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RESEARCH ARTICLE

Leukotriene D4 Levels In Patients With Breast Cancer

Sevgi AKAYDIN[*°], Sümeyye RAMAZANOĞLU[**], Ece MİSER SALİHOĞLU[***], Hasan KARANLIK[****], Semra DEMOKAN[*****]

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SUMMARY

Leukotriene D4 (LTD4) is an inflammatory mediator synthesized from LTC4 via gamma-glutamyltransferase (GGT) enzyme in the arachidonic acid pathway and has been reported to induce cell proliferation and survival in cancer. In recent studies, it has been shown that there is an increase in GGT enzyme activity in breast cancer. In recent studies, it has been shown that there is an increase in GGT enzyme activity in breast cancer. The aim of this study is to determine whether there is a change in serum LTD4 levels in patients with breast cancer and to examine the relationship between LTD4 and GGT. For this purpose, serum samples were taken from 43 patients diagnosed with breast cancer and eight healthy controls. Patients were divided into five subgroups, Luminal A, Luminal B, Luminal B-HER2(+), HER2(+), and triple-negative. LTD4 levels were measured by the ELISA method. Mean levels of LTD4 in the patients were significantly higher than in healthy controls ($p < 0.05$). Based on the molecular subtypes, serum LTD4 levels were found to be considerably higher in the Luminal A, Luminal B, and Triple (-) subgroups than in the controls ($p <0.01$, $p <0.005$, and $p <0.005$, respectively). Higher levels of LTD4 have been observed in post-menopausal patients than in premenopausal patients ($p <0,05$). A statistically significant positive correlation was observed between GGT activity and LTD4 levels in the whole study group and post-menopausal patients ($R=0.349$, $p=0.014$, and $R=0.437$, $p=0.042$, respectively). According to the literature, this study is the first to examine LTD4 levels in breast cancer and supports other studies showing the role of leukotrienes in cancer. Because of LTD4's ability to induce proliferation and inhibit apoptosis, increased levels of LTD4 in our study may be associated with cancer development, especially in post-menopausal women.

Key Words: *Leukotriene D4, Gamma-Glutamyltransferase, Breast Cancer, Post-menopausal Status*

Meme Kanseri Hastalarında Lökotrien D4 Düzeyleri

ÖZ

Lökotrien D4 (LTD4) araşidonik asit yolağında gama-glutamiltransferaz (GGT) enzimi aracılığı ile LTC4'ten sentezlenen inflamatuar bir aracıdır ve kanserde hücre çoğalmasını ve sağ kalımını indüklediği bildirilmiştir. Son yıllarda yapılan çalışmalarda, meme kanserinde GGT enzim aktivitesinde artış olduğu gösterilmektedir. Bu çalışmada, meme kanserli hastalarda serum LTD4 düzeylerinde de bir değişiklik olup olmadığını ve LTD4 ile GGT arasındaki ilişkiyi incelemeyi amaçladık. Bu amaçla meme kanserli 43 hasta ve 8 sağlıklı kontrolden serum örnekleri alındı. Hastalar, Luminal A, Luminal B, HER2 (+), Luminal B-HER2 (+) ve üçlü negatif olmak üzere beş alt gruba ayrılmıştır.

Lökotrien D4 seviyeleri ELISA yöntemi ile ölçülmüştür. Hastalardaki ortalama LTD4 seviyeleri sağlıklı kontrollerle karşılaştırıldığında anlamlı derecede yüksek bulunmuştur ($p<0.05$). Moleküler alt tiplere göre serum LTD4 düzeyleri; Luminal A, Luminal B ve Üçlü (-) alt gruplarında kontrollere göre anlamlı derecede yüksek bulunmuştur (sırasıyla $p <0,01$, $p <0,005$ ve $p <0,005$). Menopoz-sonrası hastalarda menopoz-öncesi hastalara göre daha yüksek LTD4 seviyeleri gözlenmiştir ($p <0,05$). Tüm çalışma grubunda ve post-menopozal hastalarda GGT aktivitesi ile LTD4 seviyeleri arasında istatistiksel olarak anlamlı bir pozitif korelasyon gözlenmiştir ($R=0.349$, $p=0.014$ ve $R=0.437$, $p=0.042$, sırasıyla). Bu araştırma literatüre göre, meme kanserinde LTD4 düzeylerini inceleyen ilk çalışmadır ve lökotrienlerin kanserdeki rolünü gösteren diğer çalışmaları desteklemektedir. LTD4'ün hücre proliferasyonunu indükleme ve apoptozu inhibe etme yeteneği nedeniyle, çalışmamızda bulunan artmış LTD4 seviyelerinin, özellikle menopoz sonrası kadınlarda kanser gelişimi ile ilişkili olabileceği düşünülmektedir.

Anahtar Kelimeler: *Lökotrien D4, Gama-Glutamiltransferaz, Meme Kanseri, Post-menopozal Durum*

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[*] ORCID: 0000-0002-0927-5188, Department of Biochemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey.
[**] ORCID: 0000-0003-3475-6554, Department of Biochemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey.
[***] ORCID: 0000-0003-0681-3566, Department of Biochemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey.
[****] ORCID: 0000-0001-6156-7260, Department of Surgery, Institute of Oncology, University of Istanbul, Istanbul,Turkey.
[*****] ORCID: 0000-0002-8066-8419, Department of Basic Oncology, Oncology Institute, Istanbul University, Istanbul, Turkey.

[°] Corresponding Author; Sevgi AKAYDIN,
Phone: +90-312-2023155, e-mail: sevgiy@gazi.edu.tr

INTRODUCTION

Despite many advances made in research in recent years, cancer remains the leading cause of death worldwide. Changes in the tumor microenvironment, including dysregulated immunity, contribute to carcinogenesis and cancer progression. In 1863, Rudolf Ludwig Carl Virchow described the relationship between inflammation and cancer. He supposed that the inflammatory process could provide a suitable environment for tumor development and infiltrated immune cells show the place where cancer lesions start in the inflamed tissue (Korniluk, 2017). Inflammation is responsible for removing dead cells, cell proliferation, and tissue repair, this type of inflammatory response is self-limiting and ceases after the repair is completed. On the other hand, uncontrolled inflammation can become chronic, and cause sustained release of growth factors and reactive oxygen that interact with the DNA of the proliferating epithelium and result in genomic changes which lead to induce tumor initiation and triggers malignant growth in surrounding tissues (Todoric, 2016). Inflammatory cells release pro-inflammatory mediators, including eicosanoids, chemokines, growth factors, and cytokines, which can transform the microenvironment into an abnormal environment (Bellamkonda, 2016). The synthesis of leukotrienes (LTs) is initiated by 5-lipoxygenase-activating protein (FLAP), which presents arachidonic acid to 5-lipoxygenase (5-LOX) enzyme. Although the clinical value of LTs comes from their bronchoconstriction properties during allergic inflammation, they are inflammatory mediators with potent biological activities in the pathogenesis of many diseases. Commonly LTA4 and LTB4 are considered known as LTs. On the other hand, Cysteinyl leukotrienes (CysLTs) mention LTC4, LTD4, and LTE4, and these inflammatory mediators are structurally different from LTA4 and LTB4 (Almutlaq, 2017). CysLTs are mainly synthesized by leukocytes and dendritic cells such as eosinophils, basophils, mast cells and monocytes/macrophages (Peters-Golden, 2007). CysLTs activate their specific receptors ($CysLT_1R$ and $CysLT_2R$) located on the cell membrane. CysLT1R has a high affinity for LTD4 while CysLT2R has a lower affinity (Magnusson, 2011).

The main function of the gamma-glutamyl transferase (GGT) enzyme found in the extracellular membrane is to degrade the extracellular glutathione by transferring the gamma-glutamyl group to acceptor substrates thus providing the cell with intracellular cysteine to re-synthesis glutathione (Verma, 2015). Another essential function of this enzyme, also known as gamma-glutamyl leukotrienase (GGLT), is to catalyze the synthesis of LTD4 by removing the gamma-glutamyl group from LTC4 (Funk, 2001). In recent years, increased GGT enzyme levels in various types of cancer, including breast cancer have been reported (Corti, 2010). This study aimed to examine LTD4 level and its correlation with GGT to estimate the effect of chronic inflammation in breast cancer.

MATERIALS AND METHODS

Forty-three patients with breast cancer who were followed up by Istanbul University, Institute of Oncology, Department of Clinical Oncology, Oncology Surgery Unit were included in this study. Patients have been received and understood all the research-related information before the operation date, and the informed consent was obtained from them. Serum samples were taken from 43 patients before the operation, and eight healthy people admitted to the clinic for breast reduction surgery were also included in this study as controls. Serum samples were taken from healthy women before the operation. Samples were stored at -80°C until use. Baseline characteristics and laboratory results of the patients and controls are summarized in Table 1. Ethics approval for this study was approved by The Clinical Research Ethics Com-

mittee of the Istanbul Faculty of Medicine (Ethics Committee 28.03.2016/106748). Molecular classification of the patient group was performed according to the evaluation criteria of estrogen/progesterone hormone receptor, Ki67, and cerB2 (HER2) (Goldhirsch, 2011).

Accordingly, patients were classified as Luminal A (10 patients with estrogen receptor [ER] positive and, or progesterone receptor [PR] positive, human epidermal growth factor receptor 2 [HER2] negative, low Ki67 expression), Luminal B (8 patients with ER+ and, or PR+, HER2-, Ki67 high), Luminal B/Her2+ (7 patients with ER+ and, or PR+, HER2+, Ki67 high), Her2+ (8 patients with ER-, PR-, HER2+), and triple-negative/basal type (10 patients with ER-, PR-, HER2-).

Table 1. Baseline characteristics and laboratory tests of patients and controls.

Variables	Patients (n=43)	Controls (n=8)
Age (SD)	51.3 (11.9)	39.0 (14.7)
Menopausal Status		
Premenopausal (%)	20 (46.5)	7 (87.5)
Postmenopausal (%)	23 (53.5)	1 (12.5)
Cancer Stage, n (%)*		
I	3 (7.0)	-
II	18 (41.9)	-
III	21 (48.8)	-

*One missing data

Leukotriene D4 Analysis

Serum Leukotriene D4 (LTD4) levels were measured using a commercially available ELISA kit (Oxford Biomedical Research, MI 48371 U.S.A.). In the 96-well microplate, the standards and the samples were analyzed by the kit procedure. Samples were purified using extraction columns before measurement, and then the kit procedure was applied. Measurements were carried out in absorbance (A)
at 400 nm. All data are expressed as mean (standard deviation, SS).

Statistical analysis

The homogeneity of the data was evaluated with the Kolmogorov-Smirnov test. Since the data were not normally distributed, the results were compared using nonparametric tests. Mann-Whitney U test was used to compare differences between patients and healthy controls. Spearman-correlation test was used to examine the relationship between the parameters for the non-normally distributed data. P values of less than 0.05 were regarded as statistically significant. Statistical analyzes were performed using the SPSS 22 Package Program (SPSS Inc, USA).

RESULTS AND DISCUSSION

To determine whether the data from serum LTD4 analysis were distributed normally, a Kolmogorov-Smirnov test was used. According to test results, LTD4 data did not show a normal distribution ($p<0.05$). Mean LTD4 levels in total patients were significantly higher than those in healthy controls ($p<0.005$). According to the molecular subtypes of breast cancer, patients in Luminal A, Luminal B, and Triple (-) groups had significantly higher LTD4 levels than in healthy controls ($p<0.01$, $p<0.005$, and $p<0.005$, respectively) (Table 2). Patients in the Her2(+) group had lower LTD4 levels than those in

other subtypes. However, there was no statistically significant difference between the groups in terms of LTD4 levels in general. Several studies show the direct effect of 5 lipoxygenase derivatives on cellular growth, cell migration, and invasion in cancer cells (Bishayee, 2011).

Here, we demonstrate for the first time the high LTD4 levels in breast cancer patients. It is known that the proinflammatory leukotriene D4 (LTD4) has been observed to increase proliferation, survival, and cell migration (Paruchuri, 2003; 2005). One study showed a significant correlation between high expression of $CysLT_1R$ in tissue from breast cancer patients and histological grade (Magnusson, 2011). Interestingly, other studies showed that the LTD4 receptor $CysLT_1R$ is highly expressed in human colon cancer and negative correles with patient survival (Bellamkonda, 2016; Ohd, 2003). Also, LTD4 has been found to induce migration and proliferation of colon cancer cells (Salim, 2014). In addition to that, LTD4 was reported to have a growth-stimulating effect on human gastric cancer cells (Shimakura, & Boland,1992). In contrast, one study showed that LTB4 and LTD4 inhibit MCF-7 breast cancer cell growth, and a leukotriene receptor antagonist and a 5-LO inhibitor were able to abolish the inhibitory effect of LTB4 and LTD4 on cell growth, suggesting that the leukotriene receptors mediate this effect (Przylipiak, 1998). Increased 5-LO expression and the cysteinyl leukotriene (CysLT) pathway prepare the tumor microenvironment could be by activating different signal pathways and producing inflammation (Tsai, 2021). Findings from studies seem heterogeneous, so more studies are needed to understand the role of the LTD4 in cancer.

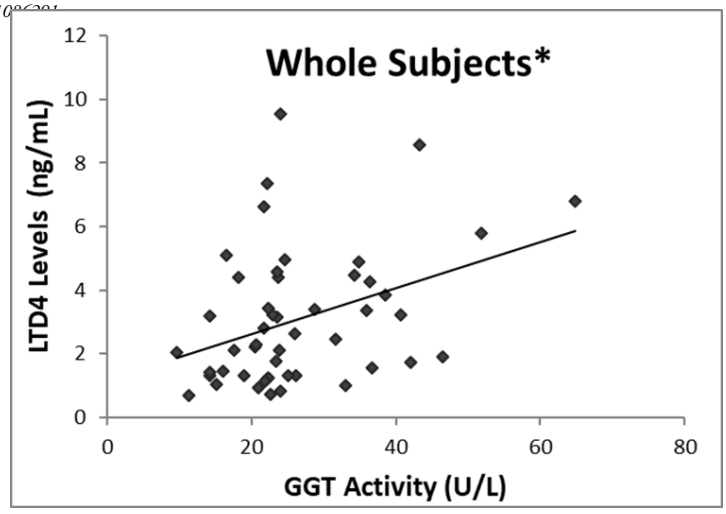
Table 2. Levels of serum LTD4 in both patients and healthy controls.

			LTD4 Levels	
		N	**Mean(SD)**	**P**
Controls		8	1.30 (0.39)	--
Total patients		43	3.42 (2.13)	0.002*
Patients in molecular subtypes of breast cancer	Luminal A	10	3.11 (2.18)	0.006*
	Luminal B	8	4.11 (2.56)	0.002*
	Luminal B+Her2	7	3.68 (2.56)	0.104
	Her2(+)	8	2.70 (2.12)	0.114
	Triple(-)	10	3.58 (1.52)	0.003*

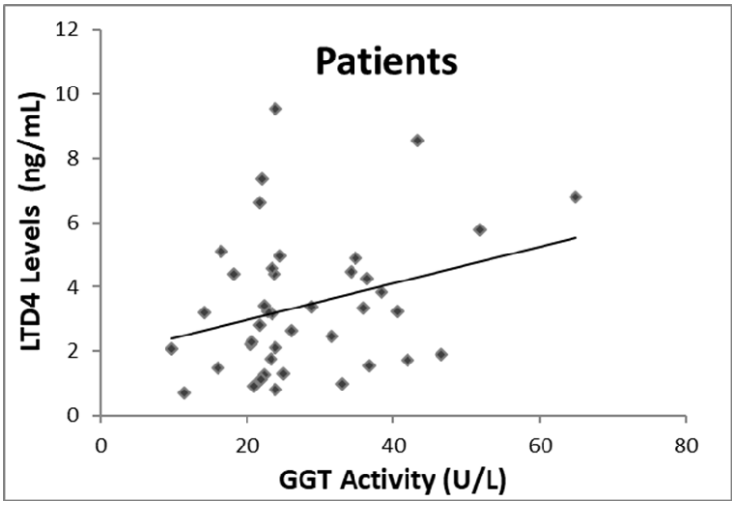
* compared with controls

In our study, post-menopausal patients had higher levels of LTD4 than pre-menopausal patients [4.13 (2.35) ng/mL vs 2.61 (1.51) ng/mL, respectively] ($p< 0.05$). Although there is no study examining leukotriene levels in breast cancer, one study has shown the role of inflammation in the pathogenesis and progression of post-menopausal estrogen-dependent breast cancer (Madeddu, 2014). Another study reported that regular use of aspirin, ibuprofen or other NSAIDs may have a significant protective effect against the development of breast cancer in post-menopausal women (Harris, 2003).

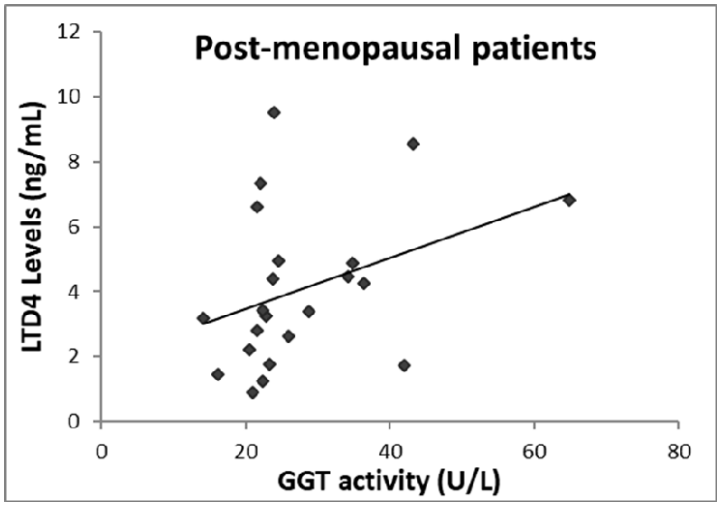
The mean GGT activity was found to be significantly higher in total patients than in healthy controls [27.89 (11.21) vs. 18.85 (4.41), respectively] ($p<0.05$).



(a)



(b)



(c)

* patients + controls (n=51)

Figure 1. Correlations between LTD4 and GGT in whole subjects (a), only patients (b) and, post-menopausal patients (c).

When the whole study group was examined, a statistically significant positive correlation was observed between GGT activity and LTD4 levels (R=0.349, p=0.014) (Figure 1a). However, a positive but not statistically significant correlation was observed between GGT activity and LTD4 levels in patients (Figure 1b). There was also a positive correlation between these parameters in post-menopausal patients (R=0.437, p=0.042) (Figure 1c). A significant correlation between high GGT activity and LTD4 levels in breast cancer patients shows the role of GGT in producing LTD4. Data from previous studies demonstrated the function of the GGT family could be catalysis LTC4 to LTD4 (Hanigan, 2014). Here we indicate that GGT participates in the inflammatory response in breast cancer patients. Our findings are consistent with the studies that showed the importance of the GGT family in the inflammation process in mice (Shi, 2001), and pulmonary epithelial cancer cells (Lukic, 2016).

CONCLUSION

In conclusion, our study is the first to examine LTD4 levels in different molecular subclasses of breast cancer, higher LTD4 levels were observed in patients with both hormone-dependent and triple (-) breast cancer than controls. The participation of GGT in LTD4 synthesis, and the correlation between GGT and LTD4 indicates the important role of these two parameters in breast cancer. More studies with larger numbers of molecular subgroups may reveal the effect of inflammation in groups.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

AUTHOR CONTRIBUTION STATEMENT

Conception or design of the work (SYA), sample collection and clinical data (HK, SD), experiments and interpretation (SYA, EMS, SR), drafting the article (SYA, SR), critical revision of the article (SYA), final approval of the version to be published (SYA, SR, EMS, HK, SD)

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