REPUBLIC OF TURKEY
BINGOL UNIVERSITY
INSTITUTE OF SCIENCE

CARDIAC RISK AND OXIDATIVE STRESS EVALUATION IN TRASTUZUMAB TREATED HER-2 POSITIVE BREAST CANCER PATIENTS

MASTER THESIS

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This dissertation was accepted by the following committee on 16.1.2018 wıth the vote unity .

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Dedication

I would like to dedicate this thesis to my wife Shilan, who has been a constant source of support throughout the years of my postgraduate study. Also, I would like to thank my family, especially my mother. To my brothers, sisters, and my sons, daughter Rahand, Lawand and Ronya.

Hashm Hamad ABDULLAH

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LIST OF ABBREVATIONS

ABC	:	Avidin-Biotin-Peroxidase Complex
AMI	:	Acute myocardial infarction
BC	:	Breast cancer
BCT	:	Breast conservation therapy
BMI	:	Body mass index
CHER	:	Cholesterol esterase
CHOD	:	Cholesterol oxidase
CM	:	Chylomicron
CNAs	:	Center for a New American Security
CSCs	:	Cancer stem cells
DCIS	:	Ductal carcinoma in situ
EDTA	:	Ethylene diamine tetra acetic acid
EGFR	:	Epidermal growth factor receptor
eNOS	:	Endothelial nitric oxide synthase
ER	:	Estrogen receptor
FDA	:	Food and Drug Administration
GLUTs	:	Glucose transporters
HDL	:	High-density lipoprotein
HER2	:	Human epidermal growth factor receptor-2
HRP	:	Horseradish peroxidase
IGF-1	:	Like growth factor
IHC	:	Immunohistochemistry
IL1-R1	:	Interleukin 1-R1 (Il-33 receptor)

LCIS	:	Lobular carcinoma in situ
LDL	:	Low- density lipoprotein
LVEF	:	left ventricular ejection fraction
MAPK	:	Mitogen-activated protein kinase
MDA	:	Malondialdehyde
NGS	:	Nottingham Grading System
PBS	:	Phosphate buffered saline
PEGME	:	Polyethylene-glycol-methylether
PI3K/Akt	:	Phosphoinositide-3-kinase/Protein kinase B
PR	:	Progesterone receptor
PVS	:	Polyvinyl sulfonic acid
ROS	:	Reactive oxygen species
RTK	:	Receptor-like tyrosine kinase
TBS	:	Tris-buffered saline
TC	:	Total cholesterol
TG	:	Total triglycerides
TMB	:	Tetramethylbenzidine

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TRASTÜZMAB TEDAVİSİNDE HER2 POZİTİF MEME KANSERİ HASTALARINDA KARDİYAK RİSKİ VE OKSİDATİF STRES DEĞERLENDİRMESİ

ÖZET

Bu çalışma, HER-2 pozitif meme kanseri hastalarında Trastüzumab tedavisinin etkisi ile bazı önemli biyokimyasal, kardiyak belirteçler ve hematolojik değişkenlerin değişimi arasındaki ilişkiyi araştırmak amacıyla yapılmıştır. Nanakaly hastanesinde HER-2 pozitif meme kanseri olan 44 erişkin hasta - Erbil (Onkoloji ve Hematoloji hastalıkları için uzmanlaşmış hastane) çalışmaya dahil edildi. Toplama örneği, 1 Mart - 1 Kasım 2016 tarihleri arasında (9) ay içinde gerçekleştirildi. Vücut kütle indeksleri 16,41-43,76 (45-80) yıl arasında değişiyordu ve ağırlıkları (65-105) arasında değişiyordu. Tüm öykü, hazırlanan anket formu kullanılarak tüm hastalardan elde edildi ve 20 kontrol hastası (tedavi gerektirmeyen Her-2 pozitif kanser hastaları) alınarak meme kanseri için standartlaştırılmış bir vaka kontrol çalışması oluşturuldu. Hastalar, dereceler, vücut kütle indeksi, trastuzumab tedavisinin doz ve süresi gibi bir dizi ana değişken göre sınıflandırıldı. Kan numunelerinin toplanmasından sonra hematolojik analiz doğrudan Hemolyzer 5'Analyzer kullanılarak yapıldı. Biyokimyasal testlerin, oksidatif stres belirteçlerinin ve kalp belirteçlerinin değerlendirilmesi Cobas e411 ve c311 kullanılarak hastaların serumları ve kontrolleri kullanılarak yapıldı. Biyokimyasal parametreler arasında: Lipid profili, Kan glikozu, kardiyak markır kardiyak troponin ve CKMB enzimini içeriyordu. Oksidatif stres belirteçleri ise serum MDA ve serum nitriti idi. Sonuçların analizi üç ana kategoriye ayrıldı: Kontroller ve hastalar arasındaki karşılaştırma ve farklı testlerin kullanıldığı farklı dereceler arasındaki karşılaştırma, farklı VKİ için karşılaştırma, ANOVA testi kullanılarak yapıldı. Kontrol ve hastalar arasındaki karşılaştırma, kardiyak troponin, MDA, nitrit ve trombosit parametrelerinde anlamlı farklılıkların bulunduğunu gösterdi. IL-1-R1'de ve diğer tahmini değişkenler arasında aralarında önemli olmayan farklılıklar kaydedildi. Dahası, notlar arasındaki karşılaştırmada, II ve III. Sınıflar arasında IL1-R1 ve kardiyak belirteçler açısından önemli bir fark olduğu bildirilmiştir. Tedavi dozu ve süresi karşılaştırıldığında, Troponin, MDA, hemoglobin ve trombosit sayısı bakımından anlamlı farklılıklar görülmüştür. Geriye kalan parametreler önemli değilken. Kaydedilen önemli bulgular arasında, doz ve sürenin artırılması ile kardiyak Troponin düzeyinin önemli oranda azalması. Trastuzumabın kardiyotoksisitesi üzerindeki obezitenin etkileri ile ilgili olarak, nitrat ve ortalama trombosit hacmi haricinde, kardiyak toksisite ile hemen hemen tüm değişkenler ile BMI yükselmesi arasında sonuçlarla açık bir ilişki bulunamamıştır.

Anahtar kelimeler: Breast cancer, Trastuzumab, Toxicity, Hert-2 positive.

CARDIAC RISK EVALUATION IN TRASTUZUMAB TREATED HER-2 POSITIVE BREAST CANCER PATIENTS

ABSTRACT

The present study was designed to investigate the relation between the action of Trastuzumab treatment and alteration of some important biochemical, cardiac markers and hematological variables in HER-2 positive breast cancer patients. Forty-four adult patients with HER-2 positive breast cancer at Nanakaly hospital-Erbil (Specialized hospital for Oncology and Hematology diseases) were included in this study. The collection sample was performed within (9) months from 1 March to 1 November 2016. Their body mass indexes were ranged between 16, 41- 43, 76 matches (45-80) years and their weights were ranged between (65-105). The whole history was obtained from all patients using prepared questionnaire form, and a standardized case-control study of breast cancer was established through taking 20 control patients (Her-2 positive cancer patients without treatment). The patients were categorized according to a number of major variables including Grades, Body mass index, Dose, and duration of trastuzumab treatment. After collection of blood samples, the hematological analysis was performed directly using Hemolyzer 5'Analyzer. The evaluation of biochemical tests, oxidative stress markers, and cardiac markers were made using sera of the patients and controls using Cobas e411 and c311. The biochemical parameters included: Lipid profile, Blood glucose, while the cardiac marker included cardiac troponin and CKMB enzyme. While the oxidative stress markers were serum MDA and serum nitrite. The analyses of results were divided into three main categories: The comparison between controls and patients and the comparison between the different Grades performed using a t-test, while comparison of different BMI performed using ANOVA test. The comparison between the control and patients showed that the significant differences were found in cardiac troponin, MDA, nitrite and platelet's parameters. While the non-significant differences were recorded between them in IL-1-R1and other estimated variables. Moreover, thecomparison between the grades reported a significant difference between the Grades II and III regarding IL1-R1 and cardiac markers. The results of comparison for the dose and duration of treatment showed significant differences in case of Troponin, MDA, hemoglobin and platelet count. While the rest of parameters were non-significant. Among the important findings which were recorded, the significant reduction of cardiac Troponin level with increasing the dose and duration. Regarding the impact of obesity on the cardiotoxicity of trastuzumab, the results showed no obvious relation between an elevation of BMI with almost all variables related to cardiac toxicity, except in case of nitrite and mean platelet volume.

Keywords: Breast cancer, Trastuzumab, Toxicity, Hert-2 positive.

1. INTRODUCTION

Breast cancer is the most popular type of cancer worldwide (Coughlin and Ekwueme 2009). This type of cancer is more common in females. However, it also occurs in men, but it is approximately 100 times less common in men than in women (Society 2013). The National Cancer Institute and American Cancer Society evaluate that in 2012 there were nearly three million breast malignancy survivors in the United States, with an extra 226,870 new analyses expected (Siegel et al. 2012). Since the Nineteen Seventies, major advances in screening technologies and treatment options have resulted in significant declines in breast cancer mortality (Siegel et al. 2012). Recently, the molecular diagnosis of breast cancer has hut light into its heterogeneity (Network 2012). The human epidermal growth factor receptor two (HER2) is a transmembrane receptor-like tyrosine kinase (RTK) over expressed in 20%–25% of breast carcinomas (Owens et al. 2004). It's a member of the ErbB/EGFR receptor family that additionally encompasses EGFR/ErbB1, ErbB3, and ErbB4 (Lemmon and Schlessinger 2010). Previous to the prevalent utilize of targeted therapies, HER2-positive BC was linked with additional aggressive disease, poor prognosis, and resistance to chemotherapeutical agents (Slamon et al. 1987). Trastuzumab, a humanized antibody that targets the HER2 receptor, has been developed as a significant advance within the treatment of HER2-positive carcinoma. This drug has been approved by FDA through a pivotal study in 1988 (Guo and Wong 2014). Many mechanisms of action have been revealed for elucidative the action of Trastuzumab, as well as PI3K/Akt and MAPK sign inhibition, a bar of HER2 cleavage by matrix metalloproteinases, antibody reliant on cell-mediated toxicity used by the immune system, and angiogenesis reserve (Recondo Jr et al. 2016). One in every one of the challenges that faced the patients treated with trastuzumab is the cardiovascular toxicity. The mechanism by which trastuzumab causes cardiotoxicity is not completely understood, however, it's thought to be related to blocking of the traditional physiological action of HER2 on cardiomyocytes (De Keulenaer et al. 2010). In the absence of HER2 function, cardiomyocytes are unable to activate survival

pathways and reactive oxygen species (ROS) accumulate leading to cardiac dysfunction (Zeglinski et al. 2011). Similarly, cardiac stem cells seem to lose their capability for cardiogenic differentiation and formation of microvascular networks (Barth et al. 2012). The main object of this study is to determine the relation of chemotherapeutic treatment of Trastuzumab with the function of endothelial cells and also with the cardiac toxicity. However, the cardiotoxic actions of Trastuzumab have been studied before, but we intend to relate this assessment with the BMI of the patients, as well as the parameters related to angiogenesis.

Specific Aim

The purposes of performing the suggested study are:

1. To determine the relationship between angiogenesis (via measuring of the ST2or IL-33 receptor) and cardiac toxicity of Trastuzumab drug.
2. Finding the impact of obesity (BMI) on the drug cardiotoxicity and endothelial dysfunction.
3. Evaluate the role of lipid peroxidation in the whole process with correlating to obesity.

2. LITERATURE REVIEW

2.1. Breast Cancer (Background)

Cancer is a broad cluster of diseases that cause cells within the body to change and grow uncontrollably. Most kinds of cancer cells eventually a lump or mass called a tumor, and are named after the part of the body wherever the neoplasm originates. The vast majority of breast cancers begin in the components of the breast tissue that are created from glands for milk production, referred to as lobules, and ducts that connect the lobules to the nipple. The rest of the breast is made up of fatty, connective, and lymphatic tissues (Allred 2010, Pape-Zambito et al. 2014). Breast cancer is characterized by uncontrolled growth of malignant cells in the duct gland tissue. The disease affects each gender. Breast cancer is that the most frequent kind of cancer in ladies worldwide, with an incidence that rises dramatically with age. BC reportedly accounted for twenty-ninth percentage of all new cancer cases and fourteen percentage of all cancer-associated deaths among women worldwide up to 2012 (Siegel et al. 2013). Breast cancer, the foremost common type of cancer among women, additionally has the second highest morbidity rate worldwide (10,9% of all cancers). Through a calculable one and thirty-eight million hearty new cancer cases diagnosed in 2008, it's also the most common cancer in both developed and developing regions. About 69,000 new cases have been estimated in every one of those regions (population ratio 1:4). Incidence rates vary from nineteen and three per 100,000 women in Eastern Africa to 89.7 per 100,000 women in Western Europe and are high (_80 per 100,000) in developed regions of the globe (except Japan) and low (_40 per 100,000) in most developing regions. The differ of death rates is far less (approximately 6-19 per 100,000) because of the more favorable survival of breast cancer in (high prevalence) developed regions. As an outcome, breast cancer levels as the fifth cause of death from cancer generally (458,000 deaths), however, it's still the most recurrent reason of cancer death in women in both developing (269,000 deaths, 12,7% of total) and increased regions, wherever the estimated 189,000 deaths is

approximately equal to the estimated number of deaths from lung cancer (188,000 deaths) (Gangopadhyay et al. 2013). In Iraq, breast cancer cases have inflated severely, especially within the after1990s, it constituted 14.3% of all types of cancers in 1997 (Fakri et al. 2006).

2.1.1. Classification Of Breast Cancer

At the moment, beside conventional use of grade, histology, and immunohistochemical analysis, changes in gene expression during bearing tumors are used as an instrument to classify breast cancer. The capable for a better understanding of breast cancer make by Molecular profiling, more precision in determining subtypes and better prediction of clinical outcome and response to therapy. Latest devices similar to microarray kits provide the possibility for simultaneous studying of the expression of thousands of genes in a breast cancer cells and finding out the Gene expression profile. Future applications will take the same approach to proteins (proteomics), genome-wide germ line variability (single nucleotide polymorphisms), or cellular metabolism (metabolomics). On these based methods, numerous separate breast cancer subtypes have been recognized including two major subtypes of estrogen receptor (ER)-negative tumors and basal-like and human epidermal growth factor receptor-2 (HER2)-enriched, and two subtypes of ER-positive tumors as well as luminal A and luminal B. These subtypes are different markedly in prognosis and in the therapeutic targets they express. The luminal A and luminal B are luminal cancers, so called because they are characterized by expression of genes besides expressed by normal breast luminal epithelial cells, have overlap with ER-positive breast cancers. Present are in addition several subtypes characterized by low expression of hormone receptor-associated genes (ER-negative), one of which is called the "HER2-enriched" subtype (previously called HER2+/ER-) and another called the (basal-like) subtype. The basal-like subtype is named since it expresses many genes characteristic of normal breast basal epithelial cells (Ostad and Parsa 2011). Molecular markers based on gene expression profiles have been used in experimental and medical settings to distinguish cancerous tumors in the stage, grade, survival time, metastasis, and drug sensitivity. However, most significant gene markers are unstable (not reproducible) among data sets (Kim et al. 2012). BC is classified into four groups based on IHC profile ER/PR and Her2/neu expression, positive (+) and/or negative (-). The groups are:

- ER/PR+, Her2+ = ER+/PR+, Her2+; ER-/PR+, Her2+; ER+/PR-, Her2+
- ER/PR+, Her2- = ER+/PR+, Her2-; ER-/PR+, Her2-; ER+/PR-, Her2-
- ER/PR-, Her2+ = ER-/PR-, Her2+
- ER/PR-, Her2- = ER-/PR-, Her2-

The IHC classification correlates well with intrinsic gene expression microarray categorization: ER/PR+,Her2+ with Luminal B; ER/PR+,Her2-with Luminal A; ER/PR-,Her2+ and ER/PR-,Her2-with triple negative/basal-like tumors (Onitilo et al. 2009). Defining the progression of breast cancer has not been possible due to lack of markers that define hyperplasia (typical and atypical), carcinoma in situ and invasive cancer (Stingl and Caldas 2007). On the other hand, breast cancer can be broadly sorted into in situ carcinoma and invasive (Infiltrating) carcinoma. BC in situ is additional sub-classified as either ductal or lobular; growth patterns and cytological features form the basis to distinguish between the two types. Ductal BC in situ (DCIS) is noticeably more common than its lobular BC in situ (LCIS) counterpart and encompasses a heterogeneous group of cancers. DCIS has traditionally been further sub-classified based on the architectural features of the tumor which has given rise to five well-recognized subtypes: Comedo, Cribiform, Micropapillary, Papillary and Solid as shown in [Figure 2.1] (Connolly et al. 1998).

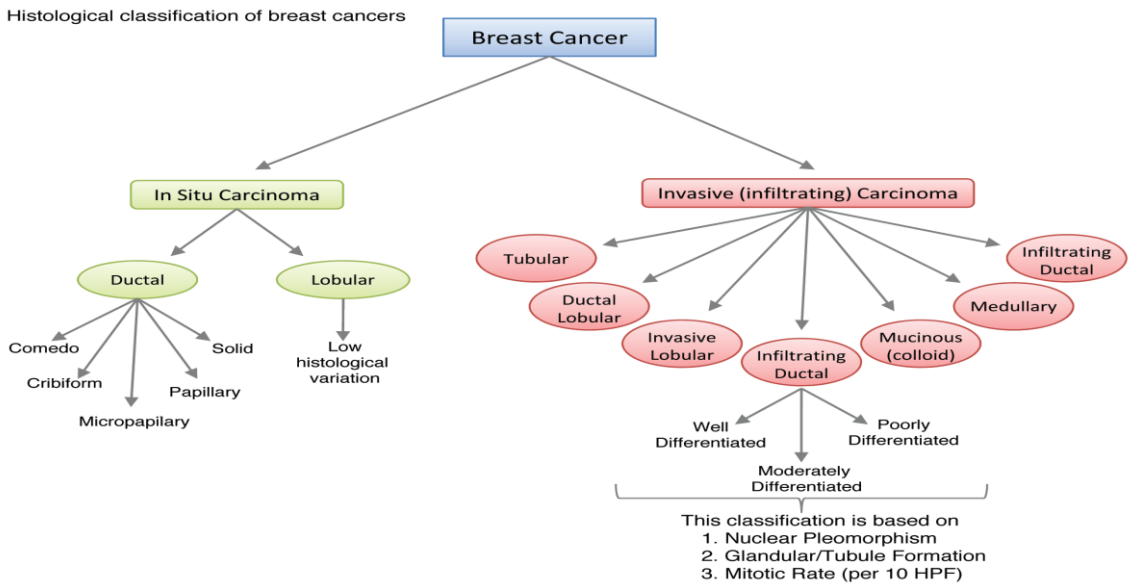


Figure 2.1. Histological classification of breast cancer subtypes. This method, currently used by clinicians, categorizes the heterogeneity found in breast cancer based on architectural characteristics and growth patterns, high power field

While the current model for breast cancer classification has prognostic value, lack of a molecular component to the classification scheme limits the ability to predict a response to newer targeted therapies. The current classification of the molecular subtypes of breast cancer has begun to address this issue. Recent studies identified several intrinsic molecular subtypes of breast cancer that were later confirmed and classified as: basal-like, ErbB2+, normal breast-like, luminal subtype A and luminal subtype B as shown in [Figure 2.2] (Perou et al. 2000, Sorlie et al. 2003).

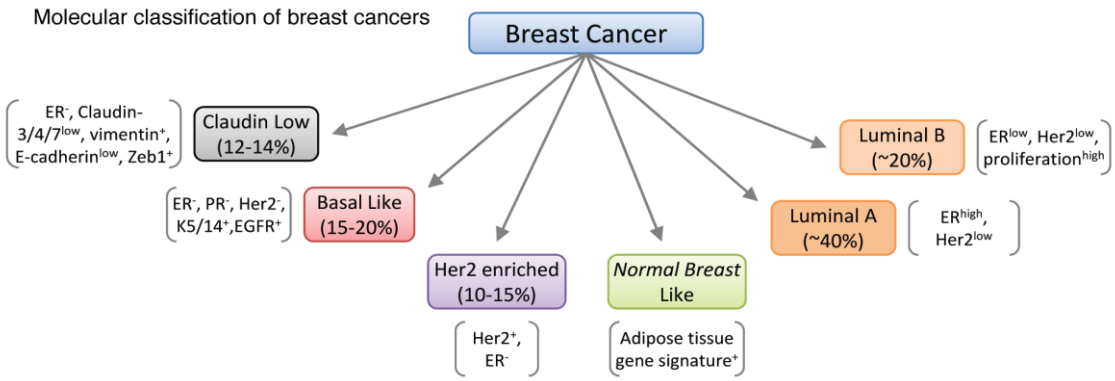


Figure 2.2. Molecular classification of breast cancer. On the based the intrinsic molecular subtypes of breast cancer identified by microarray analysis of patient tumor specimens, its classification

Individual of the best established prognostic factors in breast cancer is a histological grade, which represents the morphological assessment of tumor biological characteristics and has been shown to be able to create significant information associated to the clinical performance of breast cancers. Genome broad microarray supported expression profiling studies have unraveled numerous features of breast cancer biology and have provided further evidence that the biological features captured by histological grade are important in determining tumor behavior. (Rakha et al. 2010). NGS is based on the evaluation of three morphological features: (a) degree of tubule or gland formation, (b) nuclear pleomorphism, and (c) mitotic count, as shown in [Figure 2.3] (Elston and Ellis 1991, Ellis 2005).

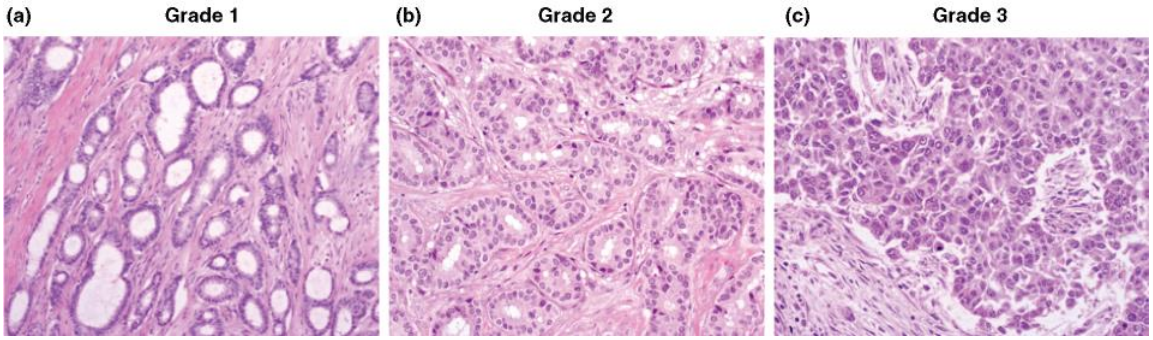


Figure 2.3. The Nottingham Grading System it's method to assessed the histological grade of breast cancer. (a) A well-distinguished tumor (grade 1) that shows high homology to the normal breast terminal duct lobular unit, tubule formation (>75%), a mild degree of nuclear pleomorphism, and low mitotic count. (b) A moderately differentiated tumor (grade 2). (c) A poorly differentiated (grade 3) tumor with a marked degree of cellular pleomorphism and frequent mitoses and no tubule formation (<10%)

A number of different staging systems are used to classify breast cancer. The TNM staging system assesses cancer in three ways: the size and extension of the tumor (T), regional lymph node involvement (N), and the presence of distant metastases (M). Once the T, N, and M classifications are determined, a stage of 0, I, II, III, or IV is assigned. The TNM staging system is commonly used in clinical settings and is used for the description of treatment patterns. Summary stage, a less complex staging system, has historically been used by central cancer registries. Cancers are classified as in situ, local, regional, and distant, based on the extent of spread, The summary stage is used to describe population-based patterns of the stage at diagnosis and survival (Siegel et al. 2012).

2.1.2. Pathogenesis Of Breast Cancer

A proliferative breast infection is related to an increased hazard of breast cancer. Proliferative breast lesions without atypical, including usual ductal hyperplasia, intraductal papillomas, sclerosing adenosis and fibro adenomas confer only a small increased risk of breast cancer development, approximately one half to two times that of the general population, Atypical hyperplasia including both ductal and lobular, usually incidentally found on screening mammography, confers a substantially increased risk of

breast cancer. Women with atypical have an approximately four and three times greater risk of developing cancer compared to the general population (Hartmann et al. 2005).

2.1.3. Sign And Symptom Of Breast Cancer

Most commonly the first sign is a lump in the breast the woman usually finds the lump. Sometimes the lump is seen on a screening mammogram before it can be felt. It presents all the time and does not get smaller or go away with the menstrual cycle. It may feel like it is attached to the skin or chest wall and cannot be moved. Also, it feels hard, irregular in shape and very different from the rest of the breast tissue usually it's not painful pain is additional frequently a symptom of a non-cancerous condition. alters in breast shape or size ,skin changes: The skin of the breast may become dimpled or puckered, redness, swelling and increased warmth, itching of the breast or nipple changes(discharge from the nipples) moreover the patients can be presented with bone pain, nausea, loss of appetite, weight loss and jaundice(Smith et al. 2009, Hertz-Picciotto et al. 2012).

2.1.4. Diagnosis Of Breast Cancer

Generality kinds of breast cancer are easy to diagnose by microscopic analysis of a sample or biopsy of the affected area of the breast. However, there are rarer types of breast cancer that require specialized lab exams. The two most commonly used screening methods are physical examination of the breasts by a healthcare provider and mammography. Can offer an approximate likelihood that a lump is cancer, as well as might also detect some further lesions, such as a simple cyst. While these examinations are inconclusive, a healthcare provider can remove a sample of the fluid in the lump fine needle aspiration and (FNA) for microscopic analysis to help establish the diagnosis. Together, physical examination, mammography, and FNA can be used to diagnose breast cancer with a good degree of accuracy (Bao and Rudek 2011).

2.1.5. Treatment Of Breast Cancer

The treatment of breast cancer depends on various factors, including the stage of cancer. Aggressive treatments are employed in accordance with the poorer the patient's prognosis and the higher the risk of recurrence of cancer following treatment (Bao and Rudek

2011). BC is usually treated with surgery, which may be followed by chemo or radiation treatment, or both, hormone receptor-positive cancers are often treated with hormone-blocking therapy over courses of several years. Monoclonal antibodies, or other immune-modulating treatments, may be administered in certain cases of metastatic and other advanced stages of breast cancer (Bao and Rudek 2011). In addition, can be administered after surgery to control cancer reappearance and metastasis. The two main types of chemotherapeutic agents are small molecule therapeutic agents and biologically specific targets, biologically specific agents are targeted at specific receptors on the surface of cancers such as ER, PR, and HER2. VEGFR (vascular endothelial growth factor receptor) is another important surface receptor in tumor health, as it stimulates angiogenesis and vasculogenesis that build blood vessels specifically for the tumor when bound with VEGF (Lee and Nan 2012).

2.2. Trastuzumab (Herceptin)

It is a humanized antibody that attaches to the extracellular domain of the HER2 transmembrane growth factor receptor. Around 20% to 30% of all invasive breast carcinomas are HER2-positive, and most of those have aggressive clinical behavior along with a poor outcome. Many clinical trials have already shown that treating the HER2-overexpressing pathological process of breast cancer (BC) with trastuzumab, either alone or in combination with chemotherapy, significantly improves time to progression, duration of response, and survival. Trastuzumab has also been shown to be an effective adjuvant therapy for HER2-positive early-stage BC patients. The most common side effect of trastuzumab is cardiomyopathy, reported affecting around 2.8% to 3.3% of patients. Some researchers have found that previous treatment with anthracycline regimens, menopause status, radiotherapy, and the frequency of trastuzumab infusion might be associated with a higher incidence of cardiomyopathy. The cardiotoxicity appears to be reversible and left ventricular dysfunction often normalizes after withdrawal of trastuzumab. Each clinical trial has used a different dosage and infusion frequency of trastuzumab. The most common dosage was 4 mg/kg for the loading dose followed by 2 mg/kg per week. In the Herceptin adjuvant (HERA) trial. The dosage was adjusted to 8 mg/kg for the loading dose followed by 6 mg/kg every 3 weeks for the clinical convenience (Wu et al. 2016).

2.2.1. Mechanism Action Of Trastuzumab (Herceptin)

HERs are cell membrane-bound glycoproteins, comprising four distinct receptors: HER1, HER2, HER3, and HER4.1 (Wong 2005). HER receptors are divided into three regions: an extracellular ligand binding region, an intracellular region through tyrosine kinase activity, and a region that spans the cell membrane and anchors the receptor to the cell. Ligand binding to the extracellular domain promotes formation of dimmers, homodimers (between monomers of same receptor), or heterodimers (between the bound receptor and other members of the HER family), activating tyrosine kinase, and triggering a cascade of complex cell biochemistry that regulates various cell functions such as cell proliferation, angiogenesis, apoptosis, adhesion, and motility (Perez-Soler 2004). The HER2 gene (HER2/neu, ErbB2 gene) is located on the long arm of chromosome 17 and is amplified (over expressed) in 20%–30% of early-stage breast cancers. The activating mechanism of the majority HER receptors is binding of a mitogen to the extracellular ligand portion of the HER receptor. However, there is no known mitogen (ligand) for HER2. Overexpressed HER2 sends signals lacking mitogen arriving and binding to every receptor. These signals promote invasion, survival, and angiogenesis of tumoral cells Trastuzumab binds to domain IV of the extracellular segment of the HER2. (Menard et al. 2003). This process prevents dimerization, causing cell arrest during the G1 phase. Some of the therapeutic effects may also be due to down-regulation of HER2. These mechanisms cause disruption of receptor dimerization, which reduces signaling pathways and results in cell-cycle arrest (Gemmete and Mukherji 2011). Decreasing VEGF production, activating antibody-dependent cell-mediated cytotoxicity, G0/G1 cell cycle cytotoxicity, and inhibiting intracellular signaling pathways (Spector and Blackwell 2009).

2.2.2. Side Effect of Trastuzumab (Herceptin)

Trastuzumab behavior is mostly healthy-tolerated with the potential for trastuzumab-stimulated cardiotoxicity because of the morbidity of primary concern. The mechanism by that trastuzumab causes cardiotoxicity isn't completely understood, but it is thought to be related to blocking of the normal physiologic action of HER2 on cardiomyocytes (Onitilo al. 2014). It is induced cardiotoxicity presents with a range of severity from an

asymptomatic decline in left ventricular ejection fraction (LVEF) to symptomatic heart failure (Chien and Rugo 2010). Including initiation of β blockers and angiotensin-converting enzyme inhibitors (Perez and Morgan 2013). While the true reversibility of trastuzumab-induced cardiotoxicity is under debate, there is general agreement that conditions improve in most patients following trastuzumab withdrawal and treatment of cardiac symptoms (Onitilo et al. 2014). Episodes of trastuzumab induced cardiotoxicity may have long-lasting effects on cardiac health, as evidenced by myocyte damage and indicate by cardiac troponin I elevation (Cardinale et al. 2010). Suggesting that even if LVEF recovers, the damage may leave the patient susceptible to future insults (Telli and Witteles 2011). It is a significant part of the treatment regimen for many women with HER2- positive breast cancer and has undoubtedly resulted in a significant improvement in the prognosis of HER2-positive breast cancers. However, the risk for trastuzumab-induced cardiotoxicity is an important consideration. Understanding risk factors for trastuzumab-induced cardiotoxicity and appropriate patient monitoring during trastuzumab treatment allows for the safe and effective use of this important adjuvant therapy (Onitilo et al. 2014).

2.2.3. Cardiac Toxicity Of Trastuzumab (Herceptin)

BC is that the most typical growth in women and its prevalence is increasing, because of the population ages, further risk factors ought to be included, such as cardiovascular disease (Kalil Filho et al. 2011, Guimaraes et al. 2015). Before the advent of the human epidermal growth factor receptor 2 (HER2)-targeted monoclonal antibody trastuzumab, HER2-positive breast cancers were difficult to treat and had a poor prognosis. The currently adjuvant trastuzumab is an important part of the treatment regimen for many women with HER2-positive breast cancer and has undoubtedly resulted in a significant improvement in prognosis, but it is associated with a risk for cardiotoxicity. The prevalence, patient characteristics, and risk factors are described for cardiotoxicity associated with the use of adjuvant trastuzumab. Understanding risk factors for trastuzumab-induced cardiotoxicity and appropriate patient monitoring during trastuzumab treatment allows for safe and effective use of this important adjuvant therapy, the part of the treatment regimen adjuvant trastuzumab is a sign for many women with HER2- positive breast cancer and has undoubtedly resulted in a significant

improvement in the prognosis of HER2-positive breast cancers. However, the risk for trastuzumab stimulated cardiotoxicity is the main consideration. Understanding risk factors for trastuzumab-induced cardiotoxicity and appropriate patient monitoring during trastuzumab treatment allows for the safe and effective use of this important adjuvant therapy (Onitilo et al. 2014). The most clinically significant side effect of trastuzumab is the risk of cardiac myocyte injury, leading to the development of congestive heart failure (Mitri et al. 2012). Cardiotoxicity is an important adverse effect of adjuvant breast cancer treatment with trastuzumab and three monthly left ventricular ejection fractions (LVEF) monitoring is considered mandatory. The purpose of this learns was to gain insight into LVEF observing through adjuvant trastuzumab therapy in clinical practice. Consequently, a significant part of patients received trastuzumab, while probably being exposed to an additional risk for cardiac failure. On the other hand, a treatment shown to be suspended from a few patients in spite of adequate LVEF recovery. The effects of non-adherence to (LVEF monitoring) guidelines on clinical outcomes need attention in future studies. Most clinicians seemed to agree that some testing is required since a majority of patients had at least a few measurements. However, the testing frequency, the intervals and the consequences of a low testing result are not as obvious in clinical practice as described in national guidelines. To improve cardiac monitoring during adjuvant trastuzumab, more attention is needed for the implementation of (cardiac assessment) guidelines in clinical practice (Visser et al. 2016). Cardiotoxicity is an important adverse effect of adjuvant breast cancer treatment with trastuzumab and three monthly left ventricular ejection fraction (LVEF) monitoring is considered mandatory (Visser et al. 2016). Trastuzumab therapy has been associated with an increased risk of cardiac toxicity, especially when used in combination with anthracyclines (Mitri et al. 2012). Summaries the key components of the HER2 survival pathway, as well as the implications for the heart when HER2 receptors are inhibited as shown in [Figure 2.4] (Sandoo et al. 2014).

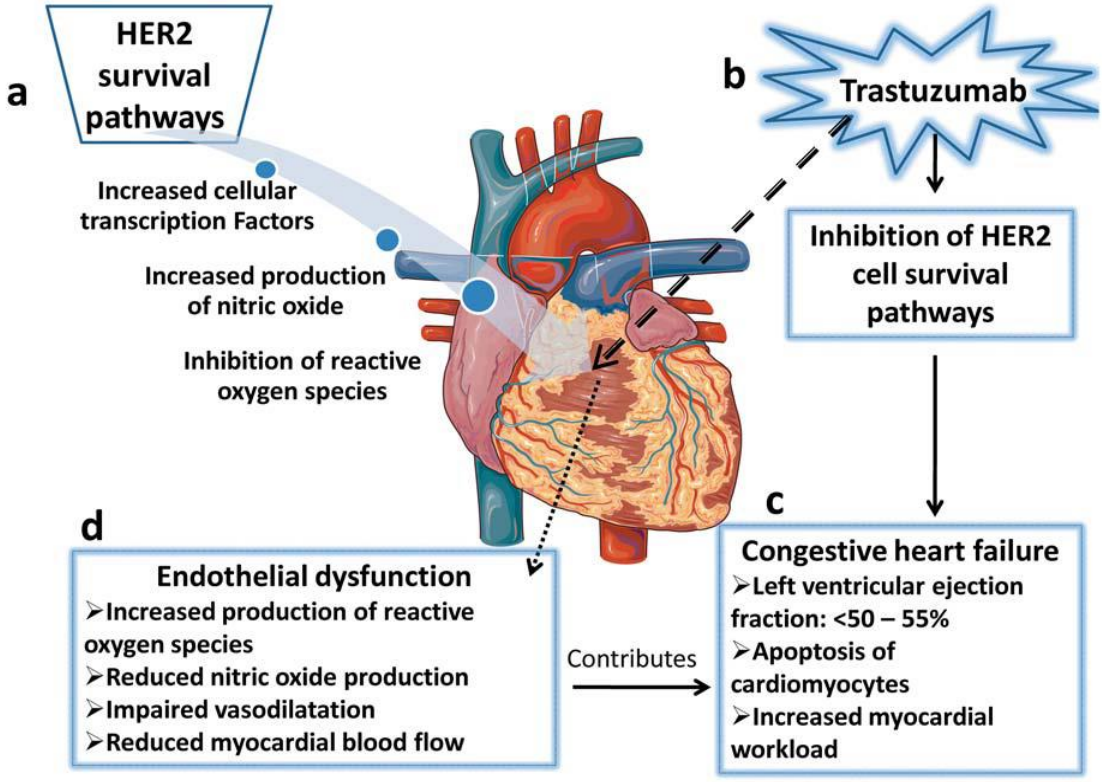


Figure 2.4. a: The cellular transcription factors which prevent apoptosis of cardiomyocytes increase by the HER2 pathways. Nitric oxide increases vasodilatation of the coronary blood vessels allowing an increase in myocardial blood flow. HER2 signaling also helps to regulate the integrity of the sarcomeres in cardiomyocytes. b: Trastuzumab inhibits HER2 cell survival pathways and may also contribute to endothelial dysfunction. c: Trastuzumab isn't specified in patients with a pre-treatment left ventricular ejection fraction of <55%, and should be halted if it's <50% when treatment has commenced. Apoptosis of cardiomyocytes reduces contractile efficiency, and this increases myocardial workload. d: Inhibition of HER2 pathways can affect the vasculature by increasing production of reactive oxygen species, which injure the endothelium and reduce the production of nitric oxide. This causes impaired vasodilatation along with a concomitant decrease in myocardial blood flow. Endothelial dysfunction is a well-established contributor to congestive heart failure

2.3. Oxidative Stress And Breast Cancer

Oxidative stress is caused by an imbalance in the redox status of the body. In such a condition, the tissue damage refers to increase of free radicals in the body. Individual of the majority important species of free radicals is reactive oxygen species (ROS) produced by various metabolic pathways, including aerobic metabolism in the mitochondrial respiratory chain. It plays a critical role in the initiation and progression of various types of cancers, it changes different signaling pathways, including growth factors and

mitogenic pathways, and controls many cellular processes, including cell proliferation, and thus stimulates the uncontrolled growth of cells which encourages the development of tumors and begins the process of carcinogenesis. The reactive species can reduce the body's antioxidant defense against angiogenesis and metastasis in cancer cells lead to increased oxidative stress. These processes are the main factors in the development of cancer lead to current processes. The free radicals in that create such compounds as malondialdehyde (MDA) and hydroxyguanosine cause by bimolecular reactions. These substances can be used as indicators of cancer. reported, free radicals as oxidizing agents, antioxidants as the immune system, and the role of oxidative stress in cancer, particularly breast cancer, have been investigated in the hope that better identification of the factors involved in the occurrence and spread of cancer will improve the identification of treatment goals (Nourazarian et al. 2014).

2.4. Breast Cancer And Obesity

In latest years, obesity has been identified as a risk factor for the development of breast cancer in postmenopausal women, and it has been associated with a poor outcome. Several factors appear to be important in the mechanism of this increased risk, including estrogen, estrogen receptors, and the adipokines leptin and adiponectin. The potent mitogen for mammary cells are called Estrogen, has long been implicated in the advance of mammary tumors. Since adipose associated aromatase action increases the conversion of androgen to estrogen, the mammary adipose tissue is thought to be an important source of local estrogen production. Leptin that will increase within the circulation in proportion to body fat stores has been demonstrated in vitro to promote breast cancer cell growth. Animal models have additionally known leptin as an important factor for the development of mammary tumors. In distinction to leptin, serum adiponectin concentrations are inversely related to body fat stores, and therefore the addition of adiponectin to human breast cancer cells reduces cell proliferation and enhances apoptosis. investigates the correlation between these factors and the development of mammary cancer in humans (Cleary et al. 2010).

2.5. Breast Cancer And Lipid Profile

The correlation between lipids and breast cancer is obscure. Until now, conflicting results are reportable on the association between lipids and risk of breast cancer in women (Shah et al. 2008). Studies suggest that there is a connection between hyper triglyceridemia, obesity, and breast cancer risk and confirms a protective role for physical activity on breast cancer risk. Study incriminates obesity and dietary fat as breast cancer risk factors. Obesity prevalence was higher in the patient group than in the control group and the difference was statistically significant. Physical action exerts an undeniable protective protecting impact on breast cancer. The analysis of the results of lipid parameters (CT, TG, HDL-C, LDL-C) used as markers of a diet based on nutrient-rich foods showed a significant disruption in the lipid profile which involved a triglyceride dosage in the group of women with breast cancer. The data gathered through this study highlight the necessity for sustainable lifestyle practices that are geared toward decreasing energy intake through a balanced diet and regular physical activity to reduce the risk of breast cancer. Prospective studies involving biomarker analysis and dietary questionnaires among populations with different dietary habits are needed to bring to light the role that nutrients, in particular, dietary lipids, play in mammary carcinogenesis among women (Laamiri et al. 2013). Clearly, breast cancer and diet meets this premise. In general, animal model evidence indicates that fat exerts its effect on the promotion and progression stages of tumor genesis, that the effect is independent of caloric intake, that the dose-response effect is nonlinear, and that the type of fat is important in this effect (Wynder et al. 1997). Blood lipid and lipoprotein levels are also influenced by environmental factors and are associated with some breast cancer risk factors. Observed whether serially evaluates of serum lipids and lipoproteins were related to breast cancer risk, these results demonstrate that serum lipids are associated with breast cancer risk in women with extensive mammographic density. The possibility that interventions for heart condition bar, that aim to reduce non-HDL-C or raise HDL-C, may have effects on breast cancer risk deserves examination (Martin et al. 2015).

2.6. Interleukin 1-R1 (Il-33 Receptor or ST2)

Interleukin-33 (IL-33) is an element of the IL-1 gene family and mostly uttered in the nucleus of tissue coating cells, stromal cells, and triggered myeloid cells. IL-33 is considered damage-associated molecular pattern (DAMP) molecule and acting an important role in many physiological and pathological settings such as tissue repair, allergy, autoimmune disease, infectious disease, and cancer. The biological roles of IL-33 consist of maintaining tissue homeostasis, attractive type 1 and 2 cellular immune responses, and mediating fibrosis throughout chronic inflammation. IL-33 exerts various functions through signaling via its receptor ST2 that is expressed in many sorts of cells including regulatory T cells (Treg), group 2 innate lymphoid cells (ILC2s), myeloid cells, cytotoxic NK cells, Th2 cells, Th1 cells, and CD8+ T cells. Cancer increase outcomes in down-regulation of IL-33 in epithelial cells but up-regulation of IL-33 in the tumor stroma and serum. IL-33 expression in tumor cells increases immunogenicity and promotes type 1 antitumor immune responses through CD8+ T cells and NK cells, while myeloid-derived suppressor cell (MDSC) and Treg lead to IL-33 in tumor stroma and serum facilitates immune suppression. Understanding the function of IL-33 in cancer immunobiology sheds lights on targeting this cytokine for cancer immunotherapy (Lu et al. 2016). The IL-33 N-terminus includes a nuclear localization signal and a homeodomain-like helix-turn-helix DNA-binding domain as well as a chromatin-binding domain (Gillibert-Duplantier et al. 2012). IL-33 mRNA is expressed in many organs − high levels of IL-33 mRNA are detectable in stomach, lung, spinal cord, brain and skin. It is expressed by a diverse range of cells with the strongest expression observed in non-haematopoietic cells including endothelial cells, epithelial cells, keratinocytes, fibroblasts, fibrocytes and smooth muscle cells (Kuchler et al. 2008). Lower expression is reported in some activated leukocytes especially innate immune cells e.g. mast cells, macrophages, and DCs. IL-33 can be induced by a variety of immune stimuli, for example, pro-inflammatory TLR ligands, cytokines and immune complexes (Zhao and Hu 2012). The IL-33 receptor (ST2) was first described in advance of the detection of IL-33 hence the potentially confusing terminology. Suppression of tumourigenicity 2 (ST2), also known as T1, DER4, and FIT-1, was originally cloned as an oncogene-induced gene from murine fibroblasts. A second similar ST2 mRNA transcript was also detected and predicted to code for a receptor, now known as the transmembrane bound

ST2L receptor (Molofsky et al. 2015). The originally identified protein is now known to be a secreted soluble form or "decoy receptor" of ST2, termed sST2. Both of these proteins contain three identical Ig extracellular domains, although sST2 lacks the transmembrane sequence it contains an additional 9 amino acids at the C terminus. Both ST2 isoforms are members of the Ig superfamily and ST2L specifically belongs to the Toll/IL-1R (TIR) superfamily, as it shows 29% homology to the IL-1R, and contains the three distinctive extracellular Ig domains and homology to the intracellular domain of IL-1R1 (Trajkovic et al. 2004).

2.6.1. Interleukin 1-R1 (Il-33 Receptor or ST2) In Angiogenesis

Angiogenesis is critical to wound repair. Recently formed blood vessels participate in provisional granulation tissue formation and provide nutrition and oxygen to growing tissues. Angiogenesis, in response to tissue injury, is a dynamic process that is highly regulated by signals from both serum and the surrounding extracellular matrix (ECM) environment. Some cytokines implicated in inflammation have been shown to induce angiogenesis and increase vascular permeability and as such play a key role in regulating inflammatory angiogenesis. Given the role of IL-33 in promoting wound healing in response to both disease and trauma-induced tissue damage, it is therefore perhaps unsurprising that clear evidence has been presented to show that IL-33/ST2 can directly drive angiogenesis. IL-33 is strongly expressed in endothelial cells (EC), with IL-33 promoting proliferation, migration and morphological differentiation of EC (Pollheimer et al. 2013). In addition, IL-33 promotes angiogenesis, increases vascular permeability, and induces activation of endothelial cells toward an inflammatory phenotype through up-regulation of IL-6, IL-8, monocyte chemoattractant protein-1, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and endothelial selectin (Aoki et al. 2010). IL-33 has also been shown to increase the production of urokinase-type plasminogen activator (u-PA) in EC (Stojkovic et al. 2014). IL-33 induces angiogenesis and vascular permeability through ST2 and multiple inflammatory angiogenic factors such as vascular endothelial growth factor (VEGF) (Choi et al. 2009).

2.6.2. Interleukin 1-R1 (Il-33 Receptor or ST2) In Tumorigenesis

The observations were that the mechanisms of wound healing and the formation of tumour stroma had similar connective tissue components, including fibroblasts, blood and lymphatic vessels, inflammatory cells, and extracellular matrix. In distinction to therapeutic wounds, chronicity of the inflammatory phase leads to uncontrolled cell proliferation, invasion, and metastasis. As outlined above, the IL-33/ST2 pathway participates in many of these processes, demonstrating clear direct effects on angiogenesis, production of matrix components, on fibrosis that can lead to tumour formation, and on modulation of immune populations which can, therefore, affect the tumour microenvironment. Unsurprisingly, therefore, links between the IL-33/ST2 signaling axis and tumorigenesis have recently been identified. In a parallel manner to the divergent roles of IL-33/ST2 reported in many of the processes associated with wound healing, both pro- and anti-tumorigenic roles have been reported for IL-33 and ST2 in cancer. Initially, the relationship between IL-33/ST2 and cancer was known in BC. Early studies utilizing ST2 demonstrated that ST2 removal inhibited breast cancer progression and increased the intra-tumoral accumulation of both innate (NK cell) and acquired immunity (CD8+ T-cells) and Th1/Th17 cytokines, indicating that an absence of IL-33 signaling through ST2L promotes a Th1 response 21484786. In addition, suppressing sST2 reduced ErbB2-induced cell motility in breast cancer cells. Furthermore, breast cancer patients with metastatic disease showed increased levels of circulating sST2 compared to patients with primary tumours (Gillibert-Duplantier et al. 2012).

2.7. Cardiac Biomarkers

Cardiac enzyme studies measure the levels of enzymes that are linked with an injury of the heart muscle. Low levels of these enzymes and proteins are normally found in your blood, but if your heart muscle is injured, such as from a heart attack, the enzymes and proteins leak out of damaged heart muscle cells, and their levels in the bloodstream rise.
The current WHO criteria for the diagnosis of AMI include the presence of two of the following criteria,

1. Clinical symptoms compatible with acute ischemia.

2. ECG abnormalities.
3. A pattern of enzyme release typically of myocardial Injury (Thygesen et al. 2007).

2.8. Creatine Kinase (CK)

The initial CK-MB rise occurs 4 to 6 hours after the onset of chest pain, peaks at 24 hours, and returns to baseline at 48 to 72 hours. One advantage of CK-MB over other markers is that it remains elevated for longer periods (Ghormade et al. 2014).

2.8.1. Troponin

Troponin is the gold standard biomarker for myocardial injury and is used usually in conjunction with creatine kinase-MB (CK-MB) and myoglobin to enable a more rapid diagnosis of acute coronary syndrome ACS. Serum levels can remain elevated for up to 4–7 days for troponin I, and 10–14 days for troponin T (Daubert and Jeremias 2010, Ahmad and Sharma 2012). Cardiac troponin is more specific than other markers for myocardial injury. Following AMI, cTnI becomes elevated at the same rate as CK-MB, but it remains elevated for 7 to 10 days. As troponin is nearly absolutely specific to myocardial tissue and exhibits high clinical sensitivity, it is the preferred biomarker for myocardial necrosis. Nowadays when all reperfusion strategies have to be instituted within minutes of patients arrival in an emergency with ST elevation the role of biomarkers is reducing since they begin to rise after 3-6hrs and the lab report can also take time (Selhub 1999, Thygesen et al. 2007). Cardiac troponins are used in the diagnosis of MI. Troponin is a complex contractile protein comprising of three subunits: C, T, and I. Troponin T and I are cardio-specific, used in MI diagnosis(Bishop et al. 2005). Troponin is a complex of globular muscle protein I band that inhibits contraction by blocking the interaction of actin and myosin; when combined with Ca+2. It so modifies the position of the tropomyosin molecules that interaction takes place. Troponins rise within a few hours on the onset of symptoms and remain elevated for 1-2 weeks. This property enables early as well as late diagnosis. The diagnostic sensitivity of troponin reaches 100% 12 hours after onset of symptoms (Gaw et al. 2013). In previous studies, they investigate troponin I, because cardiac troponin I is not expressed in skeletal muscle, so is the most sensitive for MI. Serum cardiac troponin I > 0.4 ng/ml suggests

myocardial damage (Kasap et al. 2007, Costa et al. 2008, Khan et al. 2013). (Chia et al. 2008) found that a strong correlation of troponin I with the extent of myocardial cTn I is a cardiac-specific protein, which rapidly increases after MI by a release of a loosely bound pool, as a result of degradation of myofilaments in the area of infarction, so more infarct tissue outcome in additional cardiac troponin release. Cardiac troponins are contractile regulatory peptides, and with cardiac muscle injury, they spill into circulating blood. They are used as diagnostic biomarkers, especially for the acute coronary syndrome (Apple et al. 2009).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Instruments

Equipment's that used in this study are shown in the Table 3.1.

Table 3.1. List of applied instruments

Instruments	**Original country**
COBAS INTEGRA 400 Plus System	Roche Diagnostics/U.S.A
Vortex	USA
Centrifuge	Germany
ELISA Washer and Reader	Bioteck/ U.S.A.
Handle Glass Homogenizer	Turkey
Incubator	Gallen Kamp Type/England
Autoanalyzer biochemistry	bt products, bt35i , turkey
Magnetic stirrer	Gallen-Kamp type-England
Automated Haematology Analyzer	Syamex model: K-1000/ Japan
Electronic balance	A super scientific/ China
Spectrophotometer	APEL/Japan
Water Bath	Memmert/Germany
Oven	Tafesa/ U.S.A.
Sensitive balance	NAD HF 400 type-Japan
Tornica	Turkey
Syringe (5ml,10ml)	Set Medical / Turkey
Gloves	China
Micropipette 100µl-1000µl + tips	Boeco /Germany
Chemical tube (Gel and clot act. tube)	Vacu test/ Italy
Hematology tube (K2EDTA)	Vacu test /Italy

3.1.2. Chemicals

Chemicals that used in this study are shown in the Table 3.2.

Table 3.2. List of Chemicals used in the study's experiment

Chemicals	**Original country**
$C_{6470}H_{10012}N_{1726}O_{2013}S_{42}$ (Trastuzumab)	Genentech, inc. / Swiss
CCl_3COOH (TCA)	Riedel-de Hean/ Germany
$C_4H_{10}O$ (TBA)	Riedel-de Hean/ Germany
$C_2H_{10}Cl_2N_2$ (NEED)	Riedel-de Hean/ Germany
NaOH	BDH/ England
$C_6H_7NO_3S$ (Sulfanilic Acid)	BDH/ England
$H3PO_4$	BDH/ England
$NaNO_2$	BDH/ England
C_6H_6NO6P (p-nitrophenyl phosphate)	N.S Biotic /Egypt
$C_6H_5NO_3$ (p-nitrophenol)	N.S Biotic /Egypt
H_3PO_4	Yacoo, inc. China
$ZnSO_4$	BDH/ England

3.1.3. Kits

Kits that used in this study are shown in the Table 3.3.

Table 3.3. List of Kits applied for performing the laboratory measurements

Kits	**Original country**
Commercial Kits for Determination of Interleukin 1 Receptor-like 2 IL-1RL1 / ST2 Test	Bosters Human was IL1R1 ELISA/USA
Commercial Kits for Determination of Cardiac Function Creatine kinase-MB and Troponin I Tests	Roche, Cobas kit/ Germany
Kits for Determination of Lipid Profile Tests	bt products, bt35i kit/turkey
Kits for Determination of Blood Glucose Test	bt products, bt35i kit/turkey
Kits for Determination of Complete Blood CountTests	bt products, bt35i kit/turkey

3.2. Methods

3.2.1. Sample Collection

Blood samples were collected from Epidermal growth factor receptor-positive-2 Breast cancer subjects, by using disposable syringes, 15 ml of early morning venous blood samples were drawn aseptically from each subject, 2,0 ml of this volume was collected with ethylene diamine tetra acetic acid (EDTA) tube for hematological evaluation complete blood count and the remaining volume (13,0 ml) of blood was collected in a gel tube for 15 minutes at room temperature. Serum was separated by centrifugation at 5000 rpm for five minutes, (3,0 ml) of serum samples was employed for the estimation of total cholesterol, Triglycerides, LDL-cholesterol, HDL-cholesterol, blood glucose, complete blood count and the remaining volume (10,0 ml) then each subject serum was stored and frozen at -20 C^{o}. The clear serum samples were employed for the estimation of cardiac biomarkers (Creatine kinase-MB, troponin I), oxidative stress markers (Malondialdehyde MDA, nitrite (NO_2), Interleukin 1 Receptor-like 2 (IL-1RL1 / ST2)). The collection sample was performed within (9) months from 1 March to 1 November 2016, at Nanakaly hospital for blood and oncology diseases, surgical specialty hospital Erbil Cardiac Center and Scientific Research Center of Erbil Polytechnic University at Erbil Province Erbil city were eligible for the study.

3.2.2. Design Of The Study

The present study is a case-control study. The subjects of our study were grouped into two categories:

- Breast cancer, epidermal growth factor receptor-positive-2 (HER2-positive) controls patients (group I): Twenty (20) selected subjects served as control, all patients as controls do not received trastuzumab.
- Breast cancer, epidermal growth factor receptor positive-2 (HER2-positive) patients (group II): Forty-four (44) patients received chemotherapy (Target therapy) trastuzumab.

3.2.3. Body Mass Index (BMI)

BMI was calculated as weight in kilograms divided by the square of height in meters (weight (kg)/ (height in m). We evaluated each member's standing height using anthropometry. The patient stood with the heels together and toes apart and the back of the head. We evaluated height to one decimal point (cm) with an anthropometric bar on the participant's head. Participants were weight in kilograms using a scale when gradation had stabilised after setting the scale to zero with no load. Participants were classified into five groups based on their baseline BMI at admission: underweight (BMI<18,5), normal (18,5≤BMI<23), overweight (23≤BMI<25), obese (25≤BMI<30), and extremely obese (30≤BMI).

3.2.4. Hematological Measurements

Estimation of total Leukocyte counts (WBCs), Eosinophil ,hemoglobin (Hgb) ,haematocrit (Hct) ,mean platelet volume (MPV) , mean corpuscular volume (MCV) , mean corpuscular hemoglobin (MCH) RDW%,PDW% and platelets (PLT) Parameters were made using 'Hemolyzer 5'Analyzer.

3.2.5. Biochemical Assay

3.2.5.1. Determination Of Serum Lipid Profile Tests Parameters

3.2.5.1.1. Determination Of Serum Cholesterol

Serum cholesterol has been determined by a colorimetric method using the commercial kit (bt products-turkey) according to the method of (Deacon and Dawson 1979) using the Autoanalyzer biochemistry (bt products, bt35i, turkey).

Principle

$$\text{Cholesterol Esters} + H_2O \xrightarrow{\text{C.Esterase}} \text{Cholestrol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{C. Oxidase}} \text{Cholest-4-en-one} + H_2O_2$$

$$2H_2O_2 + PHE + 4AAP \xrightarrow[\text{(Red dye)}]{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Calculation

(Abs = Absorbance)

$$\frac{\text{Abs. of Sample}}{\text{Blank}} \text{ X conc. of standard (mg /dL) = Cholesterol (mg/dL)}$$

3.2.5.1.2. Determination Of Serum Triglycerides

Serum triglycerides have been determined by a colorimetric method using the commercial kit (bt products-turkey) according to the method of (Tietz 1995). Using the Autoanalyzer biochemistry (bt products, bt35i, turkey).

Principle

$$\text{Triglycerides} + H2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{GK}} G3P + ADP$$

$$G_3P + O_2 \xrightarrow{\text{GPO}} DAP + H_2O_2$$

$$H_2O_2 + 4AP + \text{p-chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + H_2O$$

Calculation

$$\frac{\text{Abs. of Sample}}{\text{Abs.of Standard}} \text{ X conc. of standard = Triglycerides (mg/dL)}$$

3.2.5.1.3. Determination Of Serum HDL

Serum HDL has been determined by a colorimetric method using the commercial kit (bt products-turkey) according to the method of (Grillo et al. 1981) using the Autoanalyzer biochemistry (bt products, bt35i, turkey).

Principle

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methylether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. VLDL, LDL, and chylomicron (CM) reacts with PVS and PEGME and the reaction outcomes in inaccessibility of VLDL, LDL, and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER).The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Trinder reaction.

Calculation

($\Delta Abs = Abs2 - Abs1$)

$$\frac{\Delta Abs.of\ Sample}{\Delta Abs.of\ Standard}\ X\ conc.\ of\ standard = HDL - Cholesterol\ (mg/dL)$$

3.2.5.1.4. Determination of Serum LDL

Calculation of serum LDL:

Low-density lipoprotein was calculated by using the Friedewald equation as following (Friedewald et al. 1972) that is when all concentrations are in mg/dL.

[LDL]= [Total cholesterol] − [HDL] − [TG]/5

3.2.5.2. Blood Glucose Determination

Blood samples from control and treated patients were collected by vein hand by needle syringe and analyzed for glucose level employing by using the Autoanalyzer biochemistry (bt products, bt35i, turkey).

3.2.5.3. Determination Of Cardiac Biomarkers (Creatine Kinase-MB, Troponin I)

The alter triage cardiac panel is a single use fluorescence immunoassay device designed to determine the concentration of Creatine Kinase-MB **(**CK-MB), and troponin I in EDTA anticoagulated plasma specimens. The test procedure involves the addition of several drops of an EDTA anticoagulated plasma specimen to the sample port on the test device. The specimen reacts with fluorescent antibody conjugates and flows through the test, after addition of the specimen device by capillary action. Complexes of each fluorescent antibody conjugate are captures on discrete zones specific for each analysis. The test device is inserted into the Alert triage meter. The indicator is programmed to carry out the analysis after the specimen has reacted with the reagents within the Test Device. The analysis is based on the amount of fluorescence, the meter detects within a measurement zone on the Test Device. The concentration of the analysts in the specimen is directly proportional to the fluorescence detected. The meter measures the target analyst automatically. The results are displayed on the meter screen in approximately 20 minutes from the addition of specimen. Each result is stored in the meter memory to display or print when needed.

3.2.5.4. Determination Of Oxidative Stress Markers (MDA, NO_2, and IL-1RL1 /ST2)

3.2.5.4.1. Determination Of Serum Malondialdehyde (MDA)

The assessment of the lipid peroxidation process is achieved via the determination of the end product, malondialdehyde (Muslih et al. 2002). Spectrophotometrically with a TBA solution was determined the level of serum MDA. In brief: To 150 µl serum sample added the followings: 1ml trichloroacetic acid (TCA) 17.5% and 1ml of 0.66% thiobarbituric acid (TBA), mixed well by vortex, incubated in boiling water for fifteen minutes, and after that allowed to cool. One ml of 70% TCA was added and let the mixture to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, and take out the supernatant for scanning spectrophotometrically at (532nm).The concentration of MDA calculated as follow:

$$\text{MDA (Mmol/L)} = \frac{\text{Absorbance at 532nm} \times \text{D.}}{\text{L} \times \text{E0}}$$

L: light path (1cm)

E0: Extinction coefficient 1.56×105 M-1 .Cm-1

D: Dilution factor = 1 ml Vol. Used in ref./0.15 =6.7

3.2.5.4.2. Determination Of Serum Nitrite (NO_2)

Methods developed by (Moshtaghi Kashanian and Shapiro 1994) based on Griess reaction were used. For nitrite assay, samples were deproteinized by mixing of (1 ml of sample), (10µl of 10 M NaOH) and (600 µl of 0.15M $ZnSO_4$), Vortex, then let stand on ice for 15 minutes, and centrifuged 5 min at 12,000 g. In nitrite assay, Griess Reagent prepared by mixing an equal volume of 0.1% N-(1-Naphthyl) Ethylenediamine dihydrochloride (NEED) (1 mg NEED/mlH_2O) and 1% Sulfanilic Acid in 5% H_3PO_4 (10 mg Sulfanilic Acid/ml H_3PO_4), then mix equal volumes of Griess Reagent and deproteinized sample. Let it at least 10 min at room temperature and test the absorbance on a spectrophotometer at 543 nm. Sodium nitrite ($NaNO_2$) was used for preparing the standard curve through plotting absorbance versus concentration. Eight standard concentrations were used with each assay (5 µM, 10 µM, 25 µM, 50 µM, 75 µM, 100 µM, 250 µM and 500 µM) for $NaNO_2$ as a standard for nitrite (figure 3.1).

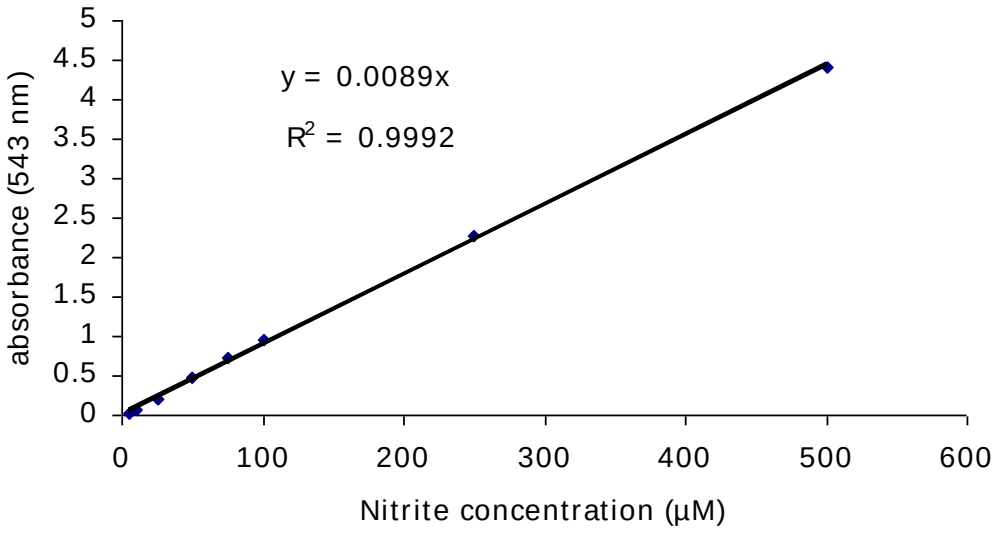


Figure 3.1: Standard curve of nitrite concentration measurement (µM)

3.2.5.4.3. Determination Of Serum Interleukin 1 Receptor-like 2 (IL-1RL1 / ST2)

Interleukin 1 receptor-like 1, also known as ILI RL1 or ST2, is a protein that in humans is encoded by the IL1RL1 gene. The protein determined by this gene is a member of the interleukin 1 receptor family. this receptor can be induced by proinflammatory stimuli that studies of the similar gene in mouse suggested, and may be involved in the function of helper T cells TL1RL1 is essential for endotoxin tolerance and, by inhibiting TLR responses, enhances Th2 responses This gene, interleukin 1 receptor, type I (IL1R1), interleukin 1 receptor, type II (IL1R2) and interleukin 1 receptor-like 2 (IL1RL2) form a cytokine receptor gene cluster in a region mapped to chromosome 2q12 (Brint et al. 2004, Schmitz et al. 2005).

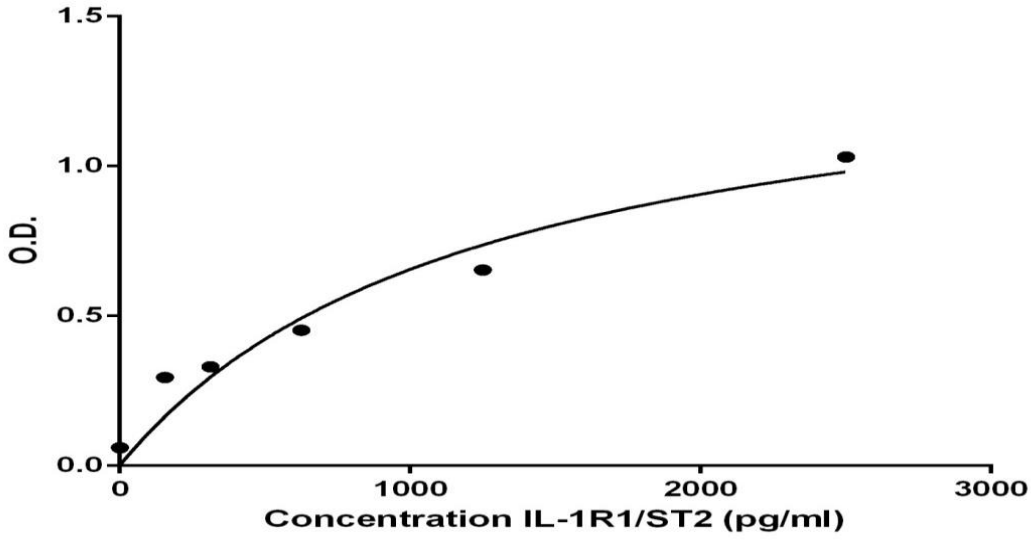


Figure 3.2. Standard curve of concentration IL1R1.1 measurement (pg/ml)

Principle

Bosters Human was IL1R1 ELISA Kit based on standard sandwich enzyme-linked immune-sorbent assay technology. Monoclonal antibody from mouse specific for IL1 RL1 has been precoated onto 96-well plates. Standars (Expression system for standard: NSO,immunogen sequence: K19-S328) and test samples are added to the wells a biotinylated detection polyclonal antibody from goat specific for IL1R1.1 is added subsequently and then followed by washing with PBS or TBS buffer incubation with Avidin-Biotin-Peroxidase Complex was added and

unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. HRP was catalyzed TMB to create a blue color creation that altered into yellow after adding acidic stop solution. The density of yellow staining intensity is proportional to the human IL1RLI amount of sample captured in plate.

Assay Procedure

The ABC functioning solution and TMB color increasing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard 1L1RL1 detection curve should be prepared for each experiment. The employer will make a decision sample dilution fold by crude estimation of IL1RL1 amount in samples. 1. Aliquot 0.1m1 per well of the 10000pg/ml, 5000pg/mI, 2500pg/ml, 1250pg/ml, 625pg/mI, 312pg/ml, 156pg/mI human IL1RL1 standard solutions into the precoated 96-well plate Add 0 1m1 of the sample diluents buffer into the control well (Zero well). Add 0.1m1 of each properly diluted sample of human cell culture supernates, serum or plasma (heparin, EDTA) to each empty well. It's suggested that each human IL1RL1 customary solution and every sample be measured in duplicate. 2. Seal the plate with a new adhesive cover provided and incubate at 374C for 90 min. 3. Eliminate the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time. 4. Add 0,1m1 of biotinylated anti-human ILI RL1 antibody working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C for 60 min. 5. Wash plate 3 times with 0,01M TBS or 0,01M PBS and each time let washing buffer stay in the wells for 1 min. Remove the washing buffer and blot the plate onto paper towels or other absorbent material (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0,3 nil PBS or TBS buffer for 1-2 minutes. Repeat this process two additional times for a total of THREE washes. Correspondence For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffers overfilling wells with PBS or TBS buffer Blot the plate onto paper towels or other absorbent material). 6. Add 0,1m1 of prepared ABC working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C for 30 min. 7. Wash

plate 5 times with 0,01m TBS or 0,01M PBS and each time let washing butter stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

3.3. Statistical Analysis

The data analyses of the present study were performed using GraphPad Prism version 6. Student t (Unpaired) test, Mann-Whitney test and one way ANOVA were applied for comparison between treatments. All data are expressed as Mean and standard error or in Median and percentile according to the nature of the data.

4. RESULTS

4.1. Comparative Analysis Of Biochemical Data Of Patients Treated With Trastuzumab And Untreated Patients

4.1. 1. Interleukin 1-R1 (Il-33 Receptor or ST2)

The data analysis revealed that trastuzumab treatment does not affect the level of interleukin (IL-1R1) in treated patients was (323 ± 28; N=44 pg/ml) which was less than controls control (357 ± 8.6; N=20 pg/ml) as illustrated in [Figure 4.1.].

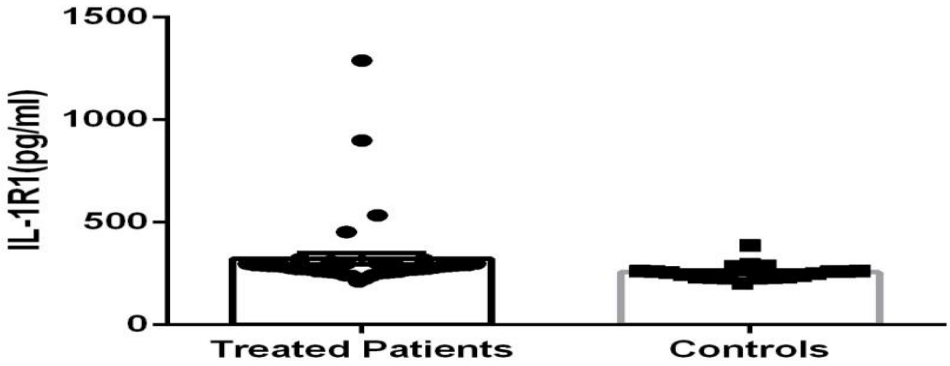


Figure 4.1. Serum interleukin 1-R1 (Il-33 receptor or ST2) patients treated with trastuzumab and control individuals. The level of IL-1R1 recorded the non-significant difference between patients and control. The result presented as means ± S.E for the treatment and unpaired t-Test was for comparison between control and treated patients

4.1.2. Cardiac Markers (Troponin and Creatine Kinase-MB)

The current results showed statistically significant differences cardiac markers (Troponin and CKMB) between trastuzumab treated patients and control patients ($p<0.05$). The level of serum troponin elevated by using trastuzumab drug (0.012 ± 0.0012 N=44 ng/ml) when compared to controls (0.0079 ± 0.00096; N=20 ng/ml) as show in [Figure

4.2.A]. The data analysis revealed that trastuzumab treatment does not affect the level of serum creatine kinase-MB (CKMB) in treated patients (1.1 ± 0.088; N=44 ng/ml) which was less than controls (1.2 ± 0.11; N=20 ng/ml) as illustrated in [Figure 4.2.B].

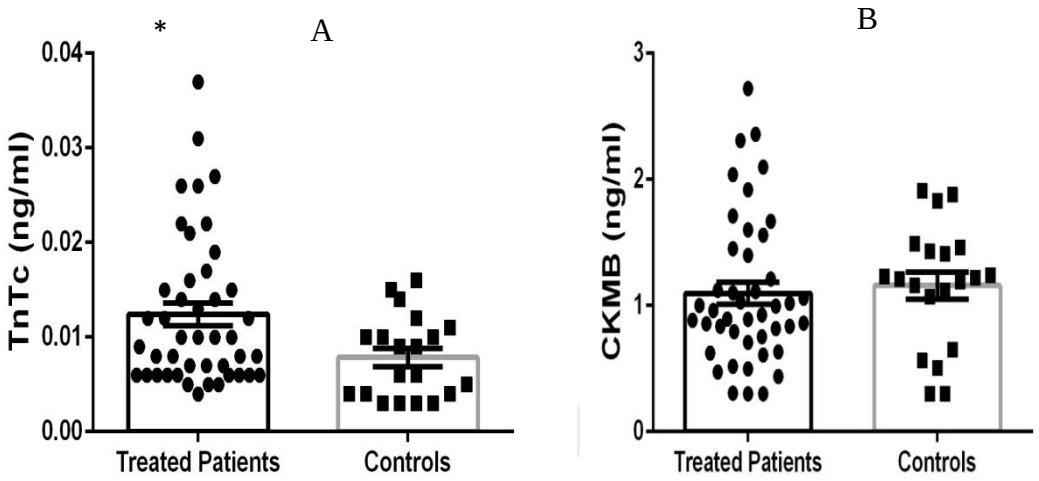


Figure 4.2. Serum cardiac markers patients treated with trastuzumab and control individuals. (A) The level serum Troponin in treated patients significantly increased compared to control ($p<0.05$). (B) The level of serum CKMB recorded the non-significant difference between patients and control. The result presented as means ± S.E for the treatment and unpaired t-Test was for comparison between control and treated patients

4.1.3. Oxidative Stress Markers (Nitrite and Malondialdehyde) (NO_2, MDA)

The present results expressed statistically significant differences oxidative stress markers (Nitrite and MDA) between trastuzumab treated patients and control patients ($p<0.0001$). The level of serum Nitrite (NO_2) elevated by using trastuzumab drug (24 ± 1.2; N=31 Mmol/l) when compared to controls (17 ± 0.94 Mmol/l; N=20) as shown in [Figure 4.3.A]. The data reported that trastuzumab treatment shows significant differences malondialdehyde (MDA) between trastuzumab treated patients and control patients ($p<0.05$). The level of serum MDA increased by using trastuzumab drug (6.1 ± 0.51; N=44 Mmol/l) when compared to controls (4.5 ± 0.23; N=20 Mmol/l) as shown in [Figure 4.3.B].

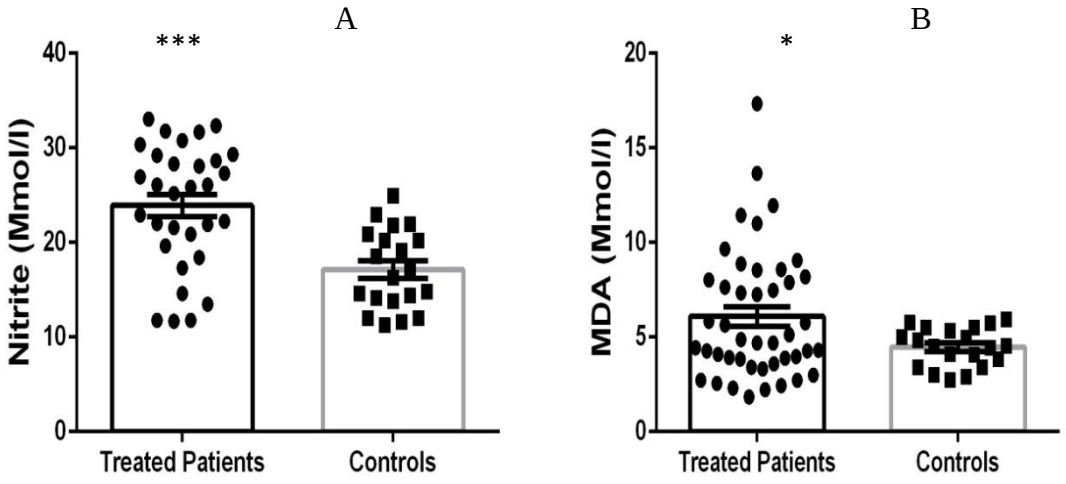


Figure 4.3. Serum oxidative stress markers patients treated with trastuzumab and control individuals. (A) The level of serum nitrite in treated patients significantly high increased compared to control ($p<0.001$). (B) The level of serum malondialdehyde (MDA) in treated patients significantly increased compared to control ($p<0.05$). The result presented as means ± S.E for the treatment and unpaired t-Test was for comparison between control and treated patients

4.1.4. Lipid Profile (Cholesterol, TG and HDL/LDL)

Data analysis detected that trastuzumab therapy does not affect the level of cholesterol in treated patients which was (202 ± 7.5; N=44 mg/dl) and was the non-significant more in comparison the control (195 ± 11; N=20 mg/dl) as represented in [Figure 4.4.A]. Similar result was found regarding TG level. The estimated serum triglyceride (TG) in patients treated with trastuzumab was (199 ± 19; N=44 mg/dl) which was non-significantly more than the triglyceride (TG) level in control patients (180 ± 21; N=20 mg/dl) as shown in [Figure 4.4.B]. Similar result was found regarding HDL/LDL level. The estimated serum HDL/LDL in patients treated with trastuzumab was (0.42 ± 0.030; N=44 ratio) which was non-significantly more than the TG level in serum control patients (0.38 ± 0.062; N=20 ratio) as shown in [Figure 4.4.C].

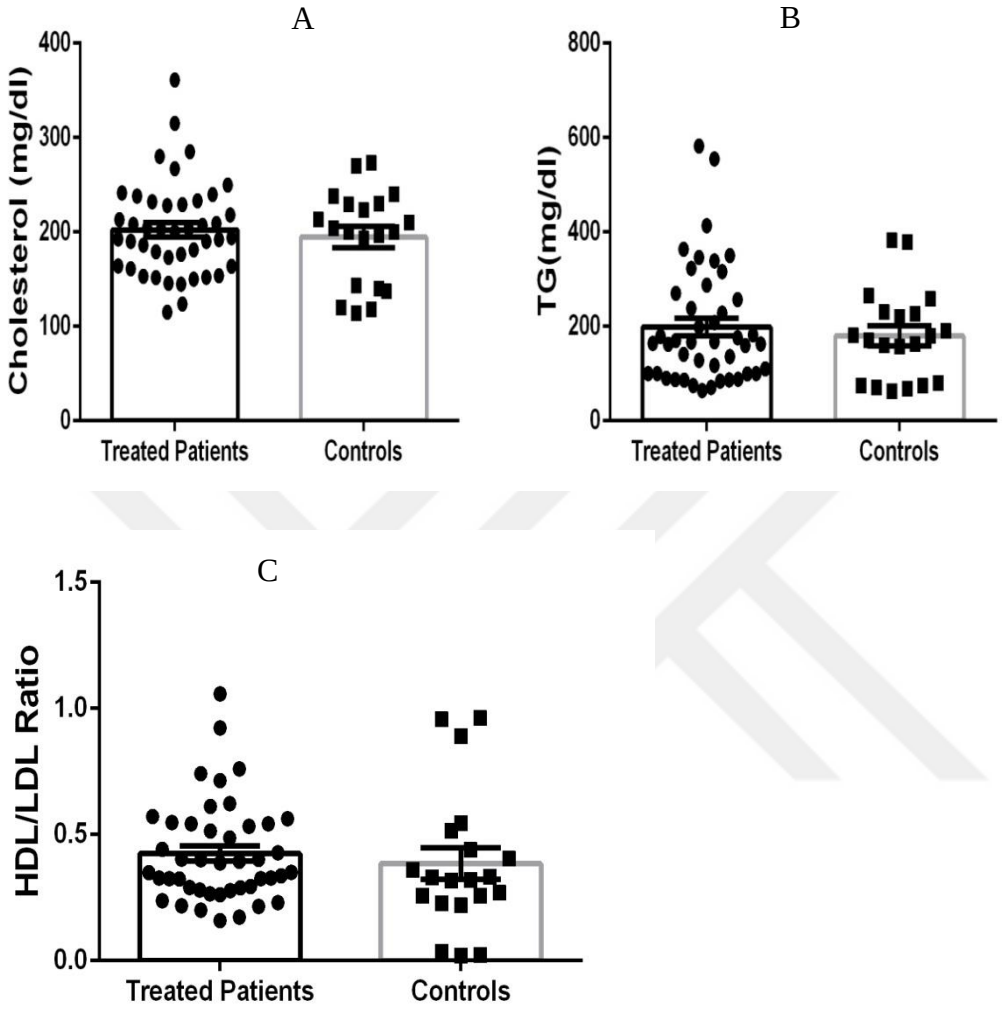


Figure 4.4. Serum lipid profile patients treated with trastuzumab and control individuals. (A) The level of serum total cholesterol which showed non- significant difference between in treated patients and control. (B) The level of serum triglyceride (TG) which showed non- significant difference between in treated patients and control. (C) The level of serum HDL/LDL which showed non- significant difference between in treated patients and control. The result presented as means ± S.E for the treatment and unpaired t-Test was for comparison between control and treated patients

4.1.5. Hematological Measurement (WBCs, HGB, PLT, and MPV)

Trastuzumab treatment does not affect the level of WBCs in treated patients, (6.3±0.46; N=44 *103) which was close to the control (6.3±0.46; N=20 *103) as shown in [Figure 4.5.A]. While trastuzumab treatment patients recorded the non-significant regarding

HGB measures (12 ± 0.19; N=44 mg/dl) when compared to control (12 ± 0.34; N=20 mg/dl) as shown in [Figure 4.5.B]. The results demonstrated statistically significant differences PLT between patients treated with trastuzumab and control patients ($p<0.05$) in PLT count. The level of PLT decreased by using trastuzumab drug (244 ± 12; N=44 10^3) when compared to controls (306 ± 37; N=20 10^3) as shown in [Figure 4.5.C]. The latest outcomes exhibited statistical significant differences in MPV between patients treated with trastuzumab and control patients ($p<0.05$). The level of MPV decreased by using trastuzumab drug (7.9 ± 0.18; N=44 μm^3) when compared to controls (8.7 ± 0.31; N=20 μm^3) as illustrated in [Figure 4.5.D].

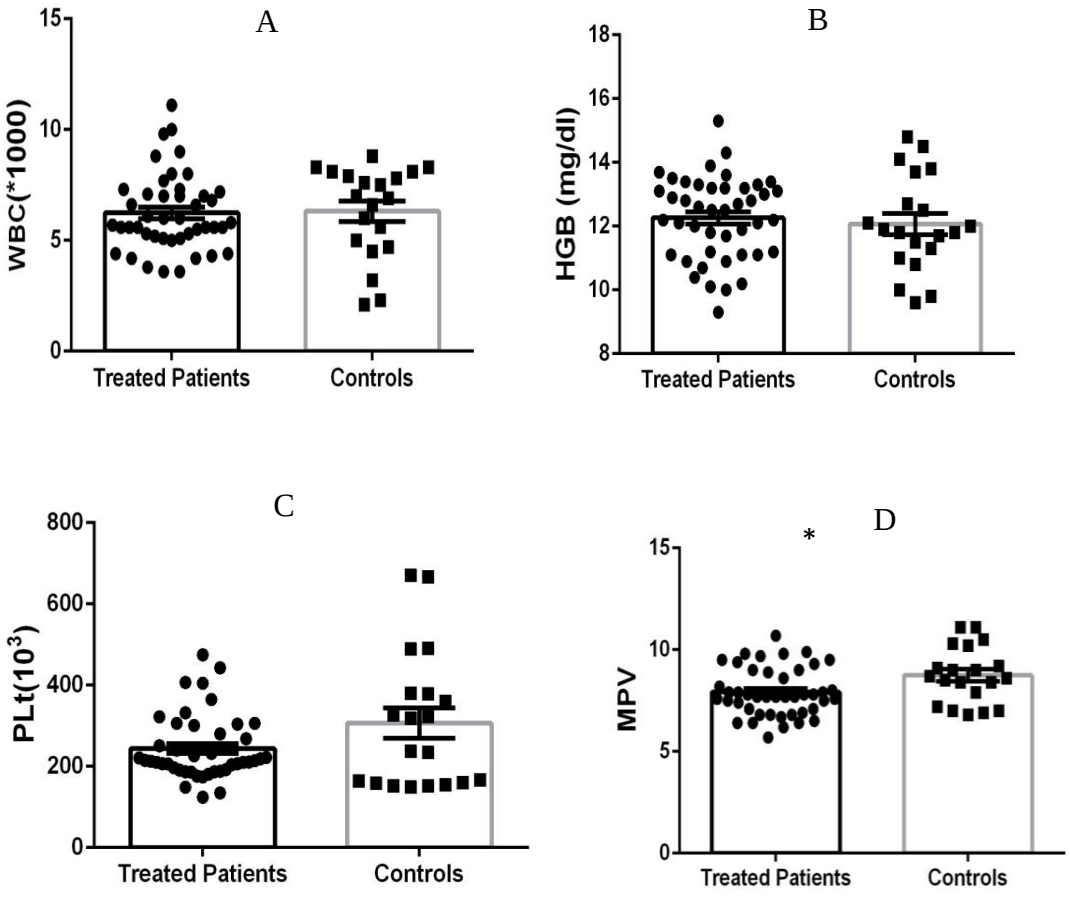


Figure 4.5. Comparison between measure Hematological parameters patients treated with trastuzumab and control individuals. (A) The level of white blood cell was the non-significant different between in treated patients and control individuals. (B) The level of hemoglobin was the non-significant difference patients compared to treated patients and control. (C) The level of platelet was statistically the non-significant difference patients compared to treated patients and control. (D) The level of MPV was the non- significant between in treated patients and control individuals. The result presented as means ± S.E for the treatment and unpaired t-Test was for comparison between control and treated patients

4.1.6. Blood Glucose (BG)

The analysis of obtained data noticed that trastuzumab therapy does not influence the level of blood glucose (BG) in treated patients which was (123 ± 5.8; N=44 mg/dl) and was close to the control (122 ± 3.7; N=20 mg/dl) as illustrated in [Figure 4.6].

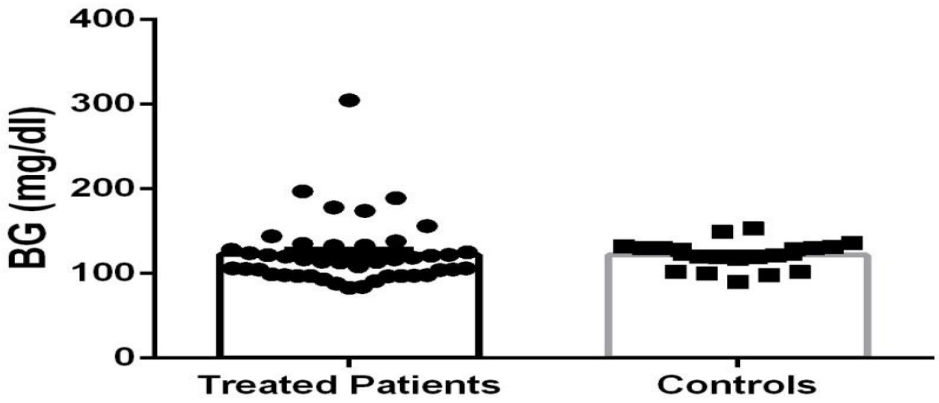


Figure 4.6. Serum blood glucose patients treated with trastuzumab and control individuals. The level of blood glucose (BG) which showed non- significant difference between in treated patients and control. The result presented as means ± S.E for the treatment and unpaired t-Test was for comparison between control and treated patients

4.1.7. Body Mass Index (BMI)

Current result observed that trastuzumab treatment does not affect body mass index (BMI) treated patients was (31 ± 0.79; N=44 kg/m2) which was dose to the control (29 ± 1.3; N=20 kg/m2) as illustrated in [Figure 4.7].

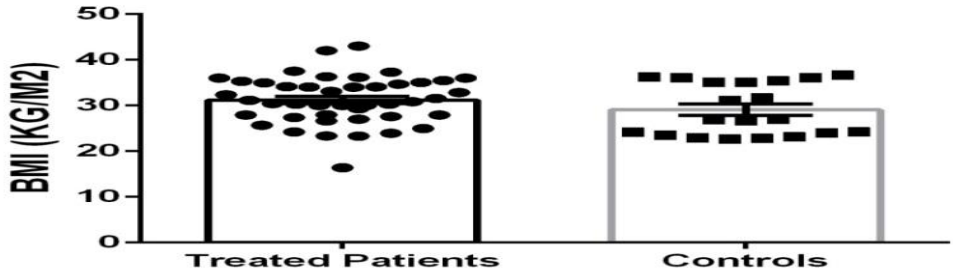


Figure 4.7. Body mass index patients treated with trastuzumab and control individuals. The level of body mass index (BMI) which showed non- significant difference between in treated patients and control. The result presented as means ± S.E for the treatment and unpaired t-Test was for comparison between control and treated patients

4.2. Comparison Patients Treated With Trastuzumab Between Grade II and Grade III of HER2-Positive Breast Cancer.

4.2.1. Interleukin 1-R1 (Il-33 Receptor or ST2)

The current results showed that the level of serum interleukin (IL1-R1) is significantly decreased ($P<0.05$) in Grade III (262 ± 7.6; N=15 pg/ml) comparing to the patients with Grade II (352 ± 42; N=28 pg/ml), however the standard deviation of the data acquired from patients with grade II were higher than expected value as shown in [Figure 4.8].

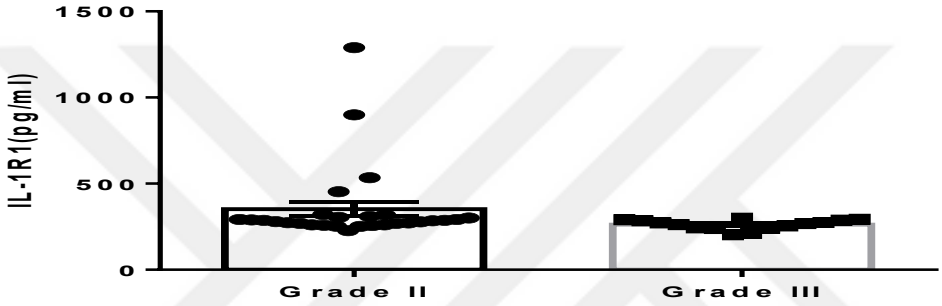


Figure 4.8. Serum of Interleukin 1-R1 (Il-33 receptor or ST2) in patients treated with trastuzumab between grade II and grade III of breast cancer. The level serum IL1-R1 in grade III treated patients significantly decreased comparing to grade II treated patients ($p<0.05$). The result presented as means ± S.E for all treatment and unpaired t-Test was for Comparison patients treated with trastuzumab between grade II and grade III of HER2-positive breast cancer

4.2.2. Cardiac Markers (Troponin and creatine kinase-MB)

The current results showed statistically significant differences cardiac markers (Troponin and creatine kinase-MB) between grade II trastuzumab treated patients and grade III trastuzumab treated patients ($p<0.05$). The level of serum troponin elevated in grade III (0.017 ± 0.0041; N=15 ng/ml) when compared to grade II trastuzumab treated patients (0.010 ± 0.0012; N=28 ng/ml) as shown in [Figure 4.9.A]. The results demonstrated statistically significant differences CKMB between grade II trastuzumab treated patients and grade III trastuzumab treated patients ($p<0.05$) in troponin count. The level of serum CKMB elevated in grade III (1.4 ± 0.18; N=15 ng/ml) when compared to grade II trastuzumab treated patients (0.96 ± 0.092; N=27 ng/ml) as shown in [Figure 4.9.B].

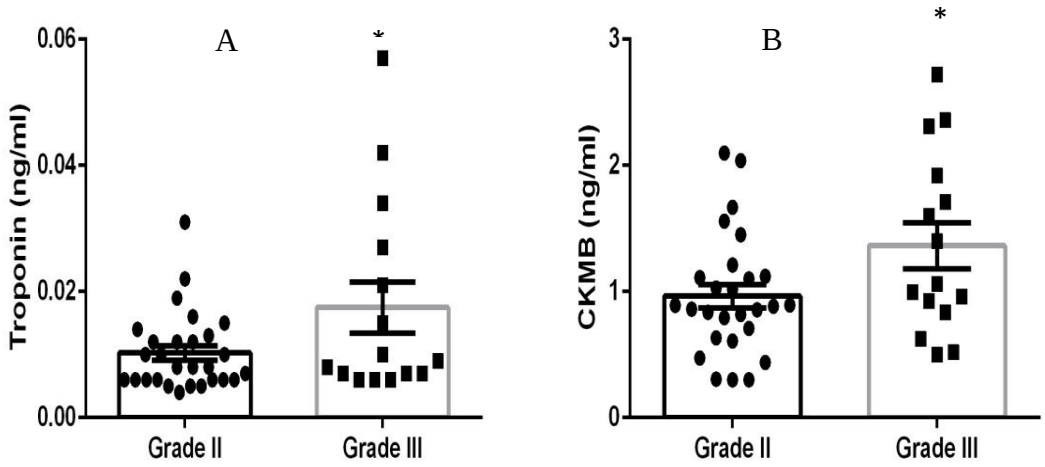


Figure 4.9. Serum cardiac markers patients treated with trastuzumab between grade II and grade III of breast cancer. (A) The level serum Troponin in grade III treated patients significantly increased compared to grade II treated patients ($p<0.05$). (B) The level serum creatine kinase-MB (CKMB) in grade III treated patients significantly increased compared to grade II treated patients ($p<0.05$). The result presented as means ± S.E for all treatment and unpaired t-Test was for Comparison patients treated with trastuzumab between grade II and grade III of breast cancer

4.2.3. Oxidative Stress Markers Nitrite (NO_2) and Malondialdehyde (MDA)

The data reported that trastuzumab treatment does not affect the level of NO_2 in grade III trastuzumab treated patients was (24 ± 2.0; N=10 Mmol/l) which was less than grade II trastuzumab treated patients (24 ± 1.4; N=21 Mmol/l) as illustrated in [Figure 4.10.A].

Trastuzumab treatment does not affect the level of serum MDA in grade III trastuzumab treated patients was (5.5 ± 0.91; N=15 Mmol/l) which was less than grade II trastuzumab treated patients (6.3 ± 0.63; N=28 Mmol/l) as illustrated in [Figure 4.10.B].

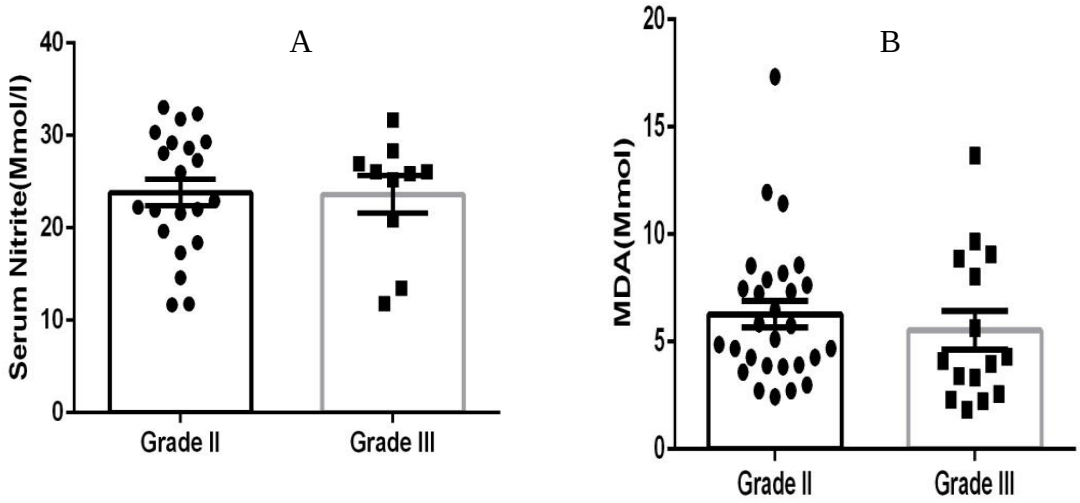


Figure 4.10. Serum oxidative stress markers patients treated with trastuzumab between grade II and grade III of breast cancer. (A) The level serum Nitrite (NO_2) in grade III treated patients which showed the non-significant difference to grade II treated patients. (B) The level serum malondialdehyde (MDA) was the non-significant difference. The result presented as means ± S.E for all treatment and unpaired t-Test was for Comparison patients treated with trastuzumab between grade II and grade III of HER2-positive BC

4.2.4. Lipid Profile (Cholesterol, TG and HDL/LDL)

The statistical analysis reported that trastuzumab treatment non-significant differences between the level of serum cholesterol in grade III trastuzumab treated patients which were (209 ± 15; N=15 mg/dl) comparing to grade II trastuzumab treated patients (198 ± 8.7; N=28 mg/dl) as illustrated in [Figure 4.11.A]. The data analysis revealed that trastuzumab treatment does not affect the level of cholesterol in grade III trastuzumab treated patients was (199 ± 26; N=15 mg/dl) which was less than grade II trastuzumab treated patients (202 ± 26; N=28 mg/dl) as illustrated in [Figure 4. 11. B]. While trastuzumab treatment patient's recorded non-significant difference regarding measures HDL/LDL in grade III trastuzumab treated patients was (0.47 ± 0.062; N=15 ratio) which was less than grade II trastuzumab treated patients (0.40 ± 0.034; N=28 ratio) as illustrated in [Figure 4. 11. C].

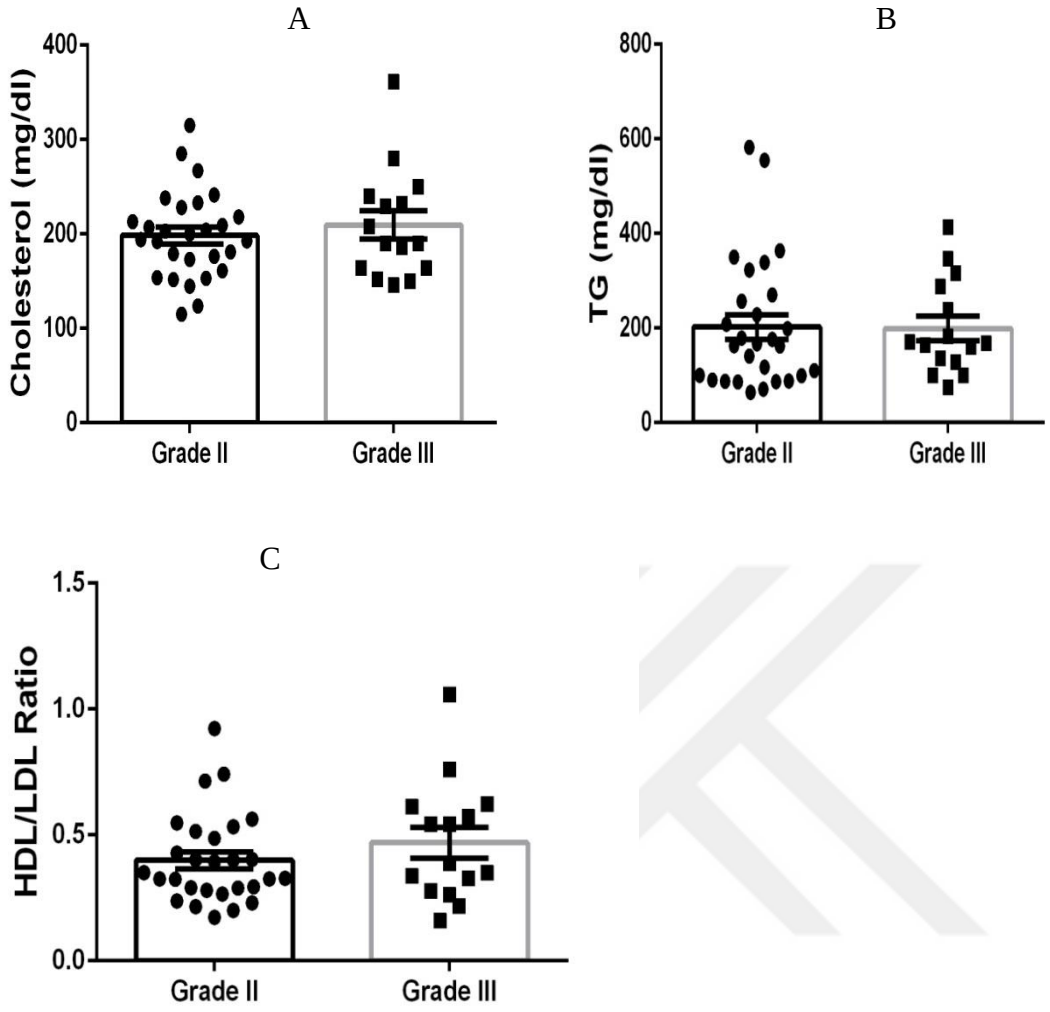
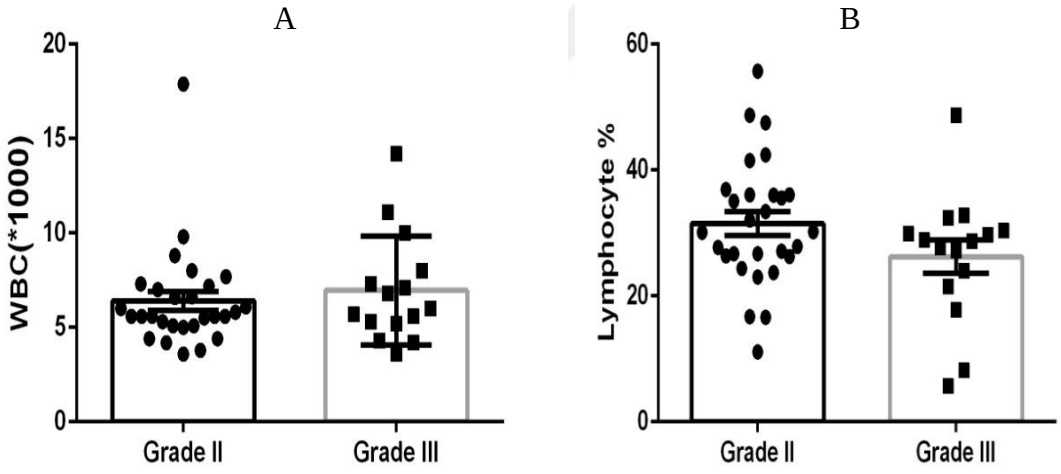


Figure 4.11. Serum lipid profile patients treated with trastuzumab between grade II and grade III of breast cancer. (A) The level serum total cholesterol in grade III treated patients which showed the non-significant difference to grade II treated patients. (B) The level serum triglyceride (TG) was the non-significant difference. (C) The level serum HDL/LDL also non-significant differences. The result presented as means ± S.E for all treatment and unpaired t-Test was for Comparison patients treated with trastuzumab between grade II and grade III of breast cancer

4.2.5. Hematological Measurements (WBCs, Lymph, PLT, Gran, MID, and MPV)

The data analysis exposed that trastuzumab treatment non-significant the level of WBCs in grade III trastuzumab treated patients was (7.0 ± 0.74; N=15 *1000/l) which was less than grade II trastuzumab treated patients (6.4 ± 0.51; N=28 *1000/l) as shown in [Figure 4.12.A]. Data analysis revealed that trastuzumab treatment does not affect the level of Lymph in grade III trastuzumab treated patients was (26 ± 2.7; N=15 %) which was less

than grade II patients treated with trastuzumab (31 ± 1.9; N=28 %) as illustrated in [Figure 4. 12. B]. Statistical analysis revealed that trastuzumab treatment does not affect the level of platelets PLT in grade III trastuzumab treated patients was (269 ± 28; N=15 $10^3/\mu L$) which was less than grade II trastuzumab treated patients (230 ± 11; N=28 $10^3/\mu L$) as shown in [Figure 4. 12. C]. The data analysis revealed that trastuzumab treatment does not affect the level of granulocytes (Gran) in grade III trastuzumab treated patients was (62 ± 4.7 N=15%) which was dose to the grade II trastuzumab treated patients (63 ± 2.0; N=27%) as illustrated in [Figure 4. 12. D]. The data analysis revealed that trastuzumab treatment does not affect the level of MID in grade III trastuzumab treated patients was (5.1 ± 0.62; N=15%) which was less than grade II patients treated with trastuzumab (7.6 ± 1.6; N=27%) as shown in [Figure 4. 12. E]. The data analysis revealed that trastuzumab treatment does not affect the level of mean platelet volume (MPV) in grade III trastuzumab treated patients was (8.0 ± 0.27; N=15 μm^3) which was less than grade II trastuzumab treated patients (7.9 ± 0.24; N=28 μm^3) as shown in [Figure 4. 12. F].



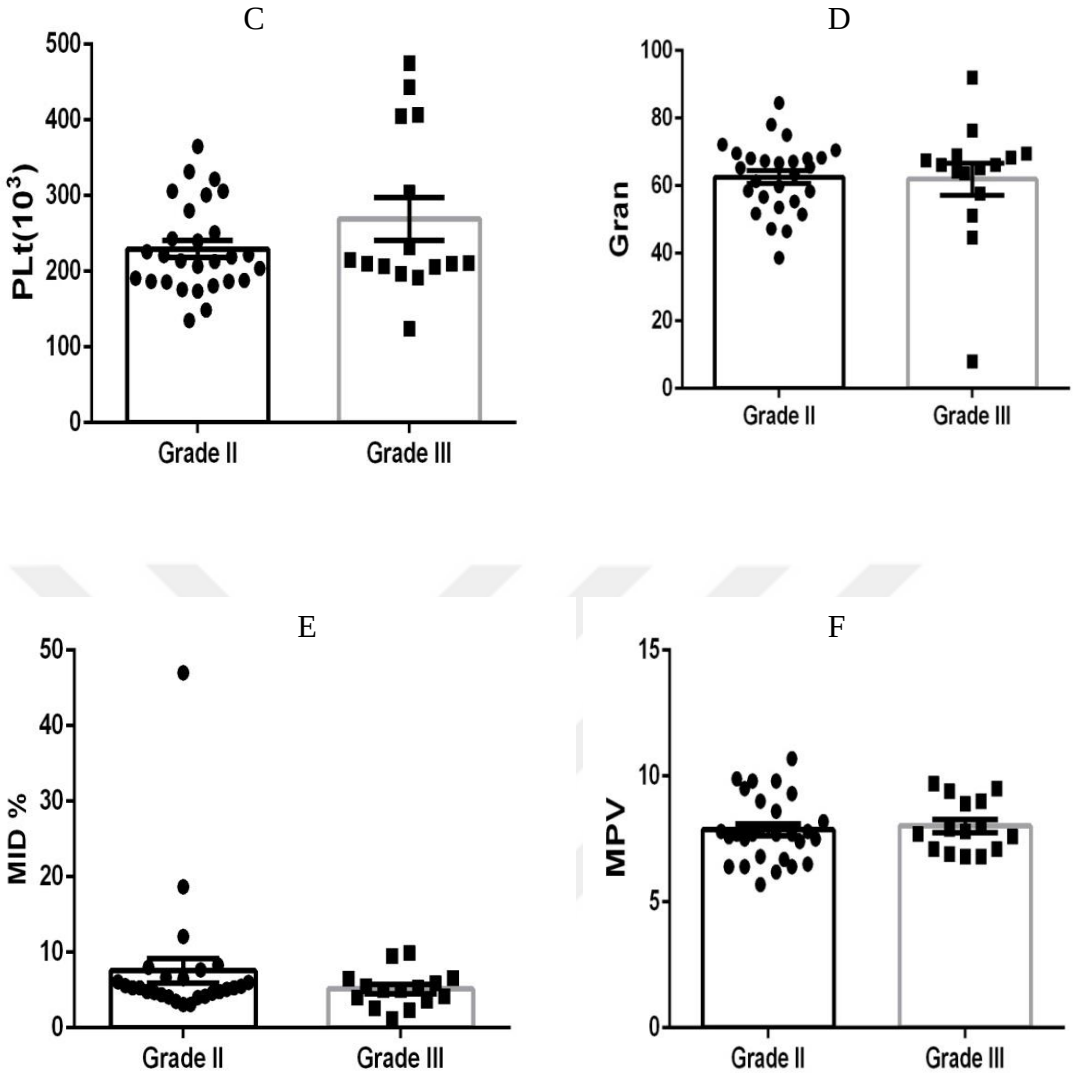


Figure 4.12. Comparison analyses between measure Hematological parameters patients treated with trastuzumab between grade II and grade III of breast cancer. (A) The level white blood cells (WBC) in grade III treated patients which showed the non-significant difference to grade II treated patients. (B) The level lymphocyte was the non-significant difference. (C) The level platelet the non-significant differences (D) The level of Gran non-significant differences (E) The level of MID % non-significant differences(C) The level of MPV also non-significant differences The result presented as means ± S.E for all treatment and unpaired t-Test was for Comparison patients treated with trastuzumab between grade II and grade III of breast cancer

4.3. Effects Of The Dose And The Duration Of Trastuzumab Treatment On Biochemical And Hematological Markers

4.3.1. Interleukin 1-R1 (Il-33 Receptor or ST2)

The current results showed statisticians the non-significant impact of the doses and duration of the therapy with the level of Interleukin 1-R1 (IL1-R1) in the patient's sera. The level of IL1-R1 recorded the highest value in the largest dose (351.8±53 pg/ml) but it was at the margin of the critical value of being significant. The values of the other groups were (308.6± 30.65 pg/ml), (278.3±5.018 pg/ml) and (293.3±20.18 pg/ml) in doses (4-2 with less than 6 cycles, 4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles), respectively, as illustrated in [Figure 4.13.].

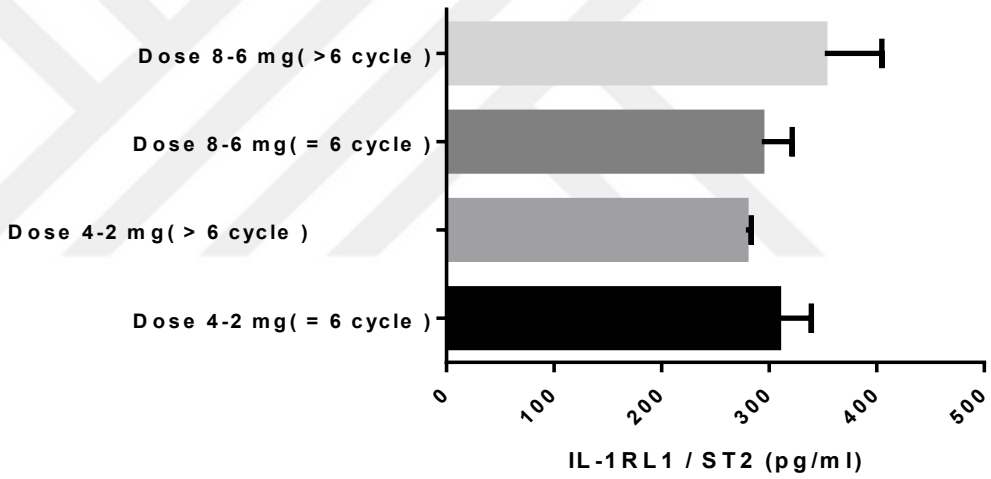


Figure 4.13. Showed non- significant impact of the doses and duration of the therapy with the level of serum Interleukin 1-R1 (Il-33 receptor or ST2) in the patient's sera. The level of serum Interleukin 1-R1 recorded the highest value in the largest dose (351.8±53) but it was at the margin of the critical value of being significant. The values of the other groups were (308.6± 30.65), (278.3±5.018) and (293.3±20.18) in doses (4-2 with less than 6 cycles, 4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles, respectively

4.3.2. Cardiac Markers (Creatine Kinase-MB and Troponin)

The data analysis revealed that deferent doses and durations of trastuzumab treatment do not affect the level of serum Creatine Kinase-MB (CK-MB) in treated patients. The level of serum Creatine Kinase-MB recorded the highest value in the lower dose (1.431 ±

0.3592 ng/ml), but it was at the margin of the critical value of being significant. The values of the other groups were (1.264±0.1650 ng/ml), (1.075±0.2299 ng/ml) and (0.7931±0.07239 ng/ml) in doses (4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles), correspondingly, as illustrated in [Figure 4.14.A]. The current results showed statisticians significant of the doses and duration of the therapy with the level of serum troponin in the patients treated with trastuzumab ($p<0.0001$). The level of serum troponin evidenced the highest value in the lower dose (0.02933± 0.007415 ng/ml) it was showed caused significant. The values of the other groups were (0.0090±0.001506 ng/ml), (0.0142±0.003245 ng/ml) and (0.008364±0.0006565 ng/ml) in doses (4-2 with more than 6 cycles, 8-6 with less than 6 cycles) and 8-6 with more than 6 cycles, respectively, as shown in [Figure 4.14.B].

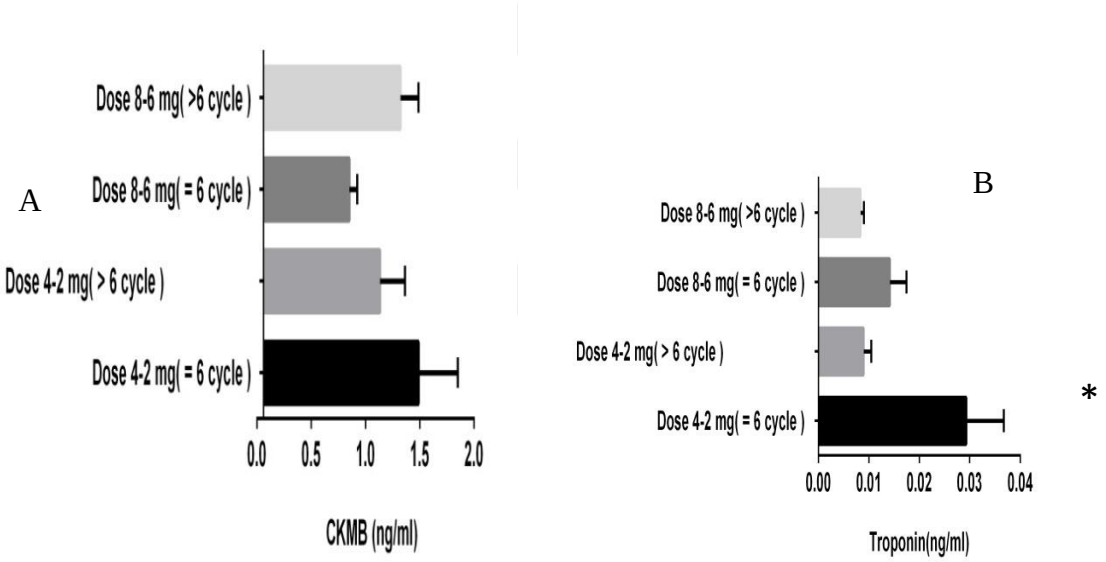


Figure 4.14. The effects of different doses and duration of trastuzumab-treated on serum cardiac markers treated patients. (A) The level of serum Creatine Kinase-MB (CK-MB) which showed the non-significant difference by using different doses and duration of trastuzumab-treated patients. (B) The level of serum troponin significant increase in using high dose and increase duration ($p<0.0001$). All the data represented as mean ± S.E and one-way ANOVA and Tukey post-hoc were performed for comparison among them

4.3.3. Oxidative Stress Markers Nitrite (NO_2) and Malondialdehyde (MDA)

Present results showed non- significant impact of the doses and duration of the therapy with the level of NO_2 in the patient's sera. The level of NO_2 recorded the highest value in the largest dose (26.88± 2.78821 Mmol/l) but it was at the margin of the critical value for

being significant. The values of the other groups were (23.28± 3.56621 Mmol/l), (18.99± 3.67521 Mmol/l) and (25.10± 2.65621 Mmol/l) in doses (4-2 with less than 6 cycles, 4-2 with more than 6 cycles), 8-6 with less than 6 cycles and 8-6 with more than 6 cycles, respectively, as shown in [Figure 4.15.A]. Present results showed statisticians significant of the doses and duration of the therapy with the level of troponin in the patients treated with trastuzumab($p<0.0001$). The level of serum troponin recorded the highest value in the largest dose (7.562±0.588021 Mmol/l) it was at the margin of the critical value for being significant. The values of the other groups were (3.807± 0.514221 Mmol/l), (4.882±0.845821 Mmol/l) and (6.473±0.458921 Mmol/l) in doses (4-2 with less than 6 cycles, 4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles), respectively, as shown in [Figure 4.15.B].

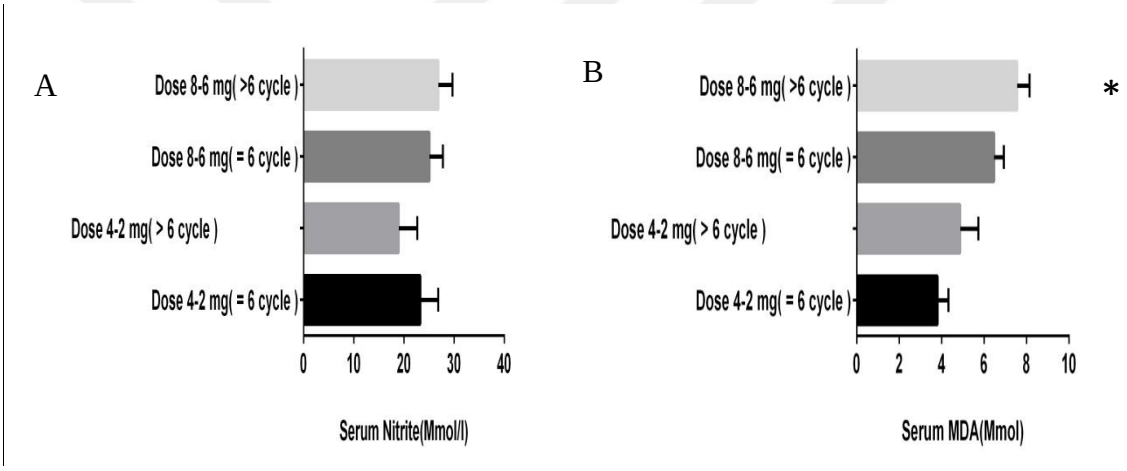


Figure 4.15. The effects of different doses and duration of trastuzumab-treated on serum oxidative stress markers treated patients. (A) The level of serum nitrite in treated patients, which showed non-significant difference by use different doses and duration of trastuzumab-treated patients. (B) The level of serum (MDA) significant increase in using high dose and increase duration ($p<0.05$). All the data represented as mean ± S.E and one-way ANOVA and Tukey post-hoc were performed for comparison among them

4.3.4. Hematological Measurements (WBCs, Lymphocyte, PLT, MPV, and HGB)

The data analysis revealed that deferent doses and durations of trastuzumab treatment do not affect the level of WBCs in treated patients. The level of WBCs recorded the highest value in the before largest dose (8.440±1.435*1000/l) but it was at the margin of the critical value of being significant. The values of the other groups were (6.233 ± 1.39*1000/l), (5.383±0.2286*1000/l) and (6.296±0.3432*1000/l) in doses (4-2 with less

than 6 cycles, 4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles), correspondingly , as shown in [Figure 4.16.A]. The data analysis revealed that deferent doses and durations of trastuzumab treatment do not affect the level of lymphocyte in treated patients. The level of lymphocyte recorded the highest value in the dose (4-2) with less than 6 cycles (32.50±2.329 %) but it was at the margin of the critical value for being significant. The values of the other groups were (24.83±3.879 %), (26.98±2.530 %) and (31.40±2.512 %) in doses (4-2 with less than 6 cycles, 4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles), correspondingly , as shown in [Figure 4.16.B]. Present results showed statisticians significant of the doses and duration of the therapy with the level of PLT in the patients treated with trastuzumab ($p<0.01$). The level of PLT recorded the highest value in the (8-6 with 6 cycles) dose (292.1± 29.99 10^3/l) it was at the margin of the critical value for being significant. The values of the other groups were (303.8± 45.33 10^3/l), (205.0±15.64 10^3/l) and (216.3±10.74 10^3/l) in doses (4-2 with less than 6 cycles, 4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles), respectively, as shown in [Figure 4.16.C]. The results shown non-significant impact of the doses and duration of the therapy with the level of MPV in the patient's sera. The level of MPV recorded the highest value in the largest dose (7.859± 0.2810 μm3) but it was at the margin of the critical value for being significant. The values of the other groups were (8.783± 0.3582 μm^3), (7.583± 25.48 μm^3) and (7.720± 37.26 μm^3) in doses (4-2 with less than 6 cycles, 4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles), respectively as shown in [Figure 4.16.D]. The current results showed statisticians significant of the doses and duration of the therapy with the level of hemoglobin (HGB) in the patients treated with trastuzumab ($p<0.05$). The level of HGB recorded the highest value in the (4-2 with more than 6 cycles) dose (12.70±29.87 mg/dl) it was at the margin of the critical value for being significant. The values of the other groups were (11.05± 0.4121 mg/dl), (11.93±0.4243 mg/dl) and (12.62±27.06 mg/dl) in doses (4-2 with less than 6 cycles, 8-6 with less than 6 cycles, 8-6 with more than 6 cycles and 8-6 with more than 6 cycles, respectively), as shown in [Figure 4.16.E].

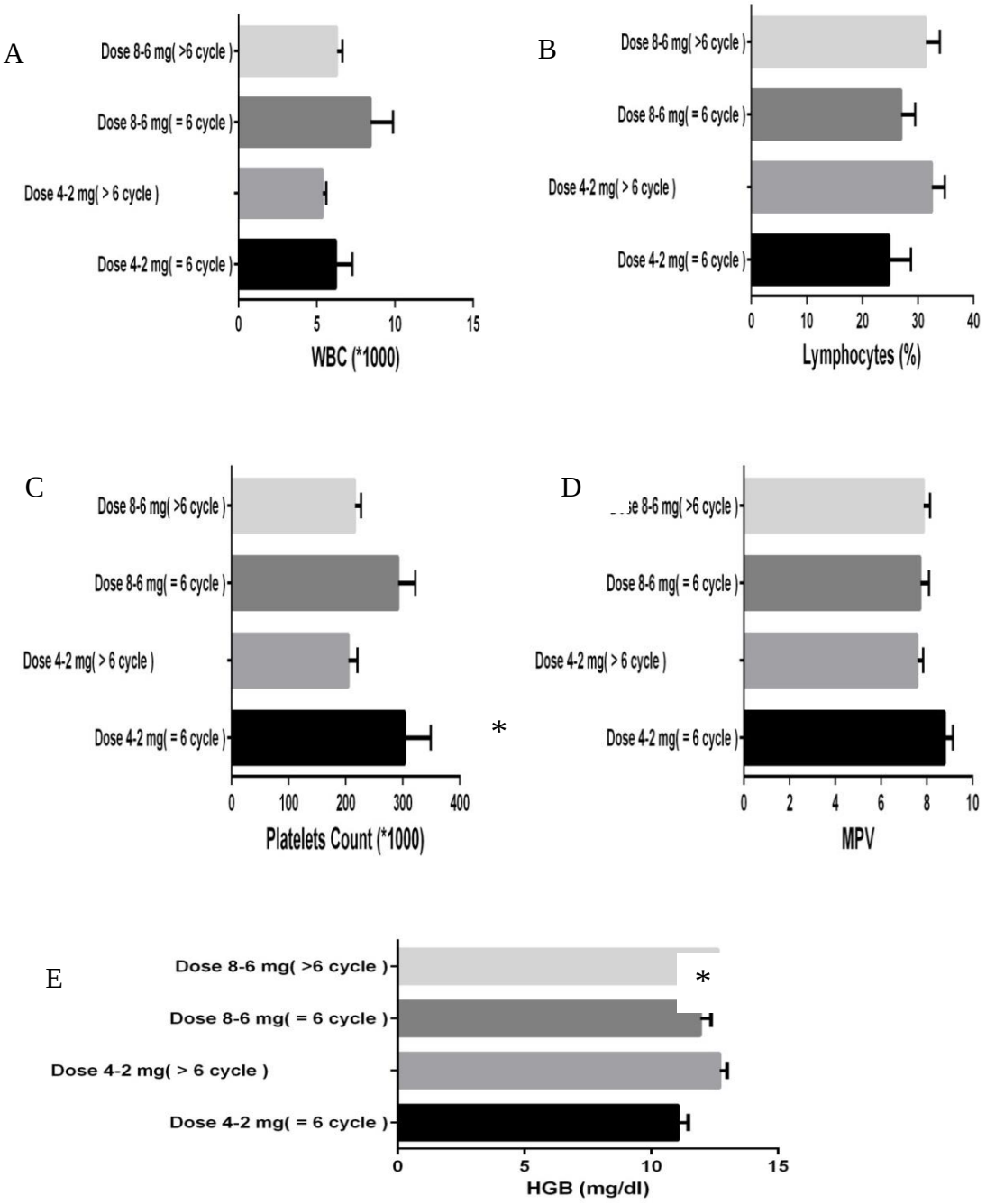


Figure 4.16. The effects of different doses and duration of trastuzumab-treated on hematological parameters treated patients. (A) The level of WBC which showed the non-significant difference by use change dose and duration trastuzumab-treated patients. (B) The level lymphocyte was non-significant difference. (C) The level of platelet significant increase in using high dose and increase duration ($p<0.01$). (D) Also, the level mean platelet volume (MPV) was the non-significant difference. (E) The level of hemoglobin (HGB) increase by using elevates different doses and duration when compared to a low dose of patients treated with trastuzumab ($p<0.05$). All the data represented as mean ± S.E and one-way ANOVA and Tukey post-hoc were performed for comparison among them

4.3.5. Blood Glucose (BG)

The current results showed non- significant effect of the doses and duration of the therapy with the level of serum blood glucose (BG) in the patient's sera. The level of blood glucose (BG) recorded the highest value in the largest dose (126.4±10.09 mg/dl) but it was at the margin of the critical value of being significant. The values of the other groups were (112.5±7.771 mg/dl), (118.5±16.77 mg/dl) and (122.9±8.386 mg/dl) in doses (4-2 with less than 6 cycles, 4-2 with more than 6 cycles, 8-6 with less than 6 cycles and 8-6 with more than 6 cycles), respectively, as shown in [Figure 4.17].

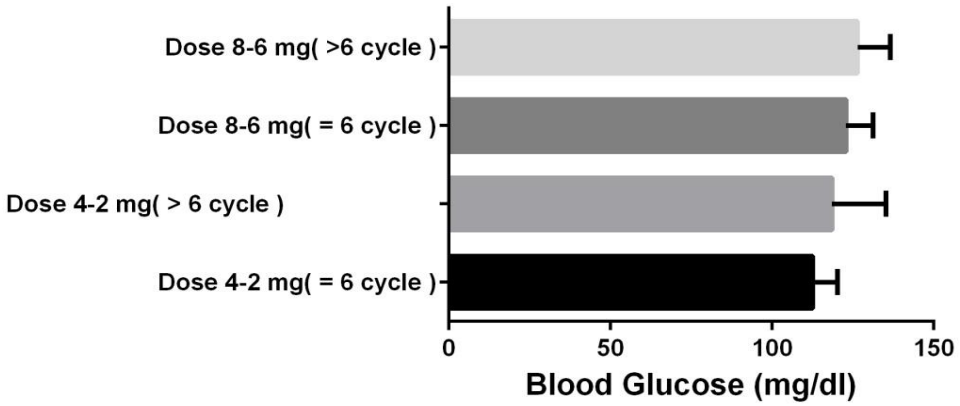


Figure 4.17. The effects of different doses and duration of trastuzumab-treated patient's serum blood glucose (BG) trastuzumab-treated patients. The level of blood glucose which showed the non-significant difference by use change dose and duration trastuzumab-treated patients. The data represented as mean ± S.E and one-way ANOVA and Tukey post-hoc were performed for comparison among them

4.4. Effect Of Body Mass Index (BMI) Of Patients Treated With Trastuzumab.

4.4.1. Cardiac Markers (Troponin and Creatine Kinase-MB)

Induction of Troponin level of treated patients affect body mass index (BMI) didn't significant. The level of serum troponin recorded the highest value in the obese group (0.01310±0.002247 ng/ml) but it was at the margin of the critical value of being significant. The values of the other groups were (0.0110±0.004033 ng/ml) and (0.01222±0.002443 ng/ml) in groups (Normal weight and over weight), respectively as shown in [Figure 4.18.A]. In the current study, level of serum creatine kinase-MB (CK-

MB) treated patients affect body mass index (BMI) non- significant. The level of CK-MB recorded the highest value in the overweight group (1.466± 0.3497 ng/ml) but it was at the margin of the critical value for being significant. The values of the other groups were (1.034±0.1942 ng/ml) and (1.082± 0.1083 ng/ml) in groups (Normal weight and obese), respectively as shown in [Figure 4.18.B].

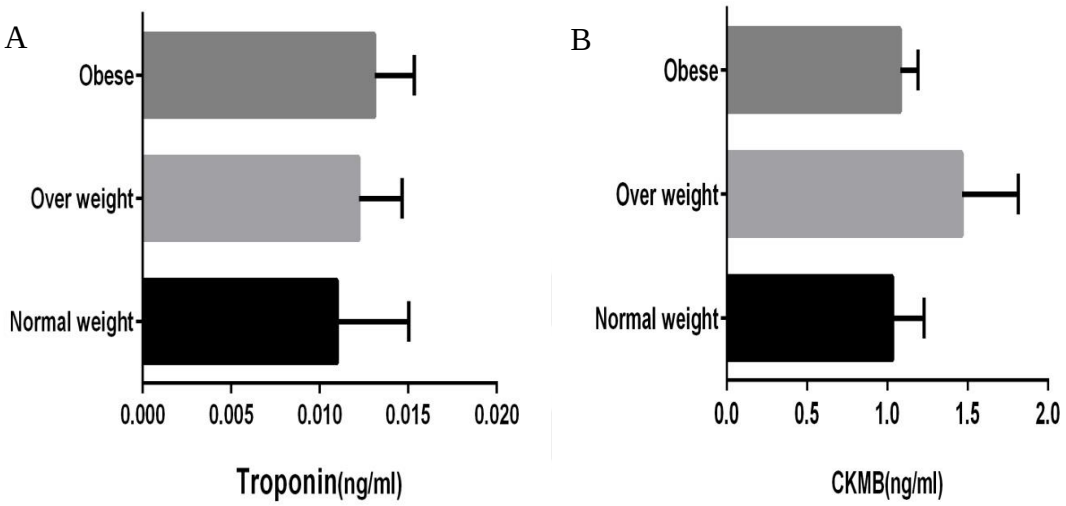


Figure 4.18. Effect of body mass index (BMI) trastuzumab-treated patients on serum cardiac markers. (A) The level of serum troponin no affect by BMI trastuzumab-treated patients (B) The level of serum creatine kinase-MB (CK-MB) no affect by BMI trastuzumab-treated patients. All the data represented as mean ± S.E and one-way ANOVA and Tukey post-hoc were performed for comparison among them

4.4.2. Oxidative Stress Markers (Nitrite and Malondialdehyde) ((NO_2, MDA)

In the current study, serum nitrite (NO_2) level of treated patients affect body mass index (BMI) caused significant. With the level of serum NO_2 in the patients treated with trastuzumab ($p<0.01$). The level of serum NO_2 recorded the maximum value in the (normal weight) group (39.96± 10.60 Mmol/l) it was increased at the margin of the critical value of being significant. The values of the other groups were (21.80± 2.612 Mmol/l) and (23.76± 1.398 Mmol/l) in groups (overweight and, obese) respectively, as show in Figure 4.19.A]. In the current study, level of serum malondialdehyde (MDA) treated patients affect body mass index (BMI) non- significant. The level of serum MDA recorded the highest value in the normal weight group (6.910± 2.337 Mmol/l) but it was at the margin of the critical value of being significant. The values of the other groups

were (6.286±1.367 Mmol/l) and (6.386± 0.8495 Mmol/l) in groups (overweight and obese), respectively as illustrated in [Figure 4.19.B].

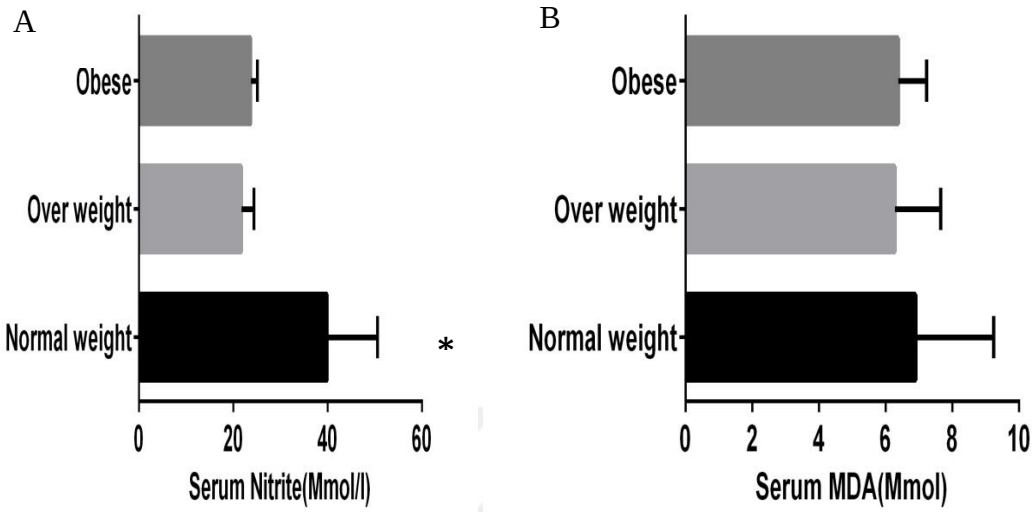


Figure 4.19. Effect of body mass index (BMI) trastuzumab-treated patients on serum oxidative stress markers. (A) The level of serum nitrite significant by BMI trastuzumab-treated patients (P< 0.01). (B) The level of serum malondialdehyde (MDA) was non-significant by BMI trastuzumab-treated patients. All the data represented as mean ± S.E and one-way ANOVA and Tukey post-hoc were performed for comparison among them

4.4.3. Hematological Measurement (WBCs, PLT, MPV, and HGB)

Induction level of WBCs treated patients affect body mass index (BMI) non- significant. The level of WBCs recorded the highest value in the obese group (6.738±0.5790 *1000/l) but it was at the margin of the critical value of being significant. The values of the other groups were (6.517±0.6789*1000/l) and (6.458±0.6728*1000/l) in groups (Normal weight and over weight), respectively as shown in [Figure 4.20.A]. The current results showed level of PLT treated patients affect body mass index (BMI) non- significant. The level of PLT recorded the maximum value in the obese group (252.2±16.22 10^3/l) but it was at the margin of the critical value of being significant. The values of the other groups were (209.0±12.76 10^3/l) and (240.6±25.30 10^3/l) in groups (Normal weight and over weight), respectively as illustrated in [Figure 4. 20. B]. The current results showed statistically significant the level of MPV in trastuzumab treated patients ($p<0.05$). The level of MPV increase in raise dose when evaluated to high BMI treated patients. The

level of PLT recorded the maximum value in the overweight group (8.756±0.4848 μm^3). The values of the other groups were (7.917±0.4362 μm^3) and (7.655±0.1904 μm^3) in groups (Normal weight and obese), respectively as illustrated in [Figure 4. 20. C]. Induction level of hemoglobin (HGB) treated patients affect body mass index (BMI) non-significant. The level of HGB recorded the highest value in the normal weight group (12.45±0.5065 mg/dl) but it was at the margin of the critical value of being significant. The values of the other groups were (12.02±0.4136 mg/dl) and (12.30±0.2505 mg/dl) in groups (overweight and obese), respectively as shown in [Figure 4. 20. D].

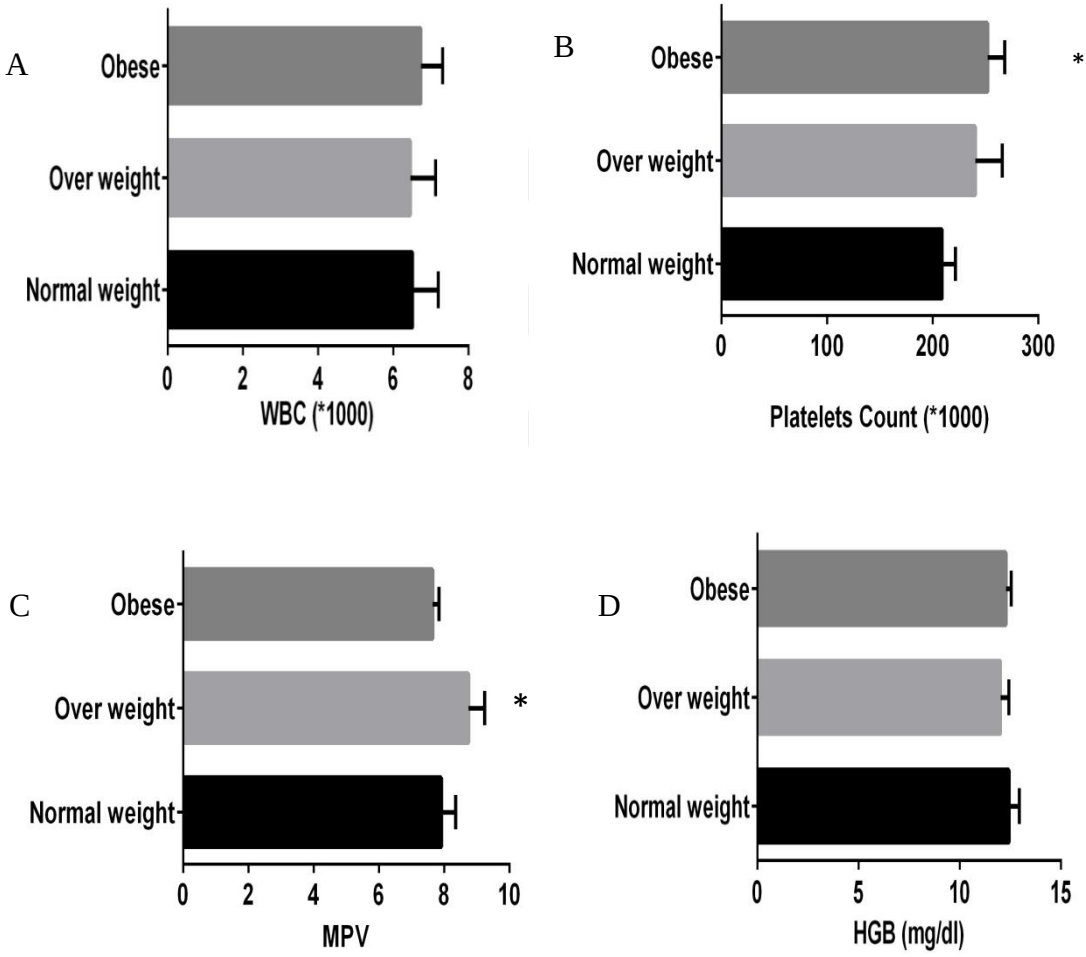


Figure 4.20. Effect of body mass index (BMI) trastuzumab-treated patients on Hematological parameters trastuzumab treated. (A) The level WBC which showed non-significant difference by BMI trastuzumab-treated patients. (B) The level platelet the non-significant differences (C) The level MPV significant differences by BMI trastuzumab-treated patients ($p<0.05$). (D) The level hemoglobin (HGB) non-significant differences by BMI trastuzumab-treated patients. All the data represented as mean ± S.E and one-way ANOVA and Tukey post-hoc were performed for comparison among them

5. DISCUSSION

The main finding of the present study is to show the relation between trastuzumab therapies with some significant variables linked to the angiogenesis, cardiac toxicity and oxidative stress markers in HER-2 positive breast cancer patients. The results found that the dose and the duration of the trastuzumab treatment failed to significantly affect the level of IL1R1 in the patient's sera. The event of trastuzumab-treated induced cardiotoxicity not only negatively affects a patient's cardiological outcome but also seriously limits her therapeutic opportunities. Troponin is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that is essential to muscle contraction in skeletal and cardiac muscle. Absent of these troponins T and I are the markers of choice for detecting the heart damage (Pasupathi et al. 2009). Troponin I (TnI) is a protein present exclusively in the myocardial cells. The TnI plasma concentration is a well-established specific and sensitive marker of myocardial injury, with both high diagnostic and prognostic value (Antman et al. 2000). In cancer patients treated with high-dose chemotherapy, troponin I (TnI) is a well-established early marker of myocardial injury, with high diagnostic and high prognostic values, Several reports showed that plasma concentration of troponin I (TnI) or a myocardial strain evaluated by echocardiography correlates with the hazard of trastuzumab-induced cardiotoxicity, and thus the measurement of these parameters may be able to predict cardiotoxicity (Suter et al. 2007). Serum biomarkers, such as high-sensitivity troponin I, and more sensitive measures of myocardial function, such as echocardiography-determined GLS, were found to be abnormal early during treatment before clinical symptoms or significant reduction in LVEF. Existing data suggest that once an elevation in troponin I is identified after high-dose chemotherapy, initiation of ACE inhibitors can prevent a decrement in LVEF and development of HF, increased troponin I in patients receiving trastuzumab had a decreased likelihood of LVEF recovery and a higher incidence of cardiac events (Cardinale et al. 2010). In trastuzumab-treated HER-2 positive breast cancer patients, an increase in troponin I compared to the controls, also soon after in elevated grade and

high-dose trastuzumab treatment is a strong predictor of late cardiotoxicity and poor cardiological outcome. This finding has important clinical implications and provides a rationale for the development of prophylactic strategies against cardiotoxicity. The current study elevated serum levels of MDA and NO_2, in other words, increased oxidative stress, was found in treated HER2 positive breast cancer patients compared to the controls. Also, levels of MDA elevated higher significant comparison between effects of dose and duration, trastuzumab-treatedHER-2 positive breast cancer patients. The present study elevated serum levels of NO_2 significant comparison between effects of BMI patients treated with trastuzumab. Thus we think that effect dose, duration, and BMI with trastuzumab-treated HER-2 positive breast cancer patient have increased oxidative stress. This finding has important clinical implications and allows the identification of patients at high risk of future cardiotoxicity in whom preventive measures are warranted. The current study elevated levels of PLT and MPV, in other words, increased oxidative stress, was found in treated HER-2 positive breast cancer patient compared to the controls. Also, levels of PLT and MPV elevated higher significant comparison between effects of dose, duration and BMI trastuzumab-treatedHER-2 positive breast cancer patients. The current study serum levels of IL1-R1 (IL-33 receptor or ST2) non-significant comparison between treated HER-2 positive breast cancer patients compared to the controls. While all factors which react with treated HER-2 positive breast cancer patients such as dos, duration, grades, and BMI didn't change the levels of IL1-R1 (IL-33 receptor or ST2) and showed no relationship between angiogenesis and cardiotoxicity of trastuzumab. In human ventricular myocytes, treatment with trastuzumab resulted in damage to cardiac myofilaments which corresponded with increased intracellular calcium, reduced diastolic relaxation time, and increased oxidative stress the endothelial nitric oxide synthase (eNOS) gene is constitutively expressed in vascular endothelial cells and releases nitric oxide (NO) a vasoactive molecule that reduces oxidative stress and prevents atherosclerosis. Through increased oxidative stress and low-grade chronic inflammation are chronic disease states characterized (such as breast cancer), endothelial cells can be damaged, leading to a reduction in eNOS expression and endothelial dysfunction (Sandoo, et al. 2015).

5.1. Comparative Analysis Of Biochemical Data Of Patients Treated With Trastuzumab And Untreated Patients

5.1.1. Cardiac Biomarkers (Troponin)

The current study revealed statistically significant differences troponin between trastuzumab treated patients and control patients ($p<0.05$) regarding the level of cardiac troponin. The level of troponin elevated by using trastuzumab drug. A similar observation was reported in the study (Varga et al. 2015) Trastuzumab therapy raise myocardial oxidative and nitrative stress and activates apoptotic pathways, important to altitudes of serum troponin-I level.In addition, it has been shown that trastuzumab increases troponin-I phosphorylation and modulate cardiac contractility in response to hemodynamic demands there reported by (Ritter et al. 2002). Similar observations were reported in other studies which are in progress to recognize biomarkers that perhaps used for near the beginning detection of trastuzumab-induced cardiotoxicity such as Troponin I (TnI) (Telli and Witteles 2011, Francis et al. 2014).

5.1.2. Oxidative Stress Markers (NO_2 and MDA)

The present results expressed statistically significant differences oxidative stress markers (NO_2 and MDA) between trastuzumab treated patients and control patients ($p<0.0001$). The level of NO_2 was increased by using trastuzumab drug. The study showed that trastuzumab treatment significantly increased the level of MDA, Treatment with trastuzumab outcome in damage to cardiac myofilaments which corresponded with increased intracellular calcium, decreased diastolic relaxation time and increased oxidative stress (Pentassuglia et al. 2007). These results are supported by another study who demonstrates that trastuzumab reduces the actions of neuregulin, which is involved in promoting NO production in the coronary microvasculature (Lemmens et al. 2007). Trastuzumab caused the main toxicity of cardiac dysfunction, with symptomatic congestive heart failure (Telli et al. 2007).

5.1.3. Hematological Measurements (PLT and MPV)

Recent results demonstrated statistically significant differences hematological measurements (PLT and MPV) between trastuzumab treated patients and control patients ($p<0.05$) regarding PLT variable. The level of PLT elevated by using trastuzumab drug. Platelets have been proposed to play an important role in cancer progression and metastasis formation (Nash et al. 2002, Bambace and Holmes 2011). The platelets provide a procoagulant surface facilitating the increase of cancer-related coagulation and can be recruited to shroud tumor cells, in this manner defensive them from immune responses, and facilitate cancer expansion and distribution (Bambace and Holmes 2011). One of the studies, in the beginning, reported the association between elevated platelet count and cancer (Tranum and Haut 1974), following studies have established this relationship for general cancers, including colorectal, lung and breast cancer, in addition to gastric, renal and most urogenital malignancies (Ikeda et al. 2002). The latest outcomes exhibited statistically significant differences between trastuzumab treated patients and control patients ($p<0.05$).The level of MPV elevated by using trastuzumab drug. Platelet mass, explained by the mean platelet volume (MPV), was suggested to reflect platelet activity, as it was revealed to be associated with platelet aggregation (Thompson et al. 1982). Cancers are one of the significant groups of thrombotic infections, by chemotherapy was induced to be increased (Mutlu et al. 2013). These results are supported by another study; increase in the risk of arterial thromboembolic incidents among patients with metastatic cancer treated with chemotherapy, and identified clinical characteristics that may be associated with an increase in this risk (Scappaticci et al. 2007). Several studies have shown that MPV is inversely correlated with platelet counts (Greisenegger al. 2004, Demirin et al. 2011, Vasse et al. 2012). MPV is a marker of platelet function, i.e., large platelets consist of more dense granules and produce additional thromboxane A2. Increased MPV has been associated with greater in vitro aggregation in response to ADP and collagen (Smith et al. 1999).

5.2. Comparison Patients Treated With Trastuzumab Between Grade II And Grade III Of HER2-Positive Breast Cancer.

5.2.1. Cardiac Markers (Troponin)

The current results showed statistically significant differences troponin between grade II trastuzumab treated patients and grade III trastuzumab treated patients ($p<0.05$). The level of troponin elevated in grade III when compared to grade II trastuzumab treated patients. Some chemotherapeutic agents inducing cardiotoxicity just after the drug is administered at high doses causes high grade, however, converted straightly value by causes LV dysfunction, systolic dysfunction, atrial fibrillation, CHF and pericarditis with chemotherapeutic agents. The current result was accommodated to the study performed by (Yeh et al. 2004). The present investigations of trastuzumab for a variety of breast cancer populations will additionally define the risk of LV dysfunction from such treatment. Trastuzumab-associated cardiomyopathy appears to be largely reversible with suitable treatment. That treatment would include ceasing the drug, administering appropriate therapy for LV dysfunction, and treating cardiac hazard issues (Lenihan et al. 2003). The reversible in study results from said trastuzumab dose, was not established to have any effect on cardiac side effects (Serrano et al. 2011).

5.3. Effects Of The Dose And The Duration Of Trastuzumab Treatment Biochemical And Hematological Markers

5.3.1. Cardiac Markers (Troponin)

The current results showed statistically significant differences troponin effects of dose and duration trastuzumab treatment on treated patients ($p<0.0001$). The level of troponin elevated in raise dose when compared to low dose treated patients. Especially, in trastuzumab-treated patients, troponin can recognize patients who recover from cardiac dysfunction; this might help us to distinguish between reversible and irreversible cardiac injure, In another study, patients who had persistently noticeable troponin after high-dose chemotherapy also had a higher incidence of cardiac actions compared to those whose troponin become undetectable (Cardinale et al. 2004). Statistics analyses suggest that additional showing examinations could reveal cardiotoxicity from chemotherapeutic

agents in a more sensitive and immediate method, as the measurement of cardiac troponin I. Cardiac troponin, a component of the myocardial contractile apparatus, is discharged after just one cycle of chemotherapy and is more frequently irregular with each cycle in patients who eventually expanded LV systolic dysfunction (Auner et al. 2003).

5.3.2. Oxidative Stress Markers (MDA)

The current results showed statistically significant differences MDA effects of dose and duration trastuzumab treatment on treated patients ($p<0.05$). The level of MDA raise in elevates doses and duration when evaluated to low dose treated patients. trastuzumab-induced cardiotoxicity may be as a result of direct binding of trastuzumab to the HER2 receptor expressed on the myocardium and raised oxidative stress (e.g., due to anthracycline use) increases the hazard for trastuzumab-related cardiotoxicity (Perik et al. 2007). Oxidative stress, described by a raised level of ROS (Jing et al. 2011). The high-dose of chemotherapy-induced excessive oxidative stress (Thevenod 2009). Oxidative stress distinguished by intracellular levels of ROS and GSH could be examined to validate our hypothesis that oxidative stress associated with various concentrations of (Wei et al. 2017). Since such, a number of demonstration analysis with the use of publicly available databases have suggested an elevated rate of trastuzumab-associated cardiotoxicity than what is reported in the breast-cancer clinical trials (Chen et al. 2012, Thavendiranathan et al. 2016).

5.3.3. Hematological Measurement (PLT and HGB)

The current results showed statistically significant differences PLT effects of dose and duration trastuzumab treatment on treated patients ($p<0.01$).The level of PLT high in increase different doses and duration when compared to low dose treated patients. Stimulated platelets are involved in cancer development and metastases (Bambace and Holmes 2011) In cellular models of both breast cancer and ovarian cancer, invasiveness has increased following exposure to platelets (Holmes et al. 2009). The current results showed statistically significant differences hemoglobin (HGB) in trastuzumab treated patients ($p<0.05$). The level of hemoglobin (HGB) increase by using elevates different

doses and duration when compared to low dose treated patients. Though there are a small number of studies that have focused on pre-treatment Hb levels for breast cancer prognosis (Zhang et al. 2014). Hemoglobin (Hb) levels are related to treatment outcomes and survival in patients with various cancers, the Hb level might alter after treatment (chemotherapy, radiation therapy, or surgery) or with diverse cancer phases, it was revealed that Hb level is a dependable indicator for response to therapy; the initial Hb level after treatment provided an accurate survival prediction, and the change in Hb level was related to the response to treatment (Lee et al. 2017).

5.4. Effect Of Body Mass Index (BMI) Trastuzumab Treated On Treated Patients.

5.4.1. Oxidative Stress Markers (NO_2)

Induction of BMI caused significant ($p< 0.01$) in the levels of serum NO_2 were noted in treated patients. The relationship between fatness and cancer hazard has been widely explored. The devices integrated insulin resistance and resultant chronic hyperinsulinaemia, increased bioavailability of steroid hormones, and localized chronic inflammation (Van Kruijsdijk et al. 2009). Healthy illustrated drugs associated with difficult events to which oxidative stress may contribute, including as of cancer therapies (Deavall et al. 2012) Obesity is significantly associated with activation of neurohormones, increased oxidative stress, increased hemodynamic load, and remodeling of the left ventricle (Engeli and Sharma 2001). In the additional study, obesity had a significant character in trastuzumab-related cardiac events, the mechanisms by which obesity might negatively influence cardiotoxicity are affected by numerous confounding factors (Kenchaiah et al. 2002). It is now clear that obesity can increase the prevalence of several cancers (Chen 2010).

5.4.2. Hematological Measurement (PLT And MPV)

The current results showed statistically significant differences effects of dose and duration trastuzumab treatment on treated patients ($p<0.05$). The level of MPV increase in raise dose when evaluated to high BMI treated patients. The present results illustrated a high MPV was found to be significantly associated with BMI factor. If the outcomes

obtained are promising, combinational targeted therapy of obesity correlated MPV. Mean platelet volume (MPV) is a marker of stimulated platelets and is related with gastric, ovarian, lung, colon, and breast malignancy (Kılıncalp et al. 2014, Gu et al. 2016). An increased mean platelet volume (MPV) is an early marker of platelet activation, MPV was also revealed to be related with the pathophysiological characteristics of different types of cancer (Yun et al. 2017). A number of medical studies reported that an elevated MPV is correlated with thromboembolic diseases, such as myocardial infarction or stroke (Kumagai et al. 2015).

CONCLUSIONS AND FUTURE ASPECTS

1. Trastuzumab exposure HER-2 positive breast cancer patients didn't induce significant differences in the level of serum interleukin 1-R1 (Il-33 receptor or ST2) compared with untreated patients (control). However, Trastuzumab exposure developed different effect on patient group distinct grades of cancer.
2. HER-2 positive breast cancer patients treated with trastuzumab shown significantly differences in the level of serum troponin compared with untreated patients (control). Recorded elevated level of cardiac troponin which confirmed it is cardiotoxic ability; however, Trastuzumab exposure didn't develope different effect on the level of Creatine Kinase-MB (CKMB) patient compared with untreated patients (control).
3. Trastuzumab exposure HER-2 positive breast cancer patients did induced significantly differences in the level of serum oxidative stress, markers malondialdehyde and nitrite (MDA and NO_2) compared with untreated patients (control).
4. Trastuzumab revelation HER-2 positive breast cancer patients according to action of dose and duration did induce significant differences in the level of serum troponin compared with low doses rather than the high doses.
5. There were no obvious relation between body mass index (BMI) of the Trastuzumab revelation HER-2 positive breast cancer patients and almost all the estimated parameters related to cardiotoxicity, oxidative stress and biochemical variables.
6. Obtained data evident that future studies are needed to be investigate the endothelial dysfunction occurred by trastuzumab treatment.
7. We recommend the further studies to focus on more investigation about the correlation of total cumulative of trastuzumab with cardiac and angiogenesis markers.

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