

ORIGINAL ARTICLE

Serum apelin levels and body composition changes in breast cancer patients treated with an aromatase inhibitor

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Summary

Purpose: The adipose tissue plays a role in carcinogenesis with the adipokines it generates. Apelin is an anti-obesogenic adipokine, and assumes roles in both vascularization and tumor cell proliferation. The present study aimed to investigate changes in apelin levels, in postmenopausal breast cancer (BC) patients receiving aromatase inhibitors (AIs).

Methods: Forty early-stage postmenopausal BC patients treated with AIs with no history of chemotherapy administration were included in the study. At the beginning, we measured serum apelin levels in postmenopausal BC patients who were receiving AIs and healthy women of similar age and normal body mass index (BMI) (control group). We evaluated changes in the body composition, serum lipid profile and serum apelin levels at the beginning and the 12th month through anthropometric measurements and bioelectric impedance analysis.

Results: Forty subjects with postmenopausal BC had a median age of 57 years (range 44-82). BC patients exhibited significantly higher apelin levels and body mass index

(BMI) scores compared to the control group ($p=0.0001$, $p=0.0001$, respectively). The 12th month's measurements indicated reduced apelin levels in 24 patients (60%) and increased apelin levels in 16 patients (40%) compared to the initial figures. With respect to the parameters, the patients with reduced apelin levels had significantly different waist-to-hip ratio (WHR) and fat mass scores compared to those with higher apelin levels ($p=0.008$, $p=0.047$, respectively).

Conclusion: This study showed that postmenopausal BC patients had high levels of apelin and high BMI scores. This finding suggests that apelin promoted carcinogenesis particularly in obese individuals. The massive and metabolic changes observed in the fat tissues of the postmenopausal BC patients receiving AIs will especially affect the BC-associated outcome.

Key words: apelin, postmenopausal breast cancer, aromatase inhibitors

Introduction

BC is the most prevalent type of cancer among women and represents the primary cause of cancer-related deaths worldwide. About 80% of primary BCs are hormone-sensitive and these patients may be treated with endocrine therapy

[1,2]. The enzyme aromatase is a member of the cytochrome P450 superfamily. Located on chromosome 15, aromatase is encoded in the CYP19A1 gene [3]. Aromatase is involved in the last step of estrogen biosynthesis that converts testosterone

into estrogens through the aromatization process. In postmenopausal women, aromatization of androgens, which are produced by the adrenal glands, serves as the main source of estrogen production after cessation of the ovarian function [4].

Today, researchers have developed specific inhibitors of aromatase. Type I inhibitors are steroidal, mimicking the structure of androstenedione which is a substrate of the aromatase. In this group, exemestane is the only molecule with current clinical use. Type II inhibitors are non-steroidal; they bind non-covalently to the heme moiety of the aromatase and saturate the binding site. Anastrozole and letrozole are clinically used molecules in this group [5,6].

Obesity is clearly identified as a risk factor in many types of cancer, with increasing prevalence of obesity detected in patients with breast cancer in all age groups [7]. Adipose tissue is the main site of estrogen biosynthesis in postmenopausal women. Adiposity is positively correlated with increased serum estrogen levels and BC [8,9]. Chronic inflammation increases the risk of several cancers and it is associated with carcinogenesis and progression [10]. Adipose tissue not only stores energy but also acts as an endocrine organ that regulates metabolism via endocrine processes [11]. Adipose tissue mass, especially white adipose tissue (WAT), produces adipokines and many inflammatory cytokines, such as leptin, tumor necrosis factor alpha (TNF α), interleukin (IL)-6, IL-8, IL-1b, as well as the prostaglandin metabolizing enzyme cyclooxygenase-2 (COX-2) [12-14]. Due to the aforementioned relationship between obesity, inflammation and cancer, increasingly more studies are committed to shed light on the carcinogenesis and explore new treatment techniques for patients with cancer.

Apelin was discovered as a peptide that acts by binding to the G-protein coupled receptor APJ, which is found in many organs including the central nervous system [15]. Its metabolic role remains unclear. Considered an anti-obesigenic molecule, apelin plays roles in insulin secretion and sensitivity, fluid homeostasis, epithelial proliferation, and cytokine regulation [16]. It increases glucose uptake and reduces insulin resistance, and is observed with high blood levels in obese individuals and patients with type-2 diabetes [17]. Apelin is required for the formation of vascular structures during embryonic development [18]. This angiogenic effect of apelin, demonstrated in human angiogenesis models [19], was also found similar in tumor neoangiogenesis studies [20,21].

Apelin plays a role in lymph node metastasis and lymphangiogenesis development by promoting lymphatic endothelial cell proliferation through ERK and PI3K pathways [22]. Some studies found significant correlations between increased apelin expression and survival outcomes in certain types of cancer [23-29]. The aim of this study was to clarify this association in postmenopausal BC patients using AIs.

Methods

Patient selection

Included in this study were 40 postmenopausal early-stage hormone receptor-positive BC patients who referred to our outpatient clinic from January 2012 to January 2014. They were receiving AIs (letrozole or anastrozole) as postoperative adjuvant endocrine treatment with no progression under this treatment, and none of them were subjected to chemotherapy. All of the patients gave verbal informed consent prior to entering the study. Each patient was followed over a period of one year. A control group of 20 healthy individuals with similar demographic characteristics and normal BMI scores was formed. All changes in body composition were recorded with anthropometric measurements and bioelectric impedance analysis, serum lipid profile, and serum apelin levels at the beginning and the 12th month.

Body composition analysis

BMI was calculated in kg/m². Anthropometric measurements and bioelectric impedance analysis of the patients were performed at the beginning (basal) and the 12th month. Body composition [total body water (TBW), fat-free mass (FFM), fat mass (FM), percent body fat] was measured by bioelectrical impedance analysis using TANITA BC-420MA scale. The waist circumference was measured half-way between the costal arc and the iliac crest during a normal respiratory position. The hip circumference was measured over the widest part of the gluteal region, and the WHR was also recorded. One nurse performed the measurements for all patients.

Apelin levels

Venous blood samples were obtained from the antecubital vein after an overnight fasting period. Serum separator tubes were allowed to clot at room temperature for 10-20 min before centrifugation. Tubes were centrifuged for 15 min at 2000 RPM, and aliquoted sera were stored at -20°C until analysis. Serum Apelin 36 was measured with enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Apelin 36 (YHB0365Hu, YH Biosearch Laboratory, Shanghai, China). The inter-

and intra-assay coefficients of variation were <12% and <10%, respectively.

Statistics

Statistical analyses were performed using the Rstudio software version 0.98.501 via R language. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk test) to determine whether they exhibited a normal distribution. Descriptive analyses of the variables were presented using means and standard error of the mean (SEM). As the variables were not normally distributed, nonparametric tests were conducted to compare these parameters. The Wilcoxon test was used to compare changes in these parameters between basal and 12th month. The Mann-Whitney U test was used to compare these parameters between control and patient groups. Correlation of apelin levels with other variables was analyzed by Spearman rho correlation test. A p value of less than 0.05 was considered to indicate statistical significance.

Results

Forty subjects with postmenopausal BC had a median age of 57 years (range 44-82). Of the patients, 15 (37.5%) had stage 1 and 25 (62.5%) stage 2. Twenty-two patients had grade 1 (55%), and 18 grade 2 (45%) tumors. Twenty patients (50%) received anastrozole, and the other half received letrozole. The clinical characteristics of the study population are summarized in Table 1.

BC patients exhibited significantly higher apelin levels and BMI scores compared to the control group (p=0.0001, p=0.0001, respectively) (Figure 1 and Figure 2). As for the relationship between basic parameters and apelin levels, we found a statistically significant and positive correlation only in the case of basic total cholesterol measurements (r=0.483, p=0.004) (Table 2). The 12th month's measurements indicated reduced apelin levels in 24 patients (60%) and increased

Table 1. Clinical patient characteristics

| Characteristics | Patients N (%) |
|----------------------------|-------------------|
| Age, years, median (range) | 57 (44-82) |
| Treatment | |
| Letrozole | 20 (50) |
| Anastrozole | 20 (50) |
| Stage | |
| Stage I | 15 (37.5) |
| Stage II | 25 (62.5) |
| Grade | |
| Grade I | 22 (55) |
| Grade II | 18 (45) |

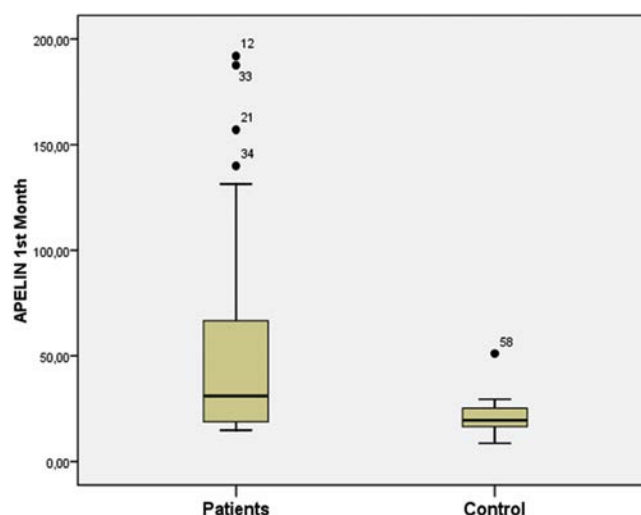


Figure 1. Difference of basic apelin measurements between control and patient groups in the first month (p<0.05).

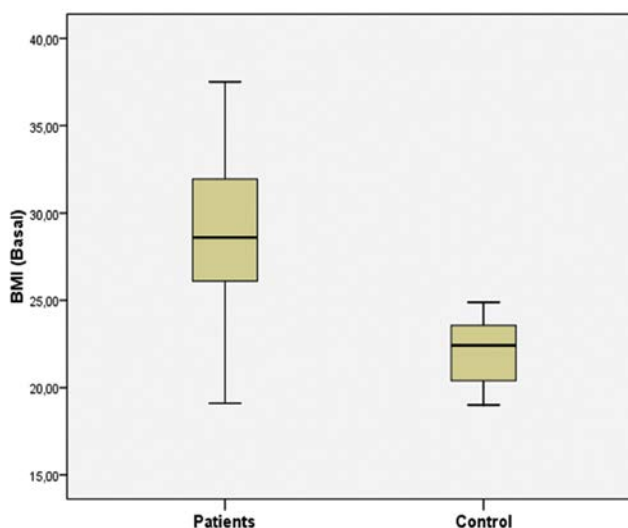


Figure 2. Basic BMI difference between control and patient groups (p<0.05).

apelin levels in 16 (40%) compared to the initial figures. With respect to the parameters, the patients with reduced apelin levels had significantly different WHR and fat mass scores compared to those with higher apelin levels (p=0.008, p=0.047, respectively) (Table 2).

Discussion

Our study showed that BC patients had significantly higher apelin levels in comparison to the control group. Some studies reported significant correlations between increased apelin expression and malignancy-associated results [23-29]. However, the data about this relationship

Table 2. Comparison of basic and 12th month apelin levels with anthropometric measurements, bioelectric impedance and biochemistry parameters

| Variables | Patients with increased apelin levels (N=16) | | | Patients with reduced apelin levels (N=24) | | |
|-------------------|---|--------------|---------|---|--------------|---------|
| | Basic | 12th. month | p value | Basic | 12th. month | p value |
| BMI | 28.0 ± 0.9 | 28.2 ± 1.0 | NS | 29.3 ± 0.9 | 29.5 ± 1.0 | NS |
| Basic weight | 71.4 ± 2.3 | 71.5 ± 2.5 | NS | 73.2 ± 2.5 | 73.4 ± 2.7 | NS |
| Upper arm circ. | 31.5 ± 0.7 | 31.8 ± 0.7 | NS | 31.5 ± 0.8 | 32.0 ± 0.8 | NS |
| Upper leg circ. | 55.8 ± 1.3 | 57.5 ± 1.3 | NS | 57.2 ± 1.0 | 57.9 ± 1.1 | NS |
| Abdomen circ. | 93.6 ± 2.5 | 95.8 ± 2.4 | 0.043 | 96.6 ± 1.9 | 100.8 ± 2.3 | 0.001 |
| Waist circ. | 106.2 ± 2.2 | 106.2 ± 2.1 | NS | 106.5 ± 1.7 | 106.3 ± 2.3 | NS |
| WHR | 0.9 ± 0.02 | 0.9 ± 0.01 | NS | 0.9 ± 0.02 | 1.0 ± 0.02 | 0.008 |
| Percent body fat | 36.3 ± 1.1 | 37.6 ± 1.1 | 0.023 | 35.3 ± 1.3 | 36.7 ± 1.6 | 0.041 |
| Fat mass | 26.1 ± 1.5 | 27.2 ± 1.6 | NS | 26.2 ± 1.6 | 27.7 ± 2.1 | 0.047 |
| Fat free mass | 43.6 ± 1.4 | 43.4 ± 1.4 | NS | 46.6 ± 1.2 | 45.5 ± 0.8 | NS |
| TBW | 33.6 ± 0.9 | 31.6 ± 1.0 | 0.0001 | 33.9 ± 0.9 | 31.8 ± 0.7 | 0.001 |
| Total cholesterol | 226.4 ± 9.0 | 237.9 ± 14.7 | NS | 234.9 ± 11.8 | 227.1 ± 10.3 | NS |
| HDL | 45.9 ± 3.0 | 49.9 ± 2.6 | NS | 46.7 ± 2.0 | 45.8 ± 2.2 | NS |
| LDL | 142.5 ± 8.8 | 158.3 ± 13.7 | NS | 162.5 ± 17.7 | 144.6 ± 7.0 | NS |
| TG | 188.0 ± 22.5 | 147.0 ± 18.9 | 0.035 | 214.6 ± 34.3 | 181.9 ± 33.1 | 0.007 |

BMI:body mass index, circ: circumference, WHR: Waist-hip ratio, TBW: total body water, HDL: high density lipoprotein, LDL: low density lipoprotein, TG: triglyceride, NS: Not significant p value. P values calculated with Willcoxon test

between apelin and BC remain immature. Peng et al. demonstrated that apelin promoted tumor proliferation and invasion via ERK1/2 in the MCF-7 cell line [30]. Apelin is also found abundantly secreted in the milk, and the mammary gland contains a high level of pre-proapelin mRNAs and apelin protein [31]. From an immunohistochemical perspective, Wang et al. showed, for the first time in the relevant literature, increased apelin expression in normal and malignant mammary tissue [32]. The present study is the first to report significantly higher serum apelin levels in BC patients in the relevant literature. Apelin is a novel angiogenic factor and recent studies suggest that apelin promotes angiogenesis, lymphangiogenesis and tumor growth in vivo and the angiogenic potential of apelin is similar to that of VEGF [33,22]. Given the increased apelin levels in milk and mammary tissue, increased serum apelin levels demonstrated in this study, and the apelin's proven important role in carcinogenesis, apelin is considered a promising molecule for the targeted treatment of BC patients [34].

After one year, we repeated the bioelectric impedance analyses, anthropometric measurements, and re-measured serum apelin levels. We found that postmenopausal patients receiving an AI did not have a history of a specific diet or exercise program. The patient serum apelin levels were changed in one year (24 patients exhibited

reduced serum apelin levels and 16 had increased serum apelin levels) and there were some changes in patient body composition as well. Body composition changes might be related with decreased plasma estrogen levels and increased plasma testosterone due to hormonal drugs, eating habits, lifestyle differences and exercising. Aromatase inhibition reduces estrogen synthesis and causes longer hyperandrogenism. As a result, AIs may drive more changes in body composition than menopause [35]. Van Londen et al. classified 82 postmenopausal women with BC after chemotherapy into two groups including those treated and not treated with AIs. At the 24th month, stable FM, increased FFM and free testosterone, and decreased sex hormone binding globulin (SHBG) were found among the group treated with AI [36]. Another previous randomized study demonstrated no change in the tamoxifen (TMX) group. However, the patients with exemestane switch after TMX had a significant reduction in FM and a higher FFM/FM ratio at the 12th month. These findings suggested that exemestane may have an effect on FM [37]. Therefore, we excluded those patients receiving TMX as it has different effects on fat tissue.

In our study, BC patients had higher BMI scores compared to the control group. A meta-analysis determined that each one-unit increase of BMI causes a 3% increase in BC development. Weight,

WHR, waist and hip circumferences, abdominal and subcutaneous adiposity were also proved to be related with increased BC development [38-41]. Besides, waist-to-hip circumference ratio has a stronger relationship with BC development than BMI. During follow-up, we detected WHR and increased fat mass in the patients with reduced apelin levels. Numerous studies reported obesity-associated changes in plasma apelin levels, and individual weight loss and apelin level reduction go hand-in-hand [16,42]. In this study, reduced apelin levels of patients with increased fat tissue might be driven by the undisclosed effects of apelin on cancer patients and AIs-driven changes in the fat metabolism. Individuals with hypercholesterolemia and hypertriglyceridemia were found to exhibit low serum apelin levels [43,44]. Higauchi et al. detected reduced triglyceride levels with intraperitoneal injection in mice [45]. However, we observed that initial apelin levels were positively correlated with total cholesterol levels. The low number of patients, uncontrolled diet and varying metabolic effects of apelin on cancer patients might have produced this result, which contrasts those reported in the relevant literature. We could not perform a survival analysis on the patients in

the first month of the follow-up period because of the early-stage BC patients' long disease-free survival and overall survival times. The patient group is being followed-up, and we will evaluate apelin's prognostic significance with a survival analysis later.

In conclusion, we showed that postmenopausal BC patients had high levels of apelin—an adipocytokine with a demonstrated role in metabolic and vascular disorders. The patient group had high BMI scores. This finding suggested that apelin promoted carcinogenesis particularly in obese individuals, with its roles in hyper-inflammation and neovascularization. Especially the massive and metabolic changes observed in the fat tissues of the BC patients receiving AI in the context of postmenopausal endocrine treatment will affect the BC-associated outcome. Further molecular and clinical prospective studies with larger patient populations may shed light on the importance of apelin for cancer patients.

Conflict of interests

The authors declare no conflict of interests.

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