RESEARCH ARTICLE

Serum Levels of G-CSF and IL-7 in Iranian Breast Cancer Patients

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Abstract

Introduction: Breast cancer cells and tumor stroma produce different cytokines and soluble factors. Cytokines, while playing crucial roles in immune responses to tumors, also favor tumor growth and progression. IL-7 and G-CSF are two cytokines that may exert influences on the pathophysiology of breast cancer. Materials and Methods: Sera were collected from 136 females with breast cancer before receiving chemotherapy or radiotherapy. The control group comprised of 60 healthy age-matched females without any acute or chronic diseases with no family history of breast cancer. Serum levels of IL-7 and G-CSF were measured by commercial enzyme linked immunosorbent assay. Results: While there was no significant difference in the level of G-CSF between patients (92.81±594.54 pg/ml) and controls (0.00 pg/ml), G-CSF level in sera of patients with advanced stages of breast cancer was elevated compared to early stages (p=0.0001). Moreover, the highest level of G-CSF was seen in patients with N3 phase tumors (p=0.0001). IL-7 was slightly but not significantly higher in the control group (0.04±0.11 pg/ml) in comparison with patients (0.02±0.10 pg/ml). Interestingly, a significant increase in the level of IL-7 in patients with skin involvement was observed (p=0.001). Conclusion: Our results showed an elevation of G-CSF in sera of patients with advanced stages of tumor, while IL-7 elevation correlated with skin involvement of breast cancer. IL-7 can be produced by keratinocytes in skin tissue and may be involved in the pathologic establishment of metastatic tumor cells in skin.

Keywords: G-CSF - IL-7 - breast cancer - stage - skin - Iranian

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Introduction

Breast cancer (BC) is a disease that affects over one million women all over the world (Montazeri et al., 2008). Despite the considerable decrease in the mortality rate of breast cancer in some countries, its rate is on the rise in Iran (Taghanv et al., 2012). The mean age of Iranian breast cancer patients is 10 years less than the rest of the world pointing to different carcinogenic exposure or genetic susceptibility in these patients (Montazeri et al., 2008). In spite of extensive clinical use of serum tumor markers like CEA and CA-125, the search for ideal tumor markers for diagnosis, prognosis or treatment of breast cancer is still ongoing. An ideal tumor marker should be tumor specific, measurable with non-invasive methods, and highly sensitive and specific; however, such a tumor marker is still missing (Dehqanzada et al., 2007).

Changes in the levels of cytokines are behavioral criteria of the immune system and tumor cells which can affect or result from the interaction between these entities. It is well known that cancer cells produce different cytokines and growth factors. In a study of 17 cytokines in a subgroup of breast cancer with no estrogen receptor expression, it was shown that the expressions of IL-6, IL-8, G-CSF, IFN-γ, MCP-1 and MIP-1β were elevated in the diseased tissue compared with normal tissue (Chavey et al., 2007). However, in the same study no overexpression of Interleukin-7 (IL-7) was observed. IL-7 is an immune regulatory cytokine that is significantly produced by tumor stromal cells and cells that exist in the inflammatory sites (Roato et al., 2006). IL-7 is essential for early development of lymphocytes and regulates peripheral T cell hemostasis (Roato et al., 2006). As a pleiotropic cytokine, IL-7 induces trophic and anti-apoptotic responses on hematopoietic tissues, particularly lymphocytes. IL-7 is known to induce the development, growth and differentiation of some hematological malignancies, including certain types of leukemias and lymphomas (Roato et al., 2006). However, little is known about its possible effects on solid tumors.

Although IL-7/IL-7R mRNA has been detected in colorectal, esophageal, renal, and head and neck (squamous cell type) carcinoma, there is not much information relating the level of serum IL-7 to the histopathological characteristics of tumors or the disease prognosis (Roato et al., 2006). Recently, it has been suggested that IL-7 exerts lymphangiogenic effects on the vascular endothelium and thus might play an important role in the lymphangiogenesis during the progression and spread of solid tumors. Production of IL-7 by human
of patients on the day before surgery. The samples were brought to Shiraz Institute for Cancer Research immediately. Samples were centrifuged and sera were preserved in -20°C till analysis was done. On the day of operation tissue biopsies were assessed by collaborative pathologist.

Cancer was staged according to tumor-node-metastasis (TNM) by American Joint Committee on Cancer Classification and stage grouping. The patients were classified according to their pathologic characteristics including histological tumor type, tumor size, in situ component, histological grade, tumor necrosis, peritumoral invasion, axillary lymph node involvement, perinodal fat infiltration and TNM staging for further analysis. Clinical characteristics of the tumors are shown in Table 1.

**ELISA assays**

Plasma level of G-CSF was measured by a commercial enzyme linked immunosorbent assay (ELISA) (ebiosciences, Austria) according to the manufacturer’s instructions. The sensitivity of this assay was 11 pg/ml and range of detection was between 39.1-2500 pg/ml.

Serum level of IL-7 was measured using a commercial ELISA assay (Abcam, UK) according to the manufacturer’s instructions. The sensitivity of this assay was less than 3 pg/ml and the range of detection was between 6.25-200 pg/ml.

**Statistical analysis**

Student’s t-test was used for the analysis of age distribution between case and control groups. One-way ANOVA or t-test was used in the comparisons between the two groups using SPSS software (11.5, Chicago, Illinois). When the data points were not enough in categories, normality of data was checked and parametric or non parametric (Kruskal-Wallis and Mann-Whitney) analyses were done. Statistically significant differences were defined as comparisons resulting in p<0.05.

**Results**

A higher level of G-CSF (92.8±594.5 pg/ml) was found in the sera of patients with breast cancer compared with healthy age/sex matched controls (0.00±0.00 pg/ml), (p=0.071). There was no significant difference between the level of IL-7 in sera of patients (0.02±0.10 pg/ml) and healthy controls (0.04±0.11 pg/ml). A higher percentage of BC patients (10 out of 136, 7.35%) had some levels of G-CSF in their sera while 0 out of 60 (0%) healthy controls were found positive for G-CSF (ROC curve cut off point=95.7 pg/ml). Conversely, a higher number of healthy controls (14 out of 60, 23.3%) had some levels of IL-7 in their sera compared to the BC patients (15 out of 136, 11.0%).

There was a significant statistical difference in the level of G-CSF (p<0.0001) between stage 3c and other stages. The mean concentration of this cytokine increased dramatically in this stage and decreased in stage 4 (Table 2). There was, however, no significant difference between IL-7 concentrations in different stages. In general, mean G-CSF concentration increased significantly from low

**Materials and Methods**

The research was approved by ethics committee of Shiraz University of medical science (SUMS). The patients were informed about the aim of this study as well as safety and security measures before their consents were obtained. Cases were selected among women with breast cancer who referred for operation to hospitals affiliated to Shiraz Medical School since April 2009 till May 2010. 136 cases with age between 25-83 years were entered to the study. None of the patients had been treated with chemotherapy or radiotherapy before sample collection.

The group of 136 breast cancer patients (all females) and 60 healthy controls (all females) were matched based on their age (Table 2). The mean age of the breast cancer patients was 49.7±12.4 years (Range=25-83 years) and the mean age of healthy individuals was 49.3±11.1 yrs (Range=21-82 years, P=0.507).

Four ml blood was collected from peripheral veins...
stages (stages I and II) to high stages (stages III and IV) while mean IL-7 concentration decreased from low to high stages (Table 2).

Although in 108/117 (92.3%) of patients no skin involvement of the tumor was observed, a significant increase in the level of IL-7 in patients with skin involvement was observed (p=0.001). The difference in the level of G-CSF between patients and controls did not reach the significant level. The mean level of IL-7 and G-CSF in patients with skin involvement was 0.13 pg/ml and 143.0 pg/ml while the levels of these cytokines in patients without skin involvement were 0.01 pg/ml and 143.0 pg/ml, respectively. We also observed a huge increase in the level of G-CSF in patients who showed some degree of lymph node involvement (190.3±872.1 pg/ml vs. 14.6±65.7 pg/ml) (Table 2). However, this difference was mostly related to an increase in the N3 phase (p=0.001, Table 2). Mean IL-7 concentration increased from N0 to N2 but decreased from N2 to N3.

In comparison with control healthy women, concentration of IL-7 was less in breast cancer patients but the difference did not reach the significant level (Table 2). Concentration of G-CSF was elevated in patients’ sera but we did not detect any G-CSF in the sera of control group. However, this difference did not reach the significant level due to the high standard deviation of the data in this group.

Although there was no significant difference between the level of IL-7 in sera of patients with right-sided and left-sided breast tumors (0.23±0.10 vs. 0.03±0.11 pg/ml), a considerable increase in the level of G-CSF in sera of patients with right-sided tumor compared to left-sided tumors was observed (218.0±976.6 vs. 27.7±102.5 pg/ml).

### Table 1. Clinicopathological Characteristics of Patients

| Characteristics | N=136 % | IL-7(pg/ml) | Mean ± SD | G-CSF(pg/ml) | Mean ± SD | Characteristics | N=136 % | IL-7(pg/ml) | Mean ± SD | G-CSF(pg/ml) | Mean ± SD |
|-----------------|---------|-------------|-----------|--------------|-----------|-----------------|---------|-------------|-----------|--------------|-----------|
| Tumor type      | In situ | 2.94 %      | 0.00±0.00 | 0.00±0.00    | 118.86    | Others          | 14.10  | 19.4       | 0.02±0.10 | 106.9±637.4 | 14.10     |
| Histological grade | Well     | 34.25 %     | 0.03±0.12 | 37.4±133.0   | 14.10     | Moderate        | 48.35  | 8.69±42.2  | 0.02±0.10 | 8.69±42.2   | 48.35     |
|                 | Poorly   | 35.25 %     | 0.02±0.08 | 312.3±1147.3 | 14.10     | Unknown         | 19.14  | 19.14      | 19.14     | 19.14       | 19.14     |
| Tumor stage     | Stage 1  | 22.16 %     | 0.00±0.00 | 22.16±16.2   | 14.10     | Stage 2         | 22.16  | 0.04±0.16  | 22.16±16.2 | 0.04±0.16   | 22.16     |
| N0              | 0.01±0.00 | 14.6±65.7   | 14.6±65.7  | 0.01±0.00    | 14.6±65.7 | N1               | 0.01±0.09 | 14.6±65.7 | 14.6±65.7 | 0.01±0.09   | 14.6±65.7 |
| N2              | 0.07±0.16 | 20.2±114.4  | 20.2±114.4 | 0.07±0.16    | 20.2±114.4| N3               | 0.01±0.03 | 858.2±1794.6 | 858.2±1794.6 | 0.01±0.03 | 858.2±1794.6 |
| High            | 13.95 %  | 0.01±0.03   | 858.2±1794.6 | 0.01±0.03   | 858.2±1794.6 | Unknown         | 13.95  | 13.95      | 13.95     | 13.95       | 13.95     |
| Tumor size (Diameter) | >2 cm    | 68.50 %     | 0.01±0.12 | 23.6±99.2    | 14.10     | Unknown         | 14.10  | 14.10      | 14.10     | 14.10       | 14.10     |
| <2 cm           | 54.37 %  | 0.03±0.12   | 166.8±832.6 | 0.03±0.12   | 166.8±832.6 | Unknown         | 14.10  | 14.10      | 14.10     | 14.10       | 14.10     |
| Tumor side      | Right breast | 49.36 % | 0.02±0.10 | 218.0±976.6 | 14.10     | Unknown         | 17.12  | 17.12      | 17.12     | 17.12       | 17.12     |
| Left breast     | 70.51 %  | 0.03±0.10   | 27.7±102.57 | 0.03±0.10   | 27.7±102.57 | Unknown         | 17.12  | 17.12      | 17.12     | 17.12       | 17.12     |
| Perivascular involvement | Is seen | 22.16 % | 0.02±0.10 | 173.7±766.1 | 14.10     | Unknown         | 15.11  | 15.11      | 15.11     | 15.11       | 15.11     |
|                | Not seen | 99.72 %     | 0.02±0.11 | 88.8±598.6  | 14.10     | Unknown         | 15.11  | 15.11      | 15.11     | 15.11       | 15.11     |
| Tumor calcification | Is seen | 32.23 % | 0.04±0.10 | 7.05±39.9   | 14.10     | Unknown         | 17.12  | 17.12      | 17.12     | 17.12       | 17.12     |
|                | Not seen | 87.64 %     | 0.02±0.09 | 142.4±739.8 | 14.10     | Unknown         | 17.12  | 17.12      | 17.12     | 17.12       | 17.12     |
| N (TNM)         | N0       | 56.41 %     | 0.01±0.09 | 14.6±65.7   | 14.10     | N1               | 32.35  | 0.02±0.09 | 20.2±114.4 | 0.02±0.09   | 20.2±114.4 |
|                 | N2       | 17.12 %     | 0.07±0.16 | 0.00±0.00   | 14.10     | N3               | 13.95  | 0.01±0.03 | 858.2±1794.6 | 858.2±1794.6 | 0.01±0.03 | 858.2±1794.6 |
|                 | Unknown  | 18.32 %     | 18.32      | 18.32       | 14.10     | Number of involved LN | 60/60 | 60/60 | 60/60 |

*Significantly elevated (p<0.001); \(^1\)p<0.0001; \(^2\)p<0.0001; \(^3\)p<0.001*
Table 2. Levels of G-CSF and IL-7

| Stage          | N   | IL-7 (pg/ml) Mean±SD | G-CSF (pg/ml) Mean±SD |
|----------------|-----|----------------------|-----------------------|
|                |     |                      |                       |
| Levels of G-CSF and IL-7 in different stages of breast cancer | | | |
| Stage 1        | 22  | 0.00±0.00            | 18.80±61.1            |
| stage 2a       | 48  | 0.02±0.10            | 8.41±58.3             |
| stage 2b       | 22  | 0.04±0.11            | 29.40±137.9           |
| stage 3a       | 18  | 0.06±0.16            | 0.00±0.00             |
| stage 3c       | 10  | 0.02±0.04            | 1094.00±2005.7*       |
| stage 4        | 3   | 0.00±0.00            | 72.10±125.0           |
| Levels of G-CSF and IL-7 in sera of BC patients with high stage compared to those with low stage | | | |
| Low stages     | 110 | 0.02±0.10            | 13.30±77.1            |
| High stages    | 13  | 0.01±0.03            | 85.80±1794.6*         |
| Levels of G-CSF and IL-7 in BC patients with lymph node involvement compared to those without lymph node involvement | | | |
| n=0            | 56  | 0.01±0.09            | 14.60±65.7            |
| n>0            | 62  | 0.03±0.11            | 190.30±872.1          |
| Higher levels of G-CSF in sera of BC patients with N3 in TNM classification | | | |
| N0             | 56  | 0.01±0.09            | 14.60±65.7            |
| N1             | 32  | 0.02±0.09            | 20.20±114.4           |
| N2             | 17  | 0.07±0.16            | 0.00±0.00             |
| N3             | 13  | 0.01±0.03            | 858.20±1794.6*        |
| Levels of G-CSF and IL-7 in sera of BC patients compared to control group | | | |
| Case           | 136 | 0.02±0.10            | 92.80±594.5           |
| Control        | 60  | 0.04±0.11            | 0.00±0.00             |
*Significantly elevated (p<0.0001) ml).

The highest level of G-CSF was observed in patients with tumors greater than 2 cm (154.8±790.6 pg/ml) as well as poorly differentiated tumors (312.3±1147.3 pg/ml) but the differences were not significant.

We did not observe any significant differences in the levels of IL-7 and G-CSF based on the tumor necrosis, tumor calcification, peritumoral lymphatic invasion, perineural invasion, vascular invasion and axillary involvement.

Discussion

In our study there was no significant difference in the levels of G-CSF and IL-7 between breast cancer patients and healthy individuals. Interestingly, there was a significantly higher level of IL-7 in tumors with skin involvement (p=0.001). Skin involvement is one of the most distressing presentations of locally recurrent breast cancer and few studies have identified effective mediators in this setting (Franchina et al., 2012). A previous study reported that IL-7 can be produced by skin tissue (Kim et al., 2011). Production of IL-7 by keratinocytes in skin is suggested to play a role in the survival of dendritic epidermal T cells (DETCs) in epidermis (Matsue et al., 1993; Takashima et al., 1995). DETCs are shown to exert both anti-tumor and immunosuppressive activities in vitro and in vivo (Kaminski et al., 1993; Cavanagh and Halliday, 1996). Specifically, a DETC line is shown to induce specific immunologic tolerance in vivo and inhibit the proliferation of naïve T cells in response to Ag-bearing dendritic cells in vitro (Love-Schimenti and Kripke, 1994). Moreover, a pathogenic role of DETCs in inflammatory skin disease as well as cutaneous cell lymphomas is suggested (Heufler et al., 1993).

We observed that patients with well differentiated tumors had the highest level of IL-7. IL-7/IL-7Rα-Fc administration inhibits tumor growth and increases survival in lung cancer by promoting afferent and efferent antitumor responses (Andersson et al., 2011). Moreover, cytotoxic T lymphocytes (CTLs) generated under IL-7 stimulating conditions can diminish the pulmonary metastatic sarcoma in mice (Jicha et al., 1991). It is possible that the attempts by the immune system to counteract the tumor growth is a reason for this increase in IL-7 level, however, as the tumor progresses to moderate and poorly differentiated phase, the immune parameters fade and are replaced by more inflammatory type of cytokines in favor of tumor (Ravishankaran and Karunanithi, 2011). This was portrayed in our observation that the level of G-CSF decreases in the transition from well to moderate differentiation phase but increases when the tumor progresses to the poorly differentiated phase. The change in the tumor environment and the factors produced by the tumors are the consequence of a dynamic process. It is definitely very simplistic to attribute the progression or regression of a tumor to only two cytokines. However, it is worth noting that the IL-7 and G-CSF showed an inverse or different pattern of elevation in relation to correlates of tumor progression and metastasis such as stage, lymph node involvement, and skin involvement. However, at the same time, both cytokines increased at the beginning of tumor expansion (T1 to T2) but this increase did not continue.

In general, serum concentration of G-CSF increased at the higher stages of breast cancer. Moreover, the patients with tumors at N3 phase had the highest level of G-CSF in their sera. These observations are in accordance with previous reports on different tumors (Yamano et al., 2007).

Elevation of G-CSF, GM-CSF and CA 15-3 plasma levels in stage II of breast cancer patients before surgery is already reported (Ławicki et al., 2009). Another study showed that G-CSF expression increases in higher stages of tumor in gastric cancer (Yamano et al., 2007). We also observed that G-CSF level in sera of patients with advanced stages of breast cancer was elevated while it was not detected in the early stages. Previously, Chavey et al. (2007) reported the same observation in estrogen receptor negative breast tumors (Chavey et al., 2007). The reported increased level of G-CSF in breast cancer may have tumor promoting or immunosuppressive effects. A study on breast cancer patients receiving G-CSF adjuvant therapy has shown an increased level of CA 15-3 in patients with resected tumors who received chemotherapy (Pentheroudakis et al., 2004). This increase which was triggered by G-CSF was suggested to be mediated by Neutrophils (Pentheroudakis et al., 2004). CA 15-3 on the other hand is an epitope of Mucin 1 (MUC1) with immunoregulatory and intercellular adhesion modulatory effects (Taylor-Papadimitriou et al., 1999). Therefore, it is possible that increased levels of G-CSF may be exploited by tumor to suppress the immune system. In this regard, it is shown that ectopic expression of G-CSF in a mammary tumor cell line can promote their growth and augment
granulocytic myeloid-derived suppressor cell (MDSC) accumulation (Waiget et al., 2011). Indeed, production of G-CSF by mammary tumor cells is already shown (Kowanetz et al., 2010). There is also one possibility that the tumor-derived G-CSF would be structurally different from that of physiologically produced G-CSF (Ghaderi et al., unpublished data).

Mean concentration of G-CSF in patients with positive peritumoral vascular invasion was more than its level in those without involvement but this difference was not statistically significant. It is suggested that peritumoral vascular invasion has a major role in prognosis of breast cancer (Sabatier et al., 2011). To elucidate the significance of G-CSF level in the peritumoral vascular invasion and prognosis of breast cancer in Iranian patients more studies are needed.

Collectively, our results showed an elevation of IL-7 and G-CSF in different stages of tumor growth and progression. The current information on the IL-7 level in breast cancer do not correspond. In one study IL-7 mRNA and protein could not be detected in breast cancer (Maeurer et al., 1997). In another study, however, the levels of IL-7, IL-7R, and its signalling intermediates were shown to be overexpressed in the aggressive breast tumors (Al-Rawi et al., 2004). Moreover, there are multifaceted aspects of G-CSF function to be considered in relation to tumor progression and defence against breast tumors. A molecular and genetic analysis of G-CSF variants in breast tumors would shed more light on the current controversy.

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References

Asano T, Morimoto S, Kitami Y, et al (2002). Bladder cancer producing granulocyte colony-stimulating factor (G-CSF): a case report. *Hinyokika Kiyo*, **48**, 495-8.

Al-Rawi MA, Rmali K, Watkins G, et al (2004). Aberrant expression of interleukin-7 (IL-7) and its signalling complex in human breast cancer. *Eur J Cancer*, **40**, 494-502.

Andersson A, Srivastava MK, Harris-White M, et al (2011). Role of CXCR3 ligands in IL-7/IL-7R alpha-Fc-mediated antitumor activity in lung cancer. *Clin Cancer Res*, **17**, 3660-72.

Cavanagh LL, Halliday GM (1996). Dendritic epidermal T cells in ultraviolet-irradiated skin enhance skin tumor growth by inhibiting CD4+ T-cell-mediated immunity. *Cancer Res*, **56**, 2607-15.

Chavey C, Bibeau F, Gourgou-Bourgade S, et al (2007). Oestrogen receptor negative breast cancers exhibit high cytokine content. *Breast Cancer Res*, **9**, 15.

Czygier M, Ławicki S, Gacuta-Szumarska E, Bedkowski E, Szmitkowski M (2010). The plasma level of S-selectin, myeloperoxidase (MPO) and granulocyte-colony stimulating factor (G-CSF) in gynecological cancer patients. *Przegl Lek*, **67**, 184-6.

Dehganzada ZA, Storrer CE, Hueman MT, et al (2007). Assessing serum cytokine profiles in breast cancer patients receiving a HER2/neu vaccine using Luminex technology. *Oncof Rep*, **17**, 687-94.

Franchina T, Adamo B, Ricciardi GP, et al (2012). Activity of pegylated liposomal doxorubicin in combination with gemcitabine in triple negative breast cancer with skin involvement. Two case reports. *Cancer Biol Ther*, **13**, 472-6.

Heutler C, Topar G, Grasseger A, et al (1993). Interleukin 7 is produced by murine and human keratocytes. *J Exp Med*, **178**, 1109-14.

Ikeda T, Ohgaki K, Miura M, et al (2005). Granulocyte-colony stimulating factor-producing gallbladder cancer without recurrence more than 2 years after resection, report of a case. *Surg Today*, **35**, 590-3.

Jicha DL, Mulé JJ, Rosenberg SA (1991). Interleukin 7 generates antitumor cytotoxic T lymphocytes against murine sarcomas with efficacy in cellular adoptive immunotherapy. *J Exp Med*, **174**, 1511-5.

Kaminski MJ, Cruz PD JR, Bergstresser PR, Takashima A (1993). Killing of skin-derived tumor cells by mouse dendritic epidermal T-cells. *Cancer Res*, **53**, 4014-9.

Kowanetz M, Wu X, Lee J, et al (2010). Granulocytecolony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. *Proc Natl Acad Sci USA*, **107**, 21248-55.

Kim GY, Hong C, Park JH (2011). Seeing is believing, illuminating the source of in vivo interleukin-7. *Immune Netw*, **11**, 1-10.

Love-Schimenti CD, Kripke ML (1994). Dendritic epidermal T cells inhibit T cell proliferation and may induce tolerance by cytotoxicity. *J Immunol*, **153**, 3450-6.

Ławicki S, Czygier M, Gacuta-Szumarska E, et al (2006). The plasma levels and diagnostic utility of granulocyte colony stimulating factor (G-CSF) and macrophage - colony stimulating factor (M-CSF) in ovarian cancer patients. *Pol Merkur Lekarski*, **21**, 465-8.

Ławicki S, Czygier M, Wojtukiewicz M, et al (2009). The plasma levels and diagnostic utility of granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage - colony stimulating factor (GM-CSF) in patients with I and II stage of breast cancer. *Przegl Lek*, **66**, 365-9.

Matsue H, Bergstresser PR, Takashima A (1993). Keratinocyte-derived IL-7 serves as a growth factor for dendritic epidermal T cells in mice. *J Immunol*, **151**, 6012-9.

Maeurer MJ, Walter W, Martin D, et al (1997). Interleukin-7 (IL-7) in colorectal cancer, IL-7 is produced by tissues from colorectal cancer and promotes preferential expansion of tumour infiltrating lymphocytes. *Scand J Immunol*, **45**, 182-92.

Mroczko B, Szmitkowski M, Niklinski J (2001). Granulocyte-Colony stimulating factor and macrophage-colony stimulating factor in patients with non-small-cell lung cancer. *Clin Chem Lab Med*, **39**, 374-9.

Mroczko B, Szmitkowski M, Okulczyk B (2002). Granulocyte-colony stimulating factor (G-CSF) and macrophagecolony stimulating factor (M-CSF) in colorectal cancer patients. *Clin Chem Lab Med*, **40**, 351-5.

Montazeri A, Vahdaninia M, Harirchi I, et al (2008). Breast cancer in Iran, need for greater women awareness of warning signs and effective screening methods. *Asia Pac Fam Med*, **7**, 6.

Matsumoto Y, Mabuchi S, Muraji M, Morii E, Kimura T (2010). Squamous cell carcinoma of the uterine cervix producing granulocyte colony-stimulating factor, a report of 4 cases and a review of the literature. *Int J Gynecol Cancer*, **20**, 417-21.

Nishimura K, Higashino M, Hara T, et al (1996). Bladder cancer progression. *Asian Pacific Journal of Cancer Prevention*, Vol 13, 2012 5311
producing granulocyte colony-stimulating factor, a case report. *Int J Urol*, 3, 152-4.

Natori T, Sata M, Washida M, et al (2002). G-CSF stimulates angiogenesis and promotes tumor growth, potential contribution of bone marrow-derived endothelial progenitor cells. *Biochem Biophys Res Commun*, 297, 1058-61.

Nasu K, Inoue C, Takai N, Kashima K, Miyakawa I (2004). Squamous cell carcinoma of the cervix producing granulocyte colony-stimulating factor. *Obstet Gynecol*, 104, 1086-8.

Penheroudakis G, Malamou-Mitsi V, Briassoulis E, et al (2004). The neutrophil, not the tumor, serum CA 15-3 elevation as a result of granulocyte--colony-stimulating factor-induced neutrophil MU1C overexpression and neutrophilia in patients with breast carcinoma receiving adjuvant chemotherapy. *Cancer*, 101, 1767-75.

Roato I, Brunetti G, Gorassini E, et al (2006). IL-7 up-regulates TNF-alpha-dependent osteoclastogenesis in patients affected by solid tumor. *PLoS One*, 1, 124.

Ravishankaran P, Karunanithi R (2011). Clinical significance of preoperative serum interleukin-6 and C-reactive protein level in breast cancer patients. *World J Surg Oncol*, 9, 18.

Shojaei F, Wu X, Qu X, et al (2009). G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proc Natl Acad Sci USA*, 106, 6742-7.

Sabatier R, Jacquemier J, Bertucci F, et al (2011). Peritumoural vascular invasion, a major determinant of triple-negative breast cancer outcome. *Eur J Cancer*, 47, 1537-45.

Takashima A, Matsue H, Bergstresser Pr, et al (1995). Interleukin-7-dependent interaction of dendritic epidermal T cells with keratinocytes. *J Invest Dermatol.*, 105, 50-3.

Taylor-Papadimitriou J, Burchell J, Miles DW, Dalziel M (1999). MUC1 and cancer. *Acta Biochim Biophys*, 1455, 301-13.

Taghavi A, Fazeli Z, Vahedi M, et al (2012). Increased trend of breast cancer mortality in Iran. *Asian Pac J Cancer Prev*, 13, 367-70.

Waight JD, Hu Q, Miller A, Liu S, Abrams SI (2011). Tumor-derived G-CSF facilitates neoplastic growth through a granulocytic myeloid-derived suppressor cell-dependent mechanism. *PLoS One*, 6, 27690.

Yamano T, Morii E, Ikeda J, et al (2007). Granulocyte colony-stimulating factor production and rapid progression of gastric cancer after histological change in the tumor. *Jpn J Clin Oncol*, 37, 793-6.