Anthracycline Induced Cardiotoxicity: A Systematic Review

A project submitted

By

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Dedicated to my parents, who inspires me in every step of my life.

Certification Statement

This is to certify that this project titled "Anthracycline Induced Cardiotoxicity: A Systematic Review" submitted for the partial fulfilment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Dr. Md. Mesbah Uddin Talukder, Associate Professor, Department of Pharmacy, BRAC University. All of the work described here is entirely my own, unless specified otherwise and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed

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Countersigned by the supervisor

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Abstract

Cancer cure currently involves a combination of integrative treatments; chemotherapy, radiotherapy, and surgery to extend life and deliver sustainability to the survivors. Among all the classes of chemotherapeutics, anthracycline group has multiple well-established therapeutic uses. Anthracyclines can be used as antibiotics and also effective over a wide spectrum of malignancies. However, they also possess undesired cardiac toxicity; being the major drawback of anthracycline chemotherapy, which restricts their clinical use. In retrospective, detrimental effects on heart associated with Anthracycline-Induced-Cardiotoxicity (AIC) is irreversible and a challenging concern in the field of cardio-oncology, considering other adverse effects. Despite its dose-limiting cardiotoxicity, anthracycline drugs are being widely used in chemotherapy because of their effectiveness over a broad spectrum of cancers and solid tumors. In addition to their versatile therapeutic activity, dose-dependent congestive heart failure and accumulation of toxic drug metabolites in cardiomyocytes may possibly be the worst consequences of cardiotoxicity caused by doxorubicin and other anthracyclines. Cardiotoxicity related to anthracyclines yet remains to be an intractable clinical conundrum for both cardiologists and oncologists. The mechanism of AIC possibly involves oxidative stress of myocardium triggered by free radicals and consequently may lead to apoptosis and lipid peroxidation, or involves immunologic responses. Notably, various other mechanisms may play an underlying role to predispose AIC. In the strategy to mitigate AIC, dexrazoxane has shown to have promising cardioprotective action and further used to lessen symptoms of cardiotoxicity. Although the impact of other cardioprotective agents such as β-blockers, ACEI (Angiotensin Converting Enzyme Inhibitors), and lipid lowering agents may reduce AIC to some degree, still significant cardiovascular complication may occur. Despite the reduction in cumulative dose of anthracyclines and concomitant use of iron chelator dexrazoxane, no other agent has absolute evidence in regard to treatment or protection against AIC. In this article, we reviewed the incidence of cardiotoxicity induced by mainly anthracyclines as well as their pathogenesis, detection, function of biomarkers and protective agents used to ameliorate cardiovascular complications. Besides, in particular, we found significant cardiovascular anomalies in ADR data of doxorubicin male patients and also a notable discrepancy in ADR data of docetaxel in different geographical regions.

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1.1. Chemotherapy and its relationship to cardiotoxicity

Chemotherapy has been effectively used to treat or taper off the growth of tumor gradually for quite a few years now. Recent advancement in tyrosine kinase inhibitors (Trastuzumab) and proteasome inhibitors (Bortezomib) adding reinforcement in the treatment of cancer (Ryberg, 2013). The cellular mechanism by which chemotherapeutic agents work follows; arresting the cell cycle at different stages or suppress the cell division, thus, resulting apoptosis or autophagy (programmed cell death). Although, new approaches have been discovered in the management of cancer but the side effects remain major drawback of such conventional therapy which can exhibit tangible and subtle long-term complications to the patients. In addition, toxicity arising from chemotherapy based treatment can affect multiple physiological systems including, hematologic, cardiac, gastrointestinal, renal, and immune system (Oudard, 2002).

Cardiotoxicity can be a consequence of numerous chemotherapy drugs, such as DNA synthesis inhibitors (doxorubicin, methotrexate), tyrosine protein-kinase inhibitors (trastuzumab), DNA alkylating agents (cyclophosphamide), anti-microtubule agents (docetaxel, paclitaxel), and protease inhibitors (bortezomib). Aforementioned drugs target cancer cells at distinctive mechanism and induce cardiotoxicity exhibiting different clinical trait and concealed mechanism. Anthracyclines, fluorouracil (5-FU), and methotrexate (MTX) have been widely used as DNA synthesis inhibitors in cancer therapy, however, anthracycline doxorubicin (DOX) among these, is the principle agent that is responsible for significant chemotherapy induced cardiotoxicity (Horacek *et al*., 2007).

Nevertheless, research regarding anthracycline induced cardiotoxicity had been emphasized frequently from the dawn of modern chemotherapy, because it is one of the major and incessant complication of many anti-neoplastic drug. It sets a barrier between effective use of those agents and quality of life and survival rate of the patients. Nonetheless, the inclination to happen cardiotoxicity has been rising continuously because of chemotherapy use by the increasing number of cancer patients (Hong, Iimura, Sumida & Eager, 2010).

However, extent to which cardiotoxicity shows burden to patients depends on some conditions and factors; such as, cumulative dose of cytotoxic agent, collateral use of other chemotherapeutics, preceding irradiation, and preexisting cardiac complication, though cumulative dose appears to be the most crucial factor contributing to anthracycline induced cardiotoxicity (Pai & Nahata, 2000). For example, a cumulative dose of 450-550 mg/m^2 doxorubicin or higher, every 3 weeks, can possibly cause cardiomyopathy and heart failure. Whereas, the typical dosage of doxorubicin is 60-75 mg/m^2 (Von Hoff *et al*., 1979). Nonetheless, in spite of this dose range, sign and severity of cardiotoxicity have been observed discrepantly in different individual, receiving a cumulative dosage less than 300 mg/m^2 (Grenier & Lipshultz, 1998; Swain, Whaley & Ewer, 2003).

Therefore, the above relationship between cardiotoxicity and cumulative dose is unambiguous, though, exact dosage range and cardiotoxicity remains skeptical**.**

Table 1.1: Cardiotoxicity associated with chemotherapeutic agents (Yeh et al., 2006)

Heart Failure	**Hypertension**
Anthracyclines +++	Bevacizumab (Avastin)++
Mitoxantrone (Novantrone)++	Cisplatinin (Platinol)++++
Cyclophosphamide (Cytoxan)-high dose++	Interferon-α++
Mitomycin (Mutamycin)++	**Bradycardia**
Trastuzumab (Herceptin)++	Paclitaxel (Taxol)+
Alemtuzumab (Campath)+	Thalidomide (Thalomid)++
Edema	**Ischemia**
Imatinib mesylate (Gleevec)+++	Fluorouracil, 5-FU (Adrucil)++

Thalidomide (Thalomid)++	Capecitabine (Xeloda)+
QT prolongation or Torsades de pointes	Interleukin-2+
Arsenic trioxide (Trisenox)++++	Cisplatin (Platinol)++
Hypotension	**Thromboembolism**
Etoposide (Vepesid)++	Bevacizumab (Avastin)++
Alemtuzumab(Campath)+++; Cetuximab(Erbitux)+; Rituximab(Rituxan)++	Thalidomide (Thalomid)++
Interleukin-2++++	
Denileukin (Ontak)++++	
Interferon-α+++	
Homoharringtonine (Omacetaxine mepesuccinate)++	

The above table 1.1, consists of anticancer drugs having potential cardiotoxic properties; Relative frequency of adverse effects: + indicates rare (<1%); ++, uncommon (1–5%); +++, common (6–10%); ++++, frequent (>10%).

The table 1.1, also highlights different groups of chemotherapeutics having similar and also overlapping adverse cardiac events.

Moreover, in the events of any sort of cardiac anomaly, there are several underlying factors that could exacerbate patient's condition and affect the course of treatment. According to Yeh (2006) points out those factors which predispose cardiac complications; they are as follows, 1) The dose of drug administered during each session, the cumulative dose, 2) The schedule of delivery/pattern, 3) The route of administration, 4) The combination of drugs given, 5) The interval of administration. 6) The age of the

patient, 7) His or her underlying cardiovascular status/ any preexisting cardiac disease, 8) Radiation therapy can also predispose to cardiotoxicity, 9) Some chemotherapeutic agents induce cardiotoxicity only when the drug is administered at high doses; examples include platinum containing drugs**,** cyclophosphamide.

1.2. Anthracyclines

The anthracyclines, originally, they are part of a wide group of antibiotics called the rhodomycins. Also, they are obtained from different species of *Streptomyces* (Brockman, 1973).

The general structure of anthracycline group resembles a planar tetracyclic molecule linked to single or multiple sugars (Figure 1.1). Consequently, structure activity relationship (SAR) is greatly depend on alteration at position C-9 side chain, and the C-4 adduct, and both the nature and number of sugar entity. However, effect of quinone ring B remain constant (Mathé & Maral, 1982).

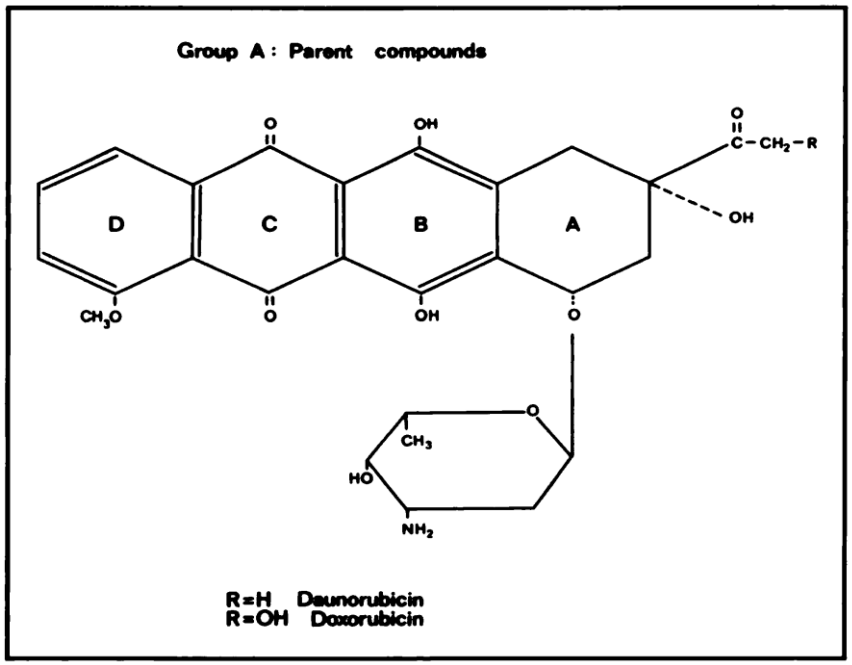

Figure 1.1: Chemical structure of Anthracycline group (Source: Mathé G *and Maral* R, 1982)

According to Mathé & Maral (1982), Anthracyline compounds, based on clinical activity, can be classified into four groups (Figure 1.2): group A: the parent compounds daunorubicin and doxorubicin or 14-hydroxy daunorubicin; group B: daunorubicin and

doxorubicin derivatives resulting from substitution on various parts of the molecule (B, C, or D ring, C-13 or -14 side chain, daunosaniine); group C: the aglycone is pyrromycinone, a glycosidic chain (di- or trisaccharide) at C-7, and acarmethoxy group at C-b; and group D: the amino sugar is linked to the D ring instead of the A ring.

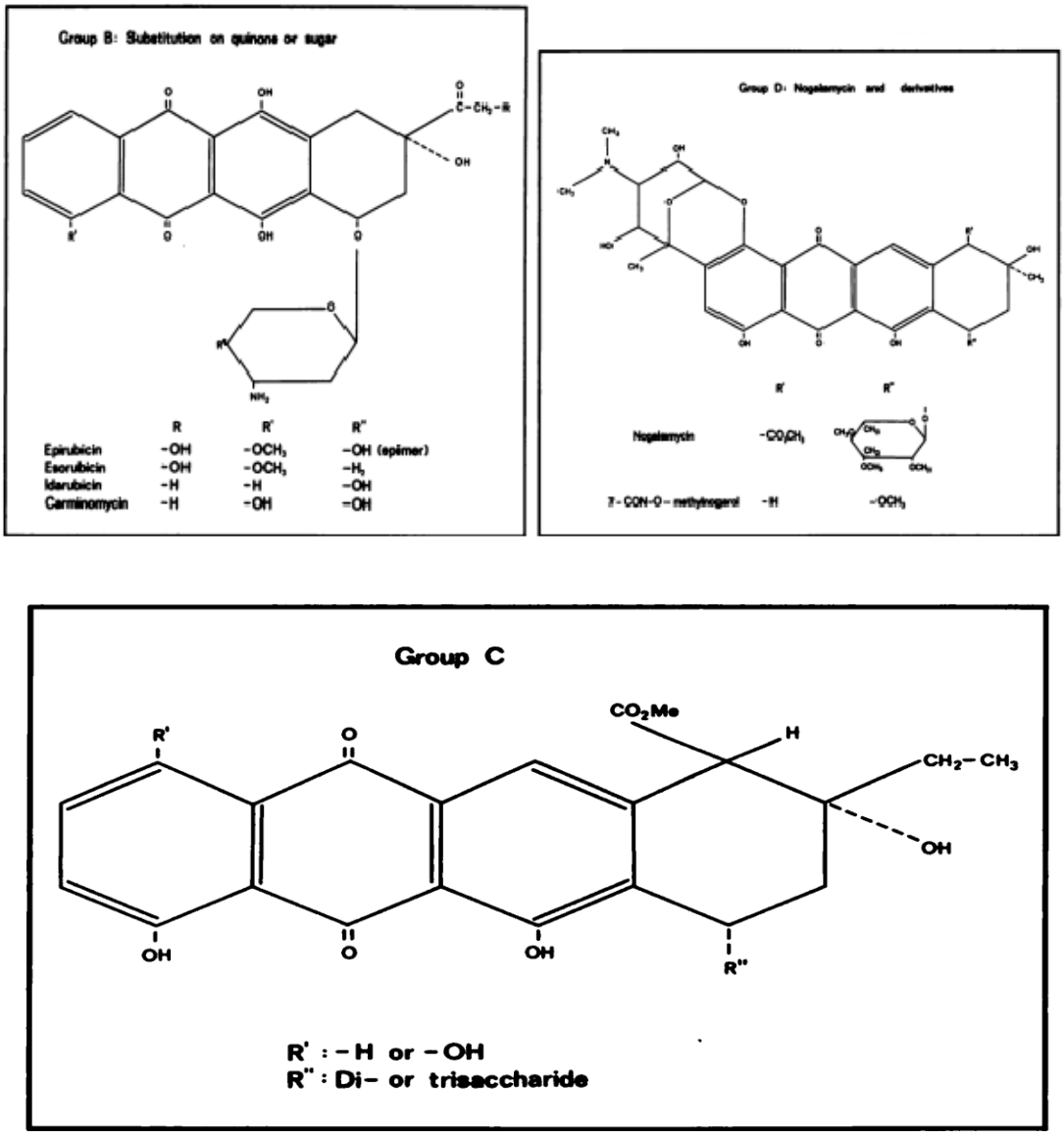

Figure 1.2: Different classes of anthracycline drugs (Wadler, Fuks, & Wiernik, 1986)

Von-Hoff *et al*. (1979) stated that the core chemical entities of anthracycline group are daunorubicin and doxorubicin (14-hydroxy daunorubicin), have been used broadly in cancer patients. In case of heamatological malignancies such as acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphoma and multiple myeloma,

daunorubicin acts as an active agent and forms foundation to fight against those type of cancers. On the contrary, he mentioned doxorubicin is effectively used in many solid tumors, some of which are: breast and stomach adenocarcinoma, sarcomas, and lymphomas.

Mechanism of action of anthracyclines:

Primarily, the exact mechanism of action by which anthracyclines showed its activity was not established. However, the first speculative mechanism of action was DNA intercalation. Surprisingly, after 20 years of clinical use, in 1984, topoisomerase IIa enzyme (TOPO IIa) was found to be precise target site for the anthracyclines (Zunino & Capranico, 1990). In addition, DNA intercalation results in cleavage of DNA double and single strand which in turn accumulates cleavage complex (protein linked with single and double-strand DNA breaks) in the cell, consequently, causing DNA damage and ultimately cell death. Inhibition of TOPO IIa enzyme and formation of cleavage complex follows few delicate steps, nevertheless, precise concept of those steps has not been addressed properly yet. Although, few hypothesizes has been postulated to support the formation of cleavage complex, and one theory speculates that, anthracyclines stabilizes topoisomerase IIa cleavage complex which may be independent of DNA intercalation mechanism. In fact, downregulation of enzyme topoisomerase could be one plausible underlying mechanism (Doroshow, 2001; Friche, Skovsgaardt & Nissen, 1989).

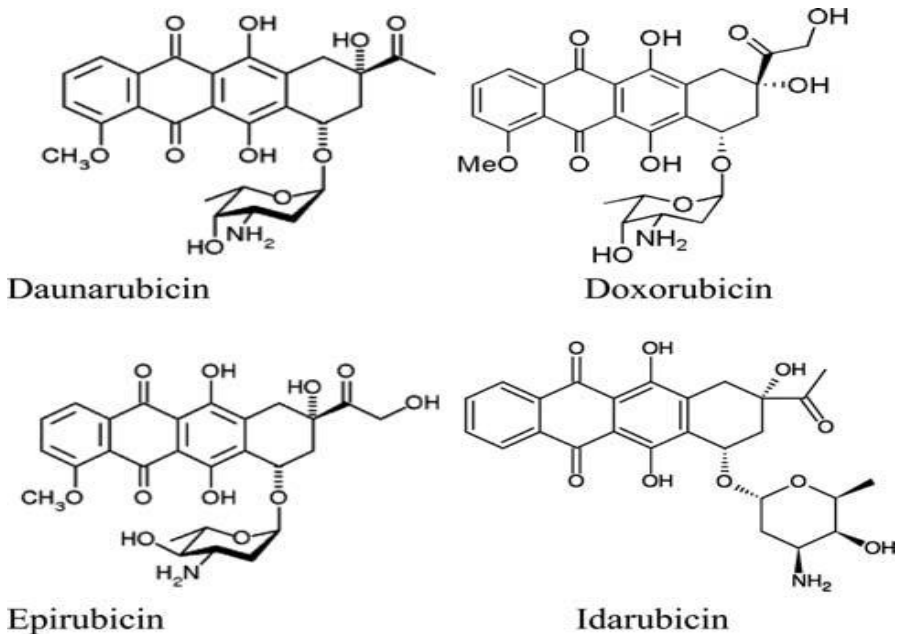

Figure 1.3: Chemical structure (a) Daunarubicin (b) Doxorubicin (c) Epirubicin (d) Idarubicin (Source: Corte´s-Funes & Coronado, 2007).

1.3. Anthracycline Induced Cardiotoxicity (AIC)

Anthracyclines are the pillar of treatment of an assortment of haematological malignancies and solid tumours. Unfortunately, the clinical utilization of these medications has been restricted by cumulative, dose-dependent cardiotoxicity which may at last exerts a serious and irreversible type of cardiomyopathy (Spallarossa et *al.*, 2016). Anthracycline-induced cardiotoxicity generally has been detected into three types; acute, early-onset chronic progressive and late-onset chronic progressive. The latter one is associated with cumulative, high dose related leading to left ventricular dysfunction and congestive heart failure. Moreover, about less than 1% patients suffer from acute cardiotoxicity instantly just after infusion, showing temporary decline in force of myocardial contraction, though it is reversible. Approximately 1.6-2.1% of patients experience the early-onset chronic progressive form of cardiotoxicity and 1.6-5% of patients exhibit late-onset chronic progressive form of anthracycline cardiotoxicity within minimum 1 year after completion of treatment (Raj, Franco, & Lipshultz, 2014).

Moreover, cardiac damage caused by anthracycline group is irreversible (Type I cardiotoxicity). On the other hand, trastuzumab, a monoclonal antibody causes reversible

cardiac damage (Type II cardiotoxicity) (Dirican *et al*., 2014). In retrospective, the pathophysiology of Type I cardiotoxicity deals with cell loss; In contrast, reversible Type II cardiotoxicity is more associated with cellular dysfunction, more specifically, mitochondrial and protein alterations, though, Type II cardiotoxicity can be exacerbated if the patient has preexisting cardiac condition and consequences can be lethal if interaction with anthracycline agent happen (Suter & Ewer, 2013).

Cardiovascular side effects can be included into 5 categories:

1. Direct cytotoxic effects of chemotherapy and associated cardiac systolic dysfunction,
2. Cardiac ischaemia,
3. Arrhythmias (especially torsade de pointes induced by QT prolonging drugs),
4. Pericarditis,
5. Chemotherapy-induced repolarisation abnormalities (Berardi *et al*.,2013).

In the course of recent years, cancer treatments have turned out to be increasingly powerful, prompting a huge decrease in death rates caused by cancer (Taylor & Francis, 2016). After the invention of effective diagnosis and treatment of cancer, life expectancy has expanded gradually in the previous two decades, in addition, subsequently more patients survived either cancer free or, on the other hand with cancer as a chronic, treatable disease (Spallarossa *et al*., 2016). Cardiotoxicity is a familiar complication of numerous anticancer agents and it remains a noteworthy restriction, emphatically affecting the quality of life and the overall survival. Because of the escalating number of patients treated by chemotherapeutics and biological medications (frequently in combination and at continuously higher cumulative doses), the rate of cardiotoxicity associated with chemotherapy treatment is consistently growing (Berardi *et al*., 2013). In retrospective, cardiotoxicity is a life-threatening irreversible obstacle in the field of clinical oncology, recurring the ubiquitous number of enduring cancer survivors, because of the necessity to utilize gradually increasing amount of cumulative doses of anthracyclines, the appearance of new anticancer drugs having potential harmful effects on the heart, and as well as other concurrent treatments having synergistic interfering

cardiotoxic effects, which is deleterious (D. Cardinale & Sandri, 2010) Inauspiciously, many anticancer drugs have been seen to develop cardiovascular problems including left ventricular dysfunction, leading to heart failure in exacerbated condition, myocardial infarction, cerebral and peripheral ischaemia, pericarditis and myocarditis, hypertension, thromboembolism, QT prolongation and arrhythmias (Spallarossa *et al*., 2016).

Apart from doxorubicin and epirubicin which are potent cytotoxic agents, 5-fluorouracil, capecitabine, cisplatin, mitoxantrone, taxoid group- docetaxel and paclitaxel and new target-mediated drug such as trastuzumab (a monoclonal antibody), which is a tyrosine-protein kinase inhibitor, are associated with substantial cardiac damage (Cwikiel, Persson, Larsson, Albertsson, & Eskilsson, 1995).

Following groups of anti-cancer drugs have been associated with cardiotoxicity in conventional cancer therapy (Berardi *et al*., 2013);

1. Anthracyclines
2. Taxanes
3. Fluoropyrimidine
4. Cyclophosphamide
5. Trastuzumab

The core chemical entities of anthracycline group, doxorubicin and daunorubicin, have been used innumerably in cancer patients. In case of heamatological malignancies such as lymphoma , ALL (acute lymphoblastic leukemia), CLL (chronic lymphocytic leukemia), and multiple myeloma, daunorubicin acts as an active agent and forms foundation to fight against those type of cancers. While on the contrary, doxorubicin is effectively used in many solid tumors, some of which are: breast and stomach adenocarcinoma, sarcomas, and lymphomas. Despite the fact that, bone marrow suppression, being the major dose dependent toxicity of doxorubicin, surprisingly, the major toxicity that confines doxorubicin use is the development of irreversible cardiomyopathy leading to recalcitrant congestive heart failure in later stage (Von-Hoff *et al*., 1991). Other minor toxiciteis are alopecia, gastrointestinal abnormalities, skin ulceration, nausea, and vomitting.

Anthracyclines have been effective against a broad spectrum of malignancies and an integral part of many anti-cancer regimens along with other cytotoxic drugs for the treatment of breast cancer, lymphoma, and sarcoma (Smith *et al*., 2010). However, despite the fact of being effective over an extensive range of malignancies and solid tumors, the clinical use of anthracyclines has been restricted because of its detrimental effects on cardiovascular system such as cardiomyopathy, heart failure and ECG alterations (prolongation of QT interval and reduction in QRS voltage). In addition, both early and late onset of cardiac adverse effects are seen in patients. Early onset of cardiac events can be seen within one year after commencement of anthracycline regimen and can manifest through acute, subacute or chronically progressive manner. However, on the other hand, late onset can occur twenty years maximum after end of the anthracycline therapy. It is also seen that, in case of children, late onset is most likely to happen than early onset (Steinherz, Steinherz, Tan, Heller, & Murphy, 1991)

As of their cardiotoxicity, anthracyclines are currently used much less frequently. Nevertheless, they are still the backbone of the treatment of many solid and haematological tumors, including breast and gastric cancer, sarcoma, leukaemia and lymphoma.

The need for and purpose of a cardiology consultation (Spallarossa *et al*., 2016):

1. There are no generally safe patients. The AHA/ACC Rules for the Diagnosis and Management of heart failure in adult's express that all patients treated with conceivably cardiotoxic medications are Class A heart failure patients
2. Unpredicted heart complications, be they recorded or, on the other hand even suspected, that happen while cancer treatment can majorly affect the integrity of therapy in a detrimental manner.
3. Late irreversible cardiotoxicity is a demanding problem for patients treated with possibly cardiotoxic drugs
4. The choice to receive prophylactic treatment with cardioprotective agents can be significantly convenient for few patients
5. Suitable alteration of cardiovascular risk factors can give noteworthy advantages towards reducing unwanted cardiovascular events and its treatment

To sum up, even if we do not have any protocol regarding necessity of integration of cardio-oncology knowledge, however, despite the fact, experts suggest to carry out a cardiology consultation for all patients who are subject to anthracycline treatment.

1.3.1. Mechanism of Cardiotoxicity

There are multiple mechanisms have been proposed by which anthracyclines shows it cardiotoxic properties. Moreover, concrete evidence had been established and conformed that cardiotoxic effects were entirely distinctive from its anti-neoplastic effects. Thus, cardiotoxic effects are including, damage to the sarcoplasmic reticulum and other cellular structures by free-radical formation, altered membrane function, altered mitochondrial ATP generation in myocardium (Myers, Muggia & Young, 1982; C. E. Myers *et al*., 1977). However, detachment of cytotoxic effect of anthracycline from cardiotoxic effect occurs when alteration of anthracycline molecule happens, with much safer therapeutic window. In support to their claim, Bertazzoli *et al*., (1982) found that screening of nine active anthracycline molecule in murine model, showed a cardioprotective effect because of demethoxylation at 4’ position on the aminosugar of those screened compounds. Furthermore, this hypothesis became more solid when both the compounds; epirubicin and esorubicin showed attenuated cardio-toxicity in preclinical and early clinical studies, while their pharmacologic effect remain unchanged. In retrospective, other plausible mechanism is pharmacokinetic profile which plays a decisive role behind this protective action on heart; for example, affinity of idarubicin is much higher in tissues other than heart. In contrast, because of higher plasma clearance of epirubicin, less cardiotoxic events occur.

Mechanism of cardiotoxicity of anthracyclines seems evidently to be distinct from their anticancer action. Despite free-radical formation triggering DNA damage and oxidative stress theory being the most accepted hypothesis, but there are few other pathophysiologic pathways that need to be considered before accounting for CTX.

Other mechanisms also have been speculated, some of which are: apoptosis; alteration of intracellular transcriptional ATP production in cardiac myocytes; downregulation of messenger RNA expression for sarcoplasmic reticulum calcium-ATPase, which reduces myocardial contraction; depressed cardiac glutathione peroxidase activity; respiratory abnormalities related to mitochondrial DNA damage; membrane damage due to lipid

peroxidation; and topoisomerase II inhibition (Zuppinger & Suter, 2010; Yeh & Bickford, 2009; Sawyer, Peng, Chen, Pentassuglia, & Lim, 2010)

Many theories have been proposed governing cardiotoxic mechanism of anthracyclines, however, oxidative stress theory is the foremost mechanism by which oxidative free radical attacks and damages sarcoplasmic reticulum and other intracellular vital structures leading to apoptosis.

Oxidative Stress Hypothesis

Oxidative Stress Hypothesis is the most shared mechanism which accounts for cardiotoxicity induced by anthracyclines. This theory involves formation and interaction of free radicals and superoxides (Rossi, Filippelli, Russo, Filippelli, & Berrino, 1994; Vasquez-Vivar *et al*., 1997).

With the free radical hypothesis, the cascade of events begins with a one-electron reduction of doxorubicin to yield a doxorubicin semiquinone radical by a decreased flavoenzyme for example, NADPH-cytochrome P450 reductase. The semiquinone radical builds a complex with iron, prompting an anthracycline-iron (Fe2+) free radical complex. This complex reduces oxygen to create superoxide and to recover doxorubicin. Consequently, hydrogen peroxide and oxygen are formed from dismutation of the superoxide (fig 1.4.). Vasquez *et al.* have demonstrated that doxorubicin ties to the reductase space of endothelial nitric oxide synthase. This causes an expansion in superoxide and a decline in nitric oxide development. The subsequent development of peroxynitrite could similarly take a part in the cardiotoxicity (Vasquez-Vivar *et al*., 1997).

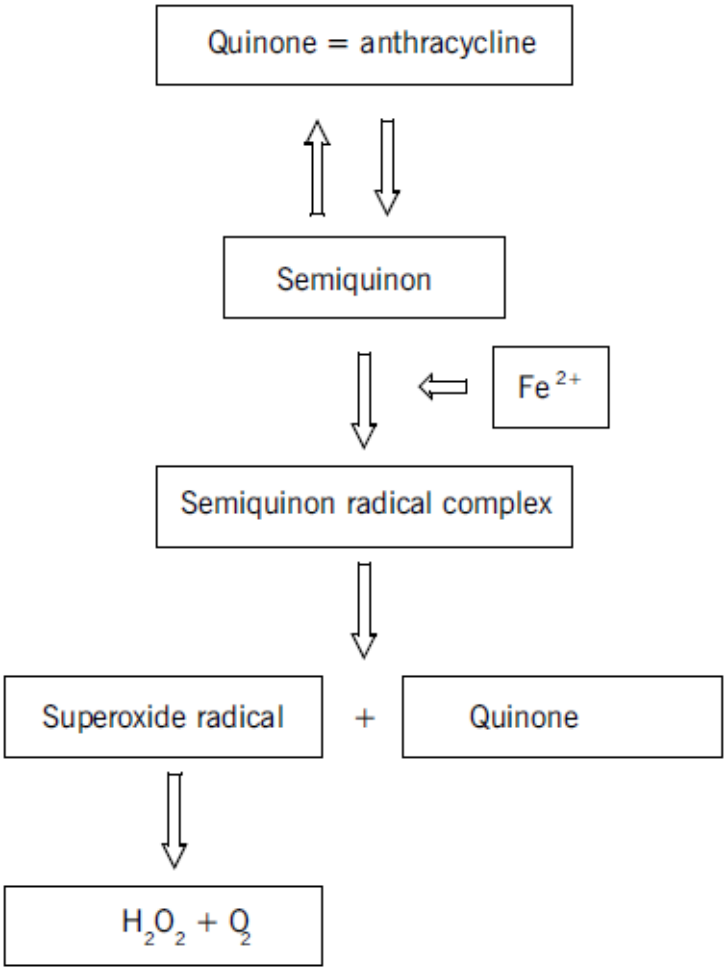

Figure 1.4: Schematic illustration of Oxidative Stress Theory (Schimmel et al., 2004)

In retrospective, concerning one or two electron reductive activation by DOX, semiquinone free radical is generated from quinone moiety after one electron reduction. However, this semiquinone free-radical became oxidized and reduces molecular oxygen to superoxide anion and hydrogen peroxide and itself converts into parent quinone form. Consequently, this produced reactive oxygen species (ROS) are involved with oxidative stress and energy depletion in cardiomyocytes. On the other hand, secondary alcohol metabolites are produced because of double electron reduction of the side chain carbonyl moiety, although, this metabolite is less active at redox cycling but markedly potent at disrupting calcium and iron homeostasis (Minotti, Menna, Salvatorelli, Cairo, & Gianni, 2004).

All these pathophysiological conditions such as oxidative stress, altered expression of cardio-specific gene and concurrent aberrant intracellular ion homeostasis eventually leads to cardiomyopathy.

Additionally, lipid peroxidation process may be started in the presence of superoxide, hydrogen peroxide and free iron. Moreover, cardiac cells are vulnerable to oxidative stress, because myocardium contains relatively low levels of the enzyme which counteracts such oxidation. This reduced, by-default low anti-oxidant capability of myocardium makes it more susceptible to oxidative stress (Doroshow, Locker & Myers, 1980). In prospective to this theory, Timao Li & Pawan K. Singal (2000) found evidence in rat's heart that, doxorubicin may have associated with low level of antioxidant enzymes. They found that doxorubicin happened to cause a further reduction in antioxidant enzymes in relative to precedent level.

Regarding oxidative stress theory, cardiotoxicity might result from other distinct mechanism. Studies stipulates that myocardial damage from doxorubicin has been associated with apoptosis or programmed cell death. To a greater extent, those oxidative free radicals have an instigating role which leads to apoptosis process. Furthermore, evidence of apoptotic death cells in cardiomyocytes of rat and aortic endothelial cells of bovine was found in the presence of doxorubicin treatment (Arola *et al*., 2000).

Metabolite Hypothesis

Lipid peroxidation, occurring frequently in an aggravated condition to cancer patients and doxorubicin most likely represses this impact in a contradictory way. It is proposed that lipid peroxidation happens when iron oxidizes not completely to the ferric form. Doxorubicin and the formed hydrogen peroxide would hamper cardiovascular lipid peroxidation by influencing the Fe(II)– Fe(III) balance of iron–oxygen complexes. This would imply cardiovascular damage not only involves semiquinone metabolites but also parent doxorubicin or its other metabolites (Minotti *et al*., 1996). Moreover, this hypothesis became formidable when evidence of a metabolite, doxorubicinol was found, which was obtained from a study demonstration. Therefore, it was found that, doxorubicinol was associated with a protein called apoprotein which regulates iron concentration. Consequently, metabolite doxorubicinol controls iron release and affect apoprotein in an undesirable manner. Furthermore, iron dysregulation also seen in human myocardium; Aconitase, also called iron regulatory protein-1 in myocardium, was inactivated irreversibly by the secondary alcohol metabolite of doxorubicin ((Minotti *et al*., 1998).

Impact on Calcium Homeostasis

One more plausible mechanism includes the impact of anthracyclines on the calcium homeostasis. Prior to apoptosis, mitochondrial permeability can be triggered by oxidative stress which affect mitochondrial calcium transport. Thus, this aberrant change in calcium transport might cause irregular cardiovascular contraction and tissue damage, followed by cell death (Schimmel *et al*., 2004).

Moreover, anthracycline can trigger the release of calcium from skeletal muscle and cardiac sarcoplasmic reticulum vesicles (Shadle *et al*., 2000). However, Rossi et al., (1994) found that verapamil could neutralize this deleterious effect of doxorubicin on mitochondrial calcium transport in mice. This impact was observed because of the drug verapamil, which is a CCB (calcium channel blocker), preventing the intracellular calcium surplus (Rossi, Filippelli, Russo, Filippelli, & Berrino, 1994).

Paradoxically, few studies undoubtedly indicated extended accumulation of doxorubicin in cardiomyocytes of rats. Further, those studies stipulate that extended accumulation was somewhat related to verapamil and condition may be exacerbated by concomitant administration verapamil in combination with doxorubicin (Lampidis, Krishan, Planas, & Tapiero, 1986). Akimoto *et al*. did not account for increased intracellular level of anthracycline but unexpectedly, correlated slight augmented cardiotoxicity by verapamil because of its specific ability to block gene expression of cardiac actin, though, a similar precedent experiment was done before with doxorubicin alone (Akimoto *et al*., 1993).

The exact mechanism of doxorubicin by which it impedes calcium regulation and its consequences for cardiotoxicity yet remains to be clarified. Despite multiple pathways that are involved to the altered mitochondrial calcium transport, but there is undeniable relationship has been established between doxorubicin and altered intracellular calcium level.

Impact of Immune System

After oxidative stress, interaction of an immunogenic response is regarded as a secondary pathway of cardiotoxicity precipitated by anthracyclines (Schimmel *et al*., 2004). However, substantial evidence found regarding association of doxorubicin with hypersensitive immunogenic reaction that might cause damage to the plasma membrane

of cardiac myocytes (Huber, 1990). Pretreatment with dexrazoxane lessened this expansion, affirming the recommendation of the inclusion of oxidative stress, trailed by an immunogenic response (Zhang, Herman, & Ferrans, 1993).

Positive analogy has been found between immunogenic reaction followed by oxidative stress. Moreover, a rise in antigen presenting dendritic cells has been found in hypertensive rats in the presence of doxorubicin, in addition, it was also confirmed that doxorubicin can trigger expression of certain antigens. Furthermore, pretreatment with dexrazoxane (an iron chelator) lessened this transient rise, supporting the connection of oxidative stress prior to an immunogenic response (Zhang, Herman & Ferrans, 1993).

Doxorubicin and Its Metabolite

Mechanisms of cardiotoxicity induced by anthracycline doxorubicin followed by formation of two reactive substances. The two harmful moieties resulted from consequently one and two electron reductions of DOX (figure 1.5). ROS semiquinone intermediate and DOXOL metabolite were formed by one and two-electron reduction respectively. In fact, DOXOL and ROS has been found to cause significant amount of early cardiotoxicity. However, DOXOL has more influence to cause late cardiac morbidities because it has the tendency to accumulate in the cell as a reservoir toxic metabolite due to its low efflux capacity. Other predisposing factors such as taxane can possibly potentiate DOXOL formation and exacerbate cardiac condition; whereas lifestyle and previous cardiac disease and other factors may play a crucial role to aggravate cardiotoxicity caused by DOX (Menna, Salvatorelli, & Minotti, 2008).

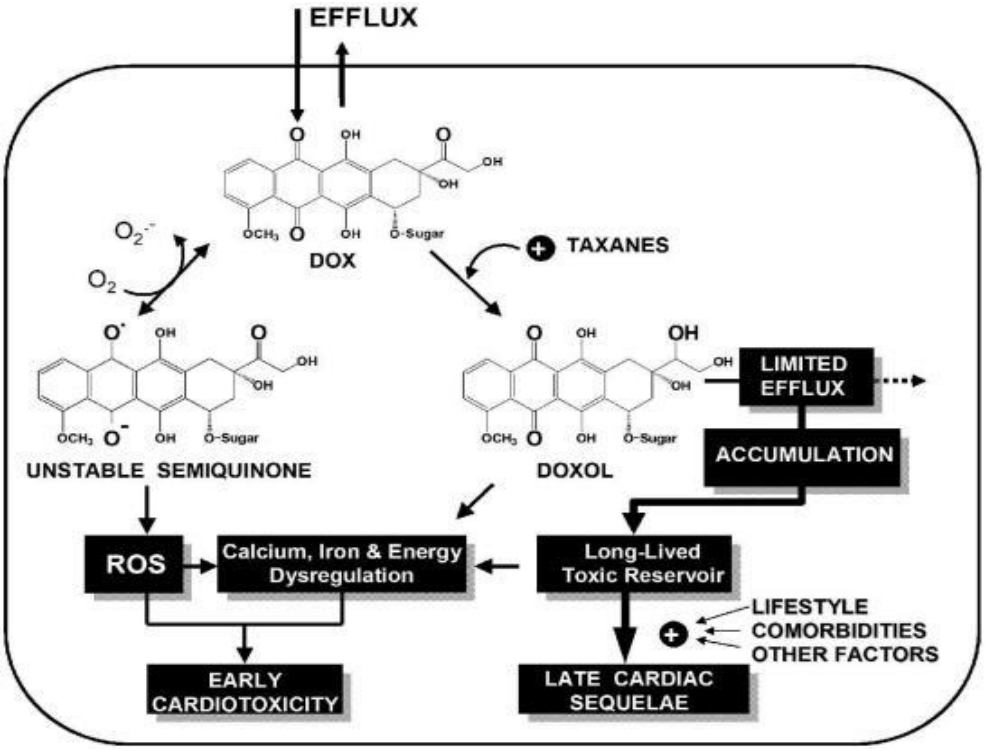

Figure 1.5: Schematic representation of cardiotoxic pathway of doxorubicin and its metabolites. (Adapted from Menna, Salvatorelli, & Minotti, 2008)

1.3.2. Diagnosis of Cardiotoxicity:

The analysis and the diagnosis of cardiotoxicity have not changed over the most recent fifty years, since the start of perception going back to the finish of the sixties. It has dependably been founded on the event of heart failure (HF) symptoms, and, from the mid-eighties, additionally on the confirmation of left ventricular ejection fraction (LVEF) drop, evaluated by echocardiography or multigated acquisition scans (MUGA) (D. Cardinale & Sandri, 2010). The most common clinical manifestation of cardiotoxicity is asymptomatic or symptomatic cardiac dysfunction. In clinical practice, regular cardiac function assessment is recommended by oncologic guidelines to detect early cardiotoxicity (Daniela Cardinale & Cipolla, 2016). An open question is when LVEF drop is viewed as noteworthy, although, a few orders have been proposed, with completely different LVEF decline thresholds for defining the first-grade cardiotoxicity, without a consensus definition. The most as often as the possible used definition in clinical trials and in clinical practice is a total reduction >10 percent units, related with a decline beneath 50%. Lately, an expert survey from the American Society of

Echocardiography and the European Association of Cardiovascular Imaging defined cardiotoxicity as a decline >10 percentage points, with a final value <53%. Moreover, from a cardiologic perspective, be that as it may, the analysis of cardiotoxicity in light of HF symptoms and LVEF drop speaks to a basic constraint. In fact, myocardial damage is distinguished just when a critical practical weakness has occurred, not letting considering any early preventive strategy (D. Cardinale & Sandri, 2010).

1.3.3. The Role of Biomarkers

Estimation of cardio-specific biomarkers can be as effective diagnostic instrument for early recognizable proof, assessment, and checking of cardiotoxicity. This method is negligible invasive, more affordable than echocardiography and LVEF, furthermore, especially atomic strategies can be avoided. In addition, the understanding of the outcomes does not rely on upon the expertise of the administrator. Troponins and Natriuretic peptides are mostly used biomarkers (D. Cardinale & Sandri, 2010).

Cardiac Troponins as Biomarkers

From 2000, for evaluation and detection of myocardial injury and acute myocardial infarction, cardiac troponins have been considered as the most typical biomarker (Alpert *et al*., 2000). In patients with intense coronary disorders, troponin assessment permits not just only for detection of myocardial damage, but additionally also serves the purpose to assess the risk of cardiac adverse events, as clinical severity and longevity of patients are associated with elevated troponin level (Cardinale *et al*., 2010).

Its utilization as a clinical biomarker of cardiovascular infection, considerably has been stretched out to an extensive variety of pathologies described via heart damage, including left ventricular hypertrophy, pulmonary embolism, congestive heart failure and cardiotoxicity related with chemotherapy treatment. Additionally, other adverse effects are blunt trauma, moderate renal insufficiency, sepsis, diabetes mellitus (O'Brien, 2008). Conventional cardiac biomarkers, including lactate dehydrogenase (LDH), creatine kinase (CK) and its isozymes, and myoglobin, were broadly utilized generally to evaluate myocardial damage and cell death (Wong, 1996). Moreover, LDH and CK isoenzyme examinations have been utilized as a part of people to evaluate ischemia-instigated cardiovascular damage since the 1960s (Cohen *et al*., 1966).

Natriuretic Peptides (NPs) as Biomarkers

To determine cardiac functional abnormalities, natriuretic peptide was found to be the most sophisticated means in this case (Cao, Zhu, Wagar, & Meng, 2017). Additionally, robust investigations have assessed their part to effectively diagnose and prognosticate cardiotoxicity responsible for antineoplastic agents (Cowie *et al*., 1997).

Notably, a correlation has been found between BNP (Brain Natriuretic Peptide) and attenuated cardiac tolerance to those agents which have cardiotoxic properties. In a study containing 27 cancer patients having hematologic malignancy, discovered that such diminished tolerance to cardiotoxic drugs had associated with constant rise in BNP level (Suzuki *et al*., 1998). Correspondingly, these peptides have been generally utilized as markers for heart failure and also to monitor treatment response and as a prognostic indicator in heart failure (Wei *et al*., 1993).

Human atrial natriuretic peptide (ANP) is encoded by the natriuretic peptide A (NPPA) gene, which is expressed principally in atrial cardiomyocytes. ANP is produced as precursor form, pro-ANP, which is changed over to the active and matured type of ANP by the cardiac transmembrane serine protease, corin, also known as atrial natriuretic peptide-converting enzyme (Yan *et al*., 1999). ANP is discharged in response to different stimuli, for example, mechanical stretching of the atrial wall, elevated sodium concentration, and increased blood volume (Song *et al*., 2015). ANP binds to ANP receptors, which are generally found in tissues, for example, the brain, heart, blood vessels, kidney, adrenal gland and receptor-agonist binding causes a lessening in blood volume as a result of induced excretion of fluids, in this way, a diminishment in cardiac output and systemic blood pressure achieved (Cao, Zhu, Wagar, & Meng, 2017).

However, in regard of this conclusive evidence, despite some positive results, in case of clinical practice, it would not be judicious to follow BNP assay to evaluate cardiotoxicity (Dolci, Dominici, Cardinale, Sandri, & Panteghini, 2008).

Other Biomarkers

Apart from cardiac troponin and natriuretic peptides, there are different substances that was anticipated as biomarkers for cardiotoxicity, which notably could be used to

determine the degree to which cardiovascular damage had occurred (D. Cardinale & Sandri, 2010).

New markers of myocardial ischemia, for example, unsaturated fat restricting protein and glycogen phosphorylase isoenzyme BB (brain) have been accounted for to likewise elevation after anthracycline induced cytotoxicity.

In addition, glycogen phosphorylase isozyme BB and fat restricting protein (unsaturated) also found to have biomarker indication in case of AIC. A spike in their level after exposure to anthracyclines was certainly related to cytotoxicity caused by anthracyclines. Moreover, elevation of other inflammatory biomarkers such as cytokines and specially interleukine-6 was found just after events of cytotoxicity. However, in a study consisting a little population, in the assessment of epirubicin induced cytotoxicity, a spike in interleukine-6 was observed just after dissecting through tissue Doppler imaging and concomitantly alteration in systolic capacity was also linked (Okumura *et al*., 2002).

Moreover, damage linked to endothelium of considerable amount has also been found in patients having treatment with agents which have cytotoxic properties (Simunek *et al*, 2005). In retrospective, presence of certain markers of endothelial damage at an elevated level was notably identified in patients who had cancers long ago.

To conclude, all these findings propose, CTX could trigger cascade of long-term events which includes endothelial injury and could induce initial processes related to atherosclerosis, acting as a precipitating factor causing increased risk for imminent cardiovascular complications. Although, no adamant relationship for long-term cardiovascular diseases was illustrated, and yet, the underlying influence of such biomarkers in cardiovascular anomalies remain to be clarified (Emdin *et al*., 2005).

1.3.4. Cardiac Protection Against Anthracycline Induced Cardiotoxicity

Many attempts and measures have been taken to come up with chemoprotectants that restrain anthracycline induced cardiotoxicity and that too without lowering the antineoplastic effect. After the free radical hypothesis, clinical trials showed that free radical scavenger antioxidants lacked significant success. Further studies revealed that acetylcysteine or tocopherol lacked cardioprotective effect and their use in clinical trial was insignificant (C. Myers *et al*., 1983). One of the investigations showed that when

melatonin was administered with other chemotherapeutic regimens, overall risk of toxicity which included cardiotoxicity was diminished. Thus, such effect is believed to be due to the antioxidant properties (Lissoni *et al*., 1999).

Dexrazoxane

Since iron and the doxorubiciniron complex have essential functions in preventing cardiotoxicity caused by anthracycline, iron chelators were developed to counteract such toxicity. Such compound work by binding to intracellular iron and thus they remove iron from the complex. The main mechanism is to eliminate the formation of free radicals (Links & Lewis, 1999).

Dexrazoxazone which was thought to be a promising agent, after investigations consisting animals showed it lowered the risk of doxorubicin induced cardiotoxicity (Herman & Ferrans, 1981). In a phase III trials, though, different cardiovascular side effects caused by doxorubicin at different dosage can be explained but dexrazoxane did not provide complete protection (Swain *et al*., 1997). Moreover, Wiseman & Spencer (1988) found, no significant indication, that it protected against late cardiovascular complications.

In another investigation, children were treated with doxorubicin (38 patients, 18 control and 20 treated with dexrazoxane) dexrazoxane demonstrated a cardioprotective action (Wexler *et al*., 1996). However, regarding paediatric oncology, the results of the studies lack reliability because a small number of clinical trials were carried out and there is insufficient evidence to draw final conclusions. The American Society of Clinical Oncology dictated that there is inadequate data to suggest the usage of dexrazoxane in the medication of paediatric malignancies (Hensley *et al*., 1999).

Notably, its use was approved by the FDA in adults if cumulative doses of doxorubicin if beyond 300 mg/m^2. Particularly, according to studies, no alteration on pharmacokinetic profile of doxorubicin was observed when dexrazoxane was administered concurrently (Hochster *et al*., 1992). A pharmacokinetic examination with epirubicin, showed an elevated clearance of the anthracycline when dexrazoxane was administered which hypothetically might prompt a diminished epirubicin exposure. Consequently, a difference in the efficacy, therefore, reduced antineoplastic effect was seen during treatment, when combined treatment was given. (Basser *et al*., 1994) In a random trial,

no statistically significant differences in survival and progression-free survival was seen between the dexrazoxane and placebo group. The route of administration of Dexrazoxane may be intravenous as either a slow or a fast injection, its dosage usually being usually 10-folds of that of doxorubicin dose. Nonetheless, the dose limiting toxicity may be leukopenia (Hochster, Wasserheit & Speyer, 1995).

Monoher

Flavonoid monoher is another radical scavenger and which acts as a cardioprotective agent by scavenging ROS (Reactive Oxidative Species). It protects the heart from doxorubicin toxicity without affecting its therapeutic effect in animal model. However, it has a low potency so the effective dose required in humans is much higher (500 mg/kg in mice). Frederine which is a recent derivative of monoher was seen to protect the heart with a smaller dosage, approximately 5-fold lower dosage required comparatively to that of monoher. Further clinical trials with this cardioprotective drug are required (van Acker et al., 2001).

Lipid lowering agents

After dexrazoxane, lipid lowering agent has also the potential to reduce adverse or may reverse cardiotoxic effects of anthracyclines noticeably (Iliskovic & Singal., 1997). In a study, concurrent administration of doxorubicin and the lipid lowering drugs and probucol (antioxidant) in rats, a favourable association between cellular antioxidants was found; the combination possibly facilitates the antioxidant enzymes glutathione peroxidase and superoxide dismutase and function and as well as a reduction in case of lipid peroxidation was also observed. Oxidative stress theory supports that, improved myocardial structure and function can be achieved by this favourable condition of antioxidant level in the heart (T. Li & P. K. Singal, 2000; Siveski-Iliskovic, Hill, Chow, & Singal, 1995).

Lately, Feleszko *et al* (2002) demonstrated a study in mice, lipid lowering agent lovastatin, which inhibits cholesterol synthesis endogenously by blocking enzyme HMG coenzyme-A reductase, can potentially increase antineoplastic activity of doxorubicin and notably, has a protective function on heart, when administered concomitantly.

Formulation Alteration

Alternatively, one particular approach to have less cardiotoxic events is the development of liposomal formulation technology. Liver and spleen tissues and other tissues with higher amount of phagocytic reticuloendothelial cells favourably receive liposomes. On the other hand, the consistent capillaries of skeletal and cardiac muscles will hence barely take up liposomes. Additionally, preclinical studies have evidently demonstrated a diminished uptake of doxorubicin in myocardium when a liposomal formulation was administered. Particularly, the ingredients of the liposome formulation itself do not seem to have a deleterious role on the heart or other tissues. Although, adverse effects such as doxorubicin toxicity or alteration bio-distribution can be observed because of significant differences in formulations of different liposomal products of doxorubicin and changes in drug-lipid ratio or subtle change in lipid composition (Gabizon, Dagan, Goren, Barenholz, & Fuks, 1982).

Pegylated Liposomal formulation of doxorubicin was studied in an analytical study of eight clinical Phase I and phase II trials containing 41 patients, about the safety of more than 500 mg/m^2 dose. The analysis revealed a surprising discovery; secondary to cardiomyopathy, none of the examined patients had suffered clinical congestive heart failure. Moreover, 10% reduction in left ventricular ejection fraction was seen in 5 patients; However, a significant advantage of liposomal doxorubicin has been found; 3 of those patients were administered non-liposomal doxorubicin prior to treating liposomal doxorubicin. Alternatively, the mean reduction in left ventricular ejection fraction was -2%, which was not clinically substantial. This study advises a significant approach that will evidently reduce doxorubicin induced cardiotoxicity by employing liposomal formulation of doxorubicin (Safra *et al*., 2000).

1.4. Non-anthracyclines have been also associated with irreversible cardiac damage.

Apart from anthracycline group, there are other groups of chemotherapeutics that can cause noticeable amount of cardiotoxicity or otherwise, can potentiate existing cardiotoxicity induced by anthracycline.

Since non-anthracycline groups are also involved in significant level of cardiotoxicity, apparently, it seems obvious to compare the cardiotoxicity of anthracycline and non-anthracycline, discussed below:

1.4.1. Alkylating agents

Cyclophosphamide, when administered at elevated doses, cause anatomical myocardial injury which eventually leads to permanent irreversible heart damage, also called type I CTX (Herrmann et al., 2014). The lesion that occurs is associated with cardiac tamponade as well as arrhythmias. CTX, which develops within 1-10 days after taking the first dose is entirely dose dependant. Reports suggest that cyclophosphamides at high dose increases the risk of heart failure between 7-28% (Pai & Nahata, 2000; Curigliano, et al., 2012).

The mechanism of how cyclophosphamide exactly causes CTX yet remains undecided/not well understood. It may cause direct damage to the epithelium. Consequently, the myocytes are then destroyed due to the exposure to the toxic metabolites. Subsequently, edema and interstitial haemmorhage may follow. Moreover, intracapillary microemboli results in ischemic myocardial damage (Jones & Ewer, 2006). It is believed that they induce a type of oxidative stress which interferes with the cellular respiration, damaging the mitochondrial membrane, (which is associated with cellular respiration) located in the inner side of the cardiomyocytes (Souid, Tacka, Galvan, & Penefsky, 2003).

Particularly, the drug is converted into acrolein. Acrolein is an unsaturated aldehyde, which is toxic in nature and is a reactive metabolite. It is responsible for vigorous modification of proteins and thus they cause damage to the myocardium. Glutathione S-transferase P is an enzyme that metabolizes acrolein and thus protects against cyclophosphamide. The function of the enzyme was studied further, the results of which showed that after treatment using cyclophosphamide, GSTP insufficiency occurred which is due to the deposition in the heart with acrolein adducts. Furthermore, the investigation revealed that GSTP partly, to some extent, it has regulatory role in cyclophosphamide CTX. Therefore, it is unambiguous that cardio-toxicity of cyclophosphamide can be prevented by the metabolism and detoxification of acrolein by GSTP (Conklin *et al*., 2015).

Ifosfamide is another anticancer chemotherapeutic drug of alkylating group which has a similar mechanism of inducing CTX as cyclophosphamide. When given at elevated doses, malignant arrhythmia and myocardial depression may arise, however these are reversible (type II CTX). Nevertheless, histopathological sign of hemorrhagic myocarditis which was a common side effect of cyclophosphamide has yet not been reported with ifosfamide (Quezado *et al*., 1993). Studies have revealed that treating with alkylating agents, cyclophosphamide and ifosfamide prevent gene expression in cardiac tissues that encode for fatty acid-binding proteins in the heart. Moreover, they also prevent the expression of protein that encodes for carnitine palmitoyltransferase I. As a result, they prevent mitochondrial transport and as well as oxidation of long chain fatty acids. In addition, serum lactate dehydrogenase, creatine kinase isozyme myocardial band, and malonyl-CoA content increased apparently. Alternatively, damage to the cardiac tissues that were observed, and such aforementioned enzymes and their corresponding pathways contributed significantly to cyclophosphamide and ifosfamide-induced CTX (Sayed-Ahmed *et al*., 2014).

Although these drugs demonstrate targeted cancer therapy and have fewer negative effects on the normal cells, these agents may occasionally lead to cardiotoxicities which include injury and permanent damage to the heart tissues, arrhythmia and other cardiac complications. The mechanisms by which they induce cardiotoxicity should be well understood in order to reduce the risk. Acrolein metabolism for example seems to reduce this risk significantly.

1.4.2. Platinum

Another example of a potent chemotherapeutic agent is platinum containing compound, cisplatin. Antineoplastic effect has been found to have a broad-spectrum activity and thus is effective against innumerable tumours. Nonetheless, the major drawback of the drug is its acute and cumulative CTX, which are manifested by EGC irregularities, angina which is associated with chest pain, arrhythmias, myocarditis, cardiomyopathy, hypertension and hypotension, acute myocardial infarction and also congestive heart failure may result (Khan *et al*., 2012; Ryberg, 2012; Dolci *et al*., 2008). Conversely, these agents also have the potential to cause or elevate the risk of thrombotic events, particularly in patients suffering from cancer (Yeh & Bickford, 2009).

The reasons as to why cisplatin CTX occurs may include a direct toxic effect on the myocytes of the heart, or production of ROS (reactive oxygen species) after which induction of oxidative stress occurs and thus leads to a prothrombic condition (Altena *et al*., 2011). In a research containing animal model, cisplatin substantially increased cardiac troponin I levels in plasma as well as increased concentrations of malondialdehyde also observed, however, concurrently a reduction in the antioxidant level was connected; that includes, low activity of superoxide dismutase and glutathione content. As a result, severe damage to the mitochondrial and nuclear DNA was observed (El-Awady el, Moustafa, Abo-Elmatty, & Radwan, 2011). Numerous other studies were carried out and one such investigation in animal study demonstrated that the drug might lead to dysfunction of left ventricle and depression of the contraction of cardiomyocytes; depressed contraction of cardiomyocytes caused by the induction of mitochondrial ultrastructural anomalies, which is manifested by apoptosis and activation of the ER stress (Endoplasmic Reticulum) response (Ma *et al*., 2010).

This class of drug may alter endothelial cell integrity which includes procoagulant activity of the monocytes and notably, the main mechanism through which these action takes place is the induction of platelet activation and aggregation (Czaykowski, Moore & Tannock, 1998). In addition, another action that is exhibited by cisplatin is the increase in the von Willebrand factor, which is primarily responsible for antiangiogenic action through hypomagnesaemia and vasospasm (Icli *et al*., 1993; Miller, Sweeney & Sledge, 2001).

Reports stated that patients who survived testicular cancer had a higher risk of myocardial infarction, following the treatment with platinum drugs such as cisplatin and patients also show a long-term presence of CTX (Carver *et al*., 2007). After the therapy is completed, cisplatin levels can be measured up to 20 years, however, it is basically plays a role in causing cumulative dysfunction of the endothelial cells eventually cutting them off from the walls of the vessels (Vaughn, Palmer, Carver, Jacobs, & Mohler, 2008). Conspicuously, it is seen that there is a correlation between the long–lasting circulating cisplatin and myocardial infarction and endothelial dysfunction. However, some variations may be seen, e.g. cisplatin is found in almost all the survivors of testicular cancer but only in 6% or fewer patients, myocardial infarction occurs (Van den Belt-Dusebout *et al*., 2006; Peng *et al*., 1997).

The adverse cardiac events associated with platinum drugs are eclectic, include- ECG abnormalities, cardiomyopathy, hypertension, arrhythmia and so on. Besides, they may significantly increase the risk of thrombotic events in cancer patients. Several mechanisms have been proposed to how it induces cardiotoxicity the most common ones being reactive oxygen species production (oxidative stress) and increase in troponin I levels in plasma and damaging the structural integrity of endothelial cells.

1.4.3. Antimetabolites

After the anthracycline group, the second most significant cause of chemotherapy-induced CTX which is triggered by fluoropyrimidines (Sorrentino, Kim, Foderaro, & Truesdell, 2012). Antimetabolites such as 5-fluorouracil is associated with chest pains such as in angina pectoris and may infrequently lead to complications such as myocardial infarction, ventricular tachycardia, arrhythmias, heart failure, cardiogenic shock, and prolongation of QT interval with torsades de pointes. (Saif, Shah & Shah, 2009; Focaccetti *et al*., 2015).

In 2009, Yeh & Bickford found that antimetabolites such as 5-fluorouracil increases the risk of CTX events between 1-68 %. In an investigation showed that the risk of CTX incidence with capecitabine was 5.5 % (Kosmas *et al*., 2008). In 2013, Kelly *et al*. in a recent investigation revealed that the CTX events linked to antimetabolites such as 5-fluorouracil, capecitabine, or raltitrexed that was mentioned in the published articles from January 1991 and August 2011; the main result found was that the overall occurrence of CTX associated with 5-fluorouracil/capecitabine ranged between 0.55 and 19% (mean: 5.0%; median: 3.85%); however, surprisingly no CTX was related with raltitrexed. Furthermore, a meta-analysis confirmed an occurrence of symptomatic CTX of 1.2 to 4.3% at some point of treatment with 5-fluorouracil and thus speculated that this risk may be further intensified through continuous infusion and simultaneous treatment with cisplatin (Polk, Vaage-Nilsen, Vistisen, & Nielsen, 2013).

The pathway through which the incidence of CTX occurs when antimetabolites such as 5- fluorouracil and capecitabine, is not entirely known or doubtful. Principally, some suggested mechanisms for the incidence of CTX include coronary thrombosis, arteritis, and vasospasm. Moreover, CTX may be associated with some metabolites of 5-fluorouracil, especially α-fluoro-β-alanine. The enzyme that is responsible for converting

capecitabine into 5-fluorouracil and also 5-fluorouracil into its active metabolites is thymidine phosphorylase; during myocardial infarction and in atherosclerotic plaques, overexpression of this enzyme occurs as it regulates angiogenesis and thus, regarded as angiogenic factor (Bronckaers, Gago, Balzarini, & Liekens, 2009). Moreover, endothelial damage may result, with increased levels of endothelin 1 which produces vasospasm and ischemia and thus, leads to vascular toxicities (Hemalatha *et al*., 2010).

Other hypothetical pathogenic mechanism of CTX include the cardiotoxic effects the medication expose the myocardium to, interference with the blood coagulation systems and also the autoimmune reactions (Kosmas *et al*., 2008). Alternatively, in cardiomyocytes and endothelial cells, 5- fluorouracil can bring about apoptosis and autophagy by means of oxidative stress, was confirmed by a cardio-oncology investigation (Saif, Shah & Shah, 2009).

Further, in 2015, Eskandari *et al*. carried out an animal-model investigation that showed CTX resulted from the treatments that involved 5-fluorouracil and capecitabine was further linked to the formation of reactive oxygen species (ROS), lipid peroxidation, and a rapid reduction in glutathione level; as a cumulative result, oxidative stress increases, thus causing mitochondrial dysfunction, which triggered caspase-3 activation. This finally instigate apoptosis or necrosis process. It was further observed that 5-fluorouracil caused leakage of lysosomal membranes when they were incubated with cardiomyocytes.

In retrospective, another investigation on animal models recommended accumulation of citrate in cardiomyocytes as another underlying mechanism leading to CTX. The possible reason was depletion of 5-fluorouracil into fluoroacetate, which interfered with the Krebs cycle (de Forni *et al*., 1992; Kosmas *et al*., 2008). Additionally, further studies reported 5-fluorouracil has the potential to induce dose and time-dependent and transient reduction in high energy phosphate level which are reside in myocardial cells (Freeman & Costanza, 1988).

In general, like other chemotherapeutic agents, they are associated with the risk of cardiotoxicity, yet there are multiple plausible mechanisms through which cardiotoxicity occurs but is not precisely understood.

1.4.4. Antibiotics

Antibiotics may act as chemotherapeutic agents and one such example of an antibiotic is mitoxantrone which has the ability to induce dose-limiting, irreversible CTX (Type I CTX). The risk of cardiotoxicity significantly increases following administration of cumulative doses of a minimum of 160 mg/m^2 of mitoxantrone. The drug has the potential to induce arrhythmias as well as chronic heart failure at later stage and without impairment of LVEF, although, a persistent diastolic dysfunction may occur (Menna, Salvatorelli, & Minotti, 2008). However, the mechanism through which mitoxantrone induces CTX is not fully known or vague, yet a proposed mechanism includes ROS formation via interaction with iron metabolism. This interaction results in injury or damage of the myocardial tissues (Xu, Persson & Richardson, 2005). Although CTX caused by mitoxantrone is similar to that of induced by anthracyclines, but has relatively less deleterious effect in heart compared to anthracyclines

L. G. Rossato *et al*. (2014) in an animal model study, observed that the drug elevated the levels of lactate and also increased the activity in the mitochondrial respiratory chain of complexes IV and V; this highlight how mitochondriopathy in mitoxantrone leads to CTX. Furthermore, Rossato *et al*. in the earlier year, carried an investigation on H9c2 cells, in that study it was seen that mitoxantrone at therapeutic concentrations, which demonstrated it altered the mitochondrial membrane potential and intracellular levels of ATP and calcium. It was found that the interference of energy metabolism was a major factor in cell damage that is induced by mitoxantrone.

Bleomycin, another vital antibiotic used in various intimidating cancers, has potentiality to have detrimental effects on heart. For example, pericarditis and coronary artery disease are complications arising from bleomycin. Acute pleuropericarditis is a common mucocutaneous toxicity of such antibiotic. On the other hand, the toxic effects of bleomycin may lead to ischemic cardiomyopathy. This pathogenic condition may develop into further complications such as endothelial dysfunction, accelerated atherosclerosis, and overt CVD (Ishii & Takada, 2002).

Besides, in a study of patients with testicular cancer, it was noted, that there was a risk of myocardial infarction, following administration of a single dose of bleomycin and sometimes even after years of bleomycin regimens (Ozben *et al*., 2007). Studies also

show that mitomycin C may lead to chronic heart failure, the risk of which increases further after cumulative doses of at least 30 mg/m^2. By following mechanisms/pathways different from those of anthracyclines, such antibiotics also contribute to the increase in oxidative stress and thus may induce cardiac damage (Menna, Salvatorelli & Minotti, 2008).

Antibiotics are associated with cardiotoxic side effects such as arrhythmia, heart failure, diastolic dysfunction and thus extensive studies and research programs are being performed to investigate the mechanism of antibiotic induced cardiotoxicity; more precisely anthracycline induced cardiotoxicity is of special concern. Until now the most accepted hypothesis is the increase in the oxidative stress which eventually contributes to heart damage in an eclectic manner. However, protective measurements following this theory fail to stand as clinical success and thus needs further studies to establish a method or intervention to completely prevent or substantially mitigate it. All cancer patients experiencing antineoplastic treatments require a comprehensive and contingent observation due to the very complicated and disparate mechanism of CTX, especially because of the susceptible nature of myocardium in the occurrence of cardiac damage by oxidative stress, and the exceptionally irregular clinical representation or occurrences that show no symptoms at initial stage.

1.4.5. Antimicrotubule agents (docetaxel and paclitaxel)

Antimicrotubule agents are those that interfere with the mitosis/cell division. Microtubules are one of the most strategic targets of anticancer therapy. They directly prevent the mitosis of cell division; as a result, the daughter cells are unable to separate, thus arresting the cell division. Despite their subtle mode of action on microtubules, they are also coupled with significant level of CTX.

Studies reported that nearly in 5% patients who were treated with paclitaxel, atrioventricular block was observed in addition to ventricular tachycardia and ischemic cardiac events. Erratically, in some patients (from <0.1 to 31%), asymptomatic bradycardia was seen (Rowinsky *et al*., 1991). Paclitaxel shows effects on the purkinje fiber system and extracardiac autonomic control, is responsible for causing arrhythmias and conduction disorders. Conversely, it was recommended by the knowledge of clinical trial and phase I studies of paclitaxel that serious, life-threatening adverse cardiac events

that include paclitaxel induced hypersensitivity reaction and patient should not take any medication that could have potentiality to interfere with cardiac conduction (Menna, Salvatorelli & Minotti, 2008).

Among all the antimicrotubule agents, paclitaxel is mainly prepared using Cremophor EL with the highest concentration per dose, which is associated with histamine release. The histamine that is released consequently, and which is specific in terms of action that stimulates certain cardiac receptors and the myocardial oxygen demand rises thus leading to coronary vasoconstriction and chronotropic effect. These consequences may induce myocardial ischemia in recipients of paclitaxel therapy (Yeh & Bickford, 2009; Rowinsky *et al*., 1991). As a matter of fact, paclitaxel differs from docetaxel in that it can delay doxorubicin catabolism, that causes decrease of LVEF, though it is not statistically significant (Biganzoli *et al*., 2003; Minotti *et al*., 2001).

However, Minotti *et al*. (2001) disclosed enhanced metabolism of doxorubicin to toxic metabolites but did not simplify whether delayed catabolism of doxorubicin tend to increase the plasma concentration and thereby possibly could cause exaggerated pharmacological action (toxic) and thus, perhaps could potentiate CTX. In contrast, Biganzoli *et al*. (2003) substantiated to the fact that paclitaxel and doxorubicin regimen can be safely used in terms of cardiac toxicity.

Statistically, data shows heart failure may occur due to docetaxel therapy which varies between 1.6 to 2%. Another effect the therapy may demonstrate is myocardial ischemia. Other adverse events include congestive heart failure in breast cancer patients (metastatic) specially with patients who are given adjuvant anthracycline therapy in prior and when in a combination therapy, docetaxel combined with trastuzumab. Moreover, in this regard, about 6-8% patients who received docetaxel therapy, LVEF in at least 15% patients decreased. This percentage is significantly higher than the above-mentioned therapies (Sessa & Pagani, 2001; Marty *et al*., 2005).

Thus, antimicrotubule agent prevents cell division (mitosis) basically by depolymerizing microtubules and therefore they aid in thwarting the functions of microtubules in mitosis phase. Despite their antineoplastic activity, they have the potential to induce cardiotoxicity. Unfortunately, Paclitaxel increases the risk of cardiotoxic effects of anthracyclines by preventing catabolism of doxorubicin and thus acts as synergistic

manner in the course of chemotherapy induced cardiotoxicity which is undesirable. Docetaxel may induce the risk of LV dysfunction and myocardial ischemia. Conversely, paclitaxel is associated with the risk of bradycardia and ischemia. Notably, several other risk factors of the different antimicrotubule agents have been identified.

Cardiotoxicity associated with chemotherapy has been an intensifying problem since the beginning of the cancer treatment. However, long term cardiovascular morbidity which confines clinical effectiveness of such cytotoxic drugs and affects quality of life of cancer survivors. Anthracycline doxorubicin being the most studied drug in the field of cardio-oncology, thus, different strategy and protective medications were developed to counteract such cardiotoxicity. On the other hand, cardiotoxicity caused by non-anthracyclines cannot be overlooked because they are also involved with noticeable amount of cardiotoxicity. Therefore, considering cardiotoxicity of anthracycline and non-anthracycline group of drugs, patients with previous cardiovascular condition must be treated carefully and constant monitoring scheme must be obtained on cumulative dose.

1.5 Aim of the study:

After collecting adverse drug reaction data from different sources, at the start of my study, I posed three research questions regarding doxorubicin adverse drug reactions.

1. What is the male-female ADR ratio of doxorubicin?
2. How does doxorubicin ADR cases of different organ class affect male and female patients?
3. How does cardiac organ class ADR cases of doxorubicin affect male and female patients and is there any deduction reachable?

Regarding docetaxel adverse drug reactions, I have looked for two major questions:

1. Which age group had suffered most from docetaxel ADR and is there any impact of regional variation on it?
2. What is the male-female ADR proportion of docetaxel and is there any influence of regional variation on it?

Chapter 2. Male Cancer Patients are Significantly Vulnerable to Doxorubicin Adverse Drug Reactions of Cardiac Disorder Organ Class.

2.1. Introduction

Doxorubicin ADR reports were taken from an open source government healthcare database of Canada which named as “Canada Vigilance Adverse Reaction Online Database”. The search result of doxorubicin ADR showed 921 cases in total. We have analysed and tried to find discrepancy between those cases.

2.1.1. Doxorubicin ADR proportion of female is markedly higher.

Including all type of organ class ADR, Total 921 cases found. Among which, 264 ADR cases resemble male and 657 ADR cases female respectively.

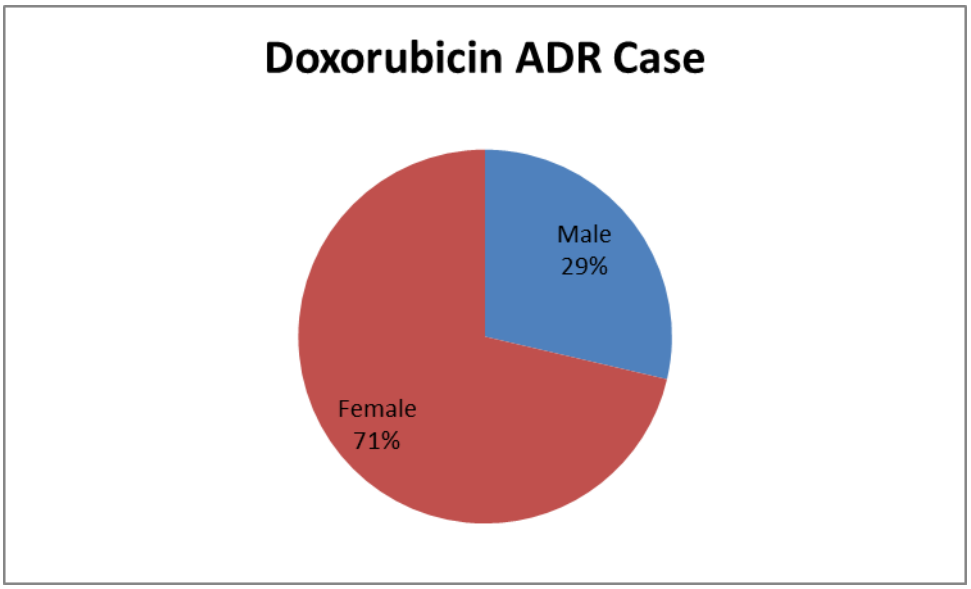

Figure 2.1: Male-female doxorubicin ADR ratio. (Data were taken from "Canada Vigilance Adverse Reaction Online Database").

Interpretation: From the figure 2.1 above, doxorubicin male-female ADR ratio is 29:71.

2.1.2. Mortality and morbidity rate of male cancer patients are significantly higher in cardiac disorder doxorubicin ADR compared to doxorubicin ADR of all organ class.

After analyzing 921 doxorubicin ADR cases of all organ class, female ADR cases were 657 and male 264 cases. Among 657 ADR cases of female, 23.3% of cases were found “Death” or “Unresolved”; On the other hand, 264 doxorubicin ADR cases of male cancer patients were found and among which 27.3% found “Death” or “unresolved”.

(Cumulated percentage value of “Death cases” and “Unresolved cases” represent Mortality and Morbidity rate)

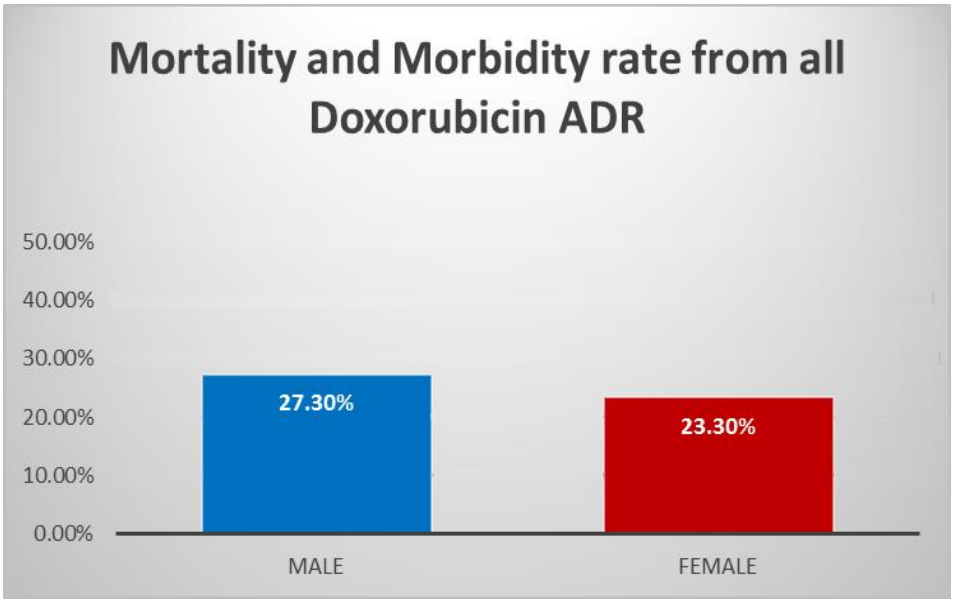

Figure 2.2: Male-female doxorubicin ADR mortality and morbidity rate; including all organ class. (Source: Canada Vigilance Adverse Reaction Online Database)

Interpretation: From the figure 2.2, we can observe that mortality and morbidity rate of male cancer patients is slightly higher than female cancer patients in terms of all organ class ADR of doxorubicin. Although, this 4% increased mortality and morbidity rate in male cancer patients is acceptable, however, there are other unaddressed factors that could be accountable for this; such as physiological vulnerability, lifestyle, genetical susceptibility and type and stage of cancer.

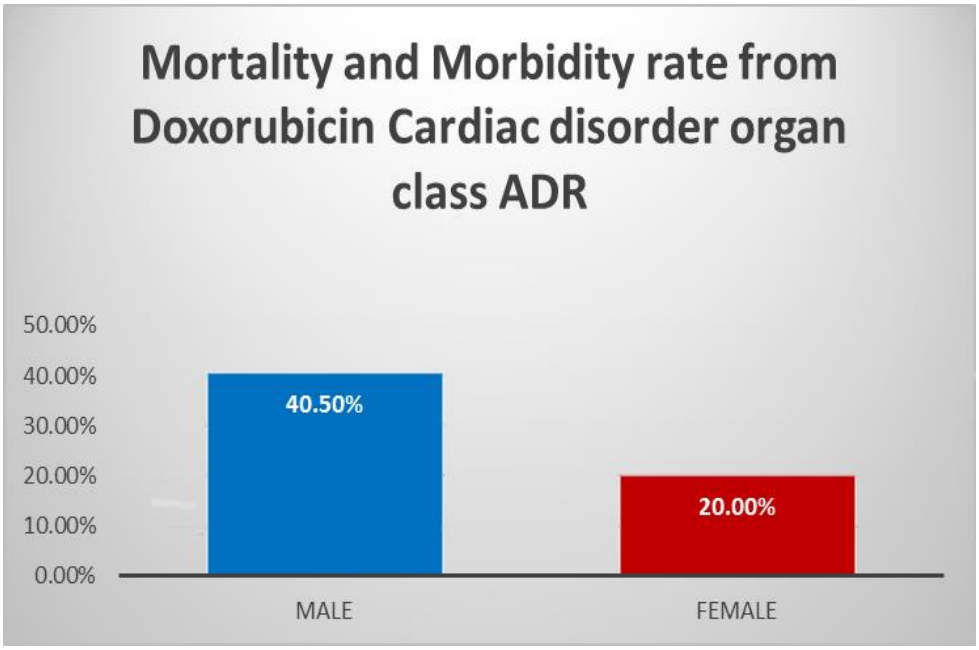

Figure 2.3: Male-female doxorubicin ADR mortality and morbidity rate; including only cardiac disorder organ class (Canada Vigilance Adverse Reaction Online Database).

Interpretation: From the figure 2.3, a deduction can be concluded; for some underlying etiologies, in the events of cardiac ADR, male cancer patients who were treated with doxorubicin; cardiac death or irreversible cardiac damage in the male patients were substantially larger relative to female patients. Although, there could be other underlying mechanisms that made male patients considerably vulnerable in terms of cardiac ADR events, however, this male-female discrepancy remains to be disputed.

2.2. Results

From the above two figures 2.2 and 2.3, following two subsequent figures (2.4 & 2.5) can be developed into new category by combining doxorubicin ADR of all organ class and cardiac disorder organ class together, distinctly for male and similarly to female. Correspondingly, figure 2.4 for male and figure 2.5 for female, illustrates similitude and dissimilitude of mortality and morbidity rate between all organ class and cardiac disorder organ class.

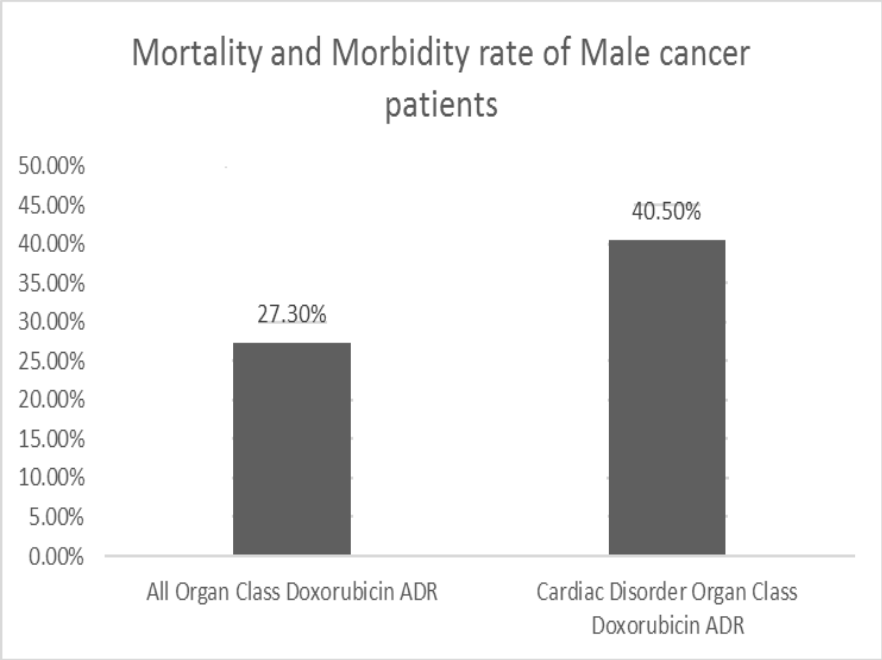

Figure 2.4: Comparison of male mortality and morbidity rate between all organ class doxorubicin ADR and cardiac disorder organ class doxorubicin ADR.

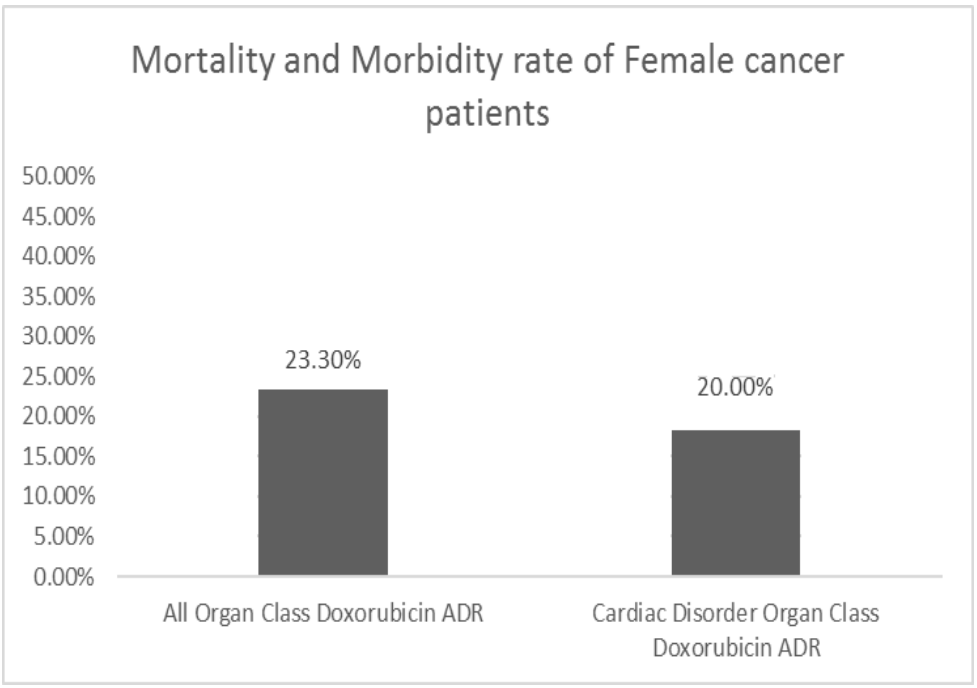

Figure 2.5: Comparison of female mortality and morbidity rate between all organ class doxorubicin ADR and cardiac disorder organ class doxorubicin ADR.

Interpretation: The figure 2.4 illustrates the differences between doxorubicin ADR of all organ class and cardiac organ class of male cancer patients; Whereas, on the other hand,

figure 2.5 clarifies the difference between doxorubicin ADR of all organ class and cardiac organ class of female cancer patients.

Male cancer patients are significantly vulnerable to doxorubicin ADR of cardiac disorder organ class.

Comparing the figure 2.4 and figure 2.5, it has been clearly found that,

1. Mortality and morbidity rate of male patients is significantly higher in cardiac disorder organ class of doxorubicin ADR (40.5%), on the other hand, it is relatively low in case of all doxorubicin ADR cases (27.3%).

   Therefore, undoubtedly it has been speculated that male cancer patients treated with doxorubicin were vulnerable to cardiac ADR's which further led to death or intractable cardiac complications.

2. Mortality and morbidity rate of female patients in all type of doxorubicin ADR (23.30%) and cardiac organ class is (20.00%), which is acceptable.

   However, unlike male patients, incongruously, mortality and morbidity rate of female patients in terms of cardiac ADR's (20.00%) are slightly lower compared to ADR's of all organ class (23.30%).

   However, this slight discrepancy in percentage value seems to be subtle but a significant assumption can be made. It can be unambiguously stated that *i*) female patients are more tolerable to cardiac ADR's of doxorubicin and *ii*) the difference in mortality and morbidity rate of cardiac doxorubicin ADR between male and female are contradictory.

3. Including all types of doxorubicin ADR's, mortality and morbidity rate of male cancer patient's (27.30%) is slightly higher than those of female cancer patients (23.30%). However, this slightly increased rate in male patient's mortality and morbidity rate appears to be expected.

Despite vulnerability of male cancer patients to doxorubicin ADR of cardiac organ class, however, two other noticeable incongruity has been also found.

2.3. Discussion

Initially, it was expected that doxorubicin ADR ratio of female would be greater than male, which was 71:29 and assumed to be normal. However, ADR's were organized into two category *i*) cardiac disorder organ class and *ii*) all organ class; both category was analyzed in terms of male and female patients.

In case of cardiac disorder organ class of male doxorubicin ADR, noticeable anomaly was detected. Their vulnerability to cardiovascular morbidity and death was significantly high compared to death related to other types of ADR's. Surprisingly, cardiovascular death or morbidity rate of male patients was 40% and whereas, in case of all organ class of ADR, it was 27%, which was low.

The result became more substantial when compared to female ADR's of cardiac disorder organ class. The cardiovascular death and morbidity rate of female patients on the other hand was only 20%, which was even lower than their corresponding all organ class ADR (23%). However, this seems to be contradictory, considering the cardiovascular death and morbidity of male patients against female patients but rises significant assumptions. It can be unambiguously stated that, female patients are more tolerable to cardiac ADR's of doxorubicin than male; otherwise, male cancer patients who were treated by doxorubicin are markedly vulnerable to cardiovascular death and complications. Lastly, the result of the study also can be concluded as. the difference in mortality and morbidity rate of cardiac doxorubicin ADR between male and female are contradictory.

Chapter 3. Docetaxel Adverse Drug Reaction Discrepancy Among Different Regions.

3.1. In Europe, significantly low percentage of cancer patients had experienced docetaxel ADR among 18-64 age group compared to other regions.

3.1.1. Introduction

Docetaxel, another anticancer drug which is antimicrotubule agent (non-anthracycline), being widely used in combination with chemotherapeutics. ADR cases of docetaxel were taken from three different open sources and compared.

ADR cases of Europe, Canada and worldwide were analyzed and compared. There are few revealing facts and statistics found regarding age group of 18-64 and male-female ADR ratio, among those different regions.

- **World:** Docetaxel ADR cases were taken from "VigiAccess", consisting of ADR cases from all over the world. table 3.1.1, shows that how docetaxel ADR cases are distributed among all patients and also demonstrates the percentage of ADR affected patients of different age group worldwide.

Table 3.1.1: Worldwide docetaxel ADR cases of different age group (Data were taken from "VigiAccess").

Age Group	Cases	%
Not Specified	9,513	14.00%
0-27 Days	15	0.00%
28 Days to 23 Months	21	0.00%
2-11 Years	40	0.00%
12-17 Years	68	0.00%
18-64 Years	**41,233**	**64.00%**

65-74 Years	10,907	17.00%
More than 75 Years	2,959	5.00%
Total	64,756	100.00%

Interpretation: From the table 3.1.1, it is observed that the most affected age group over worldwide which experienced docetaxel ADR is 18-64 years and approximately 64% of all ADR cases belongs to that age group.

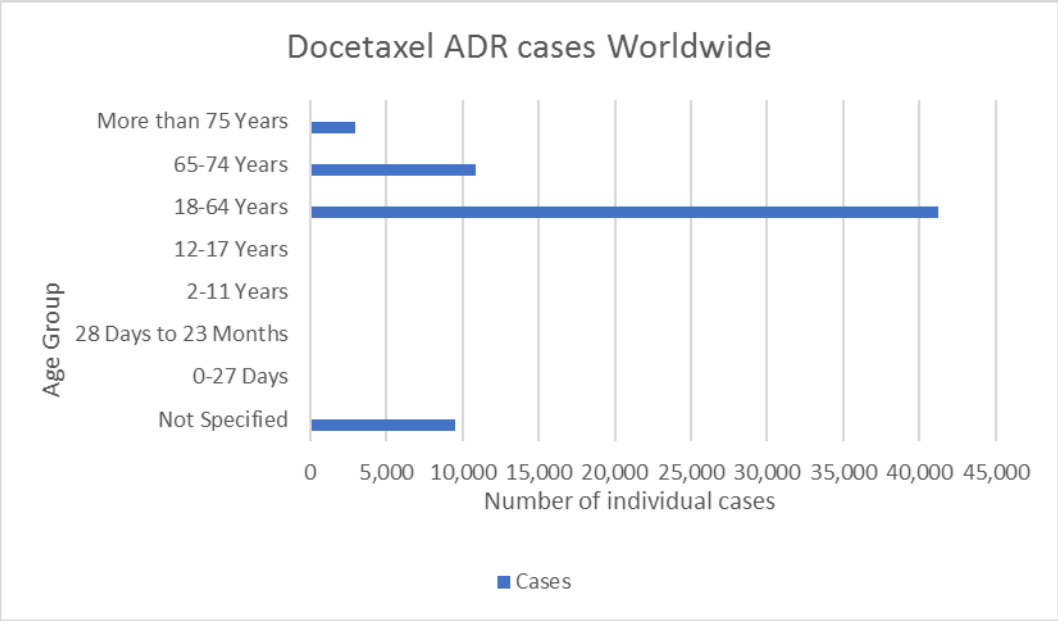

Figure 3.1.1: Graph of docetaxel ADR affecting different age group worldwide.

The above figure 3.1.1 contains graphical representation, explains that how the number of docetaxel ADR cases affect different age group of cancer patients worldwide.

- **Europe:** Docetaxel ADR cases were taken from "EudraVigilance", containing ADR cases from all over the Europe. table 3.1.2, shows that how docetaxel ADR cases are distributed among all patients and also illustrates the percentage of ADR affected patients of different age group over Europe.

Table 3.1.2: Docetaxel ADR cases of different age group of Europe (Information were taken from "EudraVigilance")

Age Group	Cases	%
Not Specified	5,433	22.9%
0-1 Month	13	0.1%
2 Months- 2 Years	7	0.0%
3-11 Years	35	0.1%
12-17 Years	97	0.4%
18-64 Years	**12,014**	**50.7%**
65-85 Years	6,005	25.4%
More than 85 Years	83	0.4%
Total	23,687	100.0%

Interpretation: From the table 3.1.2, it is clearly seen that the most affected age group in Europe which experienced docetaxel ADR is 18-64 years and this group accounts for approximately 50% of all ADR cases.

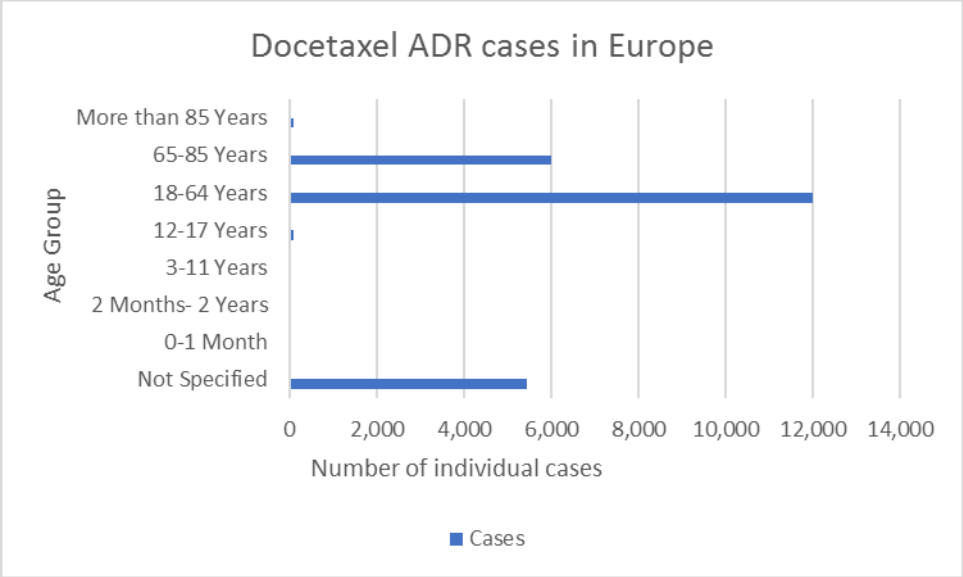

Figure 3.1.2: Graph of docetaxel ADR affecting different age group of Europe.

The above figure 3.1.2 contains graphical representation, elucidates that how the number of docetaxel ADR cases affect different age group of cancer patients in Europe.

- **Canada:** Docetaxel ADR cases were taken from "Canada Vigilance Adverse Reaction Online Database", consisting of ADR cases from all over the Canada. The table 3.1.3, displays that how docetaxel ADR cases are distributed among all patients and also represents the percentage of ADR affected patients of different age group in Canada.

Table 3.1.3: Docetaxel ADR cases of different age group of Canada (Data were retrieved from "Canada Vigilance Adverse Reaction Online Database")

Age group	Cases	%
0-17 Years	0	0%
18-64 Years	**759**	**61%**
More than 65 Years	476	39%
Total	1,235	100

Interpretation: From the table 3.1.3, it is observed that the most affected age group in Canada which experienced docetaxel ADR is 18-64 years and approximately 61% of all ADR cases belongs to that age group.

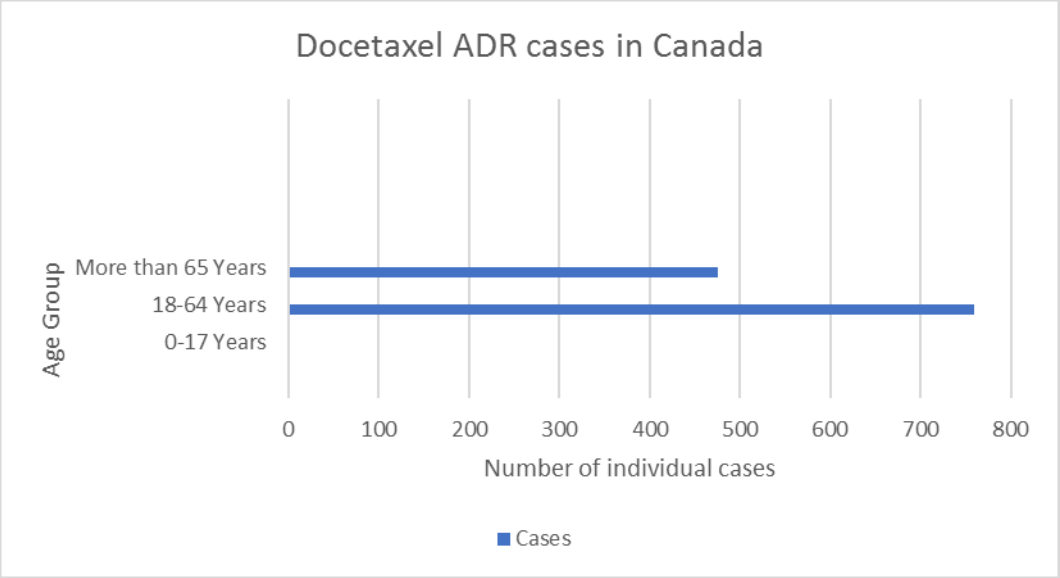

Figure 3.1.3: Graph of docetaxel ADR affecting different age group of Canada.

The figure 3.1.3 contains graphical representation, which explains that how the number of docetaxel ADR cases affect different age group of cancer patients in Canada.

3.1.2. Result

In Europe, significantly less percentage of people (age 18-64 years) had experienced docetaxel ADR compared to Canada and Global.

Comparing data from the table 3.1.1, 3.1.2, and 3.1.3; following relationship can be made by relating ADR data of Canada, Europe and World. From the following graphical interpretation, it will be more clarified that how docetaxel ADR cases (18-64 age group) vary over different regions.

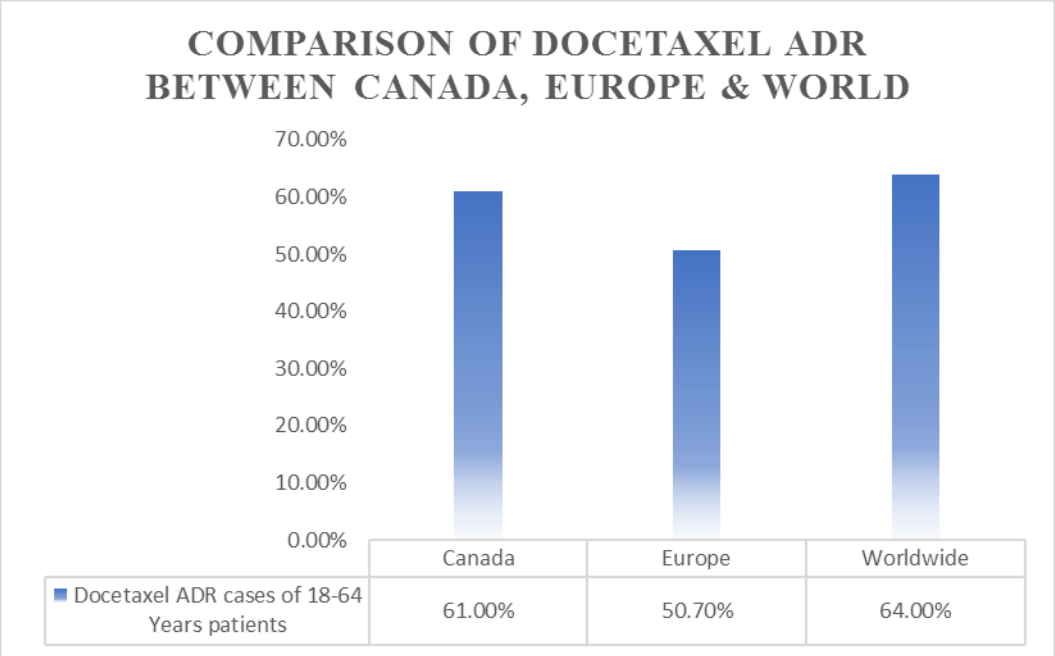

Figure 3.1.4: Graph showing docetaxel ADR (18-64 age group) affecting different region.

Interpretation: From the figure 3.1.4, in Europe, it has observed that relatively low percentage of the patient (18-64 age group) had suffered from docetaxel ADR, which is a noticeable fact. Although, 18-64 years patients have been the most affected age group in all region, however, it was significantly low in case of the European region.

3.1.3. Discussion

Generally, among different age groups, the most ADR affected age group was 18-64 years; which was not surprising because all the regions had the same precedent and notably, 18-64 age group covers the vast range of age group corresponding to ADR cases. However, regarding age group of 18-64 years, incongruity has been found in Europe. The research indicated that, in Europe, relatively low proportion of cancer patients (18-64 years) experienced docetaxel ADR. However, this slight discrepancy has been found to be noteworthy, after comparing and analyzing the same docetaxel ADR to that of Canada and worldwide.

The result of the study indicated that approximately 50% of the cancer patients in Europe who were treated with docetaxel, belongs to 18-64 years age group. Whereas, in Canada and world, the percentage was 61% and 64% respectively. Roughly, 10% reduction in

such percentage can possibly correspond to 2,370 ADR cases. This suggests that, in Europe at least 2,370 ADR cases of docetaxel should have been in 18-64 years age group.

According to population pyramid data 2017 and European Census 2011, closely 64.1% of total population of Canada belongs to age group 18-64 age group and 62.6% of total population of Europe belongs to 18-64 age group.

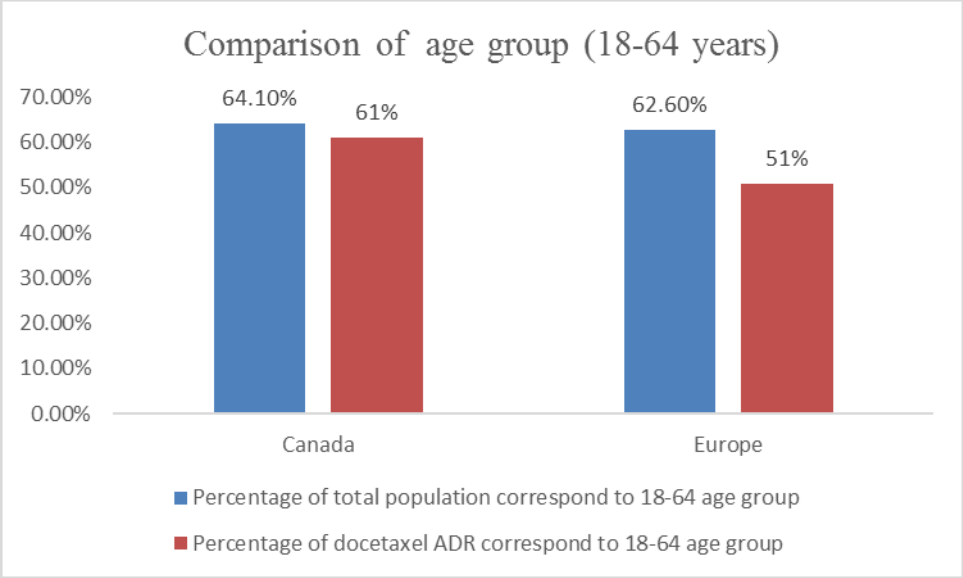

Figure 3.1.5: Comparison of age group (18-64 years) between Canada and Europe in terms of their total population and docetaxel ADR cases. **(**Information was retrieved from populationpyramid.net).

Interpretation: From the above figure, it is clarified that Europe has significantly less percentage of ADR cases of age group (18-64 years) in regard to total population that represents age group (18-64). Similarly, unlike Europe, the statistics of Canada does not show such discrepancy.

However, considering the above statistics of population pyramid of Canada and Europe, which supports our hypothesis. Presumably. we can conclude a definitive answer; European docetaxel ADR lacks almost 10% of cases in terms of 18-64 age group.

Nevertheless, one probable drawback of this study could be the sample size of ADR of Canada; which is comparatively low (1,235 cases) than the ADR size of Europe (23,687 cases) and World (64,756 cases). However, considering the population of Canada and

Europe, statistically, it has seen that in Canada, at least 1 person among 29,400 persons, suffered from docetaxel ADR. On the contrary, at least 1 person among 31,300 persons, suffered from docetaxel ADR to some point in the European region. Therefore, the sample size argument of Canada can be overlooked and supports our study to incorporate data from Canada and Europe alongside with worldwide. (Information was retrieved from “populationpyramid.net”)

3.2. In Canada, significantly low proportion of female cancer patients had experienced docetaxel ADR compared to male patients.

3.2.1. Introduction

Typically, in case of ADR's of anthracycline (doxorubicin) or non-anthracycline group (docetaxel), it has frequently been seen with both the groups that, the burden of ADR cases or the majority portion of the ADR cases belongs to female cancer patients. However, in Canada, a conspicuous inconsistency has been found in the proportion of male-female ADR ratio of docetaxel.

- **World**: Considering 64,756 ADR cases of worldwide, male-female docetaxel ADR ratio of world is 25:69, however, which is not surprising. Still, 6% of the ADR cases remained unaccounted.

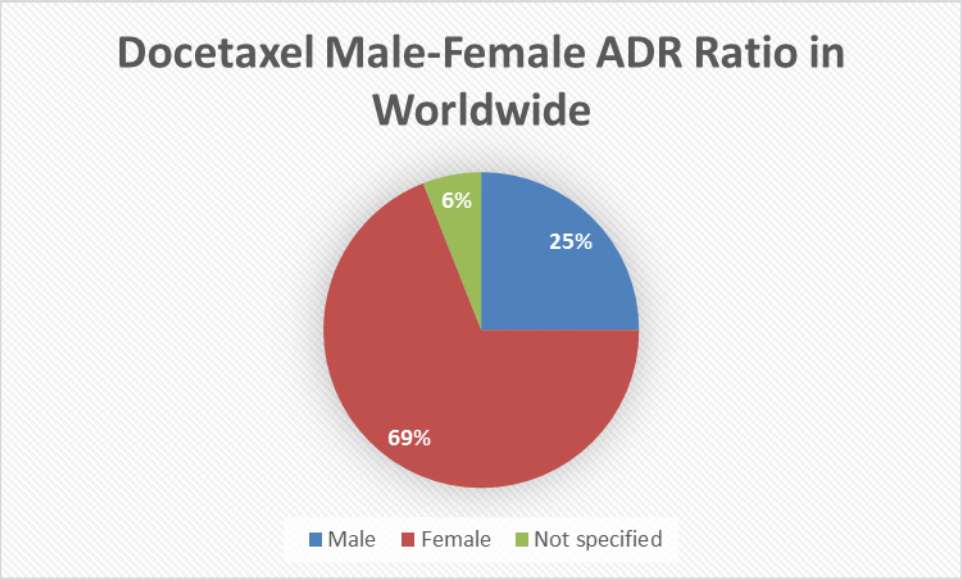

Figure 3.2.1: Percentage of male-female ADR of docetaxel over worldwide (Data were adapted from "VigiAccess").

Interpretation: From the figure 3.2.1, worldwide docetaxel ADR ratio of male-female was found to be 25% and 69% respectively; 6% was unspecified.

- **Europe:** Including 23,687 ADR cases all over the Europe, male-female docetaxel ADR ratio of Europe is 28:64, however, which is not aberrant. Yet, 8% of the ADR cases account for "not specified".

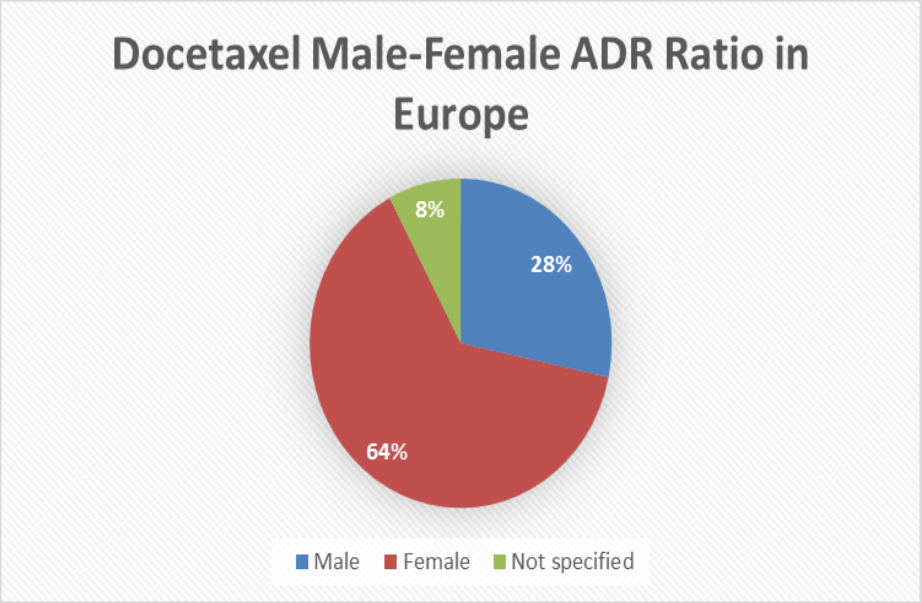

Figure 3.2.2: Percentage value of male-female ADR of docetaxel in Europe (Data were taken from "EudraVigilance").

Interpretation**:** From the above figure 3.2.2, docetaxel ADR ratio of male-female in Europe was found to be 28% and 64% respectively; 8% was not specified.

- **Canada:** Including 1,235 ADR cases in Canada, male-female docetaxel ADR ratio of Canada is 84:14, however, which seems to be aberrant because of the high percentage value of female and significantly low percentage value of male ADR patients. Notably, in the other two regions (World and Europe), that same male-female ADR ratio was predictable but in case of Canada, though only 2% of the ADR cases account for "not specified", 84% of the female ADR proportion and only 14% of the male ADR patients can rise few definite speculations.

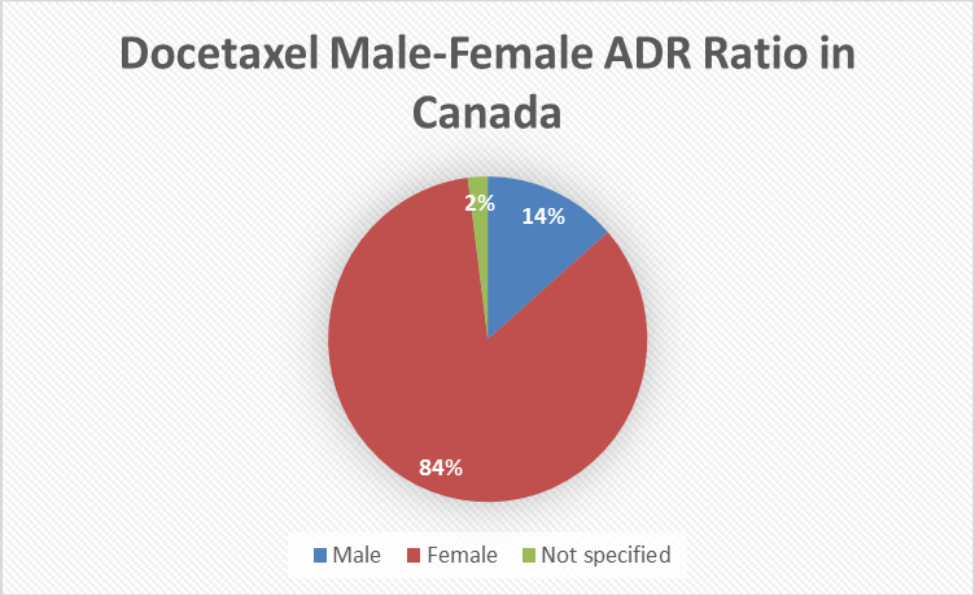

Figure 3.2.3: Percentage of male-female ADR of docetaxel in Canada (Data were taken from "Canada Vigilance Adverse Reaction Online Database").

Interpretation: From the figure 3.2.3, docetaxel ADR ratio of male-female in Canada was found to be 25% and 69% respectively; 6% was unspecified.

3.2.2. Result

In Canada, significantly high percentage of female cancer patients had suffered from docetaxel ADR relative to male cancer patients.

Comparing male-female ADR percentage data from the figure 3.2A, 3.2.B, and 3.2C; following graphical representation can be developed which simplifies high percentage ADR value of female cancer patients who were treated with docetaxel.

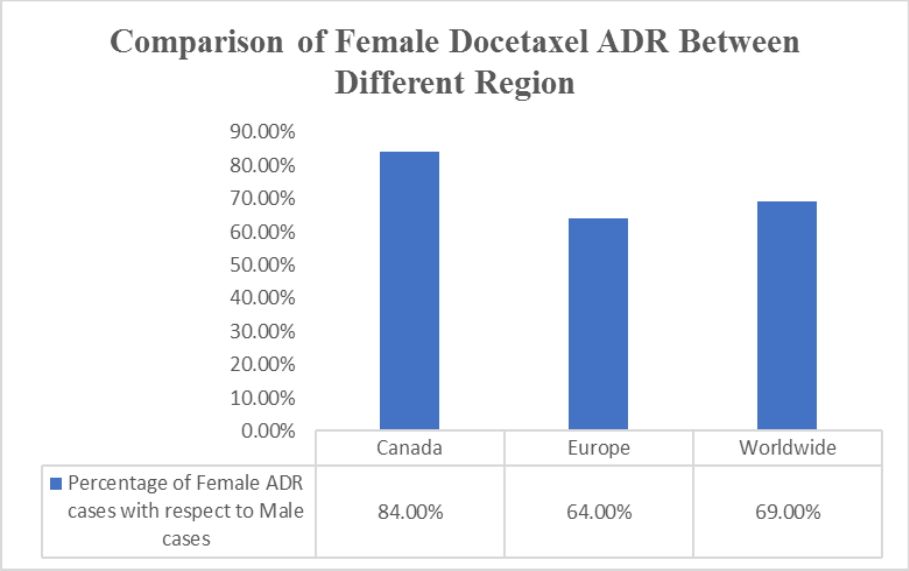

Figure 3.2.4: Percentage value of female docetaxel ADR in regard to male; affecting Canada, Europe, and World.

Interpretation: The above figure 3.2.4 specifies that all the three regions (Canada, Europe, and Global) have a high percentage of female cancer patients who are treated with docetaxel, usually represents the majority proportion that suffered from docetaxel ADR compared to that of male patients. However, in Canada, it is observed that about 84% of the female patients suffered docetaxel ADR which is significantly high.

3.2.3. Discussion

Initially, it was expected that the proportion of female docetaxel ADR patients would be larger than that of male patients. However, in Canada, it has found to be significantly high; that was beyond expectation and does not coincide to our initial hypothesis. The purpose of the study was to investigate that increased proportion of female patients are whether significant or not. Moreover, after comparing that same docetaxel ADR in different regions, it can be unambiguously stated that the increased proportion of female ADR of Canada is a noteworthy fact.

Multiple theories can be concluded based upon this finding; First plausible speculation that can be made; In Canada, either majority of the cancer patients are female, or male population does not get affected by cancers in substantial extent as much as females do. However, according to population pyramid 2017, male-female sex ratio of Canada is almost equal, 50.2% of total population is female and 49.5% is male (1.01 f/m);

Secondly, females are more susceptible to those factors which predispose to cancer compared to males, for some underlying reasons; Lastly, females are more vulnerable to docetaxel ADR particularly, which leads to long term morbidity and mortality, because of their nature of susceptibility or their lifestyle which could possibly precipitate those factors.

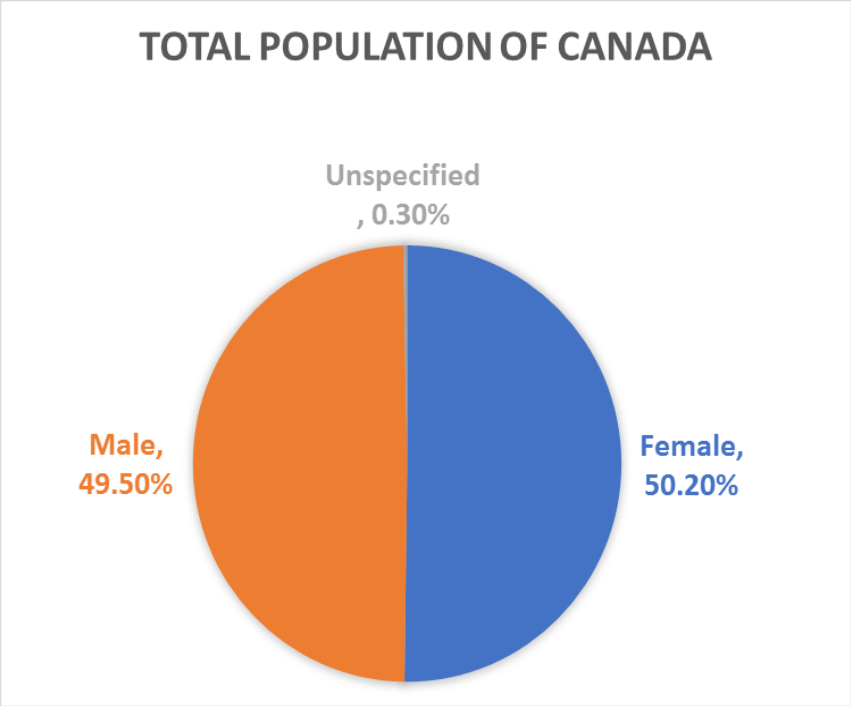

Figure 3.2.5: Male Female gender ratio of Canada (populationpyramid.net/canada/2017)

Interpretation: Considering total population of Canada, Male female gender ratio is almost equal (1.01 f/m). However, this ratio of total population abrogates the argument of male female gender discrepancy among total population.

Nonetheless, one possible disadvantage of this study could be the sample size of ADR of Canada; which is relatively low (1,235 cases) comparing the ADR size of Europe and world. However, considering the population of Canada and Europe; in Canada, at least 1 person among 29,657 persons, suffered from docetaxel ADR. On the contrary, at least 1 person among 31,207 persons, suffered from docetaxel ADR to some point in the European region. Therefore, the argument of sample size of Canada can be ignored and supports our study. (Information of population was retrieved from "populationpyramid.net")

Another decisive fact that could be the number of ADR cases that had no gender specification. Auspiciously, in case of Canada, such ADR cases account for only 2%, which is relatively low compared to that of World and Europe. That negligible fraction of ADR cases could be vital since it was low in Canada, thus more accurate male-female ADR data was obtained from docetaxel ADR cases of Canada. As a result, which facilitates our study in a substantive conclusion

Chapter 4. Conclusion and Future Directions

Chapter one is a literature review on anthracycline induced cardiotoxicity. The purpose of this chapter is to provide a thorough and comprehensive knowledge on this topic in terms of prevalence and severity of AIC. This chapter emphasizes the following aspects of AIC; cardiotoxic pathogenesis, detection, function of biomarkers and protective agents used to attenuate cardiovascular complications. In contrast, chapter one also provides an alternative approach which consolidates the knowledge of anthracycline and non-anthracycline induced cardiotoxicity. Furthermore, mechanism of cardiotoxicity of non-anthracycline such as alkylating agents, platinum based substances, antimetabolites, antibiotics, and antimicrotubule agents also have been briefly discussed and compared with cardiotoxicity caused by anthracycline.

Chapter two introduced the notion of doxorubicin ADR related to cardiotoxicity. This chapter has one significant finding based on statistical data of doxorubicin ADR. In case of cardiac disorder organ class of male doxorubicin ADR, their vulnerability to cardiovascular morbidity and death was significantly high compared to death related to other types of ADR's. Surprisingly, cardiovascular death or morbidity rate of male patients was 40% and whereas, in case of all organ class of ADR, it was 27%, which was low.

Chapter three has two parts which govern two significant contributions of docetaxel discrepancy based on statistical data of ADR. First one is about docetaxel ADR among 18-64 age group and the latter one corresponds to the proportion of female docetaxel ADR cases. The first result of this chapter indicated that approximately 50% of the cancer patients in Europe who were treated with docetaxel, belongs to 18-64 years age group. Whereas, in Canada and world the percentage was 61% and 64% respectively. Roughly, 10% reduction in such percentage can undoubtedly correspond to 2,370 ADR cases. This suggests that in Europe at least 2,370 ADR cases of docetaxel should have been in 18-64 years age group. To conclude, the research indicated that, in Europe, relatively low proportion of cancer patients (18-64 years) experienced docetaxel ADR. On the other hand, regarding the proportion of female docetaxel ADR cases, in Canada, significantly high proportion of female cancer patients were victim of docetaxel ADR compared to European region and world.

We look forward to using different data set to improve the accuracy of the result and make our study open to multiple interpretations. Moreover, our study will make valuable

contribution in future aspects of pharmacovigilance and overall public health. In order to make our study more robust, we have further decided to use a sophisticated methodology for better elucidation. Lastly, we have an inclination to use this study to compare it to the perspective of Bangladesh.

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