The possible Biochemical Modulatory Role of Vitamin D3 and B6 In Angiogenic Balance and Redox Homeostasis in Breast Cancer patients

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Summary

The complexity of cancer in addition to its strong relation with oxidative stress make analysis of one single marker or based on one strategy in treatment as the hypothesis assumed that antioxidants may impair chemotherapy induced cytoxicity, is unlikely to be precise. Oxygen radicals are continuously generated within mammalian cells causing high level of oxidatively modified DNA and increase tumor cell production of the angiogenic factors leading to increase risk factor and aggressiveness of BC. Angiogenic switch is considered as a hallmark of the malignant process since it is required for tumor progression and plays a critical role in the invasion and metastasis of tumor cells. Losing of angiogenic balance in cancer directed several studies to focus on inhibition of angiogenesis as the most promising novel approaches for the treatment of cancer. Among numerous cytokines participate in regulation of angiogenesis, VEGF was the key proangiogenic factor while ES was the master anti-angiogenic factor. The role of VEGF in progression of angiogenesis and drug resistance makes it a target for the most advanced anti-angiogeneic therapy. In contrary, the role of ES in inhibition of angiogenesis makes its recombinant one of the most promising anti-angiogenic therapy. Assessment of CECs, the target for VEGF-activation and ES-inhibitory actions, provide more precisely assessment of angiogenic balance and considered as the advanced marker of angiogenesis.

Since cancer patients suffer from persistence oxidative stress besides vitamins deficiency as a result of exceeding the production of free radicals in combination with nearly completely utilization of endogenous antioxidants. Several cellular and animal studies using nutrients that include vitamins as single agents or in combination with chemotherapy, radiation showed the same effect, no interference, increased protection of normal tissues, tumor killing, survival rate and may improve the outcomes in addition to limiting both the drug resistance and adverse effect of chemotherapy and radiotherapy. All of these may be suggested that protection from free radicals attack is warranted.

Therefore, the present study hypothesized that the benefits of vitamins D3 and B6 supplementation in combination with anti-cancer therapies may be improve outcomes by amplifying the anti-angiogenic effect of endogenous anti-angiogenic factor as ES against the pro-angiogenic factor VEGF, besides keeping redox homeostasis in BC patients throughout the treatment time intervals proceeding. Also, study the mechanism of their action either through oxidant balance or not, in addition to monitor the optimum time intervals for administration through periodical biochemical investigations follow up BC patients throughout treatment course; before mastectomy, after mastectomy, after patients had finished the chemotherapy course and for three months later. Thus the present study covers approximately the three phases of wound healing and treatment strategy by assessment of advanced prognostic and predictive markers for about seven months from surgical operation and then clinically follows up BC patients for one year. This may provide insight on beneficial of combination therapy and endogenous factors that may modulate the response to therapy and their pattern with time. This relationship may provide two lines of evidences, first, assessment of the extent of tumorgenic and angiogenic markers related to redox state may add benefits as a prognostic and predictive markers, second, it may be emerged the role of antioxidants as an adjunct therapy may improve the therapy outcomes. Thus researches are needed to better determine the most rational and

effective combination of redox-active and anti-inflammatory agents to other routine drugs in the treatment of cancer. Especially, the anti-tumor and anti-angiogenesis role of vitamins D3 and B6 through the induction of cancer cell apoptosis, cell cycle arrest, differentiation, improving immune system and the inhibition of cell invasiveness were evaluated.
Blood samples from 20 breast cancer patients were collected before mastectomy, after mastectomy, after completion of chemotherapeutic cycles and at the end of three months later in addition to 15 healthy women as control.

Biochemical Investigation:

1- Redox state markers:
* Profiling the total serum antioxidants activity.
* Profiling oxidative stress by assessment serum melandialdehyde level.

2-Tumorgenic markers:
* Quantitative analysis of serum level of circulating free DNA.

3- Angiogenic markers:
* Assessment of serum level of VEGF.
* Assessment of serum level of ES.
* Quantitative analysis of CECs in peripheral blood sample.

In Gp-II, serum MDA, cfDNA, VEGF, ES and CECs were significantly elevated compared to Gp-I while serum TAS was significantly lower in Gp-II than that in Gp-I. This mean that BC patients have higher oxidative stress and angiogenesis than healthy women.
After mastectomy, whereas serum MDA, cfDNA, CECs were significantly increased, serum TAS and VEGF were significantly decreased while serum ES level didn't show significant change compared to before mastectomy. Consequently, surgery has impact in enhancement oxidative stress which is an integral part in activation of angiogenesis progression. Therefore, BC patients need vitamins at this time to avoid progression of angiogenesis.
After completion chemotherapy cycles, serum TAS, cfDNA, VEGF and CECs significantly decreased while serum MDA and ES didn't show significant change comparing to after mastectomy but still showed higher level comparing to the matching physiological level. Moreover, Gp-IIb showed lower MDA, cfDNA, VEGF, CECs than those in Gp-IIa while serum TAS and ES were higher in Gp-IIa than those in Gp-IIb. Therefore, administration of vitamins D3 and B6 restrict not only oxidative stress accompanied with chemotherapy but also amplyfing the anti-angiogenic effect of chemotherapy angiogenesis process which was resulted from tumorgenesis itself or micrometastasis resulting from surgical process.
Finally, serum MDA, cfDNA, VEGF, CECs significantly decreased while serum TAS and ES were increased significantly in both Gp-IIa and Gp-IIb compared to after mastectomy. In addition, Gp-IIb showed lower serum MDA, cfDNA, VEGF, CECs than those in Gp-IIa while serum TAS and ES were higher in Gp-IIa than those in Gp-IIb. This means vitamins D3 and B6 play an important role in maintenance redox homeostasis near the physiological level and angiogeneic balance and without interference radiotherapy.

Collectively, the present results expand knowledge about the antiangiogenic role of Vitamins D3 and B6 supplementation was carried out by amplifying the apoptotic action of ES on ECs with

strict migratory action of VEGF via keeping the redox homeostasis throughout the treatment stages (surgery, chemotherapy and/or radiotherapy). Whatever vitamins D3 and B6 have an antiangiogenic effect directly via decreasing migration of CECs or indirectly via limiting oxidative stress-induced VEGF and enhancing ES, it is noticed their antiangiogenic effect in BC patients. Also, decline of cfDNA and ECs in response to supplementation of vitamins with chemotherapy may be good predictive markers for response to therapy. Moreover, Supplementation of vitamins may improve the outcomes and reduce the recurrence in BC patients. Moreover, supplementation BC patients with vitamins at the beginning of chemotherapy course and continued after that for three months later improve the clinical outcomes corresponding to those who weren't supplemented. From the view of improving treatment outcomes as reduce or delay recurrence of the cancer after the initial therapy, the present data suggests vitamins D3 and B6 supplementation with the beginning of chemotherapy course maintain redox homeostasis and subsequently DNA integrity in addition to maintain angiogenic balance to level nearby the physiological level. Additionally, the central finding from this study is that enhancing raising TAS level with consequently prevention of DNA damage and striking angiogenesis progression, associated with reduced risk of BC recurrence, enhance response to anti cancer drugs, improve outcomes and overcome the side effect of chemotherapy and radiotherapy.

In contrast to the researchers believed that the antioxidants supplementation can prevent oxygen free radical injury and subsequently may reduce apoptosis in cancer cells and so interfere with chemotherapy and radiotherapy. The present study revealed that integration of vitamin D3 and B6 in therapy strategy from the beginning is recommended since it is observed improving some adverse effect of chemotherapy symptoms as vomiting and nausea in BC patients who received chemotherapy with vitamins D3 and B6. This information may be useful in the development of new combination therapies and in the design of future clinical trials.

The results of the present study lead to the following considerations:

1- Anti-tumorgenic and Anti-angiogenic effect of vitamins D3 and B6 were strongly considered and parallel to their anti-oxidants role.
2- Vitamins D3 and B6 may be not only reduce aggressiveness of breast carcinoma but also didn't interfere with chemotherapy and radiotherapy.
3- Vitamins D3 and B6 could be amplified the anti-angiogenic effect of chemotherapy and radiotherapy.
4- Anti-angiogenic effect of vitamins D3 and B6 may be via their antioxidant effect not via regulatory site of VEGF C/T 936.
5- Anti-angiogenic effect of vitamin D3and B6 appeared clearly via enhancing anti-migratory effect of ES.
6- Anti-angiogenic effect of vitamins D3 and B6 on ECs may be partially through limiting oxidative stress.