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Abstract

**Background:** Recent studies indicate the immune dysfunction in cancer patients in comparison with healthy individuals. The quality and quantity of this dysfunction are not equal in all patients even with similar cancer type.

**Aim:** This study aims to correlate health locus of control (HLC) beliefs with CD4$^{+}$ helper T ($T_{helper}$) cells, T regulatory ($T_{reg}$) cells, NK cells, IL-1$\beta$ and TNF-$\alpha$ in breast cancer patients.

**Patients and methods:** The study included 30 early diagnostic breast cancer patients who responded to Form C of the MHLC questionnaire that assessed internal (IHLC), chance (CHLC), doctor (DHLC) and other person’s (OHLC) control of the patient’s health status. Peripheral blood samples were collected to analyze the numbers and phenotype of $T_{helper}$ cells, $T_{reg}$ cells and NK cells by flow cytometry and to measure gene expression of IL-1$\beta$ and TNF-$\alpha$ with real time PCR.

**Results:** A significant positive correlation was found between IHLC with $T_{helper}$ cells and NK cells. However, a significant inverse correlation was found between DHLC with NK, $T_{helper}$ and $T_{reg}$ cells.

**Conclusion:** There is strong probability that the quality of immunity in cancer patients is related to their MHLC beliefs. Further research is recommended for studying whether MHLC beliefs of patients with other types of cancer can improve their immune responses and how beliefs control immune system.

**Key words:** Breast cancer; $T_{helper}$ cells; Cytokines; locus of control; Immune; NK; $T_{reg}$ cells; IL1$\beta$; TNF-$\alpha$.

Introduction

Both preclinical and clinical studies showed that immunity towards cancer is a critical factor in shaping the overall anti–tumor responses. The type of immune cells plays a dual role in cancer as it can suppress tumor growth while it can also promote breast tumor progression (1). For instance, NK cells and $T_{helper}$ cells are known to play an important role in prevention of cancer (2) which orchestrates a broad range of immune mechanisms capable of inducing and maintaining immune responses against tumor antigens (3). There is strong indication that these roles of NK cells and $T_{helper}$ cells shaped by the type of inflammatory stimuli in the host microenvironment in particular cytokines such as IL-1$\beta$ and TNF-$\alpha$ (4, 5). However, when inflammatory stimuli are chronic (i.e., sustained for long time), this leads to exhaustion of immune cells and recurrence of cancer (6). In breast cancer, $T_{reg}$ cells promote tumor progression by suppressing effective antitumor immunity (7).

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Beside the importance of immunological function, a patient’s health-related beliefs are very important in facing cancer as there is a paper suggesting that patients’ health locus of control orientations are significantly associated with good adjustment to living with cancer (8). Health locus of control (HLC) is a set of beliefs regarding whether a person can control his/her health by his/her own efforts (internal health locus of control; IHLC) or by external factors such as chance (CHLC), doctors (DHLC), or powerful other people (OHLC) (9). IHLC might also be used as an early predictor of psychological adjustment to cancer (10). For instance, the longitudinal effect of psychological sense of IHLC at 8 months post cancer diagnosis was found to be associated with less cancer recurrence (11). Additionally, an IHLC belief has been found in cancer survivors to have a positive association with health behaviors such as exercise and diet. By contrast, higher OHLC beliefs are negatively correlated with doing exercise (12).

No previous research has studied the relation between general locus of control (LOC) beliefs and immune response in breast cancer, but there is a previous study that examined NK cell activity in a population of depressed patients. Results showed that depressed internal locus of control (I-LOC) patients exhibit nearly normal NK activity but depressed external locus of control (E-LOC) patients have lower NK cell activity (13). All in all, the above findings suggest that evaluation of immunological function and HLC beliefs of breast cancer patients can possibly act as predictors of incidence of cancer, response to treatment and survival in breast cancer. Also, it is possible that there is a relationship between HLC and immune function in breast cancer patients.

In this study, NK cells, T helper cells, T regulatory cells (Treg), IL-1β and TNF-α were assessed to study the relation between HLC beliefs and immune response to cancer as several studies suggested the important anti-tumor role of NK cells and T helper cells in immune response to cancer, in addition to the negative effect of T reg cells, IL-1β and TNF-α on the immune response against cancer.

Patients and Methods

Patients

This study used a cross-sectional design. Thirty early diagnostic women with stage 1 (n=7), stage 2 (n=15), or stage 3 (n=8) breast cancer, with ages ranging from 40 to 60 years, were recruited at the outpatient surgical oncology clinic at Tanta University Hospital in Tanta, Egypt. The aim of the research was explained and their consent was obtained. Patients with psychological disorders, neurological disorders, immunological disease, diagnosed with another type of cancer or undergoing any anti-cancer treatment (chemotherapy, radiation, surgery) and any drugs that affect the immune system were excluded. Two patients declined because they had no time to participate in the study.

Early diagnostic women with breast cancer completed the questionnaire by interview. A sample of blood was taken on the same day for analysis of immunological parameters. The recruitment of the participants started in April 2014 and ended in December 2015. The research was approved by the ethical committee at Cairo University, Egypt.

Multi-dimensional health locus of control (MHLC) scale Form C

Form C of the MHLC Scale is a condition specific measure of health locus of control beliefs (9). In this study the word “condition” was replaced by “cancer,” thus making it a measure of locus of control beliefs over cancer. MHLC-C has four subscales: internal health locus of control (IHLC, 6 items), powerful other health locus of control (OHLC, 3 items), doctor health locus of control (DHLC, 3 items), and chance health locus of control (CHLC, 6 items). Each dimension is scored separately. Scores on IHLC and CHLC can range from 6 to 36, while scores on DHLC and OHLC can range from 3 to 18.

Blood sample collection

Peripheral blood was withdrawn from patients. All blood samples were coded and submitted to biological analysis. Blood samples were divided equally into two tubes which contained sodium heparin (25µl/1 cm blood) as an anti-coagulant. The 1st tube was used for analysis of the numbers and phenotype of T helper cells (T helper), T regulatory cells (Treg) and NK cells by flow-cytometry and the 2nd tube was used for extraction of RNA on the same day then stored at –80ºC until use. The RNA was used for measurement of gene expression of the pro-inflammatory cytokines IL-1β and TNF-α by real time–PCR.

Reagents and antibodies

Ammonium chloride potassium (ACK), phosphate buffer saline (PBS) and antibodies (shown in Table 1) were used. Reagents for real time PCR, including lysis buffer, wash buffer 1 and wash buffer 2, water, nuclease free, GenJet™ RNA purification columns pre-assembled with 2 ml collection tubes, 1.5 ml collection tubes, β-mercaptoethanol and Ethanol (96–100%) and primers (see Table 2) were purchased from (Biosearch Technologies, USA). RNA was quantified in a single step assay by thermo scientific verso SYBER Green 1-step QRT–PCR kit plus ROX vial, #AB–4104/A, Lithuania.
Phenotypic analysis of immune cells by flow cytometry

For CD4+ T helper cells and Treg cells analysis, aliquots of blood were stained with anti-CD4 and anti CD25 mAbs. CD4+ T helper cells were characterized as CD4+CD25<sub>low</sub> and Treg cells were characterized as CD4+CD25<sub>high</sub>. For NK cells analysis, another aliquot was stained with anti-CD56. After staining for 30 minutes at 4ºc, cells were washed and fixed with paraformaldehyde until assessment. To determine the total number of NK cells, T helper cells and Treg cells, the stained cells were acquired by a FACS Calibur™ or Partec (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (BD Biosciences).

RNA extraction

Briefly, 500 µl blood was centrifuged, the supernatant was discarded and the pellet was lysed in lysis buffer. The lysate cells were washed by washing buffers, then RNA was dissolved in RNA free water and stored at 80ºc until use.

Real–time PCR

The cycling conditions were cDNA synthesis at 50ºc for 15 min, Thermo–start activation at 95ºc for 15 min, Denaturation at 95ºc for 15 min, Annealing at 95ºc for 15 min and Extension at 72ºc for 30 sec. The relative level of m RNA expression of a specific gene was calculated based on ΔΔCT method and normalized to m RNA level of the housekeeping gene β2-microglobulin.

Table 1. List of antibodies used in flow cytometry

<table>
<thead>
<tr>
<th>Sequence name</th>
<th>Company</th>
<th>Cat. NO.</th>
<th>Source</th>
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<td>555346</td>
<td>Mouse Anti–Human</td>
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<tr>
<td>β2–microglobulin R–3</td>
<td>Biolegend, California</td>
<td>302606</td>
<td>Mouse Anti–Human</td>
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</tr>
<tr>
<td>IL–1Beta R–3</td>
<td>CCACATTGCACAGGACTC</td>
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<td></td>
</tr>
<tr>
<td>TNF–α F–3</td>
<td>CAGCCCTCTGGGCCAGCAG</td>
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<tr>
<td>TNF–α R–3</td>
<td>GGTGAGGAGCACATGGTTGG</td>
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</tr>
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</table>

Table 2: Primers used for QRT–PCR.

<table>
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<tr>
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<tr>
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<td>Biolegend, California</td>
<td>318304</td>
<td>TNF–α R–3</td>
<td>Mouse Anti–Human</td>
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</table>

Statistical analysis

The present data were analyzed by IBM Statistical Package for the Social Sciences version 23 (IBM SPSS software, US). All the raw data were normally distributed according to Kolmogorov–Smirnov test. The Spearman correlation coefficient was used to evaluate the relationships between the studied parameters. Regression analysis was used to fit the relationships between the studied variables. All the results were expressed as a mean ± standard error of the mean (SEM).

Results

Correlations between MHLC scores and Indicators of Immune Response

This research studied the correlation between MHLC and immunological parameters in breast cancer patients. Results (see Table 3) show that: (a) there were significant positive correlations between IHLC and both T helper cells and NK cells; (b) significant positive correlation between T helper cells and NK cells; and (c) significant inverse correlations between DHLC with T helper cells, Treg cells, and NK cells.

Discussion

Our findings confirmed the relation between health locus of control beliefs of breast cancer patients and their immune response towards breast cancer. Assessment of the beliefs of patients towards breast cancer was accomplished by using Form C of the MHLC scale which assesses whether the patient might control her cancer by her own effort (IHLC) or whether external factors control such as chance (CHLC), doctors (DHLC), other people (OHLC). In our study we found that two external beliefs (chance and other people) were unrelated to the women’s immune parameters, but internality (IHLC) and the belief in doctors’ control of cancer (DHLC) were both significantly associated, in opposite directions, with T helper cells and NK cells; and (c) significant inverse correlations between DHLC with T helper cells, Treg cells, and NK cells.

Perceiving a threat such as cancer can create stress and depression as a result of negative thoughts and feelings of little control over the disease. Our findings indicate that having low IHLC beliefs has a bad effect on...
immune response to cancer. But what is the effect of the same threat if it occurs in a person with a high IHLC belief towards breast cancer? Our results suggest that higher IHLC beliefs improve the immune response to cancer. On the other hand, higher beliefs by these women that control of breast cancer rests with doctors appears to be associated with lower levels of critical immune function parameters.

This study suggested that high IHLC is related to high NK cells. Moreover, high IHLC is related to high T\textsubscript{helper} cells. To our knowledge, no previous paper studied this relation between IHLC with both NK cells and T\textsubscript{helper} cells in breast cancer patients. But this relation suggests a reason why IHLC beliefs are related to managing cancer as Goldzweig et al. (2016) found in their study of cancer patients. Their results support the hypothesis that perception of cancer as life threatening is an important factor in determining the level of depression among cancer patients. Their results also support a significant relationship between perceived threat and depression only among participants reporting low levels of internal locus of control and suggest that an internal LOC may be more relevant than an external LOC orientation in managing perceived health threats (14).

Importantly, we found that high DHLC belief are related to low T\textsubscript{reg} cells, low NK cells and low T\textsubscript{helper} cells. No previous paper has studied this relationship. But if DHLC is related to low T\textsubscript{reg} cells which have a great role in inhibiting the immune system, we can infer that decreasing T\textsubscript{reg} cells would lead to an improved immune response. DHLC was also related to low NK cells and T\textsubscript{helper} cells, both of which lead to decreased immune response. Undoubtedly there are factors affecting these relationships that were not studied in this paper; in particular, clinical examination and periodic follow-up is recommended to show the effects of both IHLC and DHLC orientations in healing among cancer patients.

Incidentally, our results (Table 3) also confirmed the positive correlation between T\textsubscript{helper} cells and NK cells in these women with early breast cancer, showing that T\textsubscript{helper} cells recruit and activate inflammatory cells (macrophages, granulocytes, eosinophils and NK cells) around the tumor. Also, during early infections, activated NK cells modulate dendritic cells functions thereby able to promote T cell responses. Moreover, NK cells enter into lymph nodes and localize in close to T cells. Then, NK cells influence T cells activation and shape the quality of T cell responses (16).

### Study limitations

The sample size of our study is very small. This is a cross-sectional study that only examined what could be a temporary association between locus of control beliefs and indicators of immune status. Therefore, longitudinal studies with larger samples of patients are required to confirm the relationships we observed. Comparison between the study findings and other studies is limited owing to the lack of previous studies investigating the relation between LOC and immune status. The women in our study were asked about having other diseases that might affect their immune status. Although they said they did not, we relied on their self-reports, which may not have been accurate. Another limitation is the location where the MHLC questionnaire interview was done. It was conducted in the hospital, which was very crowded with

<table>
<thead>
<tr>
<th></th>
<th>IHLC</th>
<th>CHLC</th>
<th>DHLC</th>
<th>OHLC</th>
<th>T\textsubscript{helper}</th>
<th>T\textsubscript{reg}</th>
<th>NK</th>
<th>IL1-\beta</th>
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<tr>
<td>IHLC</td>
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<td>-----</td>
<td>-----</td>
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<tr>
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<tr>
<td>DHLC</td>
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<td>-----</td>
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<td>-0.17</td>
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<td>0.04</td>
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<tr>
<td>T\textsubscript{reg}</td>
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<td>-0.50*</td>
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<tr>
<td>NK</td>
<td>0.82**</td>
<td>-0.21</td>
<td>-0.66*</td>
<td>0.14</td>
<td>0.79**</td>
<td>0.43</td>
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<tr>
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<tr>
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<td>0.15</td>
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<td>0.16</td>
<td>-0.37</td>
<td>0.26</td>
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Table 3. Correlation coefficients (r) between MHLC scores and indicators of Immune Response in Egyptian Women with Breast Cancer

Significant correlations at: * P<0.05 and ** P<0.01
patients’ family around them, which might have inhibited patients from replying freely to the items on the MHLC scale. Many patients appeared to be bothered by the length of the interview, and that also should be kept in mind as a potential limitation of these findings. Finally, this study was conducted with an Egyptian translation of Form C of the MHLC scale and it is possible that different results would be found with other versions of this scale in other cultures.

Conclusion

An internal locus of control orientation regarding cancer has a positive relationship with T helper cells and NK cells which play a role in immune response against cancer. Further research is recommended for studying whether the health locus of control beliefs of patients with breast cancer and, perhaps, other types of cancer can improve their immune responses. Answering these questions will help in the development of both psychotherapy and immunotherapy for cancer patients.

Acknowledgment

We are grateful to all breast cancer patients and the medical team who took part and helped in the research study. Grateful acknowledgement to Prof. Mohamed Attia, professor of clinical pathology, Faculty of Medicine, Tanta University, Egypt, for his assistance and finally to Prof. Kenneth A. Wallston for his supervision in writing this paper.

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