Serum IL-24 Levels in Gastric and Breast Cancers and Non-cancerous Inflammations

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Abstract

Background: Human IL-24 is a cytokine belonging to the IL-10 family of cytokines, also known as melanoma differentiation-associated gene 7, due to its discovery as a tumor-suppressing protein. A tumor-suppressing protein, interleukin-24 is produced by a variety of cells, including cancerous and non-cancerous healthy cells. The aim of the present study was to evaluate serum interleukin-24 concentrations in different cancers and compare them with non-cancerous inflammations.

Method: In this case-control study, we divided a total of 200 subjects into five groups of 40: control subjects without cancer and without H. pylori infection, patients with gastric cancer and H. pylori infection, patients with H. pylori infection without cancer, and patients with breast cancer and without H. pylori infection. We measured the serum IL-24 level using specific enzyme-linked immunosorbent assay (ELISA) kit; we analysed the data with SPSS software.

Results: The level of IL-24 was significantly higher in breast cancer group (160.65±55pg/mL) (mean± SD) followed by gastric cancer with (76.2±16.27 pg/mL) (mean±SD) and without (72.5±17.84 pg/mL) (mean± SD) H. pylori infection groups. The level of IL-24 in H. pylori infected patients and controls were (32.78±12.96 pg/mL) (mean±SD) and (27.4±8.5 pg/mL (mean±SD)), respectively.

Conclusion: The mechanisms by which IL-24 is produced may be different between immune and cancer cells, and serum IL-24 is more likely generated by immune cells than tumor cells. In breast cancer patients, estrogen or other sex hormones may provoke IL-24 production.

Keywords: Interleukin- 24, Breast cancer, Helicobacter pylori, Gastric cancer
**Introduction**

IL-24, a member of the IL-10 family of cytokines, is identified as a gene induced during terminal differentiation in human melanoma cells. Therefore, it was primarily called melanoma differentiation-associated protein 7 (MDA7) and later renamed IL-24. It can function as either an intracellular cell-death-inducing factor or a classical cytokine through its cell surface receptors. With respect to its anticancer actions, IL-24 selectively and efficiently induces cell death in a wide variety of cancer cells. In contrast, through receptor binding, IL-24 has been reported to induce the expression of proinflammatory cytokines. IL-24 was also found to play a role in wound healing and autoimmune diseases. It also provided protection against a number of infectious bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Mycobacterium tuberculosis*.

IL-24 is expressed by immune and non-immune cells, and it is produced by spleen, thymus, TH2 cells, B cells, natural killer (NK) cells, dendritic cells, monocytes, and melanocytes. Furthermore, certain condition such as treatment with IFN-β plus mezerein was found to induce the transient expression of IL-24 mRNA in some cancer cell lines. Myeloid cells also generate IL-24 in response to microbial products, such as lipopolysaccharides (LPS), through activating Toll-like receptors (TLRs). Among the various cytokines, IL-1β exerted significant effects on IL-24 induction. TNF-α, IL-17, and LPS weakly stimulated IL-24 expression, and this stimulation is negligible when compared to IL-1β. Furthermore, the combination of IL-1β with TNF-α induced strong IL-24 expression as compared to IL-1β alone; this suggests that IL-24 might be induced at inflamed lesions where both IL-1β and TNF-α are expressed.

Interleukin-24 is produced in various amounts by cancer and normal cells; it could also be potentially used as a biomarker as its expression is associated with disease prognosis. However, very few studies have been conducted on serum IL-24 levels in cancers and non-cancerous inflammatory conditions. The objective of the present study was to evaluate serum interleukin-24 concentrations in different cancers and compare them with non-cancerous inflammations. We evaluated serum IL-24 in patients with breast cancer, gastric cancer, *H. pylori* infection, and gastric adenocarcinoma with *H. pylori* infection.

**Patients and Methods**

**Patients**

In a case-control study, 160 patients and 40 age-matched healthy control subjects (without any history of cancer and autoimmune diseases) were enrolled. The study was approved by the Ethics Committee of IAU of QOM branch. Informed consent was obtained from all participants prior to sample collection. From May 2015 to November 2018, 200 subjects were examined; they were divided into five groups: group I (control group) consisted of 40 subjects whose endoscopic examination demonstrated no gastritis, neoplasm, or *H. pylori* infection as confirmed by the urease rapid test and serologic tests; group II comprised *H. pylori* infected patients without gastric adenocarcinoma as confirmed by the histopathological study, the urease rapid test, and serologic tests; group III was comprised of 40 patients with gastric adenocarcinoma and without *H. pylori* infection as diagnosed and confirmed through histopathological study, urease rapid test, and serologic tests; group IV included 40 cases of breast carcinoma without *H. pylori* infection as confirmed by the urease rapid test and serologic tests; group V consisted of gastric adenocarcinoma with *H. pylori* infection patients as confirmed by means of histopathological study, urease rapid test,
and serologic tests. All subjects signed an informed consent form before inclusion. We excluded patients with chronic diseases, immunosuppressed, on hormonal therapy, using non-steroid anti-inflammatory, antibiotics, proton pump inhibitor, previous radiotherapy/chemotherapy, and H2 blockers. In group I (controls), the 20 patients (50%) were male and 20 (50%) were females. In group II (H. pylori infected patients), 25 patients (62.5%) were male and 15 (37.5%) were females. All patients in group IV (breast cancer) were women (100%). In group V (gastric cancer with H. Pylori infection), the distribution was 23 male patients (57.5%) to 17 females (42.5%).

If women with breast cancer also had H. pylori infection, interleukin-24 levels might be increased in response to infection and mistakenly attributed to cancer.

**Histopathology**

The gastric tissue samples were fixed in 10% formal-saline. Sections were stained with hematoxylin and eosin and Giemsa for the assessment of tumor type and H. pylori infection. Histological classification was done according to the Lauren classification; the tumors were staged according to the TNM criteria (tumor, node, metastasis) by a team of pathologists.

In the breast cancer group, tissue processing was done by fixing the tissues in 10% buffered formalin overnight. The tissues were grossed and representative sections were taken and submitted for processing. The processed tissues were embedded into paraffin wax blocks; sections were placed on the slides, and routine H&E staining was done for histological diagnosis. Grading was done according to Bloom Richardson grading system and the tumors were staged according to the TNM criteria (Tumor, Node, Metastasis) by a team of pathologists.

**Rapid urease test**

One specimen of each patient was tested by rapid urease test (RUT) to detect *H. pylori* that was performed with a Gastro urease kit (Bahar-Afshan Co, Tehran, Iran) according to manufacturer’s instructions.

**Detection of anti-*H. pylori* antibodies**

One blood sample was obtained from each patient to determine the serum levels of IL-24 and anti H. pylori IgG titer. From each blood sample, serum was prepared and stored at -70ºC until used. We utilized the ELISA method (LDN, Nordhorn, Germany), and sera with titers >11 international units (IU) were considered positive (test cut-off point: 10 IU, <9 IU: negative, 9–11 IU: doubtful, >11 IU: positive); we considered doubtful results as negative.

**IL-24 assessment**

Serum IL-24 levels were measured using specific enzyme-linked immunosorbent assay (ELISA) kit (BOSTER BIOLOGICAL TECHNOLOGY, USA) according to the manufacturer’s protocol. Cytokine concentrations were specified with a standard curve derived from known amounts of standards using absorbance readings at 450 nm on an ELISA reader (Range of standard vials 62.5pg/ml–4000pg/ml Sensitivity <10 pg/ml).

**Statistical analysis**

The statistical analyses were performed by SPSS 16.0 (SPSS, Chicago, IL) software. The data were expressed as mean ± SD. The patients and controls were compared using ANOVA and Games-Howell test in the post-hoc analysis. A P-value of < 0.05 was considered significant. Leven’s test was used to test the homogeneity of the variance.
Results

Table 1 depicts the demographic characteristics of the study groups. Table 2 shows the clinical features of the cancer patients.

Figure 1 compares the serum levels of IL-24. The results showed that IL-24 was detectable in the sera of all subjects and controls with different concentrations (Figure 1). There were no associations between gender or age and serum levels of IL-24 for neither patients nor controls. There was no significant difference between IL-24 concentration and cancer stage at \( P < 0.05 \).

The mean (SD) levels of IL-24 in controls, *H. Pylori* infected patients, gastric cancer group, breast cancer group, and *H. Pylori* infected with gastric cancer group were 27.4(8.5) pg/mL, 32.78(12.96) pg/mL, 72.5(17.84) pg/mL, 160.65(55.00) pg/mL, and 76.2(16.27) pg/mL respectively. The level of IL-24 significantly increased in breast cancer group (160.65±55pg/mL) (mean± SD), gastric cancer with (76.2±16.27 pg/mL) (mean±SD) and without (72.5±17.84) (mean± SD) *H. pylori* infection groups versus controls (27.4±8.5). However, *H. pylori* infected patients (32.78±12.96) (mean± SD) and controls were not different regarding the mean levels of IL-24. Similarly, the mean level of IL-24 was not significantly different between gastric cancer group and gastric cancer with *H. pylori* infected patients.

Discussion

In this study, we investigated the serum levels of IL-24 in breast cancer patients, gastric cancer patients with *H.pylori* infection, and *H.pylori* infected patients. The aim was to compare the levels of IL-24 in cancerous conditions and non-cancerous inflammatory conditions. Our study showed that different cancers had different IL-24 concentrations. We found significantly higher serum levels of IL-24 in patients with breast cancer compared with other groups. Moreover, we found an increase in IL-24 concentration in response to inflammatory conditions.

IL-24 was discovered by the subtraction hybridization of cDNA libraries prepared from melanoma cells treated with IFN-\( \gamma \) and mezerein and termed melanoma differentiation-associated protein-7,\(^{15}\) and it was later renamed IL-24.\(^{16}\) IL-24 is generated by myeloid and lymphoid cells of immune system.\(^{17}\) In monocytes, the production of IL-24 is induced by LPS, concanavalin A, or cytokines.\(^{18}\) IL-24 is strongly expressed in leukemic memory-type B cells. It is also expressed in human follicular B cells; it is more abundant in CD27\(^+\) B cells and CD5\(^+\) B cells whereas it is low to undetectable in centroblasts and plasma cells.\(^{19}\) Inflammatory cytokines stimulate various cells to produce IL-24. Melanocytes produce IL-24 in response to IL-1\( \beta \), monocytes, and IL-17; Furthermore, TH2 cells secrete IL-24 in response to IL-22. IL-24 is generated by monocytes, macrophages, endothelial cells, keratinocytes, melanocytes, and subepithelial myofibroblasts. Transcription factors STAT6 and GATA Binding Protein 3 (GATA3) regulate the expression of IL-24 in TH2 cells.\(^{20}\) Moreover, IL-24 is expressed in the villi, decidual tissue, villous column, trophoblasts, stroma, and blood vessels.\(^{21}\) In addition, certain condition such as treatment with IFN-\( \beta \) plus mezerein was found to induce the transient expression of IL-24 mRNA in some cancer cell lines. In breast-derived epithelial cells, IL-24 mRNA expression was not detected de novo in six cell lines; however, it was inducible by IFN-b+ mezerein treatment in normal HBL-100 cells and in p53 mutant MDA-MB-231 and p53-null MDA-MB-157 cells.\(^{22}\)

Our study showed that IL-24 production was the highest among subjects with breast cancer. Therefore, the type of cancer might affect the production of IL-24. On the other hand, inflammation alone did not have a
major effect on IL-24 production as there was no significant difference between the control group and the *H. pylori* infected group. It is probable that the mechanisms provoking the expression of IL-24 are different in cancers and non-cancerous inflammations. In non-cancerous inflammation, inflammatory cytokines seem to play a role in the production of IL-24. However, IL-24 production in cancers is influenced by factors other than inflammatory cytokines.

A recent report showed that IL-1β stimulation-induced IL-24 expression in cultured normal human keratinocytes was strongly dependent on p38 Mitogen-activated protein kinase (MAPK) activation. Inhibition of this pathway accelerated IL-24 mRNA destabilization mediated by the 3' UTR of IL-24 mRNA, suggesting that p38 MAPK regulated the IL-24 expression at the post transcriptional level. Estrogen activates multiple signaling cascades, including the MAPK pathway. Seval Y showed that estradiol significantly increased p38 MAPK phosphorylation in endometrial stromal cells in culture within two minutes (*P* < 0.05), and this phosphorylation was blocked by a specific p38 MAPK inhibitor. Immune cells including B cells, T cells, NK cells, plasmacytoid DCs, and monocytes can express estrogen receptors; thus, more interleukin-24 production is possible in patients with a high level of estrogen. In the current study, serum estrogen concentration was not measured.

IL-24 expression in the inflamed mucosa of patients with inflammatory bowel disease (IBD) was reported, and the molecular mechanisms responsible for its expression in human colonic SEMFs were identified. Similar to differentiated melanoma (HO-1) cells, AP-1 and C/EBP binding to the IL-24 promoter was reported in these cells upon IL-1β stimulation; this resulted in enhanced IL-24 mRNA and protein expression. IL-1β also led to increased IL-24 mRNA stabilization in subepithelial myofibroblast (SEMF). *H. pylori* infection further induced the production of IL-1β; this protein is a powerful inhibitor of gastric acid secretion that promotes hypochlorhydria and favors more colonization of *H. pylori* in the stomach, leading to more severe gastritis and eventually gastric cancer.

In the clinical studies based on the immunohistochemical analysis of patients’ tumors, a strong decrease was observed in IL-24 transcript in breast cancer; also, the level of decrease correlated with poor prognosis, low survival, and lymphatic metastases. In rectal cancer, IL-24 expression had a significant inverse relationship with N stage, overall stage, and the number of involved lymph nodes, but the status of IL-24 expression did not affect survival. In our study, there was no correlation between serum IL-24 concentration and disease stage, metastasis, or lymph node involvement. These results indicate that the measured IL-24 is probably produced by immune cells rather than cancerous cells. Umeda S et al. showed that the level of IL-24 was stronger in the early stages of lung cancer while it was weaker in advanced stages; they suggested that IL-24 could emerge as a suppressor in the early stage and disappear in the advanced stage; it is also a common player in the KRAS and EGFR-mediated oncogenic pathway. Other driver oncogenes such as ALK, RET, and ROS might share the common pathway to induce IL-24 expression. In inflammatory state, IL-24 is preferentially expressed by TH2 cells where STAT6, GATA-binding protein 3 (GATA3), and JUN have been proposed to participate in transcriptional regulation. The serum level of IL-24 is probably independent of the cancer stage and likely to be produced by immune cells.

In conclusion the mechanisms by which IL-24 is generated are different from immune and cancer cells. P38 MAP kinase activation is
likely to happen in inflammatory conditions and gastric and breast cancers. Nonetheless, its activation is pronounced in patients with breast cancer. Therefore, it seems that estrogen activated MAPK pathway, resulting in higher serum levels of IL-24 in our breast cancer patients. Serum IL-24 concentration is independent of the cancer stage, and serum IL-24 is likely to be produced by immune cells. All said, more research should be done on other cancers as well as on larger sample sizes. If it is shown that estrogen can increase interleukin-24 concentrations in breast cancer patients compared to other cancers, it may be possible to detect breast cancer in early phases through measuring this cytokine.

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Conflicts of Interest
None declared.

References


Table 1. The demographic characteristics of the study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>F</th>
<th>M</th>
<th>Age (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Controls)</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>48.4±11.5</td>
</tr>
<tr>
<td>II (H.P)</td>
<td>40</td>
<td>15</td>
<td>25</td>
<td>44.2±13.6</td>
</tr>
<tr>
<td>III (GC)</td>
<td>40</td>
<td>14</td>
<td>26</td>
<td>52.5±9.1</td>
</tr>
<tr>
<td>IV (BC)</td>
<td>40</td>
<td>40</td>
<td>0</td>
<td>50.8±14.9</td>
</tr>
<tr>
<td>V (GC with H.P)</td>
<td>40</td>
<td>17</td>
<td>23</td>
<td>43.2±11.6</td>
</tr>
</tbody>
</table>

N: Number; M: male; F: female; BC: Breast cancer; GC: Gastric cancer; H.P: Helicobacter pylori; SD: standard deviation

Table 2. Clinical features of the cancer patients

<table>
<thead>
<tr>
<th>Cancer stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>11(27.5%)</td>
<td>7(17.5%)</td>
<td>22(55.0%)</td>
</tr>
<tr>
<td>Gastric</td>
<td>12(30.0%)</td>
<td>10(25.0%)</td>
<td>18(45.0%)</td>
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<tr>
<td>Gastric with H.P</td>
<td>10(25.0%)</td>
<td>6(15.0%)</td>
<td>24(60.0%)</td>
</tr>
</tbody>
</table>

Figure 1. Mean serum levels of IL-24 in study groups. The same letters show a nonsignificant difference between groups, and the non-identical letters show a significant difference between groups.