Impairment of liver synthetic function and the production of plasma proteins in primary breast cancer patients on doxorubicin-cyclophosphamide (AC) protocol

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Abstract: Doxorubicin and Cyclophosphamide (AC protocol) combination is usually considered as a first line therapy in newly diagnosed breast cancer patients. Thus, a retrospective observational study was conducted to monitor the effect of AC protocol on liver synthetic functions and production of plasma proteins in breast cancer patients, reporting to specialized cancer care hospital of Lahore, Pakistan. A total of 75 patients (n=75) on AC protocol with breast cancer were observed in this study. The patient data including age, gender, body surface area, dosage, disease status and laboratory biochemical values were recorded by reviewing historical treatment records. Pre-treatment values were taken as baseline values for albumin, globulin, blood urea nitrogen (BUN), albumin/globulin (A/G) ratio and total proteins. The baseline values were compared after each cycle of by applying ANOVA using statistical tool SPSS® version 21. The plasma levels of blood urea nitrogen (BUN), total protein and globulin dropped significantly ($p<0.05$) in patients of all age groups. However, the albumin levels were not significantly changed ($p>0.05$). The A/G ratio level increased ($p<0.05$) as a result of reduction in globulin levels. Significant changes in plasma protein levels were observed in the elderly patients (50 to 65 years) than patients between 20 to 50 years of age. AC protocol impairs liver synthetic functions as observed by decreased blood urea nitrogen (BUN) and plasma protein levels.

Keywords:Doxorubicin-Cyclophosphamide, primary breast cancer, hepatic proteins, blood urea nitrogen (BUN).

INTRODUCTION

Liver is a pivotal organ of the body because it is involved in the regulation of vital functions including biosynthesis, biotransformation and excretion of foreign chemicals (Pavek and Dvorak, 2008). One of its crucial function is to synthesize important proteins which are involved in normal functioning of the body (JE, 2011). Main proteins synthesized by hepatocytes are albumin and globulin. Albumin is the most abundant protein found in the extra cellular fluid (EFC) and accounts for 60% of the total protein in plasma (Turell *et al*., 2013). Additionally, albumin is the most valuable prognostic and diagnostic laboratory marker of liver synthetic functions (Doweiko and Nompleggi, 1991). Major function of albumin is to bind various physiological compounds including acidic drugs (Shargel *et al*., 2005). Albumin also regulates colloidal osmotic pressure of the blood (Boldt, 2010). Globulin proteins, synthesized from liver, are divided into α1-globulins and β-globulins while γ-globulin is synthesized in lymphocytes (Murray *et al*., 2009, Miller *et al*., 1951). Beside binding basic drugs, these proteins regulate transport of cholesterol and iron as well as play their structural role in forming mucoproteins, plasminogens, prothrombin and other clotting factors (Murray *et al*., 2009). Decreased level of these proteins is used as a hallmark to diagnose liver disorders (Hauser *et al*., 1996). It has been shown by number of studies that the liver diseases affecting liver synthetic functions could contribute to hypoalbuminaemia, low total protein and a change in A/G ratio(Dubois *et al*., 2006, Throop *et al*., 2004). Likewise, impaired protein synthesis by the liver could lead to diverse clinical manifestations such as, coagulopatheies, disseminated intravascular coagulopathy (DIC), increased blood cholesterol concentrations, ascites and peripheral edema (McPherson and Pincus, 2011). Moreover, a decrease in plasma protein levels has been suggested to increase the free plasma drug concentration (unbound drug) - leading to increase volume of distribution of the drug and significantly higher probability of unwanted toxic effects (Rolan, 1994, Blaschke, 1977). Liver also play its role in converting toxic ammonia molecules into urea which can easily be excreted through kidney (Landsberg *et al*., 2005).

Literature evidences suggest a close link between an organ damage and chemotherapy. (Hashmi *et al*., 2012). Drug-induced hepato-toxicity is mainly due to direct involvement of liver in the metabolism of foreign chemicals, including drugs (Maurel and Rosenbaum, 2012, Holt and Ju, 2006). AC protocol is used as a first line therapy in the treatment of newly diagnosed patients with breast cancer (Evans *et al*., 2005, Fisher *et al*., 1990). Doxorubicin, an anthracycline antibiotic, can cause subclinical hepatotoxicity in patients during course of

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therapy (Rashid *et al*., 2013, Injac *et al*., 2008, Klasco, 2009). Hepatotoxic effect of doxorubicin is more pronounced in the cancer patients using doxorubicin in combination therapy (Kalender *et al*., 2005). Out of 71% total serum binding (Chassany *et al*., 1996), 62% of doxorubicin bind to albumin (Eksborg *et al*., 1982). Cyclophosphamide, an alkylating agent, is a pro-drug activated by hepatic cytochromal P450 enzyme system (Chen *et al*., 2004). Cyclophosphamide minimally binds to serum proteins, however, its metabolites bind considerably (>60%) to serum proteins (Grochow and Colvin, 1979). Cyclophosphamide has also been shown to cause hepatotoxicity with altered liver synthetic functions (Subramaniam *et al*., 2013), owing to significant oxidative stress and generation of free radicals (Catimel *et al*., 1994, Meyer *et al*., 2012). During the course of cancer treatment many patients die owing to severity of the disease, nevertheless, many of these are related to anti-cancer therapy (Swerdlow *et al*., 2007), thus warrant regular monitoring (Galpin and Evans, 1993, Steinherz *et al*., 1992). These literature evidences suggest that among others, effect of anti-cancer therapy on liver synthetic functions pose significant risk of drug induced hepatic toxicity in cancer patients. However, little attention is paid towards the effect of AC protocol on hepatic synthetic functions during anticancer therapy. In this study, we evaluated the overall production of plasma proteins, albumin globulin and blood urea nitrogen (BUN) in breast cancer patients under AC protocol. Interestingly, total plasma protein, globulin and blood urea nitrogen (BUN) levels were significantly reduced in all patients undergoing anti-cancer therapy. However, in the elderly patients (51 to 65 years) total plasma protein, globulin and blood urea nitrogen (BUN) reduction was more significant compared to patients between 20 to 50 years of age.

Aim of the study

The aim of the study was to assess the liver synthetic functions focusing blood urea nitrogen (BUN) and protein synthesis - total, albumin and globulin protein levels, in breast cancer patients of different age groups

METHODS

Study design

A retrospective observational study was designed to see the effect of AC protocol on liver synthetic functions in synthesizing liver plasma proteins and urea. Patients on AC-protocol for primary breast cancer were selected. The patient's medical records were checked to evaluate changes in the laboratory values during course of therapy. Pre-treatment baseline values of albumin, globulin, albumin/globulin (A/G) ratio, blood urea nitrogen (BUN) and total protein were checked before initiating and after drug therapy during 4 different cycles (cycle 1, cycle 2, cycle 3 and cycle 4) with the span of 21 days. This retrospective study was designed to check the correlation between AC chemotherapy protocol and its effect on liver protein synthesis.

Data source

Data was collected from specialized cancer care hospital in Lahore Pakistan after the approval of ethical committees of the hospital and University College of Pharmacy, University of the Punjab, Lahore, Pakistan, reference number EC/UCP/092/2013.

Sample size and distribution

Total population size of this study was 75 patients (n=75). Out of this total population, 18 patients (24%) were in age group 20-35 years of age, 40 patients (53%) were in age group 36-50 years of age and 17 (23%) patients were in age group 51-65 years of age.

Inclusion criteria

The inclusion criteria were as follows; a) female patients diagnosed with primary breast cancer, b) patients treated with doxorubicin and cyclophosphamide. c) patients administered with 4 cycles of chemotherapy agents repeated after every 21 days, d) Patients from 20 to 65 years of age, e) patients with ECOG (eastern cooperative oncology group) performance status 0-2 were selected (Sørensen *et al*., 1993), f) history of no clinical hepatic disease.

STATISTICAL ANALYSIS

Data was analyzed by using SPSS version 21.0. All the parameters were noted as standard deviation of the mean. The baseline values were compared after each cycle of doxorubicin-cyclophosphamide (AC) cycle by applying ANOVA using statistical tool SPSS® version 21. A statistical value of $p \leq 0.05$ was considered significant.

RESULTS

Chemotherapeutic agents usually exhibit narrow therapeutic index – making tissues and organs susceptible to chemotherapeutic toxicity (Alnaim, 2007). Thus, we aimed at examining the effect of AC protocol on liver's ability to synthesize proteins, i.e., albumin and globulin. As shown in table. 1 average baseline profiles for total protein, albumin and globulin remain unchanged in combined age group and in split age groups intended in an ascending order (table 1 & fig. 1B-C). Similar trend was observed for albumin/globulin ratio (A/G ratio) and blood urea nitrogen (BUN) (table 1). However, compared to baseline, mild reduction in total protein (4%) and marked reduction in globulin levels (8%) were observed in all age groups with every programmed cycle, nevertheless, intra-cycle differences were negligible (table 2). However, there is highly significant reduction (21%) in the level of blood urea nitrogen (BUN) after completion of therapy in

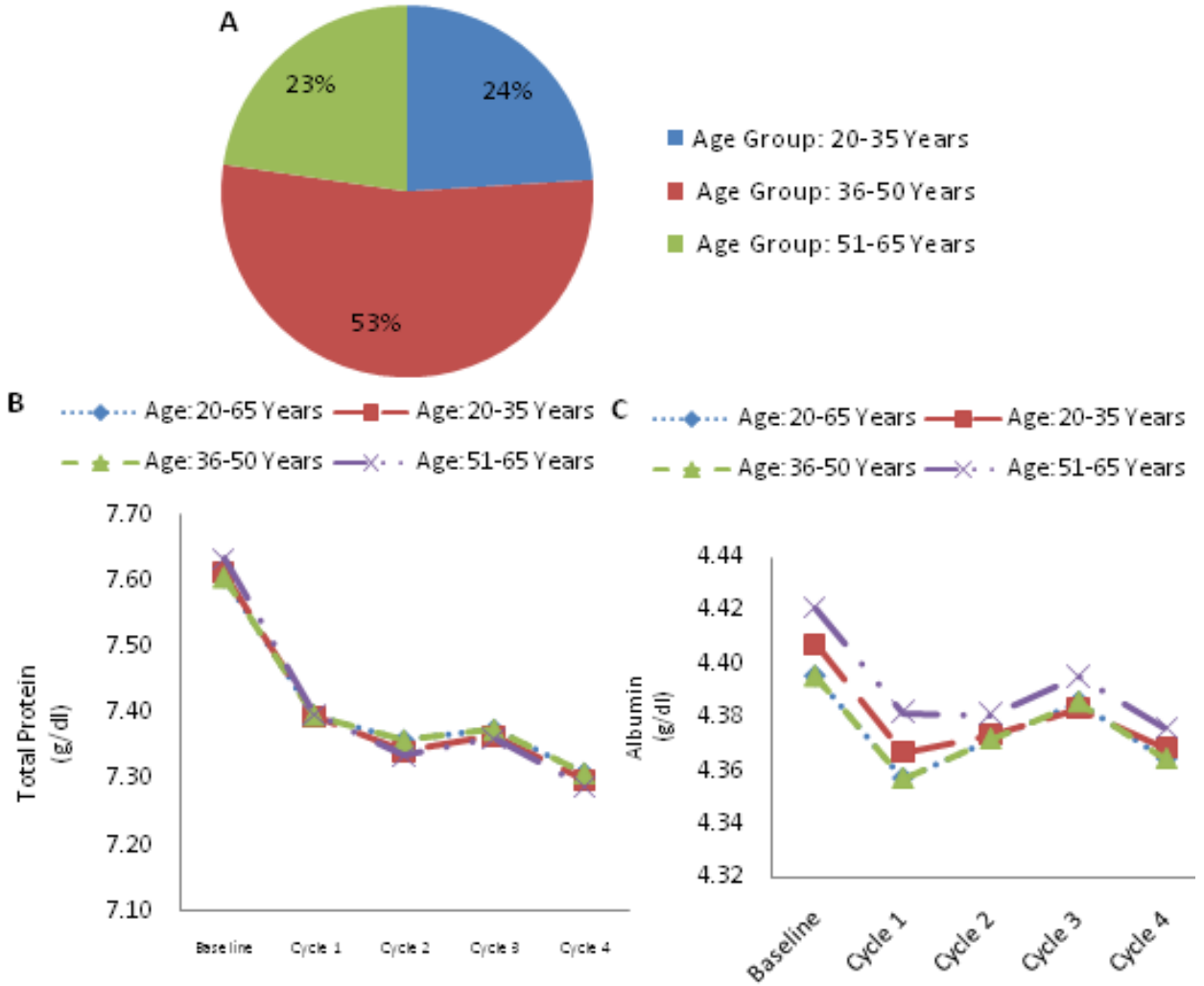

Fig. 1A): Pei chart showing percentage age distribution of female breast cancer patients. **B)** Macro-graph showing total plasma protein levels in different age groups at baseline and after every programmed AC chemotherapy cycle. **C)** Macro-graph showing plasma albumin protein levels in different age groups at baseline and after every programmed AC chemotherapy cycle.

total population (tables 1 & 4). Strikingly, compared to baseline, albumin levels were not significantly affected by AC protocol with every programmed cycle in all age groups (tables 1 & 3).

Aging is a progressive, consistent and time related loss of body's functional units (Crooks *et al*., 1976, Turnheim, 2003, Mangoni and Jackson, 2004). Age related decline in the concentration of plasma protein levels, albumin and α-1 glycoproteins, have not been reported (Fu and Nair, 1998). Additionally, there is scanty of literature evidences that point towards unwanted effects of AC protocol on plasma albumin and globulin levels. Our data suggest that breast cancer is more prevalent in females in 4 and 5th decade of life (36-50 yrs) as compared to other age groups (fig. 1A). Interestingly, compared to baseline values, the effect of AC protocol was more pronounced on aged patient's (51-65 yrs) globulin levels ($p<0.001$), while there were no intra-cycle differences, in globulins levels, in younger patients compared to significant reduction, after cycle 4, in aged patients (9%) (tables 1 & 2, fig 2A). Similarly, compared to baseline A/G ratios, the A/G ratios were higher in aged patients ($p\leq0.003$) than younger patients after cycle 4 therapy, while intra-cycle A/G ratios remained unchanged (table. 3 & fig. 2 B). The average baseline value of blood urea nitrogen (BUN) in patients of age group 51-65 years was 11.29mg/dl and declined to 8.65 mg/dl after cycle 4. It showed that it was the most significant reduction (23%) in the level of blood urea nitrogen (BUN) in elderly patients (tables 1 & 4, fig. 2C).

DISCUSSION

For women, with metastatic breast cancer, the median survival range is within or about 2 years. Therefore, a number of drug regimens have been employed, such as, AC (doxorubicin and cyclophosphamide), CMF (cyclophosphamide, methotrexate and 5-flurouracil), CAF

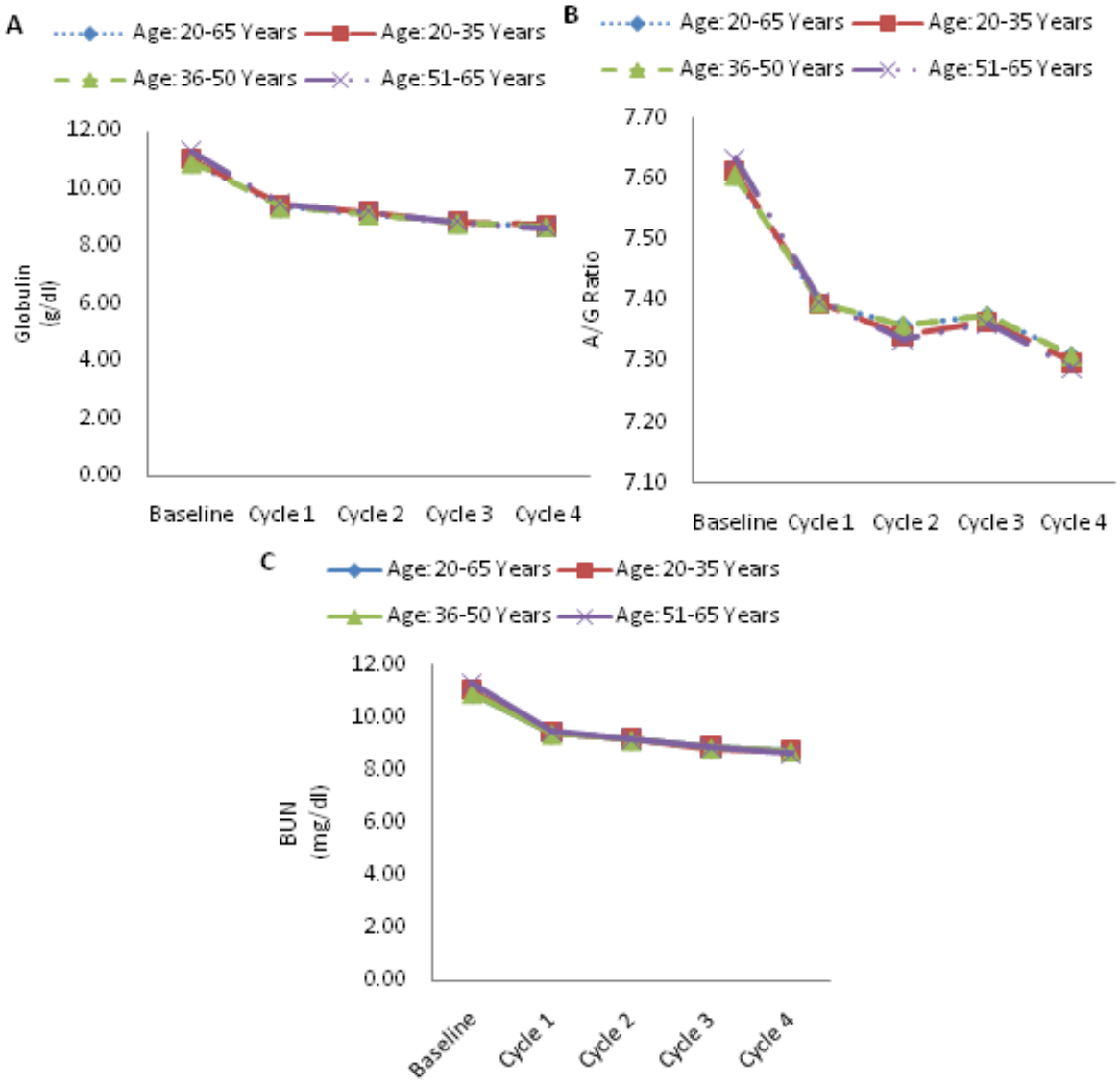

Fig. 2A: Macro-graph showing plasma globulin levels at baseline and after every programmed AC chemotherapy cycles. B) A/G ratio, calculated from plasma albumin and globulin values, at baseline and after each chemotherapy cycles. C) Macro-graph showing blood urea nitrogen (BUN) levels at baseline and after every programmed AC chemotherapy cycles.

(doxorubicin, cyclophosphamide and 5-flurouracil) and doxorubicin plus paclitaxel combination, as a first line chemotherapy in breast cancer (Tranum *et al*., 1982, Buzdar *et al*., 1989, Jassem *et al*., 2001). Despite clinical benefits, in form of prolong survival to breast cancer patients, these drug regimens are prone to cause organ/tissue toxicity, thus, beg further investigations. The frequency of breast cancer in developing countries is lower compared to western countries and is attributed to younger population pyramid (Bibhusal Thapa *et al*., 2013). However, recent trend seems alarming, suggesting that the frequency of breast cancer is increasing in younger females in Asia region, including Pakistan (Yoo, 2010). Our data point towards the similar trend showing significant breast cancer prevalence in relatively younger (18%) and middle age groups (53%) rather than older patients (17%).

In this study we focused on the effect of AC chemotherapy protocol on total plasma protein, plasma albumin, globulin and urea levels produced by liver. Data suggest that AC (Doxorubicin and Cyclophosphamide) chemotherapy protocol significantly affected the total plasma proteins, globulin and blood urea nitrogen (BUN) levels in all age groups with more significant reduction in globulin protein and blood urea nitrogen (BUN) levels in elderly patients, in 5th and 6th decade of life. However, no changes in albumin protein levels were observed. It is beyond doubt that liver plays an important role in protein and drug metabolism (Barle *et al*., 1997) and profound

Table 1: Mean baseline and post therapy values of plasma proteins in different age group

Lab Values	Age (Years)	Baseline Value	After Cycle 1	After Cycle 2	After Cycle 3	After Cycle 4
Total Protein (g/dl)	20-65	7.60±0.470	7.40±0.469	7.36±0.412	7.38±0.390	7.31±0.370
	20-35	7.61±0.481	7.40±0.390	7.34±0.350	7.37±0.410	7.30±0.395
	36-50	7.60±0.510	7.40±0.470	7.36±0.380	7.38±0.425	7.31±0.405
	51-65	7.63±0.525	7.40±0.495	7.33±0.470	7.36±0.412	7.29±0.350
Albumin (g/dl)	20-65	4.40±0.319	4.36±0.327	4.37±0.315	4.39±0.319	4.36±0.313
	20-35	4.41±0.325	4.37±0.319	4.37±0.316	4.38±0.318	4.37±0.320
	36-50	4.40±0.318	4.36±0.312	4.37±0.310	4.39±0.319	4.36±0.315
	51-65	4.42±0.322	4.38±0.319	4.38±0.318	4.40±0.319	4.38±0.314
Globulin (g/dl)	20-65	3.20±0.296	3.04±0.286	3.00±0.278	3.00±0.286	2.95±0.296
	20-35	3.20±0.231	3.03±0.296	2.98±0.276	2.98±0.299	2.94±0.291
	36-50	3.20±0.296	3.04±0.279	3.00±0.281	3.00±0.296	2.95±0.231
	51-65	3.20±0.233	3.02±0.296	2.97±0.231	2.96±0.289	2.91±0.296
A/G Ratio	20-65	1.39±0.148	1.45±0.158	1.48±0.161	1.48±0.158	1.49±0.158
	20-35	1.39±0.155	1.45±0.159	1.48±0.159	1.49±0.158	1.50±0.161
	36-50	1.39±0.158	1.45±0.163	1.48±0.153	1.48±0.151	1.49±0.158
	51-65	1.40±0.149	1.47±0.158	1.50±0.157	1.50±0.156	1.52±0.162
BUN (mg/dl)	20-65	10.92±0.45	9.35±0.40	9.12±0.39	8.80±0.37	8.68±0.35
	20-35	11.03±0.43	9.42±0.41	9.20±0.38	8.84±0.38	8.74±0.39
	36-50	10.92±0.47	9.35±0.39	9.12±0.39	8.80±0.39	8.68±035
	51-65	11.29±0.44	9.48±0.39	9.21±0.37	8.85±0.40	8.65±0.38
Lab Values	Age (Years)	Baseline Value	After Cycle 1	After Cycle 2	After Cycle 3	After Cycle 4
Total Protein (g/dl)	20-65	7.60±0.470	7.40±0.469	7.36±0.412	7.38±0.390	7.31±0.370
	20-35	7.61±0.481	7.40±0.390	7.34±0.350	7.37±0.410	7.30±0.395
	36-50	7.60±0.510	7.40±0.470	7.36±0.380	7.38±0.425	7.31±0.405
	51-65	7.63±0.525	7.40±0.495	7.33±0.470	7.36±0.412	7.29±0.350
Albumin (g/dl)	20-65	4.40±0.319	4.36±0.327	4.37±0.315	4.39±0.319	4.36±0.313
	20-35	4.41±0.325	4.37±0.319	4.37±0.316	4.38±0.318	4.37±0.320
	36-50	4.40±0.318	4.36±0.312	4.37±0.310	4.39±0.319	4.36±0.315
	51-65	4.42±0.322	4.38±0.319	4.38±0.318	4.40±0.319	4.38±0.314
Globulin (g/dl)	20-65	3.20±0.296	3.04±0.286	3.00±0.278	3.00±0.286	2.95±0.296
	20-35	3.20±0.231	3.03±0.296	2.98±0.276	2.98±0.299	2.94±0.291
	36-50	3.20±0.296	3.04±0.279	3.00±0.281	3.00±0.296	2.95±0.231
	51-65	3.20±0.233	3.02±0.296	2.97±0.231	2.96±0.289	2.91±0.296
A/G Ratio	20-65	1.39±0.148	1.45±0.158	1.48±0.161	1.48±0.158	1.49±0.158
	20-35	1.39±0.155	1.45±0.159	1.48±0.159	1.49±0.158	1.50±0.161
	36-50	1.39±0.158	1.45±0.163	1.48±0.153	1.48±0.151	1.49±0.158
	51-65	1.40±0.149	1.47±0.158	1.50±0.157	1.50±0.156	1.52±0.162
BUN (mg/dl)	20-65	10.92±0.45	9.35±0.40	9.12±0.39	8.80±0.37	8.68±0.35
	20-35	11.03±0.43	9.42±0.41	9.20±0.38	8.84±0.38	8.74±0.39
	36-50	10.92±0.47	9.35±0.39	9.12±0.39	8.80±0.39	8.68±035
	51-65	11.29±0.44	9.48±0.39	9.21±0.37	8.85±0.40	8.65±0.38

changes in the rate of protein synthesis by the liver can occur in response to hepatic insult, affecting drug protein binding and disposition (LEVELS, 2000, Sallie *et al*., 1991, Wolfe *et al*., 1989). Furthermore, according to research literature, concurrent administration of anticancer drugs can induce oxidative stress in addition to drug metabolic workload on the liver maximizing hepato-toxic prospects (Pieniążek *et al*., 2013). Thus, routinely, the most common biomarker employed for liver function test (LFT) include, ALT and albumin nevertheless, inclusion of other proteins and markers, such as total globulins (LeBlond *et al*., 2004) and A/G ratio could also provide a mean to assess liver synthetic functions (Pratt and Kaplan, 2000, El–Serag and Rudolph, 2007). In our study, we found marked reduction in the total plasma protein and globulin levels in all age groups – compared to baseline values, however, no change in albumin levels was observed. There is considerable controversy regarding the inclusion of total protein in liver function test, despite the fact that 73% of hospital laboratories in USA and UK use total protein in liver function profiles

Table 2:Multiple comparison of total protein and globulin levels between baseline and every chemotherapy cycle and intra-cycle comparison

Multiple Comparisons		Total Protein			Globulin		
Cycles (I)	Cycles (J)	Mean Difference (I-J)	Std. Error	Sig.	Mean Difference (I-J)	Std. Error	Sig.
Baseline	Cycle1	.20711*	.06887	.023	.16267*	.05220	.017
	Cycle2	.24493*	.06887	.004	.20467*	.05220	.001
	Cycle3	.22907*	.06887	.009	.20453*	.05220	.001
	Cycle4	.29467*	.06887	.000	.24920*	.05220	.000
Cycle1	Cycle2	.03783	.06887	.982	.04200	.05220	.929
	Cycle3	.02196	.06887	.998	.04187	.05220	.930
	Cycle4	.08756	.06887	.709	.08653	.05220	.462
Cycle2	Cycle3	-.01587	.06887	.999	-.00013	.05220	1.000
	Cycle4	.04973	.06887	.951	.04453	.05220	.914
Cycle3	Cycle4	.06560	.06887	.876	.04467	.05220	.913

Table 3: Multiple Comparison of albumin and A/G ratio between baseline and every programmed chemotherapy cycles and among chemotherapy cycles

Multiple Comparisons		Total Protein			Globulin		
Cycles (I)	Cycles (J)	Mean Difference (I-J)	Std. Error	Sig.	Mean Difference (I-J)	Std. Error	Sig.
Baseline	Cycle1	.20711*	.06887	.023	.16267*	.05220	.017
	Cycle2	.24493*	.06887	.004	.20467*	.05220	.001
	Cycle3	.22907*	.06887	.009	.20453*	.05220	.001
	Cycle4	.29467*	.06887	.000	.24920*	.05220	.000
Cycle1	Cycle2	.03783	.06887	.982	.04200	.05220	.929
	Cycle3	.02196	.06887	.998	.04187	.05220	.930
	Cycle4	.08756	.06887	.709	.08653	.05220	.462
Cycle2	Cycle3	-.01587	.06887	.999	-.00013	.05220	1.000
	Cycle4	.04973	.06887	.951	.04453	.05220	.914
Cycle3	Cycle4	.06560	.06887	.876	.04467	.05220	.913

(Hayden and van Heyningen, 2001). Aging has been associated with decrease serum albumin levels with no change in total globulin levels in females irrespective of menstrual function (Sokoll and Dawson-Hughes, 1989). The unchanged serum albumin levels in our study could be attributed to prolong half life of albumin (20 days) and re-synthesis plus normalization of albumin in 14 days, therefore, measuring serum albumin levels after every 21 days of chemotherapy cycle is uninformative (Beck and Rosenthal, 2002). However, unchanged albumin also suggests normal drug protein binding, especially for doxorubicin (25). Reduced serum albumin levels in cancer patients, have been associated with poor survival while its normal or higher values serve as a powerful prognostic variable and are associated with patient survival time (Lis *et al*., 2003). However, reduced serum total globulin levels in all age groups point towards the immune-suppressive effect of AC chemotherapy protocol in breast cancer patients and could be employed as an important supplementary parameter of prognostic value in advanced stage breast cancer patients (Fatima *et al*., 2013, Al-Joudi, 2005). Besides, more significant changes with aging could be attributed towards an age dependent decline in immune utility, in aged cancer patients susceptible to cyto-toxic chemotherapeutic agents because of the decrease hepatic blood flow and reduce drug clearance through kidney (Klotz, 2009, Shi and Klotz, 2011, Corsonello *et al*., 2010, Triantafyllou *et al*., 2010, Aapro *et al*., 2011). We also observed significant increase in A/G ratio (7.46 %) in all age groups, compared to base line values with more pronounced changes after cycle 4. According to literature evidences, increased A/G ratio could be associated with improved survival in patients with normal albumin levels and normally higher A/G ratio is observed in pre-treated breast cancer patients with lower albumin levels and higher globulins levels a compensatory mechanism to counter albumin reduction and to further improve immune competence (Azab *et al*., 2013). Decline in urea levels indicate an incomplete conversion of ammonia into urea. So as a result of impair liver synthetic function to synthesize urea through urea cycle, severe hyperammonemia induced hepatic encephalopathy can further worsen the condition of the patient (Ong *et al*., 2003).

In conclusion, our data suggest that AC protocol opted for breast cancer patients affect total plasma protein, blood urea nitrogen (BUN) and globulin levels, in all age groups with more significant effect in the elderly globulin levels, after every programmed chemotherapy cycle. However,

Table 4:Multiple Comparison of BUN between baseline and every programmed chemotherapy cycles and among chemotherapy cycles

Multiple Comparisons		BUN		
Cycles (I)	Cycles (J)	Mean Difference (I-J)	Std. Error	Sig.
Baseline	Cycle1	1.56227*	.47395	.009
	Cycle2	1.79867*	.47395	.002
	Cycle3	2.11307*	.47395	.000
	Cycle4	2.23080*	.47395	.000
Cycle1	Cycle2	.23640	.47395	.987
	Cycle3	.55080	.47395	.773
	Cycle4	.66853	.47395	.621
Cycle2	Cycle3	.31440	.47395	.964
	Cycle4	.43213	.47395	.892
Cycle3	Cycle4	.11773	.47395	.999

no changes in albumin levels were observed due to albumin physiological and biochemical regulation. Moreover, confirming other reports, our data suggest profound prevalence of breast cancer in younger and middle age patients. Decreased globulin levels with normal albumin levels supporting increased A/G ratio, could possibly predict better survival and long term mortality in breast cancer patients, nonetheless, further studies are required to further understand the concept.

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