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Research article

The Possible Protective effect of Melatonin In Iraqi Breast Cancer Patients Taking Chemotherapy

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ABSTRACT
Breast cancer is the most common cancer that lead to death in the world. The most common type of breast cancer is ductal carcinoma. Chemotherapy that used in the treatment of breast cancer is associated with adverse effects like cardio toxicity, especially with doxorubicin use, due to increase free radical formation like reactive oxygen species. To evaluate the protective effect of melatonin in Iraq breast cancer taking chemotherapy, 40 volunteers, 10 normal subjects served as control, 30 volunteers were divided into two groups randomly first 10 patient named group A taking only chemotherapy without melatonin. The second 20 patient named group B taking melatonin + chemotherapy. In the current study, we measured serum malondialdehyde (MDA), liver function test (ALT, AST and TSB) and cardiac enzyme (CPK and LDH). The results showed that chemo therapy increase serum MDA, AIT, AST, CPK, LDH and reduction in serum TSB. Patients who taking extra supplement with melatonin (group B) showed normalized of these biochemical parameters. Melatonin has a role in protecting against toxicity that produced by chemotherapy.

KEYWORDS: Breast cancer; Chemotherapy; melatonin.

INTRODUCTION

Breast cancer is a malignant tumor of breast cells, especially epithelial cells that lining the ducts or lobules of breast[1]. It is the most common cancer in Iraq, it ranks the first in all years from 1986-2010. It is also the most common cancer among females with an incidence rate about 21.75 per 100,000 female populations in 2010 compared 16.65 per 100,000 female populations in 2008[2]. A Combination of two or three chemotherapy drug is used in treatment breast cancer to avoid resistance that occurred during treatment and for good response example on chemotherapeutic combination is a driamycint cyclophosphomide + 5-fluorouracil)[3]. Doxorubicin is cytotoxic anthracyclin antibiotic, cyclophosphomide is alkylating agent related to nitrogen mustard while 5-fluorouracil is anti metabolite cytotoxic drug. These cytotoxic drug that used against breast cancer is limited by number of toxicities including cardiotoxicity like

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(CHF and cardio my path), hepatotoxicity, myelosuppression and blood disorders. Much of these toxicities due to induction of free radicals formation, especially reactive oxygen species (ROS) by this protocol especially by adriamycin[4].
Melatonin iss also known as N-acetyl –S-methoxytryptamine is produced within pineal gland and many extra pineal tissues such as GIT and lymphocyte[5]. Melatonin production in pineal gland is ultimately under control of light/ dark cycle through the retinal supra chiasmic nucleus with a peak production during dark phase[6]. Beside its function as synchronizer of biological o'clock, it is power full free radical scavenger and wide spectrum of antioxidant. These scavenger activity protect cells against oxidative stress that produced by chemotherapy[7].

METHODOLOGY

Drugs used in this study involve Doxorubicin, Cyclophosphamide, 5-Fluorouracil (from Ebewe, Pharma,Austria), melatonin (Vtane pharmaceutical USA).This study was carried out on (40) females with age between (22-60) years. The age distribution is presented in table 1.

Table 1. The age distribution of patients participated in the study.

Age interval	Number of patients
20-29	4
30-39	8
40-49	13
50-59	15

10 subjects served as a control group and 30 female with different stages of breast cancer after mastectomy treated with either of the following systemic chemotherapeutic regimens.

REGIMEN NO 1

Doxorubicin 60mg/m^2 + cyclophosphamide 600mg/m^2 in i.v infusion, once every 21 days.

REGIMEN NO 2

Doxorubicin 60mg/m^2 + cyclophosphamide 600mg/m^2 in I.V infusion + 5-fluorouracil 600mg/m^2 in I.V infusion once every 21st day.
Certain exclusion criteria were followed to avoid interference of any other factor like drug or pathological condition with treatment.

1. Patients with history of clinical disorder like hypertension, congestive heart failure or ischemic heart disease, diabetes mellitus and those with hepatic or renal disorder.
2. Patient with history of thyroid disorder.
3. Patient with known sensitivity to melatonin and selenium.
4. Smoking female.
5. Advance breast cancer.

Patients were diagnosed and treated in oncological hospital in Baghdad. Under follow up of specialist physicians.
The 30 treated patients were allocated in two subgroups as follow.
Group A: 10 breast cancer patients, who don't receive any antioxidant drug during chemotherapy.
Group B: 20 breast cancer patients, treated with 3mg melatonin at night for 42 days during chemotherapy.

SAMPLE COLLECTION

Venous blood sample (10ml) was obtained from the forearm of each patient by vein puncture at baseline before the initiation of therapy, after 21st days of treatment and at the end 42nd day for all patient groups. Each blood sample was placed in a gelatine tube to be centrifuged for 10 minutes at rate 3500rpm. Serum was then divided into several 1.5 ml eppendrof tubes and stored at (-30°C) until time of assay of hepatic activity (AST, ALT), total serum bilirubin, cardiac enzyme (CPK,LDH) and serum malondialdehyde. Colorimetric determination of AST and ALT was described by Reitman and Frankle[8]. Colorimetric determination of TSB was based on Tietz N.W[9]. MDA was analyzed according to method of Buege, And Aust (1978)[10].

STATISTICAL ANALYSIS

Results were expressed as mean ± standard error of means (SEM). Student's unpaired t-test and ANOVA test were used to examine the degree of significance and P values < 0.05 were considered significant.

RESULTS

A double-blind approach was carried out to perform the clinical parameters in this study. Neither the patient nor the observer knew about the patient treatment during the course of treatment.

Effect of treatment with melatonin on oxidative stress serum malondialdelyde (MDA) level in Iraqi

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breast cancer patient treated with chemotherapy. Table 2 showed the patients with breast cancer taking chemotherapy produce significant increase ($p< 0.05$) in serum MDA level after 21st days and 42nd days when compared to baseline values.
Regarding therapy with melatonin, there was a significant decrease ($p<0.05$) in serum MDA after 21st day and 42nd day of therapy when compared to baseline value of melatonin group, also the value of serum MDA in group B significantly lower than group A at 21st day and 42nd day age interval Number of patients

Table 2. Effect of treatment with melatonin oxidative stress serum malondialdelyde (MDA) level in Iraqi breast cancer patient treated with chemotherapy.

MDAmean ±SD Mmol/L

Group	Baseline	After 21st day	After 42nd day
Chemotherapy A	C2.59±0.29b	B3.29±0.29a	A4.57±0.27a
Melatonin B	A2.79±0.26a	B2.28±0.20b	C1.85±0.17b

Values are expressed as mean ± standard error of mean.

- Means with different capital letters in the same row differ significantly ($p < 0.05$).
- Means with different small letter in the same column differ significantly ($p <0.05$).

Effects of treatment with melatonin on liver function parameters (serum AST, ALT, and TSB) in Iraqi breast cancer patients treated with chemotherapy.
Effect of treatment with melatonin on ALT level in Iraqi breast cancer patients treated with chemotherapy.

Table 3. Showed the following results.

ALT U/L±SD

Group	Baseline	21st day	42nd day
Chemotherapy A	C 20.60±7.89 a	B 27.30±7.84 a	A 34.80±6.17 a
Melatonin B	A 23.46±6.24 a	A 23.45±6.45 a	A 25.90±6.70 b

Effect of treatment with melatonin on a serum AST level in Iraqi breast cancer patients treated with chemotherapy.
Table 4, showed the following results:
In the chemotherapy group, there was a significant increase at $p<0.05$ in serum AST level after 21st day, but there was no significant change at $p<0.05$ after 42nd day in comparison to 21st day.

The differences among group in baseline were not significant. The chemotherapy group showed that ALT increased significantly $p<0.05$ after 21st, and 42nd days when compared to baseline. Concerning therapy with melatonin, there was no significant change during therapy compared to baseline but in comparison to chemotherapy group ALT level significantly lower $p< 0.05$ in melatonin at day 42nd of therapy.
Regarding treatment with melatonin, there was no significant change at $p<0.05$ after 21st, 42nd day, respectively, but in comparison between melatonin group at day 21st and day 42nd to chemotherapy group the level of serum AST significantly lower than chemotherapy group.

Table 4. Showed following results.

AST Mean±S

Group	Baseline	21st day	42nd day
Chemotherapy A	B 24.90±3.63 a	A 31.40±10.79 a	A 32.50±3.34 a
Melatonin B	A 24.11±5.17 a	A 25.80±5.07 b	A 26.92±9.92 b

Effect of treatment with melatonin on a serum TSB level in Iraqi breast cancer patients treated with chemotherapy.
Table 5, showed the following results:

There was a significant reduction $p<0.05$ in serum TSB level after 21st and 42nd day in the chemotherapy group. Regarding therapy with melatonin there was no significant change during therapy. In Comparison between chemotherapy

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group and melatonin Confirmed that the level of serum TSB was significantly higher *p<0.05* in melatonin group at 21st and 42nd day.

Table 5. Effect of treatment with melatonin on serum TSB level in Iraqi breast cancer patients treated with chemotherapy.

TSB mg /dl ±SD

Group	Baseline	21st day	42nd day
Chemotherapy A	C0.54±0.20a	B0.44±0.21b	A0. 3±0.27b
Melatonin B	A0.69±0.13a	A0.55±0.14a	A0.62±0.16a

Effect of treatment with melatonin on serum cardiac function in Iraqi breast cancer patients treated with chemotherapy. Effect of treatment with melatonin on a serum CPK level in Iraqi breast cancer patients treated with chemotherapy Table 6, revealed that in the chemotherapy group, there was significant *p<0.05* increase in serum CPK after the 21st day.

Effect of treatment with melatonin on a serum LDH level in Iraqi breast cancer patients treated with chemotherapy.

Table 7 showed the following. In the chemotherapy group, there was a significant *p<0.02* increase in serum LDH level after 21st and 42nd days compared to baseline. In the melatonin group, there was no significant change in serum LDH during therapy at 21st day. In Comparison between chemotherapy group and melatonin Confirmed that the level of serum TSB was significantly higher *p<0.05* in the melatonin group at 21st and 42nd day.

In the melatonin group, there was no change in serum CPK a long time of therapy. But in comparison between chemotherapy group and melatonin, at 21st and 42nd day respectively the level of CPK was significant *p<0.05* lower than group A.

Table 6. Effect of treatment with melatonin on a serum CPK level in Iraqi breast cancer patients treated with chemotherapy.

CPK U/L±SD

Group	Baseline	21st day	42nd day
Chemotherapy A	B98.00±48.71a	A128. 50±53.67	A151.60±44.10a
Melatonin B	A100.55±38.90a	A102. 70±26.26b	A113.50±25.08b

Effect of treatment with melatonin on a serum LDH level in Iraqi breast cancer patients treated with chemotherapy.

Table 7 showed the following. In the chemotherapy group, there was a significant *p<0.02* increase in serum LDH level after 21st and 42nd days compared to baseline. In the melatonin group, there was no significant change in serum LDH during therapy at 21st day.

In Comparison between chemotherapy group and melatonin Confirmed that the level of serum TSB was significantly higher *p<0.05* in the melatonin group at 21st and 42nd day.

In the melatonin group, there was no change in serum CPK a long time of therapy. But in comparison between chemotherapy group and melatonin, at 21st and 42nd day, respectively the level of CPK was significant *p<0.05* lower than group A .

Compared to baseline, but there was significant increase in serum LDH at 42nd day compare to 21st day.When comparison between chemotherapy group and melatonin selenium group at 21st and 42nd day, the level of LDH was significant *p<0.05* lower than group A.

Table 7. Effect of treatment with melatonin and selenium on a serum LDH level in breast cancer patients treated with chemotherapy.

LDH U/L ±SD

Group	Baseline	21st day	42nd day
Chemotherapy A	C 168.00±54.78a	B200.30±74.67a	A261.00±94.68a
Melatonin B	B170.65±88.88a	B 180.15±77.03b	A202.60±73.54b

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DISCUSSION

Oxidative stress has been linked to breast cancer risk[11]. When oxidative stress, increased (ROS and other radicals levels is high), may lead to damage DNA, producing the mutation that initiates tumors. This may lead to breast cancer[12]. The data presented in this study show that serum MDA level significantly *p<0.05* increased in breast cancer patients compared to normal control, which may be attributed to over production of free radicals or the state of systemic oxidative stress or deficiency of antioxidant, these results consistent with that reported by A. Nath, et al.[13] that who found a significant increase in plasma MDA level in breast cancer patient compared than normal subject. Doxorubicin and its iron chelate undergo redoxcycling, resulting in generation of free radicals and reactive oxygen species (ROS)[14]. Cyclophosphmide metabolized in the liver to produce phosphoramide mustard and acrolein, the later responsible for free radicals production[15]. 5 flourouracil has been proved to induce oxidative stress by increase intracellular ROS[16] The hypothesis of Safinas Seta, Durak et al. and Conklin KA were supported by our study, in which there was a significant elevation of the MDA level after 21st day and more significant after 42nd day when compared to baseline in of chemotherapy. Melatonin act direct scavenges hydroxyl radicals single Oxygen and peroxynitrites[24-26].Melatonin dose in this study 3mg once daily at night[22]. In this study addition, melatonin to chemotherapy show significant reduction in serum MDA level after 21st and 42nd days respectively compared to baseline. Also, when compared to a group of chemotherapy, the level of serum MDA significantly lower than group A. These results were consistent with that reported by Qiw. That who found the administration of melatonin decrease serum MDA level(17) . Although chemotherapy (Doxorubicin , cyclophosphomide and 5-FU) are extensively metabolized in liver, their hepatotoxicity are uncommon since liver antioxidant capacity may protect again free radical injury[18-20]. The current study, revealed that treatment with chemotherapy increase serum AIT level significantly *p<0.05*, But there is moderate elevation in serum AST after 21st day and no significant change after 42nd day. The result of this study is consistent with that of Deep PR et al. who found doxorubicin elevated serum AIT significantly[21]. Therapy with melatonin produce no significant change in serum AST, and AIT level, but when made comparison to chemotherapy group ,the level of serum AST, ALT significant lower than chemotherapy level at 42nd day. These results consistent with. Dr. Mustafa Ghazi Alabassi that found melatonin has a protective role for hepatocyte[22]. In the current study, TSB level significantly decrease with chemotherapy after 21st day and 42nd day as compared to baseline, The decrease in TSB level may attributed to its potent antioxidant properties of bilirubin which prevent the oxidative damage triggered by a wide range of oxidant related stimuli[23], these results consistent with Bshir Abdul Razzaq et al. that found Doxorubicin, cyclophosphomide decrease level of bilirubin[23] also consistent with the study of Khalid Al Khazaji , that found CAF protocol reduce total serum bilirubin[24]. In melatonin, there was no significant change a long time of therapy, and there was a significant increase in serum TSB level when compared to chemotherapy group as shown in table 5, these result suggest the reduction in persistent consumption of endogenous bilirubin antioxidant activity by melatonin and selenium, these results consistent with a study that's done by Khalid AlKhazaji[24]. Treatment with chemotherapy may induce cardio toxicity. Cardio toxicity more frequent more than 20% in patient treated with doxorubicin and 5-FU and less frequent with cyclophosphamide[27]. The incidence of doxorubicin induced cardio toxicity varies depend on medication and cumulative dose. The rate of toxicity 4%-36% in patient receiving 500-550 mg/m2. In this study the cardio toxicity manifested by significantly (*p<0.05*) increase serum CPK and LDH level, which suggest cell destruction lead to leakage from these cells. Our study consistent with Kamal Adel Amin, et al. that five (5) found patients with breast cancer treated with FAC chemotherapy regimen significantly increase serum CK , and LDH that consist chemotherapy induced cardio toxicity[25]. In our study treatment breast cancer patients with melatonin produce no significant *p<0.05* change in serum CK but when make a comparison with the chemotherapy group at 21st and 42nd day, respectively, the level of serum CK in melatonin was significantly lower than the level of CK in the chemotherapy group . When see serum LDH level in melatonin group there was slight significant elevation in serum LDH after 42nd day when compared to baseline. But when make comparison between melatonin and chemotherapy, the level of serum LDH in melatonin was significantly lower than level of LDH in chemotherapy group at 21st day and 42nd

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day. This result are consistent with Ayca Bilginoglu[26] that found melatonin has cardio protective effect against cardio toxicity.

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