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EVALUATION OF ADRIMYCIN AND CYCLOPHOSPHAMIDE NEPHROTOXICITY USING URINARY KIDNEY INJURY MOLECULE -1 IN BREST CANCER PATIENTS

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ABSTRACT

Nephrotoxicity is a common side effect of chemotherapy treatment. Traditional blood and urine markers for diagnosis of nephrotoxicity are insensitive and non-specific. This study aimed to investigate the efficiency of kidney injury molecule-1 (KIM-1) as a sensitive biomarker for early kidney injury in chemotherapy treated cancer patients. This study included fifteen female breast cancer patients treated with adrimycin (ADR) and cyclophosphamide (CP). Urinary markers such as KIM-1 and microalbuminuria (MALB) were measured. Glomerular Filtration Rate (GFR), serum levels of creatinine (SCr), blood urea nitrogen (BUN), superoxide dismutase (SOD), catalase (CAT), malonaldehyde (MDA) and electrolytes were analyzed. All markers were detected before and after 24h of treatment. A significant increase in MALB ($P < 0.001$) and KIM-1 ($P < 0.000$) levels were observed after chemotherapy treatment while no significant differences in the mean value of GFR, SCr, BUN, and electrolytes levels were found. The activities of SOD and CAT showed significant decrease ($P < 0.001$) while significant increase of MDA ($P < 0.001$) levels were detected. In conclusion, quantitation of urinary KIM-1 is likely to be a sensitive biomarker for the evaluation of early kidney injury.

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Introduction

Complications such as acute kidney injury (AKI) and destruction of renal function are caused in people with cancer by the treatment with chemotherapy drugs or from the disease itself [1,2]. A combination of Adriamycin (ADR) and cyclophosphamide (CP) is used to treat breast cancer patients. ADR is an effective antineoplastic agent used in the treatment of a variety of hematologic and solid malignacies, such as breast cancers [3]. CP is a cytotoxic alkylating agent, which is usually used in the treatment of acute and chronic lymphocytic leukemia, Hodgkin's disease, multiple myeloma, soft tissue sarcomas and other benign diseases [4,5]. Adriamycin induced nephropathy is one of the most experimental models used in progressive kidney disease. A single dose of this drug induces a progressive and irreversible proteinuria that progresses to focal segmental glomerulosclerosis and tubulointerstitial lesions [6]. CP can result in glomerular dysfunction and tubular dysfunction, glomerular proteinuria, tubular proteinuria, and reduction of glomerular filtration rate [7, 8, 9]. Renal function assessment is carried out to allow safe administration of medication and monitor the effects of treatment on the patient. The classical markers of evaluating renal function involving the measurement of serum creatinine (SCr) and blood urea nitrogen (BUN) are insensitive and nonspecific, especially in the setting of AKI. It is also important to recognize that changes in serum creatinine and blood urea nitrogen concentrations primarily reflect functional changes in filtration capacity and do not indicate renal injury until a significant degree of renal function is lost [10, 11, 12]. Accordingly, there is a need for better biomarkers to diagnose acute kidney injury (AKI) for the prediction of severity and for the monitoring of proximal tubule injury in AKI as well as chronic kidney disease. Urinary kidney injury molecule -1 (KIM-1) also known as TIM-1—T-cell

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immunoglobulin and mucin-containing molecule is a discovered early biomarker for renal damage. KIM-1 expression is induced in a variety of renal diseases, whereas in healthy kidney tissue KIM-1 is virtually undetectable [13,14]. In kidney damage, KIM-1 is expressed on the apical membrane followed by cleavage of the ectodomain which is released in the urine in rodents [13,15,16,17] and in humans [14,18]. The aim of the current study is to evaluate the utility of urinary KIM-1 as an earlier and equally sensitive biomarker for detection AKI in patients treated with ADR together with CP in comparison with routinely used markers.

Subjects and methods

Subjects and Study design

This study was approved by Unit of Biomedical Ethics committee, King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia. After obtaining informed consent from each participant, subjects were interviewed using structured questionnaire to collect data including: age, weight, height, life style. This study included 15 newly diagnosed female patients with breast cancer aged (35-60) with no previous history of renal disease attending King Abdulaziz university hospital in Jeddah. Fifteen healthy female individuals were enrolled in the study as controls. Controls were matched for age and gender and consider healthy if they had no history of renal disease and use no medications. The medical history of the patients including the site of cancer, chemotherapy regime and dose of chemotherapy were recorded. The dose of chemotherapy drugs ADR/CP was between (90 mg/ 900mg – 120mg/ 1000mg) respectively.

Biochemical analysis for Kidney Function

Blood and urine samples were collected 24 hours before chemotherapy and 24 hours after chemotherapy. Blood and urine sample where withdrawn from control subjects. Whole blood was collected in plain tubes from all participants for measurement of Cr, BUN, SOD, CAT, MDA, Na, K and Cl. All serum samples were stored at -80Cº. Creatinine was detected at 510 nm using the kit (Cat. No. K1033). BUN was spectrophotometrically measured at 340 nm using the kit (Cat.No. K1021). Antioxidant parameters catalase (CAT), superoxide dismutase (SOD) were determined according to the method quantitative sandwich enzyme immunoassay technique using the kits (Catalog No CSB-E13635h) (Catalog No CSB-E16845h) respectively. Malondialdehyde (MDA) was detected using (Catalog No CSB-E08557h). Electrolytes, sodium (mEq/L), potassium (mEq/L), and Cl (mg/dL) were detected using Dimension Clinical Chemistry System. Urine samples were centrifuged at 6000 rpm for 15 min. Supernatants were collected and stored at -80Cº. Samples were used for the analysis of Microalbuminuria (MALB) and Kidney Injury Molecule-1 (KIM-1). The MALB method used on is an in vitro diagnostic test (Cat No. K7062) intended for the quantitative determination of albumin in human urine. KIM-1was determined according to the method quantitative sandwich enzyme immunoassay technique (Catalog No CSB-E08807h). Subsequently, the Glomerular filtration rate (GFR) was calculated according to the Cokcroft-Gault formula [11,19].

Statistical analysis

Data was analyzed using Social Package for Social Science software (SPSS version 13.0). Data was represented as mean ± standard error of the mean. The significance of the difference between samples was determined using simple student t test. The difference was regarded as significant when p-value was $p \leq 0.05$, highly significant when $p \leq 0.0001$ and non-significant when $p > 0.05$.

Results

Table 1 shows comparison of serum Cr, BUN and urinary MALB, KIM-1 and GFR in breast cancer patients before and after treatment with ADR and CP. Compared with healthy controls, the level of creatinine did not show any significant change before and after 24h of administration of ADR and CP. Meanwhile, BUN levels was decreased significantly in cancer patients post treatment and no different in its level after 24h of ADR and CP infusion compared to the level before. Urinary MALB levels increased significantly in cancer patients before treatment compared to controls and a very highly significant increase were seen in MALB levels (2-folds) 24h after treatment. Urinary Kim-1 in the base line of the analysis in patients before treatment was 6- folds higher than healthy controls. After 24h of treatment with ADR and CP a very highly significant increase in the mean value of Kim-1 was registered as compared to before treatment. In contrast, urinary Kim-1 level after chemotherapy administration was higher 14- fold than control. No significant changes were observed in the range of GFR in cancer patients following treatment, it seems to be in the normal rang as compared to healthy control.

Table 2 summarizes the effect of ADR and CP on the activities of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and the activity of lipid peroxidation malondialdehyde (MDA) in breast cancer patients before and after treatment. There was no significant decrease in the activities of CAT and SOD in cancer patients before treatment as compared to control. Following treatment, cancer patients showed significantly lower CAT and SOD activities and significantly higher MDA levels compared to a control group of healthy control.

Table 3 shows comparison of sodium (Na), potassium (K) and chloride (Cl) levels in Breast Cancer Patients treated with ADR and CP. There was no statistically significant difference in the level of sodium, potassium and calcium in the serum of cancer patients compared to control and following ADR and CP administration.

Table 1: Comparison of Serum Cr, BUN and Urinary MALB, KIM-1 and GFR in Breast Cancer Patients treated with ADR and CP and Control Group ($\overline{X}$ ± SE).

Groups / Parameters	Control	Base line before ADM/CP infusion	After 24h of ADM/CP infusion
SCr (mg/dl)	0.63 ± 0.02	0.65 ± 0.04	0.60 ± 0.02
P-value		NS	NS
BUN (mmol/L)	4.21 ± 0.21	3.26 ± 0.47	3.74 ± 0.18
P-value		0.01	NS
MALB (mg/dl)	4.26 ± 0.31	6.25 ± 0.54	15.81 ± 1.92
P-value		0.01	0.001
Kim-1 (ng/ml)	1.22 ± 0.58	6.10 ± 0.44	14.84 ± 0.46
P-value		0.000	0.000
GFR (mg/min)	88.20 ± 5.33	90.56± 6.66	89.23 ± 7.12
P-value		NS	NS

- SCr: serum creatinine, BUN: blood urea nitrogen, MALB: Microalbuminuria KIM-1: Kidney Injury Molecule-1, GFR: Glomerular filtration rate
- P value is significant at < 0.05 NS: non-significant, *P* value > 0.05.

Table 2: Comparison of Catalase (CAT), Superoxide dismutase (SOD), Malondialdehyde (MDA) Activities in Breast Cancer Patients treated with ADR and CP and Control Group ($\overline{X}$ ± SE).

Groups / Parameters	Control	Base line before ADM/CP infusion	After 24h of ADM/CP infusion
CAT (pg/ml)	1255.57 ± 11.82	1240. 60 ± 4.24	902.42 ± 30.21
P-value		NS	0.000
SOD (pg/ml)	456.64 ± 25.75	443.18 ± 15.86	351.02 ± 5.32
P-value		NS	0.01
MDA (µg/ml)	9.49 ± 1.25	15.46 ± 1.73	33.69 ± 5.32
P-value		0.01	0.001

- P value is significant at < 0.05
- NS: non-significant, *P* value > 0.05

Table 3: Comparison of sodium (Na), potassium (K) and chloride (Cl) levels in Breast Cancer Patients treated with ADR and CP and Control Group ($\overline{X}$ ± SE).

Groups / Parameters	Control	Base line before ADM/CP infusion	After 24h of ADM/CP infusion
Na (mmol/L)	136 ± 0.96	140 ± 0.51	139 ± 0.61
P-value		NS	NS
K (mmol/L)	3.5 ± 0.03	4.08 ± 0.08	3.91 ± 0.09
P-value		NS	NS
Cl (mmol/L)	103.50 ± 1.50	100 ± 1.55	99 ± 1.59
P-value		NS	NS

- P value is significant at < 0.05
- NS: non-significant, *P* value > 0.05

Discussion

The results of the present study revealed that after 24h of treatment with ADR and CP, there was no significant difference in serum levels of Cr and BUN and in the mean values of GFR. Meanwhile, a highly significant increase was registered in the levels of urinary MALB and KIM-1. ADR nephropathy is a well-established model for interstitial fibrosis, characterized by the gradual development of proteinuria, due to direct toxicity on glomerular structure responsible for changes in glomerular permeability due to oxygen free radicals [20,21,22,23]. Reabsorption of leaked proteins was followed by tubular activation characterized by the production of growth factors and cytokines. This may lead to tubular injury characterized by proliferation, apoptosis, inflammation, and increased extracellular matrix production [24,25]. Abraham et al. [26] observed a decrease in the activities of lysosomal enzymes in the kidneys of rats treated with CP. Lysosomes play an important role in cell death and tissue damage due to drugs and toxins [27,28]. Lysosomal dysfunction can result in lack of digestion of proteins that are regularly degraded by the lysosomes there by resulting in increased half-life of proteins and cause an accumulation of abnormal amount of proteins within the cell [29,30].

Our study indicated that urinary KIM-1 levels elevated after 24h of exposure to ADR and CP while GFR, SCr and BUN did not show significant difference from the results before treatment. Zhou et al. [17] found that urinary KIM-1 was elevated within 24 h after exposure to nephrotoxicants and remained elevated through 72 h. At 72h, after treatment with the nephrotoxicants, there was increased KIM-1 immunoreactivity and necrosis involving about 50% of the proximal tubules; however, only urinary KIM-1 was significantly increased, while BUN and SCr were not different from controls. Also similar results were reported by Vaidya et al. [31] when administrate ten toxicants to rat including ADR and found that urinary KIM-1 outstripped SCr and BUN, as predictors of kidney tubular damage scored by histopathology. Sugumar et al. [9] demonstrated that CP induces renal damage histologically, but the plasma Cr remains unaltered in rat model. Some other studies showed that CP could be nephrotoxic, both in humans and animal models, which result in glomerular dysfunction and tubular dysfunction, glomerular proteinuria, tubular proteinuria, reduction of glomerular filtration rate [7,8,9]. Tonomura et al. [32] measured the levels of twelve urinary nephrotoxic biomarkers including KIM-1, SCr and BUN in rats treated intravenously with alkylating agents, which known to induce glomerular injury or proximal tubular injury. The authors registered KIM-1 large magnitude of early alteration and its high accuracy and sensitivity of detection. In addition, Prozialeck et al. [33] reported that, urinary KIM-1 levels increased severely earlier than the increases of BUN and plasma creatinine. Urinary KIM-1 has been shown in additional studies to be a sensitive and early diagnostic indicator of renal injury in a variety of acute and chronic rodent kidney injury models resulting from drugs [17,34], environmental toxicants [13,17,35], ischemia [16], and protein overload [36]. Han et al. [37] demonstrated marked expression of KIM-1 in kidney biopsy specimens from six patients with acute tubular necrosis, and found elevated urinary levels of KIM-1 after an initial ischemic renal insult, prior to the appearance of casts in the urine. Chaturvedi et al. [38] have reported that KIM-1 is not expressed in normal kidney but specifically expressed in injured proximal tubular cells, and such an expression can persist until the damaged cells have completely recovered. Moreover, the rapid and integrated cleavage of its ectodomain into the lumens of kidney tubules can make it detectable in urine.

Oxidative stress, refers to the cytological consequence of imbalance between the production of free radicals and the ability of the cell to defend against them [39]. It occurs when the generation of reactive oxygen species (ROS) increases or the capacity to scavenge them and repair of oxidative modified macromolecules decreases, or both [40,41]. The oxidants that are not scavenged by antioxidant defense system attack cellular components producing useless molecular debris and sometimes cell death. The antioxidant enzymes represent a first line of defense against toxic reactants by metabolizing them to innocuous byproducts [42,43]. In this study, a highly significant decrease in the activity of both CAT and SOD are accompanied by intensification of lipid peroxidation processes, which is confirmed by elevated MDA serum levels after 24h of treatment with ADR and CP. Venkatesan et al. [44] reported that in kidneys exposed to ADR, there was a significant increase in lipid peroxides and total lipids. Different researches have reported an increase in MDA level and reduction in the activity of SOD which consider an indicator of oxidative stress in the kidney after CP administration [45,46]. Cyclophosphamide, is metabolized into active metabolites that form ROC which can modify the components of both healthy and neoplastic cells in circumstances of decreased antioxidative abilities. That leads to the dysfunction of organs, including the kidneys [47]. Lipid peroxidation is widely used as an indicator to reflect oxidative stress and cell membrane damage [48]. The decrease in the activities of antioxidant enzymes lead to a buildup of oxidative stress and could cause tissue damage. SOD detoxifies the superoxide radicals giving rise to hydrogen peroxide (H_2O_2). However, H_2O_2 is itself a potent free radical generator and can generate toxic hydroxyl radicals by reacting with ferrous ions, which can induce lipid peroxidation of cell membranes. In addition, cellular CAT and GSH-Px detoxify H_2O_2 [49]. Catalase is a heme enzyme that has a predominant role in controlling hydrogen peroxide concentration in human cells, by converting H_2O_2 into H_2O and O_2. Normally, the balance between reactive oxygen species (ROS) produced by pro-oxidant and that scavenged by antioxidant is maintained, and cellular damage arises when this equilibrium is disturbed [50,51]. Premkumar et al. [52] found that a one-time administration of CP (40 mg/kg, i.p.) induced significant oxidative stress and decreased levels of SOD, CAT, and increased LPO. Similar results were found by Estakhri et al. [28] who observed that administration of CP over a period of time causes an elevation in plasma level of MDA in rats. Other study reported that in cancer patients treated with ADR alone or associated with other anticancer drug, there was lipid peroxidation and antioxidant status [53]. Administration of antineoplastic agents during cancer chemotherapy results in a much greater degree of oxidative stress than is induced by cancer itself [53,54,55]. Oxidative damage to membrane lipid and other cellular components is believed to be a major factor in the ADR toxicity. ADR and its iron chelate undergo redox cycling, resulting in the generation of free radicals and reactive oxygen species ROS [56]. Several mechanisms have been proposed for the anticancer activity of anthracyclines. ADR alters membrane function and undergoes a one-electron reduction to its semiquinone, which can donate an electron to molecular oxygen resulting in superoxide generation. Although generation of hydroxyl radicals from superoxide is an explanation for the cytotoxicity of ADR and cause kidney injury [57]. Electrolytes in body fluids occur either as free ions or partly bound to proteins. The maintenance of correct electrolyte concentrations in body fluids is vital and disorders in electrolyte balance, which use to diagnose changes in renal and metabolic function [58]. A variety of renal disease and electrolyte disorders can result from the drugs that used to treat malignant disease. In our study, the results of treated patients showed no significant alteration in serum levels of sodium, potassium and chloride. In agreement with our study, Defronzo et al. [59] observed no changes in serum levels of K and Cl during cyclophosphamide demonstration also, no decrease in Na excretion. Blackburn et al. [60] reported that in patients with cancer, the metabolic relationships between electrolytes, minerals, and cancer show no general abnormalities. In a study on animal model, Mimnaugh [61] found that the levels of Na, K and Cl remained within normal limits after treated with ADR.

In conclusion, the present study demonstrates that a single injection of ADR and CP to breast cancer patients caused glomerular and tubular injury. In addition, it resulted in renal lipid peroxidation and a decrease in antioxidant activities after 24h of treatment. The traditional laboratory tests of renal functions were insensitive and nonspecific for early detection of

renal damage. A role for MALB would be in line with KIM-1 in protein overload induced proteinuria. Quantitation of urinary KIM-1 is likely to be a sensitive biomarker for the evaluation of kidney injury. Its level can be detected in the urine of treated patients after 24h of treatment, which make it useful for monitoring the therapeutic effects of AKI.

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