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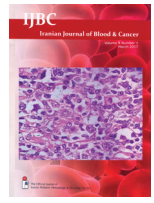

Review Article

Leptin in Breast Cancer: Its Relationship with Insulin, Estrogens and Oxidative Stress

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ABSTRACT

Breast cancer is the most common cancer in women. Several risk factors such as age, family history of breast cancer, marital status, early menarche and late menopause are related to breast cancer. Obesity is also a main health problem associated with breast cancer incidence and subsequent mortality. Association between obesity and expansion of breast cancer may be due to excessive sex steroid hormone production, particularly estrogen. Moreover, adipose tissue is not only a source of estrogen secretion, but also a producer of certain "adipocytokines" including leptin. Leptin is a neuroendocrine hormone with 167 amino acid produced predominantly by white adipose tissue. Leptin after binding to receptor activate JAK/STAT/MAP. Leptin also increased expression of cyclin D1 and cdk2 and induces proliferation. It may also develop mammary tumor growth via multiple mechanisms like pro-inflammatory, oxidative, and anti-apoptotic proangiogenic effects. Leptin can increase aromatase activity in MCF-7 cell line which may increase estrogen production and subsequently induce tumor cell growth. Hyperinsulinism through enhanced leptin production by adipose tissue can affect poor breast cancer prognosis.

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Introduction

Breast cancer (BC) is the most common cancer in women.[1,2] It affects one of every 8 women in the United States. Also, it is one of the most frequent malignancies among Iranian women.[3] Several risk factors such as age, family history of breast cancer, marital status, early menarche and late menopause are related to development of breast cancer.[4] Obesity, as a main health problem, is associated with increased breast cancer incidence and subsequent mortality.[5] However, the mechanism of how obesity relates to the development of breast cancer remains unknown.[4] Studies have shown that the association between obesity and breast cancer may be due to excessive sex steroid hormone production, particularly estrogens.[6] A group of studies showed that obese individuals have high level of serum leptin that is linked to breast cancer development. In fact, obesity is characterized as a leptin resistant process.[7] Moreover, adipose tissue is not only a source of estrogen secretion, but also a producer of certain ''adipocytokines" including leptins.[8] Adipokines, particularly leptin, may have a major role in breast cancer biology.[5] It is suggested that leptin could stimulate mammary glands' growth via multiple mechanisms.[4]

Leptin

After identification of the obese (*OB)* gene, "leptin" was discovered and it is now considered as a member of adipokines. It is a 16 KDa neuroendocrine hormone that acts as a multifunctional protein with 167 amino acids, produced predominantly by white adipose tissue.[8,9] Leptin is secreted into the blood, where it circulates in both bound and free forms.[10] Stomach, placenta, ovary, liver, pituitary and skeletal muscles are among tissues

that expression of leptin mRNA have been reported. Leptin gene expression can be regulated by epigenetic mechanisms. Also there is a reverse relationship between DNA methylation and leptin expression. This relationship was associated with lower methylation density in visceral adipocyte fraction compared to the stromal vascular fraction of white adipose tissue and liver.[7] The principal role of leptin is the regulation of energy homeostasis via controlling energy intake and expenditure, by its function on the arcuate nucleus of the hypothalamus.[9] Obesity is associated with high levels of leptin. In fact, obesity is associated with leptin resistance.[7] However, it is difficult to separate the independent effects of BMI and leptin because of their close biological association.[11] There is minimal leptin production in normal conditions which increases in certain pathological processes such as inflammation and malignant transformation.[12]

Leptin has also contributions to the endocrine and immune systems including reproduction, glucose homeostasis, bone formation, tissue remodelling, inflammation, and angiogenesis.[4] Leptin may also play a main role in the growth of mammary tumors via modulation of the extracellular environment, down-regulation of apoptosis and/or up-regulation of anti-apoptotic genes.[4] It also promotes proliferation and angiogenic differentiation of endothelial cells in vitro and in vivo.[7] It is recognized that leptin is expressed in the vicinity of breast cancer cells and leptin receptors are expressed on the cells of ductal and lobular breast carcinomas.[6] Breast cancer cell lines MCF-7, T47D and MDA-MB-231 and non-malignant cell line MCF10A also express leptin. It can stimulate the proliferative activity of breast cancer cell lines via the presence of a leptin receptor detected on these cell lines by different signaling pathways.[13] Researchers have reported that leptin and its receptors (a member of the cytokine receptor family with two cytokine domains and a single transmembrane domain) are overexpressed in breast tumors.[14] In obese mice, the incidence of mammary tumors is correlated with high level of leptin and leptin receptors. Moreover, leptin may exert its capability in breast cancer development via cell proliferation or tumor progression.[11]

Leptin, Insulin and Breast Cancer

A variety of metabolically active factors such as insulin and glucocorticoids can influence circulating level of leptin.[9] Insulin stimulates leptin secretion following meals and leptin is decreased during insulin deficiency.[9] Leptin also decreases insulin secretion through direct action on pancreatic beta cells. This finding compared with the fact that insulin is able to increase leptin expression, reveals a negative feedback loop between insulin and leptin.[9] A number of studies have shown that there is positive correlation between leptin, obesity and insulin resistance, but other studies could not support this.[10] Insulin as a mitogenic agent stimulates the secretion of leptin and hyperinsulinism through enhanced leptin production by adipose tissue can affect poor prognosis of breast cancer patients.[9] It is hypothesized that the potential interaction between insulin and metastatic cascade is mediated through leptin.[9]

Leptin, Estrogen and Breast Cancer

Numerous studies demonstrated that leptin (OB-R) and estrogen receptors are co-expressed in breast cancers. It seems that interaction between leptin and estrogen promotes breast carcinogenesis.[7] Therefore, estrogen as well as other hormones and growth factors can act as intermediates or biological effectors for leptin's mitogenic activity and stimulates breast cancer.[9] Chezet and colleagues reported that leptin can promote breast cancer development in obese women via enhancing estradiol production in situ, not only via adipose tissue but also via epithelial breast cells.[6] Another study also reported that estrogen production can be promoted by leptin or follicular estradiol secretion may be limited by it. Leptin can increase aromatase activity in MCF-7 cell line which may increase estrogen production and subsequently induce tumor cell growth. Moreover, leptin receptors expressed in T47D breast cancer cell line induced proliferation of T47D cells by leptin.[11] When leptin binds to its receptor Ob-R1 (Obesity receptor), tyrosine phosphorylation and transactivation of signal transducer and activator of transcription 3(STAT 3) were enhanced at the same time with expression of estrogen receptor (ER).[15] On the other hand, leptin-induced STAT3 activation acts as a key event in ER α dependent development of malignant diseases and estrogen receptor alpha expression increases the activity of leptin-induced STAT3 in breast cancer cells.[15]

Impact of Leptin in Angiogenesis

Leptin acts as a positive regulator of vascular endothelial growth factor (VEGF) in breast cancer and blockage of leptin signaling, decreases VEGF expression and tumor growth in mouse xenografts.[16] Another study reported that leptin signaling plays a major role in the growth of both ER positive and ER negative breast cancer that is associated with regulation of pro-angiogenic factors (VEGF/VEGF-R2) as a biomarker of poor prognosis in invasive breast cancer and pro- proliferative molecules.[9] The data supported potential use of leptin-signaling inhibition as a novel treatment for Breast Cancer.[7]

Leptin, Oxidative Stress and Breast Cancer

Leptin may play a main role in "reactive oxygen species" (ROS) production. It is interesting that leptin decreases production of mitochondrial ROS; therefore, it can have protective role for cells, but in many cases it increases the oxidative damage in the cells. This mechanism is not clearly understood, but there is some evidence of modulation of the NADPH oxidase enzymes which cause production of several compounds directly involved in cell survival or cycle disruption.[13]

Impact of Leptin in Apoptosis

Leptin can regulate apoptosis via exerting anti-apoptotic effects. Therefore, it decreases apoptosis through expression of apoptosis inhibitor like survivin and Bcl2 in MCF-7 cells and by inhibiting of pro-apoptotic caspase

9 activity.[13] Therefore, leptin via pro-inflammatory, oxidative and anti-apoptotic proangiogenic effects can have a main role in the pathogenesis of breast cancer.[13]

PPAR Ligand and Leptin Signaling in Breast Cancer

Peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear receptor family of ligand dependent transcription factor.[17] Leptin after binding to receptor activates JAK/STAT/MAPK. It increases GR phosphorylation (pGR) and nuclear translocation. pGR transactivates leptin promoter by binding to GRE motif and activates breast tumor growth. Rosiglitazone (BRL) acts as a new class of antidiabetic drugs and reduces hyperglycemia and hyperinsulinemia in insulin-resistant states. In the presence of BRL, PPAR binds to GRE and as a result GR/PPAR complex is formed, which finally reduce breast tumor growth.[17]

It has been shown that PPAR ligands suppress ObR mRNA and its promoter activity and block signaling of leptin.[18-20] They also reported that PPAR-ligands may show pharmacologic properties and be employed as new therapeutic adjuvant strategies for breast cancer treatment.[17]

Other Leptin Signaling Pathways in Breast Cancer

Another study demonstrated that leptin increases cell proliferation via progression of cell cycle in MCF-7 human breast cancer cells, with up-regulation of "protein kinase C", PPARc, and PPARa, but others reported that leptin through activation of the mitogen-activated protein kinase (MAP kinase) pathway stimulates proliferation of MCF-7 cell line[6] and T47D cell line.[20] The effect of leptin on cell proliferation was decreased through inhibition of MAPK pathways, AKT and PI3K activated by leptin.[21-26]

Conclusion

According to the literature, leptin promotes mammary tumor growth via multiple mechanisms such as pro-inflammatory, oxidative, anti-apoptotic and pro-angiogenic effects. Enhanced leptin production by adipose tissue through hyperinsulinemia can affect poor breast cancer prognosis.

Conflict of Interest: None declared.

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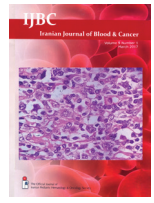

ORIGINAL ARTICLE

Paraoxonase and Arylesterase Activities in Patients with Cancer

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ABSTRACT

Background: Cancer has the highest disease-related mortality rate in Iran. Reduced activity of paraoxonase reported in patients with cancer may be due to a reduction in its antioxidant properties and a subsequent increased risk of developing cancer. We aimed to assess antioxidant and oxidative status in patients with cancer through measuring the activity of PON1 as an antioxidant enzyme and determining MDA as a marker of oxidative stress.
Methods: This case-control study was conducted on 50 patients with colon, lung, blood or breast cancer and 50 age- and sex-matched healthy controls matching during 2014-2015. Paraoxonase-1 and arylesterase activities were measured with paraoxon and phenylacetate substrates and their malondialdehyde levels and serum lipid profile were determined through spectrophotometry.
Results: Serum paraoxonase activity was lower in patients with cancer (28.52±2.77 IU/L) compared with the healthy subjects (96.57±1.49 IU/L; P<0.0001). Similarly, serum arylesterase activity was lower in patients with cancer (49.27±2.90) than the controls (66.91±2.47; P<0.0001). MDA levels were higher in patients with cancer (1.3166±0.0876) than the healthy controls (0.9008±0.0452). The Mann-Whitney U-Test showed significant differences between the two groups in terms of their triglyceride levels (P<0.05). Although serum HDL levels were higher in the control group compared with the cases, the difference was not statistically significant (P>0.05). Serum VLDL, LDL and total cholesterol levels differed significantly between the two groups (P<0.05).
Conclusion: The results obtained showed a reduction in paraoxonase activity and an increased lipid oxidation in the patients with cancer and thereby reduced the antioxidant power of paraoxonase and weakened the body's antioxidant system.

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Introduction

In many countries, cancer is the second cause of mortality.[1,2] in Iran, it is the third leading cause of death after cardiovascular diseases and accidents.[3] The etiology of cancer remains unknown and different factors have been proposed to cause it. Some studies propose genetic factors as the fundamental causes of cancer,[4,5] and other have proposed environmental factors,[6,7] nutrition,[8,9] and infections, smoking and alcohol.[10-12] Nevertheless, the most fundamental cause of cancer is known to be oxidative stress, which is inevitable for aerobic organisms,[13] and can be a major mediator in the damage of cell structures such as proteins, membranes, lipids and the DNA,[14] as an excessive reactive oxygen species (ROS). Increased oxidative stress and oxygen free radicals increase the risk of developing different types of cancer.[15] Low antioxidant levels that increase free radical activities significantly increase the risk of cancer.[16] Reactive oxygen metabolites play a major role in the pathogenesis of gastric and intestinal mucosal

inflammation and cancer.[17] Lipid peroxidation is a known indicator of free radical activity[16] and malondialdehyde (MDA) is one of the final products of lipid peroxidation with a higher rate in patients with cancer.[18-21] The antioxidant system is a set of enzymes and antioxidants that act against free radicals and oxidants; paraoxonase 1 (PON1) appears to be one of these antioxidant enzymes. Some in-vitro studies have proposed paraoxonase as a strong oxidation inhibitor that causes H2O2 hydrolysis.[22] Moreover, PON1 prevents LDL oxidation by removing the oxidized phospholipids.[23]

Paraoxonase 1 is synthesized in the liver and binds to HDL (an active site) in the blood and improves the antioxidant properties of HDL.[24] This enzyme has several different catalytic activities, including paraoxonase, arylesterase, diazoxonase and lactonase activities.[25-27] PON1 is the best and most known PON and is able to destroy lipid peroxides before their accumulation on LDL.[28] The physiological substrates of the enzyme are still unknown, but paraoxonase and arylesterase activities are both performed on single PON1 and paraoxon and phenylacetate are their synthetic substrates, respectively.[17] Oxidizers may be produced or restored in the body through the oxidation metabolic pathways or due to the consumption of oxidized fats. Given that this enzyme binds to HDL, its activity levels were measured along with the lipid profile. Changes in size significantly affect the shape of the binding and the stability of PON1 and reduce its antioxidant capacity.[29] The oxidation of low-density lipoproteins (LDL) in the artery walls is responsible for the initiation and progression of atherosclerosis. High-density lipoproteins (HDL) prevent atherosclerosis and can reduce LDL oxidation. HDLs have a variety of functions that help its protective effect against atherosclerosis, including antioxidant, anti-fibrinolytic and anti-inflammatory properties, and also inhibit matrix metalloproteinase and help keep the endothelial plaques normal and intensify endothelial restoration. As LDLs and their oxidized species are associated with the failure of various tissues followed by different diseases such as cerebral, cardiac, hepatic and renal diseases, diabetes and cancer, most recent studies has been dedicated to the antioxidant properties of paraoxonase.[30] Different studies have also confirmed the role and significance of PON1 in the pathogenesis of different diseases such as diabetes, chronic renal failure, obesity, and the metabolic syndrome, cardiovascular diseases, Alzheimer's disease, HIV infection, chronic hepatic disorder and cancer.[31,32] As a result, paraoxonase 1 is likely to reduce the risk of cancer through its antioxidant properties. Given the limited number of studies on the role of PON1 in cancer, we aimed to assess antioxidant and oxidative status in patients with cancer through measuring the activity of PON1 as an antioxidant enzyme and determining MDA as a marker of oxidative stress.

Materials and Methods

The present case-control study was conducted on 50 patients from Khuzestan province with colon, lung, blood, or breast cancer admitted to the Adult Hematology Division of Shafa Hospital in Ahvaz, Iran, during 2014-2015. The patients were diagnosed with these cancers through blood tests and histopathological findings.

A total of 50 age- and sex- matched healthy individuals were selected as the control group. The controls lacked underlying diseases, diabetes, kidney or liver failure and blood diseases and were selected from the healthy subjects referring to the hospital's medical laboratory. Fasting venous blood samples were obtained and after being transferred to the laboratory, their serum was centrifuged at 3000 rpm for 15 minutes and immediately frozen at -80 °C for the tests.

Paraoxonase Activity Measurement

Paraoxonase activity was measured by adding 20 µl of the serums (dilution 1:10) to 180 µl of paraoxon (1.2 mmol paraoxon in 1 M Tris-Hcl and 1 M NaCl buffer containing 1 M CaCl2 and PH=8.5) at 37 °C and with a wavelength of 405 nm.[33] Paraoxonase activity was expressed in $nmolmin^{-1}ml^{-1}$ in serum.

Arylesterase Activity Measurement

Arylesterase activity was measured with phenyl acetate (Fluka) according to the method proposed by Gan and colleagues using the synthetic substrate of paraoxonase 1. Phenyl acetate was purchased from the Merck Group in Germany along with some other necessary substances. The substrate solution was prepared fresh every day and stored in a closed container and shaken intensely before each use. 10 µl of the serum was then added to the reaction mixture containing 2 mmol of phenyl acetate and 2 mmol of CaCl2 in 100 mmol of Tris-HCl buffer (pH=8). The substrate hydrolysis rate was measured using a spectrophotometer at 37 °C and with a wavelength of 270 nm with UV 1250 (made by Shimadzu in Japan). The enzyme activity was calculated with an extinction coefficient of 1310 $M^{-1}cm^{-1}$ mol/liter and the results were reported in mol/min/ml of serum.[34]

Oxidative Status Measurement

Serum levels of MDA were measured as a marker of lipid peroxidation using Yagi's method, [35] and based on their reaction with thiobarbituric acid and a measurement of the solution absorption and its mixture with n-butanol by mixing 125 µl of its serum with 5.1 ml of phosphoric acid in a test tube and by adding 0.5 ml of thiobarbituric after stirring. The tube containing the mixture was then placed in boiling water for 45 minutes. After cooling, 1 ml of n-butanol was added and centrifuged for 10 minutes; the mixture's pink supernatant was then separated and its absorption was measured at a wavelength of 532 nm and the standard curve solution of MDA formed from tetraethoxypropane was thus obtained.[35]

Lipid Profile Measurement

Triglycerides (TG), Total Cholesterol (CH) and High-Density Lipoproteins (HDL) were measured with standard biochemical methods using commercial laboratory kits (made by Pars Azmoon Co., Tehran, Iran) and also with enzymatic methods using autoanalyzer BT3000; LDL

levels were calculated using Friedewald's formula or through electrophoresis.[36]

Friedwald's Formula

LDL=Total cholesterol-[HDL+TG/K], where k=5.

Very low-density lipoprotein cholesterol, VLDL-C

The samples' VLDL-C was calculated using the following equation:

VLDL-C (mg/dl)=TG (mg/dl)/5

Data Analysis

Data analysis was done using SPSS software, version 22. The quantitative variables were expressed as mean ± standard deviation and the qualitative variables as a percentage. The t-test was used to compare the groups and assess their differences. Pearson's correlation coefficient was used to assess the dependence between the variables. Non-parametric tests were used for the non-normally-distributed variables. The level of statistical significance was set at P≤0.05.

Results

Of the 50 patients examined, 14 (28%) were women and 36 (72%) were men with a mean±SD age of 54.22±13.99 years (range: 25-88 years). Of the total of 50 healthy subjects examined, 17 (34%) were women and 33 (66%) were men with a mean±SD age of 42.22±11.96 years (range: 25 to 70 years) (table 1).

Table 2 presents a comparison of the paraoxonase and arylesterase activities, the MDA levels and the lipid profile. These results suggest a significant reduction in paraoxonase and arylesterase activities in the patients and a significant increase in serum levels of MDA and triglyceride in the group of patients compared to the control group. An inverse correlation was therefore observed between paraoxonase and arylesterase activities and MDA levels (r=-0.457, P≤0.0001 and r=-0.303, P<0.002, respectively) and a direct correlation was also observed between paraoxonase activity and HDL cholesterol levels (r=0.213, P=0.039).

Discussion

Few studies have been conducted to measure PON1 activity in patients with cancer. The present study is one of the few in which paraoxonase, MDA and arylesterase levels are simultaneously measured in patients with blood, colon, lung, or breast cancer. Oxidative stress damages the biological membranes, the intracellular organelles and macromolecules such as proteins and DNA, and can lead to the production of active compounds such as aldehydes, ketones, and hydroxy acids. These radicals are produced in the body as a result of oxidation and restoring reactions within the body or else as a result of environmental factors outside the body. An imbalance in the formation and removal of these radicals, including reactive oxygen species (ROS), can cause genetic damage, interfere with cellular signals and cause neurodegenerative diseases, aging and metastasis. Cardiovascular diseases such as atherosclerosis and coronary artery disease are their long-term pathological presentation.[37] Oxidant-antioxidant balance appears to be important in the initiation and progression of cancer.[38] This study therefore measured PON1 in patients with cancer relying on the enzyme's antioxidant properties and found the enzyme's activity to be significantly lower in patients with cancer compared with the controls. Previous studies have reported similar findings. For example, Akçay and colleagues examined patients with gastric cancer and found PON1 activity to be significantly lower in them compared to in the controls.[23] Baskül[19] also obtained similar results. In line with the present findings, another study examined

Table 1: Mean age and BMI of the case and control group

Variables	Patients group		Control group	
	Men	Women	Men	Women
Sex	36	14	33	17
Age (year)	12.718±55.72	50.35±16.735	46.909±11.601	33.11±6.009
(kg/m2) *BMI	28.84±0.257	28.35±0.201	26.306±0.208	26.55±0.325

*BMI, body mass index

Table 2: Comparison of Paraoxonase, Arilesterase enzymes activity, Lipid peroxidation and lipid profile in two groups of the study (individuals suffering from cancer and healthy ones)

Variable	Patients group	Control group	P value
Paraoxonase)U/L)	28.52 ±2.77**	96.57±1.49	0.000
Aril Esteraz)U/L)	2.9**±49.27	66.91±2.47	0.000
MDA(nmol/L)	1.316±0.087 **	0.9008±0.0452	0.000
HDL-C (mg/dl)	48.91±2.72	53.98±1.56	0.281
Triglyceride (mg/dl)	137.17±12.92 *	105.12±5.0	0.02
LDL-C (mg/dl)	58.28±2.59 **	88.96±2.63	0.000
Cholesterol)mg/dl)	139.93±5.09 **	168.1±3.29	0.000
VLDL-C (mg/dl)	27.11±16.56*	21.02±7.085	0.02

TG: Triglycerides; LDL: Low-density lipoprotein; CHO: Cholesterol; HDL: High-density lipoprotein; VLDL: Very low density lipoprotein; PON Serum paraoxonase ARE: Arylesterase; MDA: Malondialdehyde; Results have been stated as±standard deviation average (values are mean±SD). *Significant difference with control group approximate to 0.05; **Significant difference with control group approximate to 0.0001

patients with esophageal and gastric malignancies and found a significant reduction in arylesterase and paraoxonase in them.[17] Another study observed a significant reduction in PON1 in patients with lung cancer.[15] Oxidative stress is also one of the main risk factors for cancer[35] that sometimes occurs in the body due to disrupted mitochondrial function or inadequate defense mechanisms.[36,37] The present study used MDA as a measure of serum lipid peroxidation. Low antioxidant enzyme activities reduce the antioxidant capacity and increase lipid peroxidation and its metabolic product, MDA, while increased antioxidant enzyme activities inhibit lipid peroxidation and thus reduce MDA production.[39] As expected, MDA levels were significantly higher in cancer patients compared to in the healthy controls, suggesting an increased lipid peroxidation in these patients. Previous studies also confirm this finding.[19,21,39,40] One of the main capabilities of HDL is that it functions as a depository of antioxidant enzymes that can reduce ample levels of oxidized phospholipids from the blood. Paraoxonase is one of the main blood plasma antioxidants that limit the accumulation of oxidized phospholipids in plasma lipoproteins.[41] Although some other HDL-binding proteins, such as apolipoprotein A1, lecithin cholesterol acyl transferase and platelet-activating factor acetyltransferase, also have antioxidant properties, the antioxidant activity of PON1 appears to be the most significant.[42]

Measuring the lipid profile yielded the following results: Triglyceride and VLDL levels increased significantly in the group of patients compared to in the controls; however, HDL cholesterol levels were lower in the patients than in the controls, although the difference was not statistically significant. A significant reduction was also observed in LDL and total cholesterol levels in the patients compared to in the controls. Similar results were reported in the study by Akçay et al.[23] Previous studies have compared PON1 and lipid peroxidation (MDA) levels in patients with gastric cancer and hepatitis and have found a significant inverse relationship between them.[19,43] In another study, PON1, ARE and MDA levels were measured in those exposed to ionizing radiation and a significant inverse relationship was observed, although not between ARE and MDA.[44] A negative correlation appears to exist between PON activity and MDA levels due to the extensive oxidative damage to PON. The binding of PON to HDL confirms the dependence of this enzyme on lipids. The hydrophobic environment of HDL is necessary to paraoxonase activity. Phospholipids, especially those with long fatty acid chains, stabilize PON and are essential to its binding on lipoprotein surfaces.[45] The mechanism of reduced PON1 activity in cancer is not yet identified. The reduced paraoxonase activity in the group of cancer patients compared to in the healthy controls could be due to several factors, including enzyme inactivation. In this process, PON1 free sulfhydryl group reacts with specific oxidized lipids and ultimately becomes inactive.[46] The attack of free radicals (ROS) on the enzyme may be responsible for its inactivation. Moreover, the reduction in paraoxonase activity may be caused by the increased oxidative stress in the patients.[47] The present study found no significant reductions in HDL levels in the group of patients compared to in the control group. Previous studies have shown that changes in HDL structure can lead to the non-binding of PON1 to HDL, thereby reducing serum PON1 levels. Another mechanism associated with the reduction of PON1 activity could be due to the suppression of the enzyme due to genetic defects[46] or perhaps due to the down regulation of its transcription in the liver. Another reason for the reduction in paraoxonase may be the disrupted liver structure and function. Since the liver is the largest and most important organelle in the body and since colon, lung and breast cancer commonly metastasize to the liver, patients with cancer tend to also develop liver damage. Since PON1 is produced in the liver, an impaired liver function can reduce the production of PON1 or cause the production of impaired HDL.[48]

Overall, paraoxonase has been shown to become inactive after the hydrolysis of lipid peroxides in patients with high levels of lipid peroxidation.[47] Moreover, paraoxonase is an HDL-dependent enzyme and any changes in the metabolism and structure of HDL can reduce paraoxonase activity.[48] Moreover, since PON1 is reduced as an antioxidant enzyme in the body, the oxidant-antioxidant balance is impaired and oxidative stress thus increases. The oxidant, inflammatory and angiogenic environments then lead to carcinogenesis and enable the progression of cancer.[23] As PON1 is an antioxidant factor, using factors that increase its activity may help with the treatment of cancer. Overall, the present study shows that paraoxonase levels reduce in patients with cancer and consequently increase oxidative stress and lipid peroxidation. This finding suggests the positive effects of antioxidants on cancer. Nevertheless, the high levels of MDA and the reduced PON1 activity suggest an impaired oxidant-antioxidant balance in patients with cancer, suggesting oxidant-antioxidant balance to have a major role in the pathogenesis of cancer.

One of the limitations of the present study is that it did not assess the many different elements involved in oxidant-antioxidant balance, thereby making the generalization of the results to the entirety of oxidant-antioxidant balance irrespective of the other factors at play a matter that should be pursued with extreme caution; the following measures are therefore recommended: To better understand the antioxidant properties of paraoxonase in patients with cancer, antioxidant factors such as vitamins, including vitamin E and C, and antioxidant enzymes such as catalase, glutathione and peroxidase are recommended to be studied along with paraoxonase. Dietary antioxidants are also recommended to be administered to patients with cancer either in combination or separately and their effects to be assessed on PON1.

Conclusion

In patients with cancer, reduced paraoxonase activity accompanied by reduced arylesterase activity indicates weak antioxidant activities in the body. Increased MDA levels as a marker of lipid peroxidation suggest the lower oxidative status potentially caused by oxidant-antioxidant imbalance (including reduced antioxidant power, more

oxidized substances or both). These findings demonstrate oxidative stress or its aggravation in patients with cancer.

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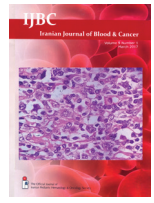

ORIGINAL ARTICLE

Expression Pattern of Interferon-γ in Human Leukemic T Cell Lines Following Treatment with Phytoheamagglutinin, phorbol myristate acetate and Lipopolysaccharide

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ABSTRACT

Background: As a T helper type 1 (Th1) derived cytokine, Interferon gamma (IFN-γ) is an important regulator of inflammatory immune responses. Furthermore, IFN-γ plays an essential role in defense against tumors and intracellular pathogens. This study was designed to assess the pattern of IFN-γ production in human leukemic (Jurkat and Molt-4) T cell lines in vitro.
Methods: Jurkat and Molt-4 cells were cultured in whole RPMI-1640 media. The cells were imbedded at a density of 2×10^6 cell/ml. The cells were stimulated with different concentrations of Phytoheamagglutinin (PHA) (2-10 µg/ml), phorbol myristate acetate (PMA) (1-25 ng/ml) or lipopolysaccharide (LPS) (1-4 µg/ml) for activation and cytokine production for 48 hours. Then the cell-conditioned media were used for IFN-γ assay. Analysis of variance (ANOVA) was done for comparing the groups statistically.
Results: PHA and PMA substantially augmented IFN-γ level in human leukemic T cells (Molt-4 and Jurkat) in a dose-dependent manner after 48 hours of incubation compared with untreated control cells, whereas LPS did not have any significant effect on IFN-γ production in human leukemic T cell lines compared with unstimulated cells.
Conclusion: human leukemic Jurkat and Molt-4 T cell lines could potentially produce IFN-γ with different amounts. PHA was a more potent stimulator of IFN-γ production than PMA. Molt-4 cell line could produce more IFN-γ than Jurkat cell line. These cells could be appropriate for studying the mechanisms of action of immunomodulators as well as screening the IFN-γ stimulators/inhibitors.

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Introduction

Interferons (IFNs) are well-studied proinflammatory cytokines.[1,2] Interferon gamma (IFN-γ), as a T helper type 1 (Th1) derived cytokine is an important regulator of inflammatory immune responses.[3] Furthermore, IFN-γ plays an essential role in defense against tumors and intracellular pathogens.[4,5] There are also crucial roles suggested for IFN-γ in the pathogenesis of numerous diseases such as vitiligo, alopecia areata and Type 1 diabetes.[6,7] Dysregulation of IFN-γ in some diseases including intracranial aneurysms and kawasaki disease have been demonstrated.[8,9] Besides decrease of IFN-γ in several diseases such as complicated respiratory viral infections, HIV, cystic fibrosis and asthma has been shown.[10-13] In addition increased level of IFN-γ in various disorders including type 2 diabetes and inflammatory bowel disease (IBD) has been reported.[14,15] Immunomodulatory effects of physical activity on IFN-γ has also been revealed.[16,17] Moreover, the beneficial effects of some drugs such as propranolol, pentoxifylline and

methotrexate in a number of cardiovascular diseases such as autoimmune myocarditis has been partly attributed to their inhibitory effects on IFN-γ production.[18,19] Upregulation of IFN-γ by some immunomodulators such as piperine, clinacanthus nutans (a medicinal plant) and leptin had therapeutic effects on immune-compromised tuberculosis, hepatoma and leishmania donovani infections, respectively.[20-22]

In this study, pattern of IFN-γ production in human leukemic (Jurkat and Molt-4) T-cell lines has been evaluated in vitro.

Materials and Methods

Microtiter plates, tubes and flasks were purchased from Nunc (Falcon, USA). Fetal calf serum (FCS) got from Gibco (USA). Phytoheamagglutinin (PHA), Phorbol myristate acetate (PMA), Lipopolysaccharide (LPS), penicillin, streptomycin, RPMI-1640 and trypan blue (TB) were purchased from sigma (USA). IFN-γ standard ELISA kit was obtained from R&D company (USA). Human leukemic Molt-4 (NCBI C149) and Jurkat (NCBI C121) T cells were purchased from the National Cell Bank of Iran (NCBI). These cells were retained in RPMI-1640 media complemented by 10% FCS in 5% CO_2 at 37°C.

Cell Culture

The leukemic cells were cultivated in RPMI-1640 media added with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at 37°C in 5% CO2. After that the cells were distributed at a concentration of 2×10^6 cell/ml and next treated with different concentrations of PHA (1-10 µg/ml), PMA (5-25 ng/ml) or LPS for 48 hours. The supernatants of cell culture media were removed and used for IFN-γ quantification. Each test was arranged in triplicates.[23]

IFN-γ Test

The extent of IFN-γ produced in cell culture supernatants of leukemic T cells (Molt-4 and Jurkat) was measured with the Quantikine human enzyme-linked immunosorbent assay (ELISA) kits (R&D organizations) as per the manufacturer's procedures. This test uses the quantifiable sandwich enzyme immunoassay method. Whole RPMI media was used also for control. In order to illustrate the standard curves, human recombinant IFN-γ was used.

Statistical Analysis

IFN-γ measurement in cell-culture medium was done in three diverse tests and the data were specified as mean±standard error of the mean (SEM). Analysis of variance (ANOVA) was used for statistical analysis between groups. $P<0.05$ was statistically designated as significant. For statistically significant variations, test of multiple comparison of Tukey was performed (5%).

RESULTS

Pattern of PHA-Induced IFN-γ Production in Molt-4 Cells

IFN-γ production was relatively low in unstimulated human leukemic Molt-4 cells, but PHA considerably increased IFN-γ production in Molt-4 cells following 48-hour treatment compared with the control cells dose-dependently ($P<0.05$, figure 1).

Pattern of PHA-Induced IFN-γ Production in Jurkat Cells

IFN-γ production was very low in unstimulated human leukemic Jurkat cells but PHA markedly increased IFN-γ production in leukemic Jurkat cells subsequent to 48-hour treatment compared with the control cells. The PHA-stimulated IFN-γ production in leukemic Jurkat cells was concentration-dependent ($P<0.05$, figure 1).

Pattern of PMA-Induced IFN-γ Production in Molt-4 Cells

Unstimulated human leukemic Molt-4 cells produced relatively little amount of IFN-γ, but again PMA considerably increased IFN-γ production in Molt-4 leukemic cells following 48-hour treatment compared with the control cells dose-dependently ($P<0.05$, figure 2).

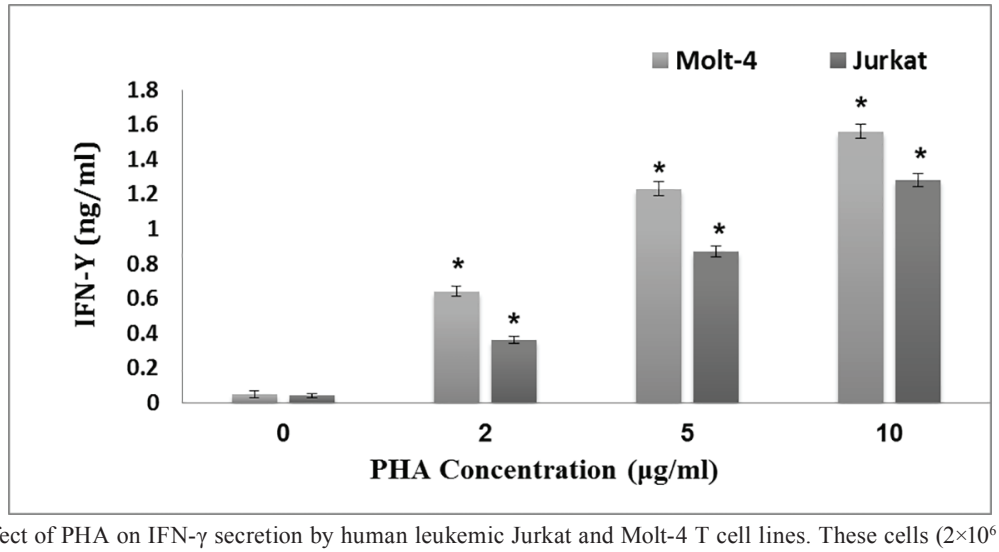

Figure 1: Effect of PHA on IFN-γ secretion by human leukemic Jurkat and Molt-4 T cell lines. These cells (2×10^6 cells/ml) were cultured in complete RPMI-1640 medium and next were stimulated with various doses of phytoheamagglutinin (PHA) (2-10 µg/ml) for 48 hours. Finally, IFN-γ levels in cell culture media was quantified by ELISA kit. Results are mean ± SEM of three distinctive tests.*$P<0.05$ was specified significant.

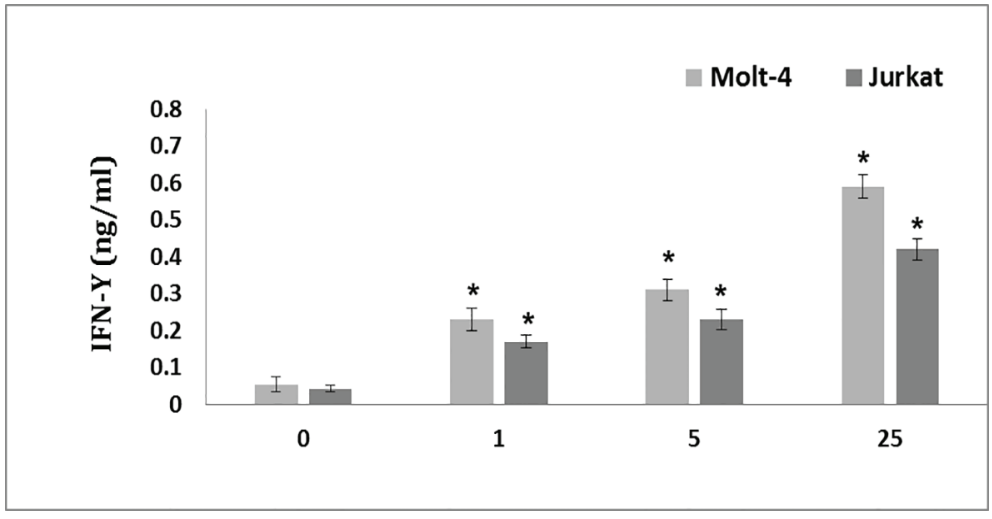

Figure 2: Impact of PMA upon IFN-γ secretion by human leukemic Molt-4 and Jurkat T-cell lines. These cells (2×10^6 cells/ml) were cultured in complete RPMI-1640 medium and next were stimulated with various doses of phorbol myristate acetate (PMA) (1-25 µg/ml) for 48 hours. Finally, IFN-γ levels in cell culture media was quantified by ELISA kit. Results are mean ± SEM of three distinctive tests.*P<0.05 was specified significant.

Pattern of PMA-Induced IFN-γ Production in Jurkat Cells

Unstimulated human leukemic Jurkat cells produced very low amounts of IFN-γ but PMA considerably enhanced IFN-γ production in leukemic Jurkat cells following 48-hour treatment compared with the control cells. The PMA-stimulated IFN-γ production in leukemic Jurkat cells was concentration-dependent (P<0.05, figure 2).

Pattern of LPS-Induced IFN-γ Production in Molt-4 Cells

IFN-γ production was relatively low in unstimulated human leukemic Molt-4 cell line and LPS had no effect on IFN-γ production following the 48-hour treatment compared with unstimulated control cells (figure 3).

Pattern of LPS -Induced IFN-γ Production in Jurkat cells

IFN-γ production was very low in unstimulated human leukemic Jurkat cells and LPS had no effect on IFN-γ production in these cells subsequent to the 48-hour treatment compared with unstimulated control cells (figure 3).

Discussion

In this study, we found that Jurkat and Molt-4 leukemic T cell lines can produce IFN-γ. We also found that PHA/PMA increase IFN-γ production in above mentioned leukemic cells but LPS did not show any significant effect on IFN-γ production. Different patterns of IFN-γ are expressed in different diseases[24-26] and different profiles of IFN-γ are produced in response to various

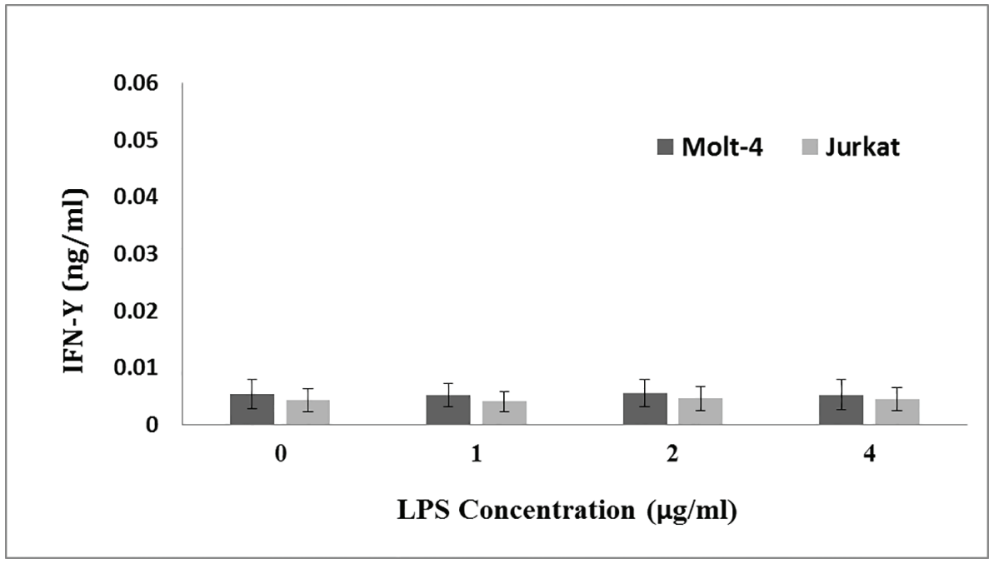

Figure 3: Impact of LPS on IFN-γ secretion by human leukemic Molt-4 and Jurkat T-cell lines. These cells (2×10^6 cells/ml) were cultured in complete RPMI-1640 medium and next were stimulated with various doses of lipopolysaccharide (LPS) (1-4µg/ml) throughout 48 hours. Finally, IFN-γ levels in cell culture media was quantified by ELISA kit. Results are mean ± SEM of three distinctive tests.*P<0.05 was specified significant.

antigens.[27-29] In the present study, PMA, PHA and LPS were used to assess their modulatory effects on IFN-γ production. Consistent to our results, induction of IFN-γ mRNA expression by PHA and PMA in Jurkat cells have been shown by Benbernou et al.[30] In their study, Jurkat cells were used at 1×10^5 cells per well and were stimulated simultaneously by PHA (10 µg/ml) and PMA (10 ng/ml). However, Benbernou and colleagues assessed IFN-γ mRNA expression and did not evaluate the level of IFN-γ production. We used 2×10^6 Jurkat or Molt-4 cell and stimulated the cells with different concentrations of PHA (1-10 µg/ml) or PMA (1-25 ng/ml).

According to our results, LPS did not display any significant effect on IFN-γ production in human leukemic cells (Jurkat and Molt-4). Different effects of LPS on IFN-γ production has been determined by different studies.[31,32] In Kabanov and colleagues' study, Rhodobacter capsulatus PG LPS stopped IFN-γ production in human whole blood.[31] Their findings are consistent with our results. We assessed the LPS effect on the unstimulated human leukemic cells (Jurkat and Molt-4) and since these cells produced very low amounts of IFN-γ in unstimulated state, therefore we could not conclude that LPS does not have any effect on IFN-γ production. It is suggested to assess the effects of LPS on IFN-γ production on cells stimulated by PHA or PMA. In another study, LPS-stimulated bone marrow mesenchymal stromal cells, showed profoundly decreased IFN-γ gene expression while co-cultured with T lymphocyte.[32]

In our study PMA increased IFN-γ production in leukemic cells dose-dependently after 48 hours of stimulation. This enhancing effect reached the maximum point at 25 ng/ml of PMA. In accordance to our results, another study showed an increase of IFN-γ production in rat whole blood cells dose-dependently which maximized 6 hours after stimulation with 25 ng/ml PMA.[33] The reason for the discrepancy between our study and the mentioned study was that we used leukemic T-cells incubated with PMA for 48 hours while the mentioned whole blood cells incubated with PMA for 0-10 hours. In addition, consistent with our data, Barten et al. have shown an augmented IFN-γ secretion in healthy whole blood cells stimulated with 25 ng/ml PMA after 5 hours.[34] Moreover, similar to our study, Keski-Nisula and colleagues revealed that PMA increased the excretion of IFN-γ in healthy whole blood cells after 24 hours incubation.[35]

Furthermore, contrary to a previous study[33] which did not observe any significant influence of PHA on the production of IFN-γ in rat whole blood cells, in our study PHA profoundly increased IFN-γ production in human leukemic T cells in a dose-dependent manner after 48 hours of incubation. This discrepancy between that study and ours may be due to the difference in cells which were used, species and incubation time. We used human leukemic T-cells incubated with PHA for 48 hours while in the mentioned study, the researchers used rat whole blood cells incubated with PHA for 0-10 hours.

Taken together, according to the results of our study, diverse profiles of IFN-γ are produced in response to various stimulating agents. Also we found that PHA and PMA are potent stimulators of IFN-γ production in human leukemic T-cells. Moreover, PHA was a more potent stimulator of IFN-γ production than PMA. In addition, Molt-4 cell line could produce more IFN-γ than Jurkat cell line. Hence these cells may be appropriate tools to evaluate the mechanism of IFN-γ induction in diseases in which IFN-γ production is dysregulated as well as screening the regulators, stimulators or inhibitors of IFN-γ induction.

Conclusion

According to our data, human leukemic Jurkat and Molt-4 T cell lines could potentially produce IFN-γ with different amounts. PHA was a more potent stimulator of IFN-γ production than PMA. In addition, Molt-4 cell line could produce more IFN-γ than Jurkat cell line. Therefore, these cells possibly will offer proper tools to assess the regulating mechanisms of IFN-γ secretion in diseases in which IFN-γ production is dysregulated as well as screening of the regulators, stimulators, or inhibitors of IFN-γ production.

Conflict of Interest: None declared.

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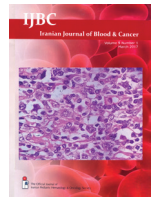

ORIGINAL ARTICLE

Clinicopathological Features of Non-metastatic Triple Negative Breast Cancer

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ABSTRACT

Background: Triple negative breast cancer (TNBC) is reported to be associated with a high risk of recurrence, poor overall survival (OS), and disease-free survival (DFS) rates. This study evaluated the clincopathological features and survival of non-metastatic TNBC women in the capital of Iran compared with other areas of the world.
Methods: In a retrospective study, 119 women with TNBC based on the criteria were analyzed in this study during 2007-2015. A number of clinicopathological variables, OS and DFS were determined in all patients. The mean follow-up was 38 months, which 6 patients lost to follow-up and 16 died of the disease and therefore were censored from the study.
Results: The mean age at diagnosis was 44.9 years (range: 21-85 years). 31.9% were older than 50 years. The 2- and 5-years OS rates were 96% and 88.1%, respectively; whereas, the 2- and 5-years DFS rates were 87% and 74.1%, respectively. Right breast tumor and lymph node involvement were more common in patients younger than 50 years, but vascular invasion was more observed in patients aged ≥50 years. There was no significant difference between menopause status, age and Ki-67 index for OS or DFS.
Conclusion: The prevalence of TNBC was more common in women younger than 50 years. Ki-67 index, menopausal status and age could contribute to prognosis and survival of patients.

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Introduction

Breast cancer (BC) is the most common malignancy and the leading cause of death among women.[1,2] This cancer is a common health problem in Iranian women,[3] and occurs about a decade earlier than women in western countries.[4] Triple negative breast cancer (TNBC) is defined by the absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)[5] receptors. Therefore, patients with TNBC do not benefit from hormone or trastuzumab-based therapies.[6] TNBC accounts for 10-17% of all BCs.[5,7] Risk of developing TNBC varies with age, race, genetics, breastfeeding patterns and parity. Some of TNBCs are very chemosensitive and most patients treated for TNBC will never relapse.[8] Proliferative index reflected by Ki-67, is a key characteristic feature of malignant tumors and could be one of the major factors associated with prognosis.[9,10] TNBC is characterized by a typical ductal histology, high grades, and high proliferation and mitotic rates.[11] It is associated with a high rate of local recurrence and poor disease-free survival (DFS).[12] We aimed to evaluate the clinicopathological features and survival of

non-metastatic TNBC women in Tehran, Iran, compared with other areas of the world.

Materials and Methods

In this retrospective study, out of all patients with breast cancer referred to a Private Clinic, Tehran, Iran, 2007-2015, 119 patients with TNBC were selected for this study. Age, laterality of the tumor, tumor size, lymph node involvement, vascular invasion, perineural invasion, stage, type of pathology, grade, margin involvement, Ki-67 index, menopausal status, radiation therapy, OS and DFS were determined in all patients. The mean follow-up was 38 months During this period, 16 women died and 6 were lost to follow-up. We included women with breast cancer with ER, PR and HER2 negativity (TNBC) aged over 18 years. We excluded women with TNBC with HER2 2+/FISH+ and metastatic TNBC at diagnosis. The characteristics of the included women such as age, laterality, tumor size, menopause status, vascular invasion, lymph node involvement, tumor grade, pathology, receiving radiotherapy, and marginal involvement were assessed.

All patients were treated with adjuvant chemotherapy. The OS was defined as from the date of diagnosis until death from any cause and DFS as the time from diagnosis to either relapse, second cancer, or death from any cause. ER and PR negativity was defined as less than 10% positive tumor cells with nuclear staining and HER2 2+ was tested by fluorescence in situ hybridization (FISH).[13,14] Meanwhile, Ki-67 index was divided into ≤20% and >20%.

Data were analyzed with SPSS version 19 software and survival data were plotted with GraphPad Prism 5 (Kaplan Meier curves and Log-rank test for analysis). P<0.05 was considered statistically significant.

Results

Mean age at diagnosis was 44.9 years (range, 21-85 years); 31.9% of patients were older than 50 years of age (table 1). Out of 119 patients with TNBC, 44.5% had right breast involvement, 47.9% showed lymph node involvement, 18.5% vascular invasion, 7.6% perineural invasion, 7.6% margin involvement and 27.7% had Ki-67≤20% and 87.4% received radiotherapy. 18.5%, 62.2% and 19.3% of the patients were diagnosed with stage I, II and III, respectively. 10.9%, 42% and 47.1% of patients had grade I, II and III tumors, respectively. Ductal carcinoma was the most common histological type (89.99%), followed by medullary carcinoma (9.2%) and lobular carcinoma (0.8%). Tumor size was <2 cm, 2-5 cm and > 5cm in 26.9%, 58% and 15.1% of the patients, respectively.

The correlation between a number of variables and age is shown in table 2. There was a significant correlation between laterality of tumor, lymph node involvement and vascular invasion with age (P=0.015, P=0.012 and P=0.003, respectively). Therefore, right breast involvement and positivity for lymph nodes were more common in patients less than 50 years old, but vascular invasion was more observed in patients ≥50 years.

Figure 1 shows the OS and DFS for all TNBC patients. The 2- and 5-years OS rates (the means) were 96% (22 months) and 88.1% (34.2 months), respectively. Also, the 2- and 5-years DFS rates (the means) were 87% (21.5 months) and 74.1% (33.5 months), respectively.

Figure 2 shows the 5-year OS and DFS in terms of Ki-67

Table 1: The characteristics of the patients with triple negative breast cancer (n=119)

Variables	N (%)	Variables	N (%)
Age group, years		Type of pathology	
≥50	38 (31.9)	Ductal carcinoma	107 (89.9)
<50	81 (68.1)	Medullary carcinoma	11 (9.2)
Laterality		Lobular carcinoma	1 (0.8)
Right	53 (44.5)	Grade	
Left	66 (55.5)	I	13 (10.9)
Tumor size, cm		II	50 (42)
<2	32 (26.9)	III	56(47.1)
2-5	69 (58)	Radiotherapy	
>5	18 (15.1)	Yes	104 (87.4)
Lymph node involvement		No	15 (12.6)
Yes	57 (47.9)	Margin involvement	
No	62 (52.1)	Yes	9(7.6)
Vascular invasion		No	110(92.4)
Yes	22 (18.5)	Ki-67, %	
No	97 (81.5)	≤20	33 (27.7)
Perineural invasion		>20	86 (72.3)
Yes	9 (7.6)	Menopausal status	
No	110 (92.4)	Premenopausal	80 (67.2)
Stage		Postmenopausal	39 (32.8)
I	22 (18.5)		
II	74 (62.2)		
III	23 (19.3)		

Table 2: The correlation between a number of variables and age in triple negative breast cancer patients (n=119)

Variables	Age<50 N=81	Age≥50 N=38	P value
Laterality (right)	42(51.9)	11(28.9)	0.015
Tumor size, cm (<2, 2-5)	24(29.6), 46(56.8)	8(21.1),23(60.5)	0.557
Lymph node involvement (yes)	45(55.6)	12(31.6)	0.012
Vascular invasion (yes)	9(11.1)	13(34.2)	0.003
Perineural invasion (yes)	4(4.9)	5(13.2)	0.115
Stage (I, II)	16(19.8),47(58)	6(15.8),27(71.1)	0.363
Type of pathology (DC*, MC**)	72(88.9), 0	35(92.1), 1(2.6)	0.210
Grade (I, II)	11(13.6),36(44.4)	2(5.3),14(36.8)	0.184
Margin involvement (yes)	73(90.1)	37(97.4)	0.153
Ki-67, % (≤20)	20(24.7)	13(34.2)	0.194
Menopausal status (Premenopausal)	56(69.1)	24(63.2)	0.328

*Ductal carcinoma, **Medullary carcinoma

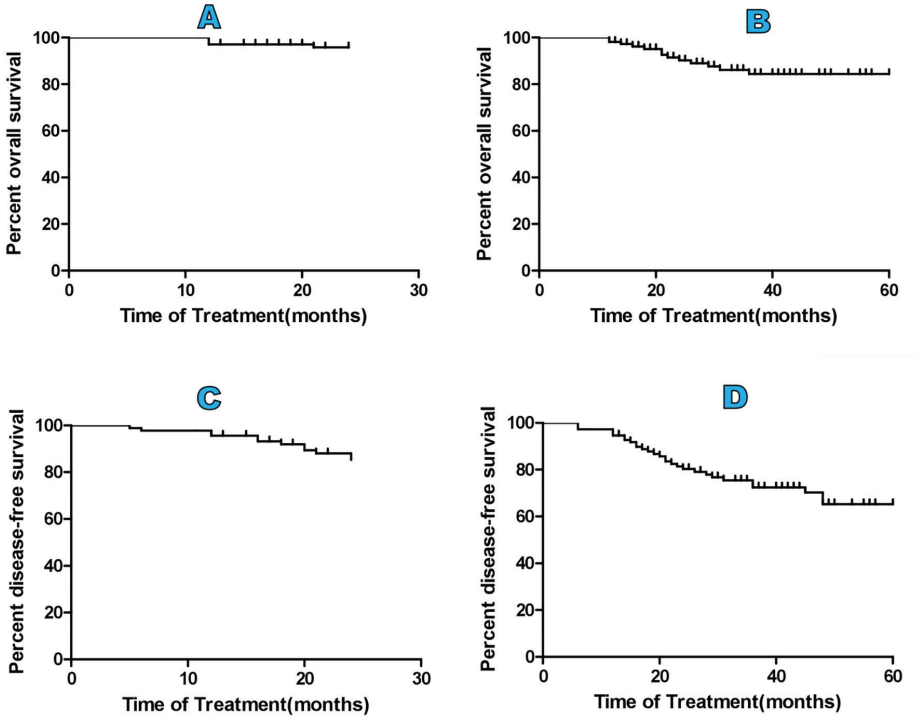

Figure 1: The overall survival rate for all patients: (A) 2-year (B) 5-year, and disease-free survival for all patients: (C) 2-year (D) 5-year

index and menopausal status in all patients. The OS and DFS rates (the means) for the patients with Ki-67≤20% were 86.7% (38.9 months) and 86.2% (38.1 months), respectively; whereas for patients with Ki-67>20% were 85% (32.6 months) and 71.6% (32.3 months), respectively. Therefore, there was no significant difference between Ki-67 index and OS (hazard ratio [HR] 0.77, 95%CI 0.26-2.24; P=0.63) or DFS rates (HR 0.48, 95%CI 0.21-1.12; P=0.09).

Also, the OS and DFS rates (means) for the patients of premenopausal were 82.9% (39.4 months) and 67.1% (35 months), respectively; whereas for the patients of postmenopausal the corresponding figures were 91.1% (32.5 months) and 85.3% (31.2 months), respectively. Therefore, there was no significant difference between menopause status and OS (HR 1.41, 95%CI 0.45-4.41; P=0.54) or DFS (HR 1.85, 95%CI 0.84-4.10; P=0.12). The OS and DFS rates (means) for the patients younger than 50 years were 87% (37.1 months) and 76.6% (34.2 months), respectively; whereas for the patients older than 50 years were 84.2% (30.1 months) and 68.4% (30.1 months), respectively. Therefore, there was no significant difference between age and OS (HR 0.62, 95%CI 0.21-1.87; P=0.40) or DFS (HR 0.62, 95%CI 0.28-1.37; P=0.24).

Discussion

This study evaluated a number of associated factors and also the OS and DFS in women with non-metastatic TNBC. In a retrospective analysis,[14] 296 patients with TNBC had a median age of 55 years old (range, 23–88.5)

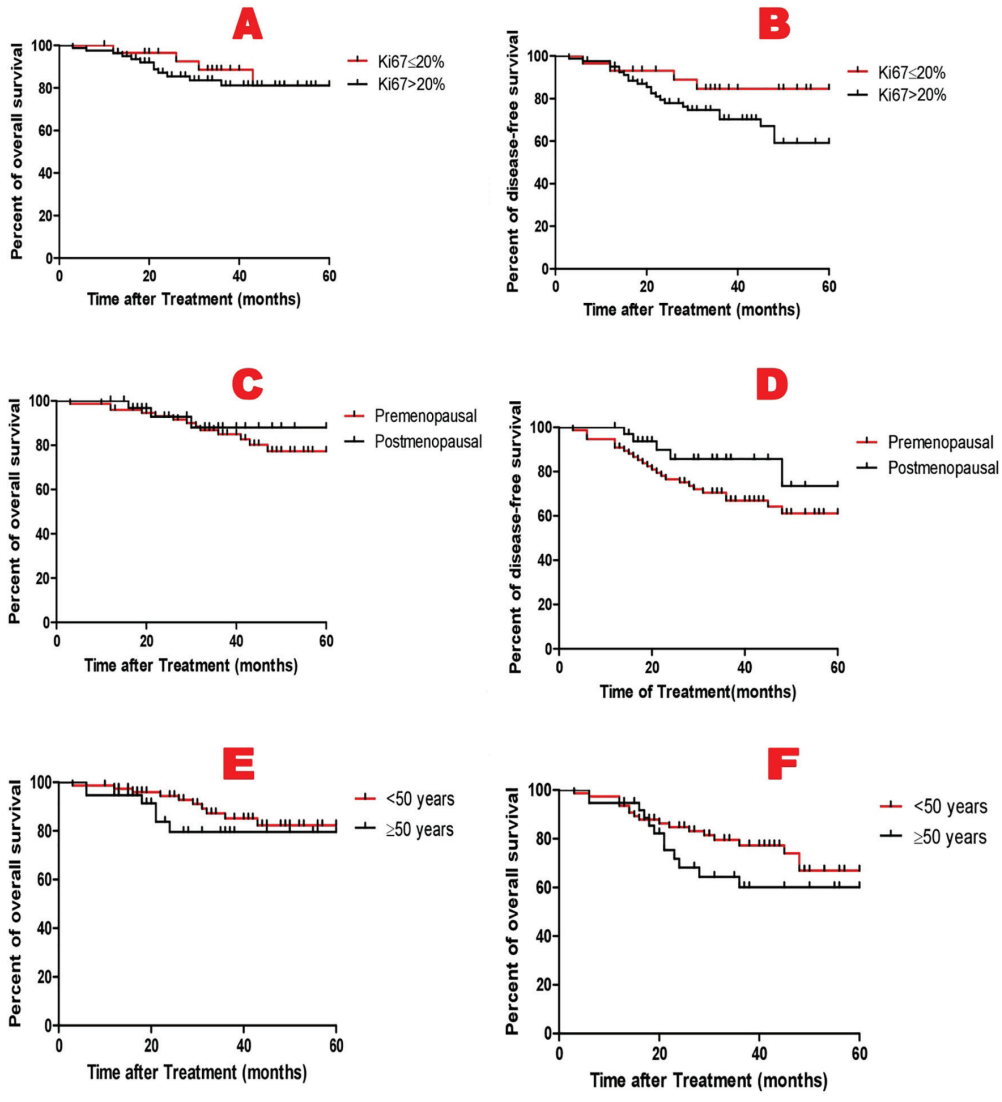

Figure 2: (A) 5-year overall survival and (B) 5-year disease free survival based on percentage of Ki-67; (C) 5-year overall survival and (D) 5-year disease free survival based on menopausal status; (E) 5-year overall survival and (F) 5-year disease free survival based on age group

at diagnosis. The median age of non-metastatic TNBC in the study of Yue et al.[15] was 57 years old (range, 28-92 years). The median age of TNBC patients at diagnosis in another research was 54.5 years old (range, 24-86).[16] The results were almost similar and showed that the mean age of patients with TNBC approximately is reported over 45 years of age in most studies. Ovcaricek et al.[14] reported that non-metastatic TNBC patients were more likely to have grade III tumors (82.5%), tumor size >2 cm was reported in almost two third of the patients. At least one axillary lymph node was positive in 46.1% of patients and one third of the tumors were positive for lymphovascular invasion and most women were postmenopausal at the presentation (60.3%). Pogoda et al.[16] showed that 4% of patients with TNBC had an evidence of metastases at initial diagnosis and 55% had axillary lymph node involvement at presentation. The most common histological type in their study was ductal carcinoma (81%).[16]

A total of 448 non-Hispanic black and white women were identified which 57% were premenopausal and 89% had grade III tumors. Stage II (47%) was the most frequent stage at diagnosis followed by stage III (28%); 32% had lymphovascular invasion. The 5-year OS and DFS rates were 68% and 60% for blacks and 65% and 63% for whites, respectively.[17] The results of this study and other studies suggest that considering various clinicopathological features in TNBC patients, genetic factors and geographical area could have a significant impact on these factors.

Christiansen et al.[18] enrolled women with different ethnics with stage I-III TNBC who had received adjuvant chemotherapy (African Americans vs. non-African Americans). Among the patients, 42.6% were African American. The African American patients had a significantly lower 5-year DFS rate (45.2% vs. 79.7%) and a higher 5-year recurrence rate (42.5% vs. 7.0%; P=0.0005), compared with the non-African American patients. In a study from Lithuania,[19] consisting of 99 TNBC patients, the OS of these patients was 97.0%, 84.9% and 66.5% following 10, 30 and 60 months of diagnosis, respectively. The study of Kaplan et al.[20] showed that 5-year relapse-

free survival and OS in TNBC patients were 84 and 81%, respectively. Aghili et al.[21] evaluated 107 patients with TNBC and found 2 and 5-year DFS rates of 68% and 63%, respectively. A study from Slovenia,[14] reported the 5-year DFS and OS rate of 68.2% and 74.5%. Van Roozendaal et al.[22] in the Netherlands, showed a 5-year DFS of 78.7% and OS of 82.3%. In a study from Poland,[16] 6-year DFS and OS rates were 68% and 62%, respectively.

Our current study identified the 2- and 5-year OS rates were 96% and 88.1%, respectively; whereas the 2- and 5-year DFS rates were 87% and 74.1%, respectively. These figures assume to be superior in comparison to studies from other areas; however, the kind of treatments the patients receive could have a contribution on the outcome. Therefore, the correlation between genetic or race with survival in TNBC patients is yet to be defined.

Kassam et al.[23] reported that TNBC patients younger than 50 years of age had an inferior outcome. In another study, age was not related to prognosis.[24] On the other hand, Ovcaricek et al.[14] showed that age>65 years was an independent prognostic factor for DFS and that the risk of recurrence was around 2-fold higher in older patients.[16] Yue et al.[15] retrieved 192 consecutive non-metastatic TNBC patients who had undergone the resection of a primary tumor that the multivariate Cox analysis identified three significant variables for survival: Ki-67, tumor stage, and nodal involvement. Proliferation marker Ki-67 was an important variable for survival in the study of Keam et al.[25] and the high Ki-67 index was associated with a higher histological grade, larger tumor size, presence of axillary lymph node metastasis, and worse outcome. Since TNBCs typically exhibit higher grades and high proliferation rates, the expression of Ki-67 was usually higher in most of TNBC patients.[15] In this study, patients older than 50 years had a poor outcome compared with younger patients, but this difference was not statistically significant. Right breast and lymph node involvement were more common in patients younger than 50 years compared with older patients. Vascular invasion was reported more commonly in patients older than 50 years. In current study; however the difference of DFS rates for Ki-67 index (≥20% vs. <20%), menopausal status (post vs. pre) and age (≥50 years vs. <50 years) was not significant, but its rate was higher in the group of patients with Ki-67<20%, postmenopausal women and those younger than 50 years old. Therefore, these variables may have a significant impact on survival of TNBC patients. In addition, age can be suggested a determining factor besides other clinicopathological factors.

Conclusion

The prevalence of TNBC was more common in women younger than 50 years of age. It might be suggested that Ki-67 index, menopausal status and age could have a contribution on prognosis and survival of TNBC patients besides geographical and ethnic factors. To confirm this, future studies with larger samples and careful analysis in the same geographical areas are needed.

Conflict of Interest: None declared.

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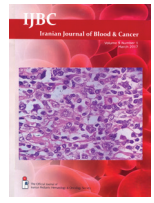

CASE REPORT

Hodgkin's Lymphoma Occurring Secondary to Autologous Stem Cell Transplantation in Plasma Cell Leukemia; A Case Report

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ABSTRACT

Survival of patients with multiple myeloma has improved substantially because of availability of new therapies including autotransplants, immunomodulating drugs and proteasome-inhibitors. Second primary cancers have emerged as an important determinant of morbidity and mortality among cancer survivors. Even though there is an increased risk of new cancers of the lymphoreticular and haematopoetic system, it is very rare for Hodgkin's lymphoma to occur as a second malignancy following autologous peripheral blood stem cell transplantation (APBSCT) for myeloma. We report a case of a female with plasma cell leukemia treated with autologous peripheral blood stem cell transplantation and lenalidamide maintenance. She developed cervical lymphadenopathy 4.5 years after the APBSCT, biopsy confirmed the diagnosis of classical Hodgkin's lymphoma, nodular sclerosis type. Since she developed allergic reaction to ABVD, she was given 6 cycles of COPP chemotherapy and is in complete remission now.

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Introduction

Secondary cancers have emerged as an important determinant of morbidity and mortality among cancer survivors recently. Survival of patients with multiple myeloma (MM) has improved substantially due to access of new therapies including stem cell transplantation, immunomodulating drugs and proteasome inhibitors. Several studies report an increased risk of new cancers especially of the lymphoreticular and haematopoetic system.[1-4] Most of these are acute myeloid leukemia, myelodysplastic syndrome and non hodgkin's lymphoma. It is very rare for Hodgkin's lymphoma to occur as a secondary malignancy following APBSCT for myeloma. We report a case of a female with plasma cell leukemia treated with APBSCT, developing classical Hodgkin's lymphoma 4.5 years later.

Case Report

A 39-year-old woman presented with history of fatigue and recurrent fever. Physical examination was remarkable for pallor, cervical and axillary lymphadenopathy. A peripheral blood smear showed 63% atypical plasma cells, serum protein was 12 g/dL, with s.globulin of 8.8 g/dl. A bone marrow examination showed 70% plasmacytoid cells which on flowcytometry demonstrated positivity for CD19, CD20, CD23 and CD138. A diagnosis of plasma cell leukemia was made. Her serum immunoglobulin G was 8085 mg/dl and β2 microglobulin was 6.15 mg/L. She did not have any evidence of bone involvement. She was staged according to "International staging system" (ISS) to stage 3 and was started on bortezomib and dexamethasone. However, bortezomib was stopped after 2 cycles due to severe peripheral neuropathy and changed to VAD chemotherapy for 6 cycles. She achieved complete remission and was assigned to go through ASCT with high dose melphalan as the conditioning regimen. This was followed by maintenance lenalidamide for one year after which she was on regular follow up.

She presented 4.5 years after the transplant with fever and left cervical lymphadenopathy. Excisional lymph node biopsy showed a pleomorphic infiltrate of large mononuclear, binucleated or occasional multinucleated cells replacing the parenchyma, suggestive of hodgkin's cells; Reed-Sternberg and lacunar cells (figure 1). On immunohistochemistry, these cells were CD20+(downregulated), CD30+, PAX5+, and occasional cells were CD15+. A diagnosis of classical Hodgkin's lymphoma, nodular sclerosis subtype was made. Computed tomogram showed bilateral cervical, pretracheal, paraaortic and celiac lymph nodes. She was staged as stage III B HL. A thorough assessment for multiple myeloma was negative in the patient. The EBV-DNA (PCR) was negative. She was started on ABVD (adriamycin, bleomycin, vinblastin, dacarbazine) protocol; however, due to severe allergic reaction the protocol was changed to COPP (cyclophosphamide, vincristine, procarbazine and prednisolone). She has completed 6 cycles of chemotherapy and currently is in complete remission. Patient has given consent to publish her case.

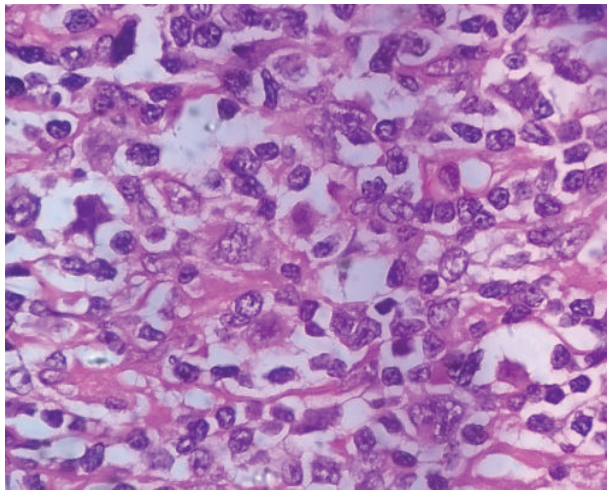

Figure 1: Section from lymph node showing Hodgkin's and Reed-Sternberg cells. (haematoxylin and eosin, magnification 100×)

Discussion

The issue of developing second malignancies has emerged as a clinical challenge among cancer survivors following successful chemotherapy for malignant disorders. According to the NCI SEER database, cancer survivors have a 14% increased risk of developing a second malignancy when compared to the general population.[5] High dose chemotherapy followed by APBSCT is considered the standard of care for patients with MM since the Intergroupe Francophone du Myeloma trial demonstrated improved survival for such treated patients compared to conventional treatment. This in conjunction with induction and maintenance therapy with newer agents such as lenalidamide and bortezomib has further improved the survival in MM, to the extent that the 10-year survival in young patients with myeloma has reached to 50%.[6]

A recent meta-analysis reported a high risk for new hematological malignancies in patients who have received lenalidamide and melphalan.[1] In a SEER based study, the authors reported an age adjusted incidence of MM with second primary cancers to be 2.2 per 1 million. There was an increased incidence of lymphatic and hematologic malignancies and a lower risk of solid organ cancer.[3] The standard incidence ratio of Hodgkin's lymphoma developing in patients with multiple myeloma was 1.17.[4]

In another study on second malignancies following APBSCT in MM, the overall cumulative incidence was 5.3% at 5 years and 11.2 % at 10 years, there was one case of non Hodgkin's lymphoma and no Hodgkin's lymphoma.[7] In another study on new cancers following autologous transplants in MM, there were 2 cases of Hodgkin's lymphoma occurring at 3-5 years post transplant.[2] In this study increasing age, male gender and obesity were associated with an increased risk of new cancers. Our patient was also treated with APBSCT with melphalan and maintenance lenalidamide which might have contributed to the development of second primary Hodgkin's lymphoma.

The factors that are attributed to development of second primary malignancies include: the primary site, histology, age at diagnosis and kind of chemotherapeutic agents given for the primary malignancy, as well as environmental and genetic factors. Similarly, in MM, the development of second malignancy may be treatment-related, myeloma-related, or due to environmental and host-related factors.

The outcome following the treatment of second malignancies in MM tend to be poor. MM patients with second cancer has 2.3-fold higher risk of death compared to MM patients without a second cancer, with a median survival of 1.1 years after diagnosis of second cancer.[8]

Conflict of Interest: None declared.

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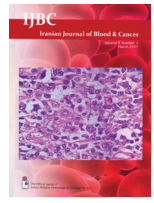

Letter to Editor

Folic Acid Supplementation: to Advise or not to Advise?

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Dear Editor

It has been well known that Folic Acid (Folicin, Folate, Vitamin B9) is from the vitamin B family and these vitamins are water soluble and do not accumulate in tissues because they can be secreted in the urine. Folate is synthesized de novo by bacteria, plants, fungi, and certain protists. Animals and human are dependent on their diets for adequate supplies of this vitamin. Food with very high folate content include dark green vegetables, orange juice, nuts, legumes, and liver. However, the reduced folates present in food are labile to light and oxidation and are partly destroyed during processing and cooking.[1] The recommended daily allowance for folate is expressed in dietary folate equivalents to take into account the greater bioavailability of folic acid form than the reduced polyglutamylated forms present naturally in foods and fruits.

The average content of body stores in adults has been estimated to be 12-28 mg folate, half of which is stored in the liver.[2] It is relevant to note that the ratio of body folate stores to recommended daily intakes is less than 100:1, whereas the ratio for cobalamin approximates 1000:1. As a result, the folate depletion necessary to produce megaloblastic anemia is achieved much more rapidly than depletion of cobalamin. Storage data are sparse in children, but infants of normal birth weight who died in the perinatal period had hepatic folate contents of 0.76 mg; low-birth-weight infants' hepatic content averaged only 0.13 mg.[3] These data, although not fully representative, suggest much lower stores to recommended intake ratios in newborns and infants than in adults.

Recognition that supplementation in the periconceptional period with folic acid results in decreased occurrence and recurrence of neural tube defects (NTDs) in children of women with no clinical evidence of folate deficiency,[3,4] led to recommendations that all women of childbearing years should take folate supplements (200 to 400 µg daily), because the critical period for prevention of NTDs is early in pregnancy before most women are aware that they are pregnant. Since January 1998, folic acid fortification of all enriched cereal-grain products has been mandated in the United States, Canada, and parts of South America.[3] As a result, serum and red blood cell (RBC) folate concentrations have increased in these populations and the incidence of NTD-affected pregnancies has decreased by 26% in the United States and by 48% in Canada.[4,5] On the other hand, concerns about folate fortification delaying the diagnosis of cobalamin deficiency, promotion of cancer growth, and issues of free choice have delayed mandating folate addition to the diet in Europe and elsewhere.[6] Although the defense against infections relies on the ability of the immune cells to proliferate and differentiate and on the effective renewal of the epithelial linings. Folates and vitamin B-12 play a crucial role in DNA and protein synthesis, which suggest that processes in which cell proliferation is essential may be impaired by poor status. Macroscopic disruption of the epithelial linings occurs with antifolate treatment, and the immune system is affected by folate and vitamin B-12 deficiency. The phagocytic and bactericidal activity

of polymorphonuclear leukocytes is poor in individuals with severe folate deficiency and improves with folate replenishment. In one clinical trial investigating the impact of folic acid and/ or vitamin B_{12} supplementation on the prevention of diarrhea and risk of infections; neither folic acid nor vitamin B-12 administration reduced the incidence of diarrhea or lower respiratory infections. In comparison with placebo, children treated with folic acid alone or in combination with vitamin B-12 had a significantly higher risk of persistent diarrhea.[7]

Although many reports of folic acid toxicity or increased risk of cancer (especially lung cancer) need confirmation in larger trials, but so do many claims of preventive benefits of folates. Exposing normal individuals especially children to chronically high dose of vitamins needs serious caution, even for folic acid. Meanwhile, the needs of selected patients with chronic hemolysis or pregnant women should not be the reason for fortification of grain for general population; even if there was no economical concern. So still there is not really any consensus regarding administering folates as supplement for general population; or even for specific populations except in peri-conceptional period in women and pregnancy.

Conflict of Interest: None declared.

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PHOTO CLINIC

Recurrent Cytomegalovirus Retinitis in a Patient with Leukemia on Maintenance Chemotherapy

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A 14-year-old boy, known case of acute lymphoblastic leukemia (ALL) on maintenance treatment presented with visual loss in lower half of visual field. Laboratory tests showed pancytopenia. ALL Relapse was ruled out by bone marrow aspiration. An ophthalmologic consultation was done and a diagnosis of "CMV retinitis" was made. CMV-PCR was positive with 3,000,000 copies/ml. CT-scan of chest and abdomen was unremarkable and fungal infection assessments were negative. He was admitted and treatment with intravenous gancyclovir 10 mg/kg/day along with intravitreal injection of gancyclovir was started for the patient. He received a course of 4-week treatment which was discontinued thereafter.

Nine months later, he referred with fever and pancytopenia while on oral maintenance chemotherapy. Due to prolongation of fever and unexplained cytopenia, a thorough work-up was performed which yielded a plasma CMV-PCR of 3,500,000 copies/ml. Ophthalmologic examination established relapse of "retinitis" (figure 1). Another course of intravenous and intravitreal injection of gancyclovir initiated. He was assigned to receive a prolonged course of maintenance with oral valgancyclovir until attaining negative PCR in order to prevent CMV reactivation.

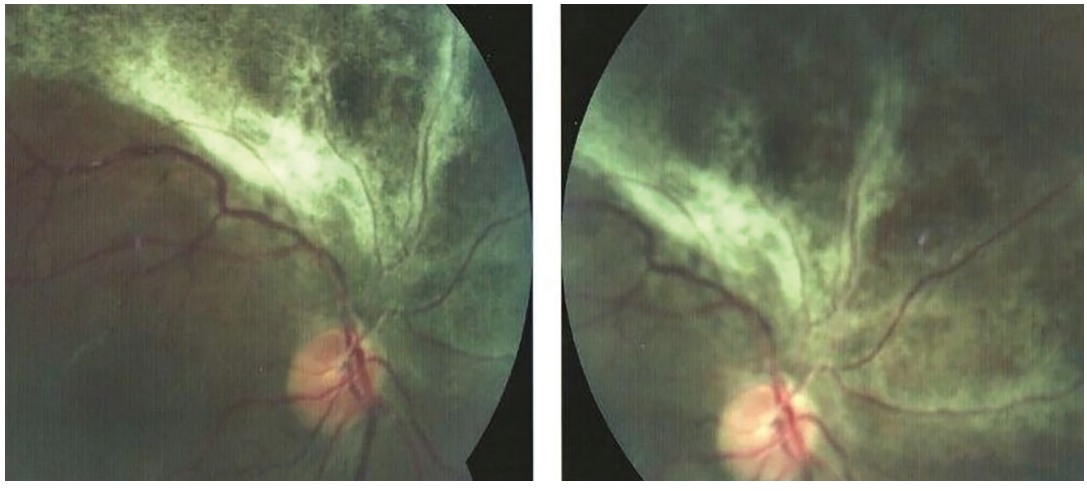

Figure 1: Retinitis in the midperiphery with a "brush fire" pattern

CMV infection is a major cause of morbidity and mortality in immunocompromised patients, particularly in transplant recipients.[1] There are major organ systems which could be involved by CMV and should be considered as "active CMV disease" in immunocompromised patients. They include respiratory, gastrointestinal, and central nervous systems and retina.[2] In pediatric patients with ALL in the setting of nontransplant, CMV disease is very rare with a few reports in the literature.[3] CMV viremia has been reported in 13.6% of patients with lymphoid malignancies who did not receive any stem cell transplantation.[4] In the nontransplant cases, incidence of CMV retinitis is reported about 3.5% in children with ALL.[5] Preemptive anti-CMV therapy (routine active screening and treating patients with progressively increasing viral titers) is the recommended strategy in the transplant settings; however, such an strategy is not yet suggested in childhood ALL patients on chemotherapy.[3]

As CD4+ T-cell lymphopenia is a major risk factor for reactivation of latent CMV infection and developing active disease, routine control for exacerbation of viremia by PCR is recommended by authors in these high-risk patients in setting of nontransplant, specifically in those with a history of previous active disease for which have been treated before. Hence; in case of increasing high copy numbers, preemptive therapy could be suggested and in patients with associated signs and symptoms such as fever and unexplained cytopenia, definite treatment is indicated.

Conflict of Interest: None declared.

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