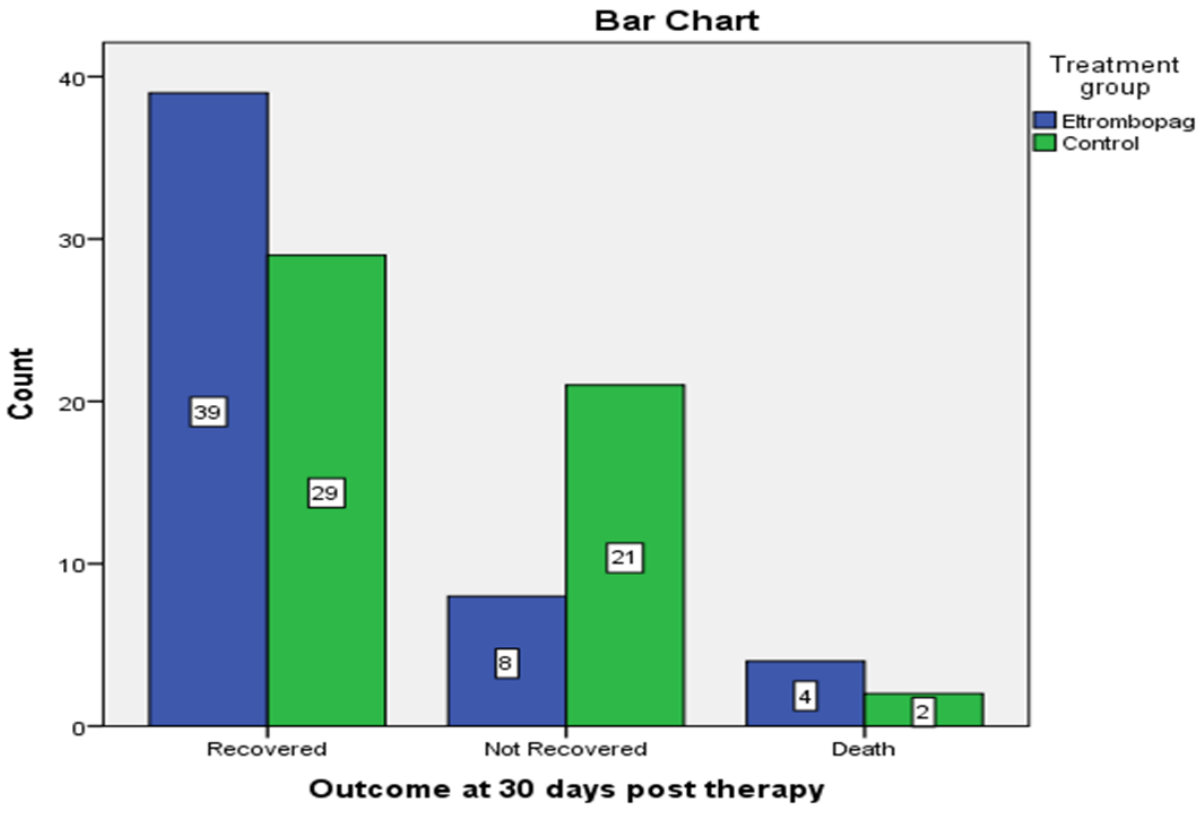


Figure 12: Comparison of outcomes

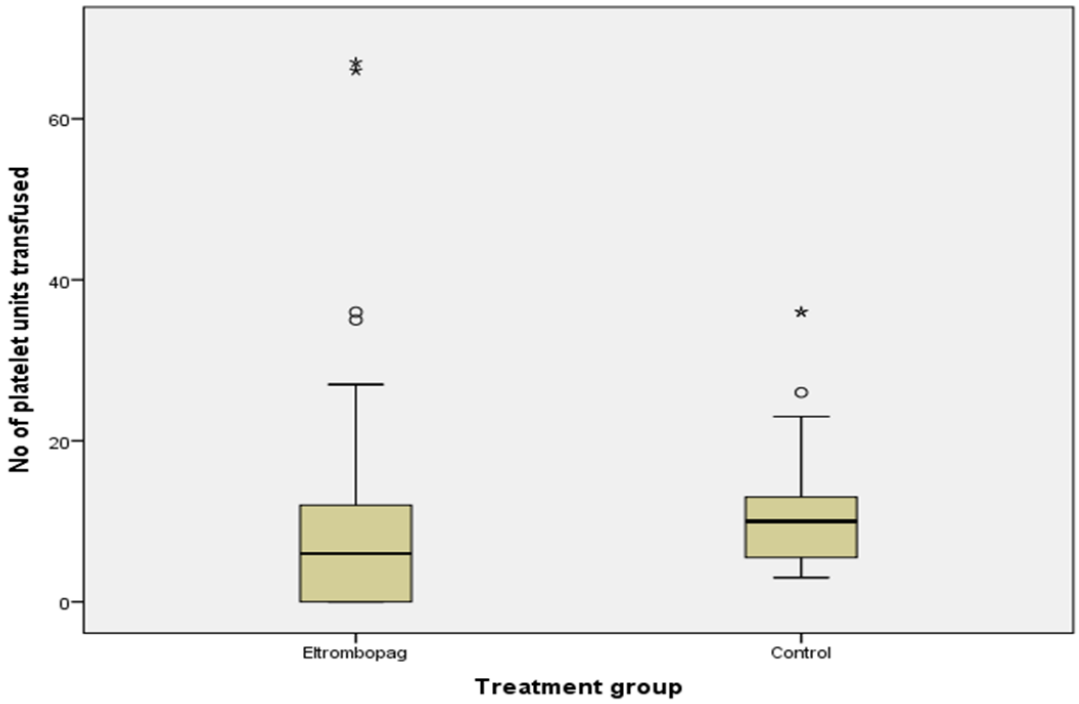


Figure 13: Comparison of number of platelet units transfused

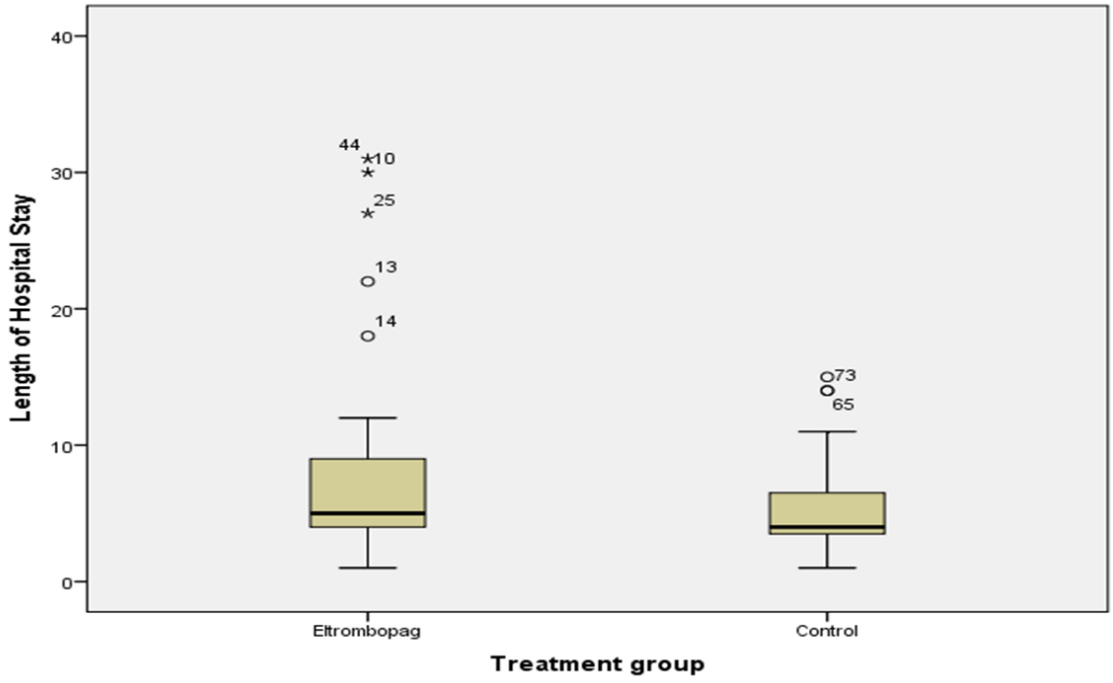


Figure 14: Comparison of length of hospital stay

The days taken for the platelets of the participants in both the groups were recorded. In the eltrombopag group, the participants took a mean of 6.83 ± 3.32 days for the platelets to cross the threshold of 50,000/µL. Whereas in the control group, a mean of 6.31 ± 2.7 days were required for the platelets recovery. The results from the two groups no statistically significant difference (p = 0.54).

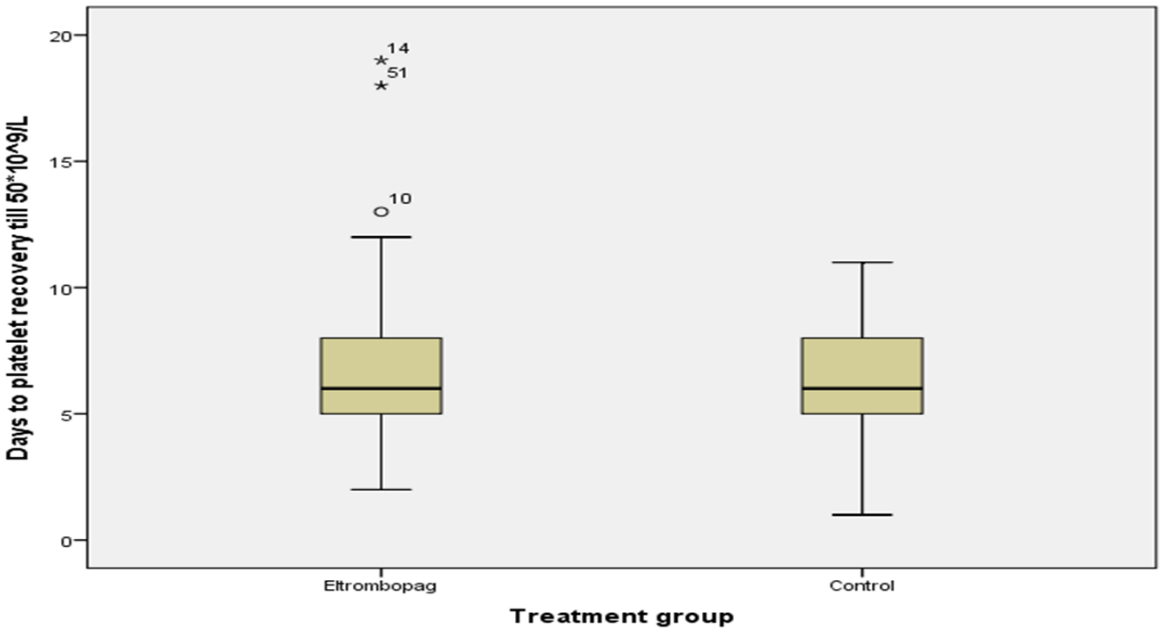


Figure 15: Comparison of days required for platelet recovery.

Results of Pearson's correlation are shown in the table 3 below. There is a weak correlation between the number of days form the last chemo administration and the days to platelet recovery. There is also a weak correlation found between platelet count at the start of the treatment with the days required for platelets recovery.

Table 3: Correlation of days to platelet recovery

		Days to platelet recovery till 50*10^9/L
Days to platelet recovery till 50*10^9/L	Pearson Correlation	1
	Sig. (2-tailed)	
	N	69
No of days from last chemo administration	Pearson Correlation	-.060
	Sig. (2-tailed)	.624
	N	69
Platelet count at start of Eltrombopag	Pearson Correlation	-.045
	Sig. (2-tailed)	.714
	N	69
No of platelet units transfused	Pearson Correlation	.341**
	Sig. (2-tailed)	.004
	N	69
No of chemo Cycles before Thrombocytopenia episode	Pearson Correlation	-.191
	Sig. (2-tailed)	.119
	N	68

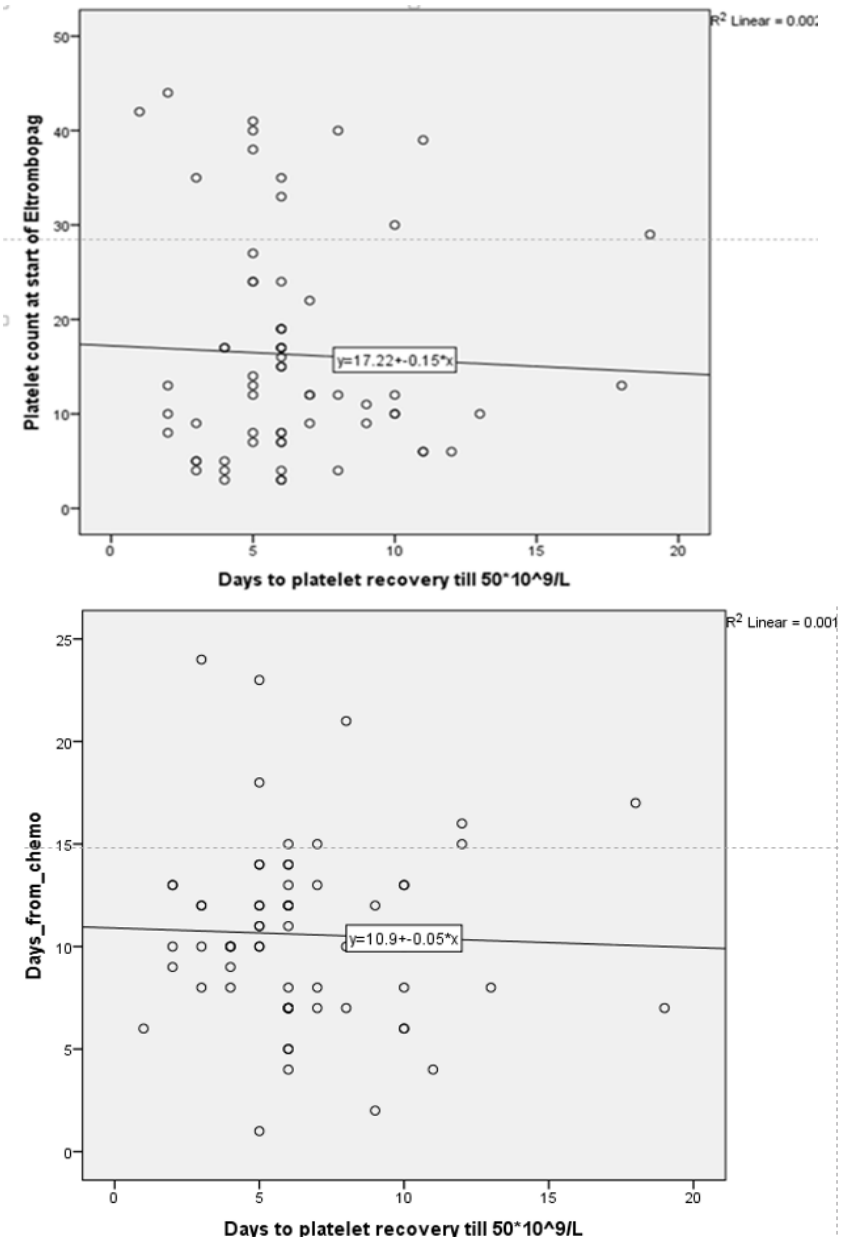


Figure 16: Correlation between days to platelets recovery, platelet count at start of therapy and days from chemotherapy administration

Binary logistic regression was applied to give a prediction model of platelet recovery outcomes based on five baseline characteristics. The results are shown in table 4.2.11. The variables used for determination of the model were number of cycles administered before thrombocytopenic episode, number of days from chemotherapy administration, platelet count at the start of therapy, number of platelet units transfused and treatment with eltrombopag. Out of these 5, number of days from chemotherapy administration, platelet count at the start of therapy and treatment with eltrombopag were found to be directly related in determining the recover outcome. They had the beta coefficient values of 0.114 (p=0.012), 0.099 (p=0.003) and -1.729 (0.004) respectively.

Table 4: Binary logistic regression

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Cycle_No	.117	.092	1.607	1	.205	1.124
Days_from_chemo	.114	.046	6.253	1	.012	1.121
Platelet_count_start	.005	.028	.030	1	.861	1.005
Platelet_Transfused	.099	.034	8.641	1	.003	1.104
Treatment_group(1)	-1.729	.605	8.160	1	.004	.177
Constant	-2.682	.870	9.503	1	.002	.068

CHAPTER 5: DISCUSSION

There is a widespread interest in the effectiveness of thrombopoietin receptor agonists drugs ever since their approval by the drug regulatory authorities and their commercial availability [1]. These agents have a proven benefit in restoring the platelet count to normal in conditions such as immune thrombocytopenia and aplastic anemia. However very limited data is available on the usefulness of these agents when given to cancer patients in the chemotherapy induced thrombocytopenia settings. Chemotherapy induced thrombocytopenia has been established as the most common cause of thrombocytopenia in cancer patients receiving chemotherapy. Therefore, it was considered relevant to the time and needs of Pakistani population to investigate this question.

Given the observational nature of the study, the confounding factors could not be strictly controlled. It was attempted to choose the control subjects as close to the cases as possible. The mean age of both treatment and control group was similar, 48.79±16.01 years vs 47.29±18.08 years. The body surface area is also an important variable as the dose of chemotherapeutic drugs is calculated according to the patients' BSA. The treatment group had BSA of 1.76m^2 whereas the control group had BSA of 1.75m^2. The mean platelet count at the starting of eltrombopag was considerably higher than that at the start of platelet transfusion, 18274±12721 vs 11000±7720 (p=0.01). This is reflective of the selective practice of treating physician in which patients screened to be at a higher risk of chemo induced thrombocytopenia or with a previous history of bleeding are started on eltrombopag treatment at an earlier stage. The mean number of days for platelet count to fall below 50,000/µL was 13.16±7.56 days. On the other hand, in the control group the platelet count fell to the lowest at 10.46±6.21 days.

The major outcome of interest was the days required for the platelet count to return to 50,000/µL or above. The results showed that there was no statistically significant difference in the days required for the platelet recovery in the eltrombopag and control group. The treatment group required 6.83±3.82 days compared to 6.31±2.70 days taken for the control group to reach platelet count to 50,000/µL (p=0.54).

Another patient oriented outcome is the need for platelet and red blood cell transfusion. In this study we attempted to explore if the patients receiving eltrombopag would require a decreased number of platelets or red blood cell transfusion. Number of platelet units transfused in the treatement group was slightly lower than that in the control group, 10.02±8.9 units vs 10.94±7.48 units (p=0.69). As for the packed RBCs, number of units in treatment group was again less than that in control group 2.02± 2.44 vs 2.29±2.25, but again this was statistically insignificant (p=0.64).

The use of eltrombopag did show a beneficial effect in reducing the length of hospital stay in patients suffering from chemotherapy induced thrombocytopenia. The mean length of stay in patients who received eltrombopag was significantly less than those who did not receive eltrombopag. In the treatment group, the mean length of stay was 5.43±3.34 days whereas in the control group it was 7.9±6.71 days (p=0.02). This aspect had not been explored in previous studies. Given the increased direct and indirect cost associated with prolonged hospitalization, a reduction in length of stay indicates an improvement in the financial burden for the patients as well as their care givers and health care providers.

The number of patients requiring delay in the next chemotherapy cycle was comparable in both the groups with 28 patients from the eltrombopag group and 23 from the control group (p=0.23). 10 patients from each treatment and control group experienced at least one episode of bleeding after thrombocytopenia.

There were also fewer number of patients requiring chemotherapy discontinuation in the eltrombopag group as compared to the control group. 13 patients in the treatment arm required the discontinuation of chemotherapy due to prolonged thrombocytopenia whereas 16 patients in the control group had to discontinue chemotherapy but the p value was not significant. The number of patients requiring no decrease or discontinuation of chemotherapy in the subsequent cycles was similar in the two groups, 25 in treatment and 27 in control.

Another major end-point is the resolution of thrombocytopenia when the patients are followed up to 30 days post chemotherapy. Our study suggested a favorable result in the eltrombopag group. 39 out of 52 patients had platelet counts recovered up to 50,000/µL till 30 days post chemotherapy. Number of patients in which the platelet count did not recover was significantly

lower in the group receiving eltrombopag compared with those not receiving the drug, 8 vs 21 (p=0.019). This result indicates that even though there might not be significant benefit in the days to plaetelet recovery with the concurrent use of eltrombopag, in patients where despite prolonged time duration and platelet transfusion, eltrombopag provides the benefit of recovery.

Out of the baseline and contributing variables, five were identified to contribute towards the outcome of platelet recovery at 30 days post chemotherapy. These variables included number of prior chemotherapy cycles received, days from the last chemotherapy administration, platelet count at the start of therapy, number of platelet units transfused and the administration of eltrombopag. A binary logistic regression model was applied. The resulting equation showed that number of days from chemotherapy administration, number of platelets transfused and administration of eltrombopag were contributing factors towards the recovery outcome of the patients, p values 0.012, 0.003 and 0.004 respectively.

The results from our study are consistent with the trends indicated in the previous studies by Garcia et al. The authors shared similar experience with the off-label use of eltrombopag in chemotherapy induced thrombocytopenia. The platelet recovery status at 30 days post chemotherapy was not investigated in the previous studies.

CHAPTER 6: CONCLUSION

The results from our prospective cohort study indicate that patients receiving Eltrombopag did not show any improvement in number of days to platelet recovery as compared to the control group. Eltrombopag does not decrease the need for platelet transfusions in patients with chemotherapy induced thrombocytopenia. There was however, a significant difference in the proportion of patients recovered in the patients receiving Eltrombopag compared to the control group ($p=0.019$).

Taking into account the high cost of the drug, the author would not recommend the off-label use of eltrombopag for the treatment of chemotherapy induced thrombocytopenia until there is emergence of new evidence from reliable studies.

There is a strong need for conducting high quality randomized controlled trial to assess the efficacy of Eltrombopag in patients with chemotherapy induced thrombocytopenia while balancing the confounding factors highlighted in our study.

CHAPTER 7: REFERENCES

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