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Fatty Acid Transport Proteins and Breast Cancer Risk by pathological subtype Novel Insights from Gene expression and Associations with Serum Fatty Acids

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ABSTRACT

Background: Breast cancer (BC) is the most prevalent cause of death in women. Malignant cells rely on increased fatty acid (FA) uptake and utilization to sustain their rapid growth and proliferation. Therefore, it is crucial to target the transporters responsible for FA uptake, such as fatty acid transport proteins (FATPs), which are upregulated in various cancers. **Aim**: The current investigation aimed to find the association and correlation between long chain fatty acids (LCFAs) and FATPs relative mRNA expression in women with BC and controls. **Materials and Methods**: In total, 136 subjects were recruited in the study; FA methyl esters were isolated and serum LCFA levels were determined by gas chromatography (GC-FID). Total RNA was isolated and relative mRNA expression of FATP gene was evaluated by using real time–quantitative polymerase chain reaction. The expression of FATPs in BC tissue sections were analyzed by IHC. Statistical analysis was carried out using SPSS 20.0; $p\ value \leq 0.05$ was considered to indicate statistical significance. **Results:** The present study revealed significant differences in serum LCFA levels between case and control subjects. Relative mRNA expression of FATPs were substantially high in BC subjects compared to control. Notable variations in the gene expression of FATPs were also found between pathological subtypes of BC. Significant association was observed between the relative mRNA expression of FATPs and that of serum LCFAs in BC subjects. Immunohistochemical analysis revealed increased FATPs expression in BC tissue. **Conclusion:** Current study highlights the significance of FATPs and serum LCFAs in BC, suggesting that increased expressions of FATPs are associated with BC.

Keywords: FATP/SLC27A, Breast cancer, Relative mRNA expression, Receptor status, LCFAs

Introduction:

Breast cancer (BC) is the most significant cause of mortality in women [1]. Almost two million new cases are reported annually [2]. Every year, more than 100,000 new cases are detected in India [3-4]. Despite progresses in early detection and treatment, nearly one-third of BC subjects will develop metastasis [2]. Factors such as obesity, genetic variations, sedentary lifestyles impact the onset and progression of BC [3-5]. Adipocytes store triglycerides (TG) as an energy source [6]. TG are then degraded to release FAs [7]. Various cellular functions in mammals are carried out by different types of FAs, including long-chain fatty acids (LCFAs), medium-chain fatty acids, and short-chain fatty acids [8].

Lipids and LCFAs are crucial for maintaining mammalian cell homeostasis and play vital roles in various physiological processes, such as membrane structure, energy storage, and cell signaling [9]. Additionally, they are involved in regulating immune response and gene expression [10]. When there is an increased need for metabolic energy, cancer cells use FAs as a fuel source. In particular, cancer cells depend on the increased synthesis of fatty acids, uptake and utilization of FAs to sustain their rapid growth and proliferation [11]. This reliance on fatty acids is a distinct characteristic of cancer metabolism. By utilizing FAs, cancer cells can generate ATP more efficiently and support their uncontrolled growth [12]. The increased uptake of FAs in cancer cells is often accompanied by variations in lipid metabolism, leading to the production of various lipid species that can promote tumor progression and metastasis [13]. LCFAs alter signaling events in cells associated with inflammation & immunity, resulting in altered pattern of gene expression [14]. Understanding the complex relationship between cancer cells & LCFAs is vital for developing targeted therapies that disrupt this metabolic pathway and hinder tumor growth. One potential approach is to target the transporters responsible for FA uptake, such as FATPs, which may be a potential therapeutic avenue to disrupt the metabolic reliance of cancer cells on fatty acids [8]. FATPs comprise six integral membrane proteins (FATP1-6) that differ in tissue expression & intracellular localization. They belong to solute carrier proteins 27 and perform dual roles of an acyl-coA synthase and as FA transporter. These proteins contain an extracellular FA binding site, intracellular ACS activity site and a functional AMP binding motif that actively aids in FA transport.

FATPs transport exogenous FAs into the cell [15-17]. Members of the FATP family exhibit distinctive tissue expression patterns [18].

They are actively involved in lipid metabolism and are most abundantly expressed in tissues such as the liver, intestine, heart, adipocytes, brain, and lungs. FATPs differ in the level of posttranslational modification, molecular mass, intracellular localization, and tissue distribution; some have a specific pattern, while others are ubiquitously expressed. For example, FATP1 is located in adipose tissue,

FATP2 in the liver and kidney, FATP3 in the lung and ovary, FATP4 in the liver and intestine, FATP5 in the liver and FATP6 in the heart and skin [8]. These proteins aid in the uptake and transport of LCFAs/VLCFAs across the plasma membrane and intracellular locations. The uptake of FAs is facilitated by FATPs localized on the plasma membrane, whereas those localized inside the cell may lower the concentration of intracellular FAs and thereby indirectly increase the uptake of FAs by vectorial acylation [19]. Variations in FATP expression or activity can cause significant changes in circulating fatty acid levels, which can have an impact on cellular lipid metabolism, energy balance, and the progression of diseases such as cancer. Evidence from previous studies reports that, FATPs can alter cell growth, FA metabolism, and cell proliferation and play a role in the development of various malignancies, such as breast cancer, melanoma, renal cell carcinoma, prostate cancer bladder cancer, hepatocellular carcinoma, and lung cancer [8, 20-28].

Recently, it has been demonstrated that FATP is critical for the uptake of LCFAs by malignant cells as well as their proliferation and invasion both *in vitro* and *pre-clinical evaluation* [29]. The breast fat pad is composed of adipose tissue, and adipocytes are known to encapsulate invasive BC cells and function as efficient lipid storage cells, providing a readily available carbon source [30]. Hence, FATPs play a crucial role in BC control. Rapid progression of BC is marked by increased FATP expression [20]. The relevance of FATPs and their relationship with LCFAs in BC is unclear, and research on the correlation between serum FATP levels and BC is scarce. As previously mentioned, each FATPs shows different tissue distributions in the human body. The increased cancer cell turnover that occurs during proliferation necrosis and apoptosis leads to the release of Ctnas (circulating tumor nucleic acid DNA and RNA) to aid in metastasis [31-33]. In the current investigation, we were interested in determining whether FATPs were detectable in the serum at the mRNA level and, if so, what are their expression patterns in BC subjects and how they are associated with serum LCFAs. Therefore, our study aimed to analyze the levels of LCFAs, gene and protein expression profiles of FATPs in women with BC and to find association between serum LCFAs and FATPs mRNA levels in women with BC.

Materials and methods:

Ethical Approval:

This case control study was conducted at the Justice KS Hegde Charitable Hospital and Central Research Laboratory, KS Hegde Medical Academy (KSHEMA) in Mangalore, India, at the Departments of Oncology and Surgery (April 2021–March 2023). The study was carried out in relation with the Helsinki Declaration and was approved by the Central Ethics Committee of Nitte (Deemed to be University) (**Ref, NU/CEC/2021/86 Dated: 26-04-2021**) and Institutional Biosafety Committee of Nitte (Deemed to be University). A total of 136 female individuals between the ages of 25 and 80 years

were recruited for the study (68 with breast cancer and 68 healthy females). The inclusion and exclusion criteria were used. Informed consent was obtained from all the participants prior to their involvement in the study.

Inclusion/exclusion criteria:

Each participant in the study underwent a clinical examination and was histopathologically determined to have breast cancer. Pregnant women, those with breast implants, or had a history of other tumors were not included in the study. The control subjects were those who visited the outpatient department and had not undergone breast-conserving surgery/mastectomy or who reported no history of cancer. 3 mL of peripheral blood was collected for the study, centrifuged to separate the serum, divided into aliquots and stored at 80°C.

Anthropometric measurements: Baseline information such as weight, age, height, body mass index (BMI), basal tumor subtypes (ER+/-, PR+/-, and HER2+/-), first-degree family history and clinical data, including grade and TNM (tumor node metastases), was collected from BC subjects.

Fatty acid analysis

Stored serum samples were subjected to lipid transesterification to generate fatty acid methyl esters (FAMEs) using a modified procedure described by Metcalfe et al.,1966 [34]. LCFA levels were measured in the presence of an internal standard (1 mg/mL methyl heptadecanoate-C17:0, Sigma Aldrich). Total serum LCFAs were extracted by hydrolyzing esters and then derivatizing esters under alkaline conditions using 14% boron trifluoride-methanol for five minutes at 100°C to form methyl esters. The LCFAs were then quantified using gas chromatography (GC-FID). FAMEs were extracted and analyzed using a 7820A Agilent GC-FID (flame ionization detector) and J and W DB-23 high-quality columns. Each LCFAs were detected by comparing their elution times to relative LCFA standards. The relative abundance of long-chain fatty acids was calculated relative to that of the internal standard. The amount of individual LCFAs was measured as a percentage of the total LCFA concentration in each sample.

RNA isolation, reverse transcription (RT) and relative quantitative real-time polymerase chain reaction (RT–qPCR):

FATPs gene expression was analyzed by RT-qPCR. Gene transcription was evaluated by mRNA quantification in clinical cases of breast cancer and controls using reverse transcription (RT). This method relies on the reverse transcriptase enzyme, which produces a single-stranded complementary DNA (cDNA) from RNA template. Real-time polymerase chain reaction (qRT–PCR) measures gene expression with precision and sensitivity, allowing relative (RQ–PCR) quantification of cDNA [35–38]. Relative (RQ-PCR) quantification of cDNA is made possible by the accuracy and sensitivity with which RT–qPCR assesses gene expression [37-38]. A housekeeping gene that is constitutively expressed in cells was used to standardize the RT–qPCR results [39].

RNA isolation: Total RNA was extracted from the serum samples obtained from the study participants. With slight modifications to the manufacturer's instructions, total RNA was extracted using RNAiso Plus (Takara Bio Inc., Japan), and cDNA was synthesized using the PrimeScript^{TM} RT Reagent Kit (Takara Bio Inc., Japan) and kept at −20°C. A Nanodrop spectrophotometer (Eppendorf) was used to check purity (A260/A280 ratio was 1.8), and quantify the RNA (total RNA yield from serum was 1000ng/µl).

Synthesis of Complementary DNA (cDNA):

Complementary DNA (cDNA) was synthesized using a Prime Script 1st strand cDNA synthesis kit (Cat. #RR037A, Takara) from 500 ng of total RNA in as per the manufacturer's instructions. The mixture was incubated in an Eppendorf thermocycler at 37°C for 15 minutes and 85°C for 5 minutes. cDNA was synthesized and refrigerated at 20°C (Thermo Fisher Scientific, USA) until further use.

Reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR):

RT–qPCR was carried out using SYBR Premix Ex Taq II (Takara, Cat-RR820A) according to the instructions from manufacturers. A real-time PCR system (AriaMx Real-Time PCR, Agilent, USA) was used for the experiment. The best thermocycler conditions were used for this real-time PCR system, which included 40 cycles of denaturation at 95°C for 10 min and 94°C for 30 s, annealing at 60°C for 60 s, elongation at 72°C for 90 s, and a final extension at 72°C for 10 min. The results are presented as the fold change in expression and were normalized to the expression of the housekeeping gene beta-actin (β-actin). The RQ=2− ΔΔCt technique was used to calculate the relative mRNA expression levels [40, 41]**.** Individuals with no other malignancy and other comorbidities were used as controls. In subjects with BC and control, the following equations were applied: ΔCt sample−ΔCt mean of healthy controls=ΔΔCt and Ct target gene−Ct βactin=ΔCt. All the samples were evaluated in triplicate. The primers used in this study are listed in **Table 1**.

Table 1: List of primers:

Gene Name	Primer Sequence 5' --> 3'	No of Bases	Reference
FATP1	Forward: 5'CAACATGGACGGCAAGGTC-3'	19	
	Reverse: 5'- CAGCAGCTCCATTGTGTCCTC-3'	21	
FATP2	Forward: 5'-GGATGTATTGTGGCTGGTGC -3'	20	
	Reverse: 5'-CCGAAGCAGTTCACCGATATAC-3'	22	
FATP3	Forward: 5'-CGTGCTTCCTGGCTTTACAAG-3'	21	
	Reverse: 5'-CCTGGCTCACCTGGAGATG-3'	19	
FATP4	Forward: 5'-CTACCTTACTGGTGATGTGCTG-3'	22	42

	Reverse: 5'- GCGGCTGAGTGTGCCTTCCAC-3'	21	
FATP5	Forward: 5'-CTGCTTAGATCTCGGAGCC-3'	19	
	Reverse: 5'-CGCCCACATACAGGATCACTG-3'	21	
FATP6	Forward: 5'-GGATGTGTTGAGTTGGGTGCC-3'	21	
	Reverse: 5'- GATTGTTTGCAAAGGTAGCGAC-3'	22	

Case selection for Immunohistochemistry:

Tumor tissue samples were obtained for the current study from the pathology department at KSHEMA,Nitte (Deemed to be University) from thirty newly diagnosed BC subjects who had undergone mastectomy (2020–2023). Tumor-adjacent and malignant tissue samples were obtained. All surgical tissues preserved in 10% buffered formalin and embedded in paraffin were cut into 5 μm thick sections for immunohistochemistry (IHC) analysis. The expression of FATP1, FATP2, FATP3, FATP4, FATP5, and FATP6 was examined by IHC staining.

Immunohistochemical analysis

To examine the expression patterns of FATP1- FATP6, immunohistochemical staining was performed using a Biomarq IHC detection kit (Hyderabad, India). Five-micrometer-thick paraffin sections were deparaffinized with xylene (X1-10 min, X2-10 min) and then hydrated in ethanol (100% − 5 min, 70% − 5 min, 50% − 5 min, 30% − 5 min) [42]. For the epitope retrieval procedure, prewarmed citrate buffer (pH 6.0) was used for 7 minutes. Endogenous peroxidase activity was inhibited for 15 minutes at RT using EP blocking. Sections were incubated with anti-FATP1, anti-FATP2 (R&D Systems) and anti-FATP3 (Biorbyt), anti-FATP4, anti-FATP5 (R&D Systems) and anti-FATP6 (Biochain Incorporated) antibodies overnight at 4°C (dilution 1:100) mixed with protein block to decrease inaccurate staining. After adding the secondary antibody coupled with horseradish peroxidase (HRP), the membrane was incubated for one hour. For IHC staining, diamethoxybenzidine (DAB) was used as the substrate. Hematoxylin counterstaining was carried out. Every step was performed at ambient temperature.

Statistical analysis:

Statistical analysis was performed using SPSS software (version 22.0, IBM, USA) and GraphPad Prism, version 8.0.2 (GraphPad Software, Inc., La Jolla, CA, USA). Categorical variables were evaluated using Chi-square test. Normally distributed data were evaluated by using an independent sample t test. Mann–Whitney U test & Kruskal-Walli's test was employed to evaluate nonparametric variables; While correlating the nonparametric variables for all subjects, Spearman's correlation coefficient test was used. Each P values were two-tailed, with a significance level of $p \leq 0.05$.

Results:

Baseline characteristics of the study population

A total of 136 participants were included in this study. There were 68 control subjects and 68 BC subjects. A summary of the demographic characteristics is summarized in Table 2**.** The mean age (±SD) of the control subjects was 48.18±7.63 years, and the mean age of the BC subjects was 50.88 ± 9.17 years, with a similar age distribution. The history of the family and first-degree relatives among the BC cases and controls revealed that the subjects with BC were more likely to have a larger proportion of first-degree relatives (mother, sisters, and daughters) **(Table 2).** A statistically significant difference was observed while comparing weight between the groups.

Table 2: Demographic characteristics of the study population

Parameters	Control (n=68)	Case(n=68)	p Value
Age	48.18 ± 7.63	50.88 ± 9.17	0.113
BMI	20.20 ± 2.80	25.06 ± 4.76	<0.01**
First-degree family history (%)			
Yes	4	10	0.218
No	96	90	

Age and weight are represented as mean ± SD. Subgroup of first-degree family history is given in percentage. Student t-test was used to calculate P value for parametric variables*p<0.01. The statistical significance of for categorical variables was analyzed using chi-square test.**

Clinical characteristics of the study population:

Among 68 BC subjects, 38 were diagnosed with invasive ductal carcinoma, and 30 were diagnosed with invasive lobular carcinoma. In terms of clinicopathological differences between the two BC subtypes, there were no significant differences in TNM stage, grade or receptor status. **Table 3** summarizes the clinicopathological features of BC subjects.

Table 3: Clinical characteristics of the study population

Tumor Characteristics	Invasive Ductal Carcinoma *n* (%) (*n*=38)	Invasive Lobular Carcinoma *n* (%) (*n*=30)	*P* Value
TNM Stage %			
I	1(2.63)	1(3.33)	**0.752**
II	10(26.31)	9(30)	
III/IV	27(71.05)	20(70)	
Receptor status %			
ER+/PR+/HER+	4 (10.52)	3(10)	
ER+/PR+/HER2-	8(21.05)	8(26.66)	
ER+/PR-/HER2+	2(5.26)	5(16.66)	**0.665**
ER-/PR-/HER2-	12(31.57)	7(23.33)	
ER-/PR-/HER2+	8(21.05)	3(10)	
ER+/PR-/HER2-	2(5.26)	2(6.66)	

ER-/PR+/HER2-	2(5.26)	2(6.66)	
ER-/PR+/HER2+	(0)	0(0)	
Grade %			
2	21(55.26%)	13(43.33%)	**0.464**
3/4	17(44.73%)	17(56.66%)	

The variables—TNM stage, receptor status, and tumor grade—are expressed as percentages. For categorical variables, the statistical significance of the two groups was tested using a chi-square test. *P≤0.05 was considered to indicate statistical significance. Abbreviations: TNM—Tumor Node Metastases; ER-Estrogen Receptor; HER2-Human Epidermal Growth Factor Receptor 2; PR-Progesterone Receptor; TNBC-Triple Negative Breast Cancer.

Comparison of Serum Long-Chain Fatty Acids between Breast Cancer Subjects and Controls

The percentages of LCFAs between the groups are represented in **Table 4.** The results showed that the levels of FAs, such as myristic acid (C14:0) and palmitic acid (C16:0); and monounsaturated fatty acids (MUFAs), such as Oleic Acid (C18:1) percentage was higher in case compared to control. Whereas, the percentage of ω3 fatty acids, such as alpha-linolenic acid (C18:3); Eicosapentaenoic acid (EPA) (C20:5); were lower in BC case subjects compared to control. Among ω6 fatty acids, cancer subjects had a higher percentage of linoleic acid (C18:2) and Arachidonic acid (C20:4) than in control which was significant. Dihomo-GLA (C20:3), and gamma linolenic acid (C18:3) was significantly lesser in case compared to healthy individuals. Along with this the omega 6/3 ratio differed significantly between the case and control groups.

Table 4: Comparison of serum LCFAs between BC subjects and controls

FAs (%)	Controls(N=68)	Cases (N=68)	p value
Saturated fatty acids (SFA's)			
Myristic Acid (C14:0)	3.48 (2.70-4.20)	2.00 (1.70-2.50)	< 0.001***
Palmitic acid (C16:0)	10.99(10.53-11.62)	14.48(12.90-16.85)	< 0.001***
Stearic Acid (C18:0)	4.24(2.13-6.22)	5.24(4.15-5.97)	0.068
Mono-Unsaturated fatty acids (MUFAs)			
MyristoleicAcid(C14:1)	0.47 (0.46-0.81)	0.54 (0.38-0.66)	0.053
palmitoleic acid (16:1)	0.64 (0.28-0.96)	1.57 (1.23-1.94)	0.112
Oleic Acid (C18:1)	9.54 (9.33-9.83)	12.71 (11.19-13.88)	< 0.001***
ω3- Fatty acids			
α - Linolenic acid (C18:3)	0.336 (0.31-0.69)	0.023 (0.017-0.32)	< 0.01 **
Eicosapentaenoic acid (EPA) (C20:5)	1.04 (0.96-1.24)	0.39 (0.28-0.49)	< 0.001***
ω6- Fatty acids			
Linoleic acid (C18:2)	8.14 (5.38-10.42)	15.63 (11.98-17.22)	< 0.001***
Dihomo-GLA (C20:3)	0.84 (0.58-1.05)	0.21(0.14-0.26)	< 0.001***
Gamma-linolenic acid (C18:3)	1.32 (1.14-1.74)	0.79(0.64-0.96)	< 0.001***
Arachidonic acid (C20:4)	4.34 (4.16-4.54)	5.23 (3.36-5.94)	< 0.01 **
Trans-Fatty acids			
Lino-elaidic Acid(C18:2)	0.72 (0.31-0.98)	0.75 (0.42-1.51)	0.087
Omega 6/3 ratio			
Omega ratio	8.55 (4.91-10.36)	34.85 (21.28-51.44)	< 0.001***

Data are shown as median (interquartile range) *Mann–Whitney U test for non-parametric variables. The quantity of each LCFA was calculated as a percentage of the total LCFAs. P value ≤0.05 was considered statistically significant. ** p < 0.01 *** p < 0.001 and * p < 0.05.

Distribution of serum LCFA levels according to receptor status in breast cancer subjects

According to receptor status, the differences in the serum LCFA levels among the BC subjects are shown in **Table 5**. When comparing the levels of serum LCFAs according to receptor status, among the 68 BC subjects, the level of palmitic acid (C16:0) and Oleic Acid (C18:1) differed significantly between the receptor status in BC subjects. Whereas, the levels of other LCFAs, such as myristoleic acid (C14:1) and linoleic acid (C18:2), moderately differed between the group. Other serum LCFAs, such as myristic acid (C14:0), α- (C18:2), Eicosapentaenoic acid (EPA) (C20:5), Dihomo GLA (C20:3), gamma linolenic acid (C18:3), AA(C20:4) and the omega ratio did not show significant difference between the receptor status of BC subjects.

Table 5: Distribution of serum LCFA levels between the receptor status of the BC subjects

FAs (%)	ER+/PR+ /HER2+ (N=8)	ER+/PR+ /HER- (N=18)	ER+/PR- /HER2 + (N=7)	ER- / PR- /HER2 – (N=20)	ER- / PR- /HER2+ (N=7)	ER+/PR- /HER2- (N=4)	ER- / PR+ /HER2- (N=4)	P value
Saturated fatty acids (SFA's)								
Myristic Acid (C14:0)	1.83 (1.44-2.33)	2.09 (1.66-2.26)	2.72 (1.80-3.34)	1.96 (1.82-2.87)	2.68 (2.44-3.56)	2.62 (1.97-3.70)	2.22 (1.80-2.64)	0.14
Palmitic acid (C16:0)	12.50 (7.67-17.48)	14.94 (13.09-17.31)	14.01 (11.96-16.09)	**16.48(16.22-20.39)**	13.58 (12.50-17.89)	7.58 (0.52-14.91)	6.50 (0.44-12.55)	**< 0.001****
Stearic Acid (C18:0)	4.61 (1.81-6.28)	4.28 (3.90-6.17)	**6.17 (3.90-6.29)**	5.24 (4.11-7.16)	5.90 (5.26-6.48)	5.24 (5.21-5.77)	5.24 (2.99-5.77)	0.620
Mono-Unsaturated fatty acids (MUFAs)								
MyristoleicAcid Acid (C14:1)	0.38 (0.35-0.62)	0.46 (0.37-0.66)	0.61 (0.57-0.72)	**1.25 (0.57-1.94)**	0.68 (0.65-0.72)	1.02 (0.36-1.85)	0.53 (0.34-0.65)	**<0.05***
Palmitolate (C14:1)	1.20 (1.10-1.80)	1.83 (1.14-1.97)	**2.44 (1.97-3.20)**	1.57 (1.25-1.87)	1.81 (1.46-1.88)	2.04 (1.57-7.00)	1.64 (1.57-6.80)	0.112
Oleic Acid (C18:1)	**13.22 (12.89-18.80)**	10.86 (5.96-12.80)	13.06 (12.82-16.00)	12.82 (12.35-13.24)	12.27 (10.11-13.22)	10.85 (10.68-12.28)	11.85 (10.55-13.28)	**< 0.001*****
ω3- Fatty acids								
ALA(C18:2)	0.032 (0.017-0.42)	0.032 (0.01-0.032)	0.023(0.023-0.421)	**0.023(0.012-0.032)**	0.023 (0.023-0.32)	0.22 (0.021-0.42)	0.020 (0.017-0.023)	0.541
EPA(C20:5)	0.34 (0.21-0.47)	0.48 (0.30-0.48)	0.48 (0.32-0.49)	**0.32 (0.28-0.47)**	0.43 (0.28-0.47)	0.32 (0.18-0.94)	0.43 (0.14-0.63)	0.371
ω6- Fatty acids								
LA(C18:2)	11.13 (7.73-14.70)	**17.42(13.54-18.81)**	15.54 (11.98-16.15)	13.66 (11.90-16.48)	9.36 (3.36-15.54)	16.75 (8.88-18.40)	9.66 (6.31-13.01)	**< 0.05***
AA(C20:4)	5.72 (1.16-6.90)	3.23 (0.25-5.66)	5.80 (1.32-9.29)	5.16 (1.71-5.31)	5.27 (4.25-5.97)	4.24 (2.48-5.76)	5.58 (3.76-6.91)	0.395
DGLA(C20:3)	0.23 (0.12-0.52)	0.21 (0.20-0.22)	0.14 (0.11-0.37)	0.21 (0.14-0.25)	0.24 (0.14-0.59)	0.23 (0.18-0.47)	0.45 (0.37-0.54)	0.210
GLA(C18:3)	0.77 (0.46-1.05)	0.60 (0.58-1.04)	0.79 (0.64-0.81)	0.74 (0.67-0.78)	0.81 (0.24-1.05)	0.82 (0.70-1.50)	0.73 (0.59-0.88)	0.769
(ω6/ ω3) Ratio								
(ω6/ ω3) Ratio	**40.59 (15.67-60.40)**	33.99 (19.0-46.29)	32.23 (14.59-50.19)	35.95 (19.88-52.05)	31.07 (19.67-60.39)	22.59 (15.18-48.43)	35.95 (19.88-52.05)	0.927

Values presented as Median (25th – 75th percentile); Kruskal-Wallis test -Non Parametric. The quantity of each LCFA was calculated as a percentage of the total LCFAs Abbreviations: AA-Arachidonic Acid; LA-Linoleic Acid; ALA-α Linolenic Acid; EPA-Eicosapentaenoic Acid. ***p<0.001, p<0.01,p<0.05***

Comparison of the relative mRNA expression of FATPs in subjects with and without breast cancer

The relative mRNA expression of differentially expressed genes, i.e., FATPs, between the case and control groups are represented in a box plot **(Figure 1).** Compared to those in control subjects, statistically significant increase in the relative mRNA expression of FATPs were observed in the case subjects.

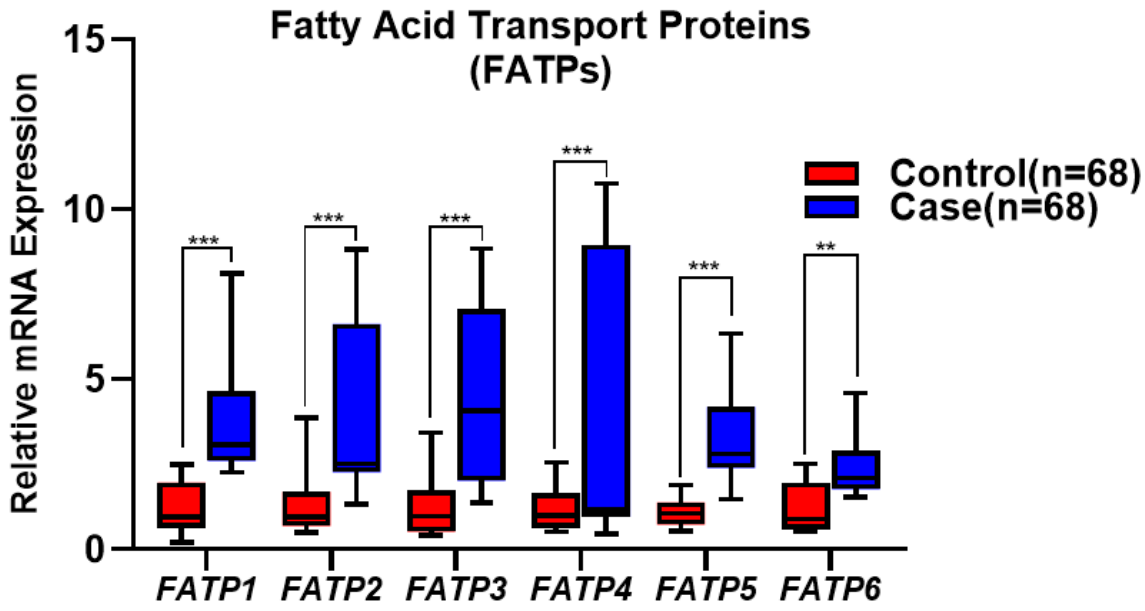


Fig 1: FATPs/SLC27A relative mRNA expression between the BC case and control subjects. Data obtained from two independent experiments performed in duplicates were normalized to β-actin expression and shown as fold changes of mRNA expression. Data were expressed as median (interquartile range). p value was calculated using Mann–Whitney U test. P<0.05 was indicated as statistically significance. ***p<0.001, p<0.01.**

Distribution of relative mRNA expression FATPs based on histological type, TNM stage, and grade of Breast cancer:

A comparison of the relative mRNA expression FATPs with respect to TNM stage, histological type and grade in BC patients is shown in **Figure 2.** Concerning tumor histology, 44.11% of the BC subjects had invasive lobular carcinoma (ILC), and 55.88% of the BC subjects had invasive ductal carcinoma. Relative mRNA expression FATP2/ *SLC27A2*, FATP3/ *SLC27A3* and FATP5/ *SLC27A5* was greater in the ILC group than in the IDC (Invasive ductal carcinoma) group. Relative mRNA expression FATP1/ *SLC27A1* was greater in the IDC group than in the ILC, but no statistically significance difference was detected among the histological subtypes of the BC subjects **(Figure 2A).**

In terms of grade, 50% of the BC subjects were Grade I&II, and 50% of the BC subjects were Grade III&IV. Relative mRNA expression FATP3/*SLC27A3* and FATP4/ *SLC27A4* were significantly greater in grades III and IV than in grades I and II, while FATP2/ *SLC27A2* Relative mRNA expression level

was also greater in III and IV than in grades I and II, but the difference was not statistically significant. However, the Relative mRNA expression of other FATPs (FATP1, FATP5, and FATP6) increased in grade I and II, but the differences were not statistically significant **(Figure 2B)**. Regarding the TNM stage, 30.88% of the BC subjects were in stage I&II, and 69.11% of the BC subjects were in stage III& IV. The Relative mRNA expression of FATP3/ *SLC27A3* and FATP4/ *SLC27A4* was significantly greater in stage III and IV subjects than in stage I and stage II patients. However Relative mRNA expression of FATP1/*SLC27A1* and FATP2/*SLC27A2* was greater in stage III and IV subjects than in stage I and stage II patients, but the differences were not significant **(Figure 2C).**

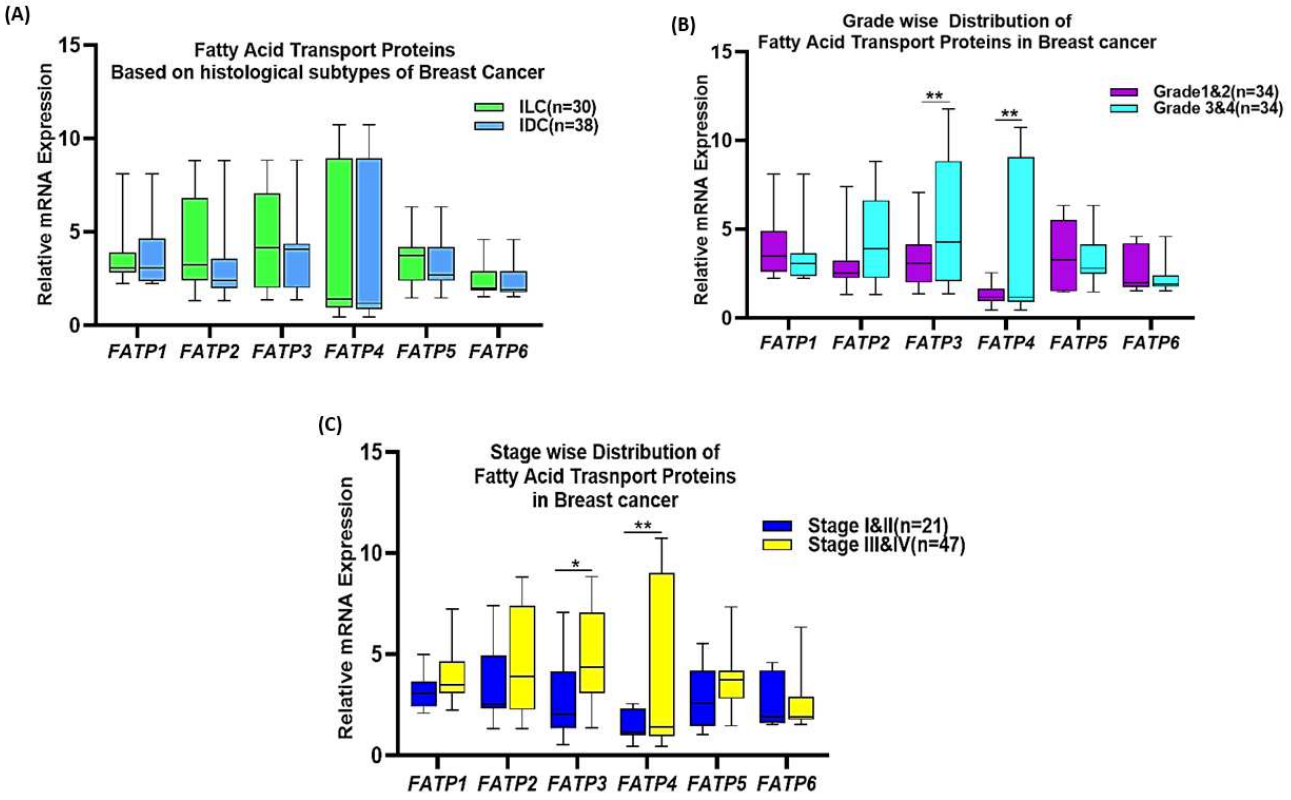


Fig 2: Comparison of Relative mRNA expression of FATPs (FATP1-6) according to histological type (2A), grade (2B) and TNM stage (2C) in BC subjects. P values were calculated using the Mann–Whitney U test for nonparametric variables. The data are shown as medians (interquartile ranges). ** p < 0.01; * *p* < 0.05 Abbreviations: FATP-Fatty Acid Transport Proteins, TNM—Tumor Node Metastases; Tumor IDC—Invasive ductal carcinoma; ILC—Invasive lobular carcinoma

Comparison of the Relative mRNA expression of *FATP*s between the receptor status of BC subjects

The differences in relative mRNA expression of FATPs (FATP1-FATP6) according to receptor status are shown in **Table 6**. The results showed significant increase in the relative mRNA expression of FATP1/*SLC27A1*, FATP4*/SLC27A4* and FATP6/*SLC27A6* in TNBC subtype. Whereas FATP2/ *SLC27A2*, FATP3/ *SLC27A3* and FATP5*/SLC27A5* was high in ER + subtypes of BC.

Table 6: Comparison of the Relative mRNA expression of FATPs between the receptor status of BC subjects

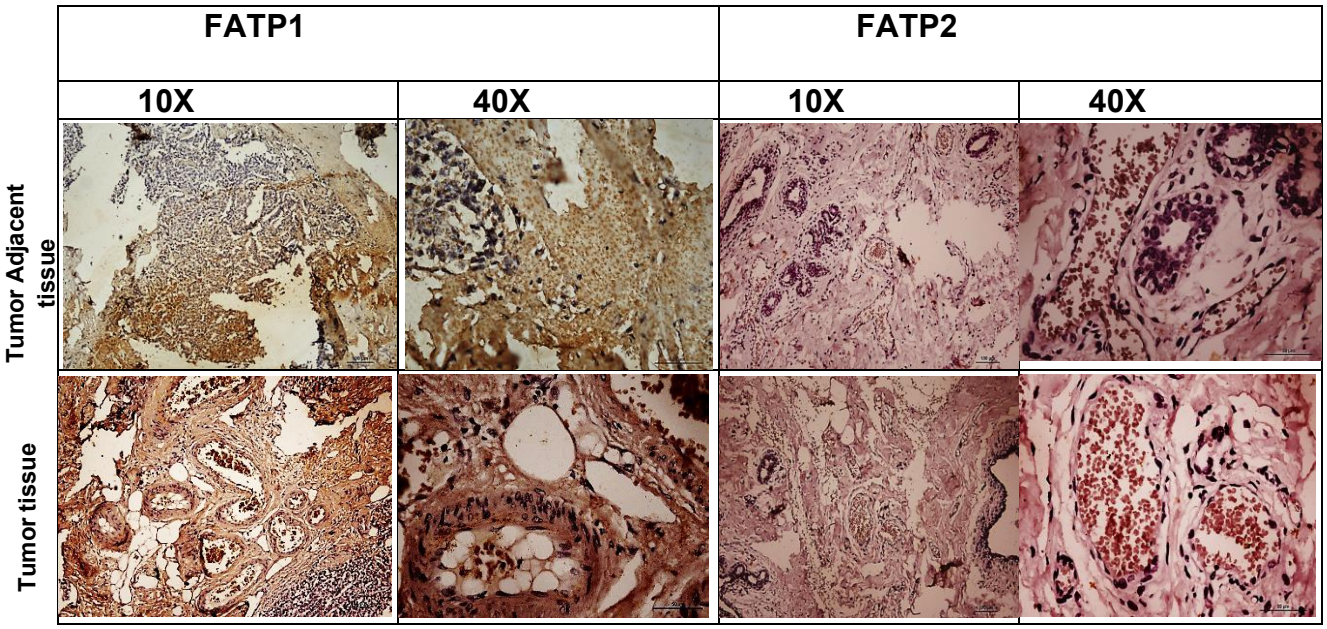
FATPs	ER+/PR+ /HER2+ (N=8)	ER+/PR+/ HER- (N=18)	ER+/PR- /HER2 + (N=7)	ER- / PR- /HER2 – (N=20)	ER- / PR- /HER2+ (N=7)	ER +/PR- /HER2- (N=4)	ER- / PR+/HER2- (N=4)	*P* value
FATP1	3.05(2.60-4.37)	3.49(2.60-3.89)	2.60 (2.37-3.49)	**6.84 (3.24-8.60)**	3.07 (2.37-4.11)	5.19 (3.98-5.62)	5.56 (5.05-5.96)	**0.001****
FATP2	2.46 [2.27-3.72]	2.52[1.72-3.41]	2.07 [1.72-2.27]	2.91 [2.27-6.63]	3.41 [2.41-7.41]	**5.25 [3.72-5.67]**	3.39 [2.45-3.74]	**0.018***
FATP3	**5.08 [2.53-8.28]**	4.08[2.01-4.41]	2.08 [1.35-8.79]	4.37 [4.08-13.43]	4.37 [4.08-7.06]	3.80 [1.90-4.39]	4.87 [2.10-5.73]	**0.050***
FATP4	1.52 [0.77-5.93]	1.17[0.96-1.65]	1.09 [0.85-2.56]	**5.25 [2.41-5.88]**	1.65 [1.06-1.90]	1.31 [0.90-1.88]	2.56 [1.10-9.20]	0.237
FATP5	3.20 [2.63-4.90]	**4.18[2.38-5.52]**	2.38 [1.51-2.57]	3.73 [2.38-4.18]	4.17 [1.46-5.52]	2.54 [1.39-2.81]	2.16 [1.59-2.73]	**0.027***
FATP6	2.09 [1.71-3.13]	1.90[1.68-4.31]	2.09 [1.77-2.42]	**3.07 [1.90-3.77]**	1.90 [1.52-2.68]	2.97 [1.92-3.38]	2.82 [1.67-3.56]	0.467

Data are shown as median (interquartile range). Kruskal Wallis test for non-parametric variables, Abbreviations: ER—Estrogen Receptor; HER2—Human Epidermal Growth Factor Receptor 2; PR—Progesterone Receptor.

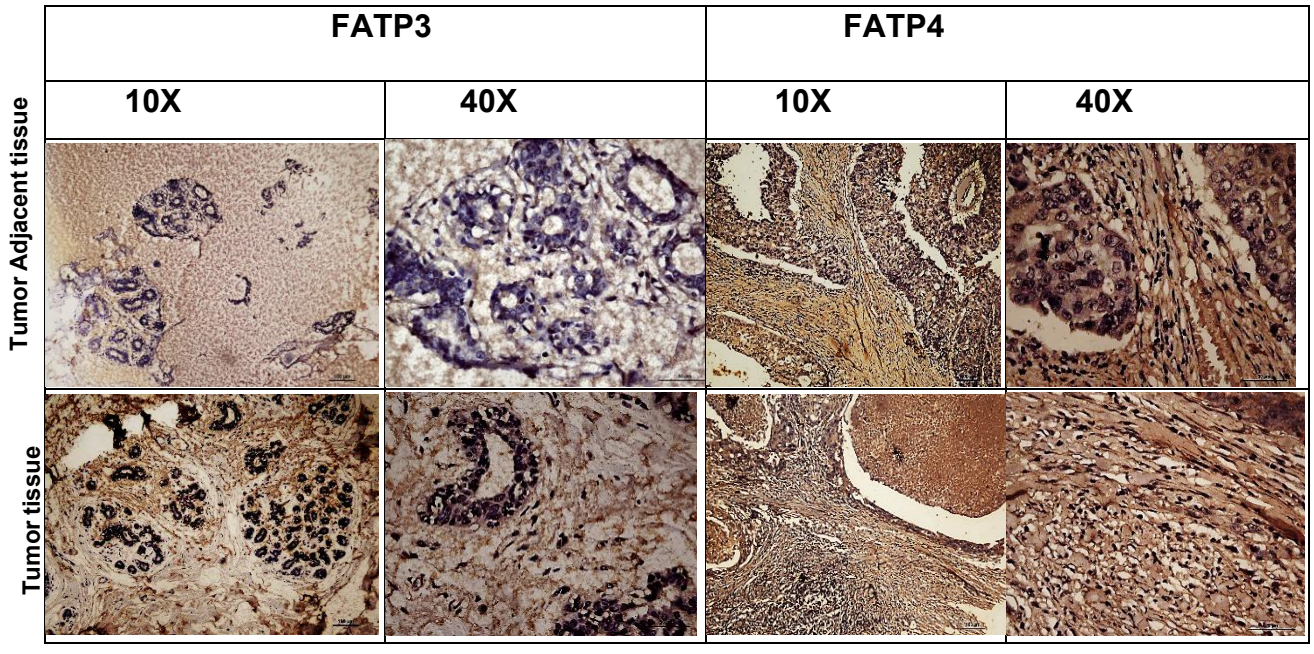
Immunohistochemical Observations of FATPs in BC Subjects:

To determine the expression of FATPs in BC tissues, we performed an immunohistochemical investigation. These proteins were found to be expressed in the cytoplasm, nucleus, or both of these domains in the examined BC tissue (Figure 3). Tumor tissues exhibited high FATPs expression.

(A)


FATP1
FATP2
10X
40X
10X
40X
Tumor Adjacent tissue
Tumor tissue

(B)


FATP3
FATP4
10X
40X
10X
40X
Tumor Adjacent tissue
Tumor tissue

(C)

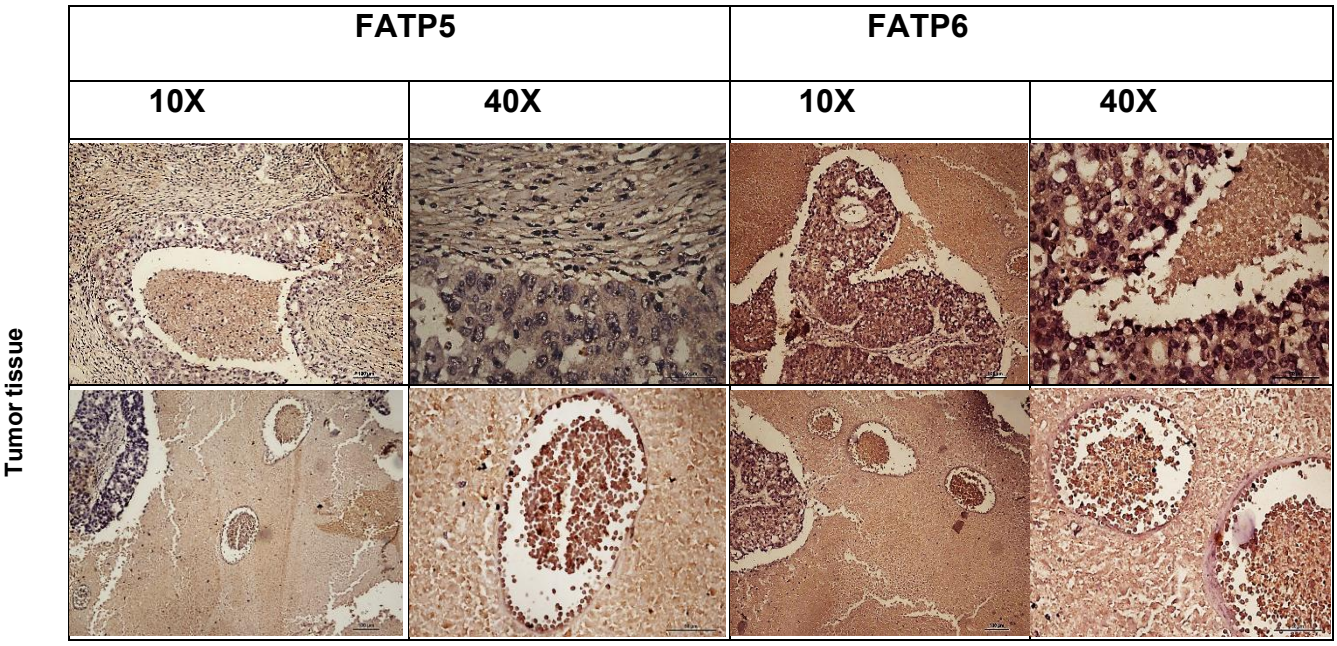


Figure 3: Representative images for Immunohistochemical detection of FATPs from tissue sections of breast cancer. Brown color indicates the expression (Scale: 10X-100µm, 40X-50µm)

Association of relative mRNA expression of FATPs with the percentage of serum LCFAs in Breast cancer subjects:

Relative mRNA expression of FATPs (FATP1-FATP6) were significantly associated with the percentage of serum long-chain fatty acids (LCFAs) in BC subjects **[Table 7]**. Significant association was found between Relative mRNA expression of FATP1/ *SLC27A1* and FATP2/ *SLC27A2* with the percentage of palmitic acid, EPA, DGLA, lino-elaidic acid and the omega-6/3 ratio in BC subjects. In addition to this, FATP3/ *SLC27A3* and FATP5/ *SLC27A5* showed significant association with myristic acid, palmitic, stearic, linoleic, DGLA and GLA. Furthermore, FATP4/ *SLC27A4* and FATP6/ *SLC27A6* showed significant association with stearic acid, palmitic acid, myristoleic acid, DGLA, GLA palmitoleic acid, and Linoelaidic acid. Conversely, FATP1/ *SLC27A1* and FATP2/ *SLC27A2* mRNA levels with palmitoleic acid, myristoleic acid, oleic acid, ALA and LA did not show significant association, FATP3/ *SLC27A3* and FATP6/ *SLC27A6* didn't show significant association with EPA, Linoelaidic acid and Omega Ratio. FATP4 and FATP5 have not showed any significant association with ALA in BC subjects.

Table 7: Association of the fatty acid transport proteins (FATP1-6) Relative mRNA expression with the percentage of serum LCFAs in BC subjects

Variables	FATP1		FATP2		FATP3		FATP4		FATP5		FATP6	
Saturated fatty acids (SFA's)	**Low(n=14)**	**High(n=54)**	**Low(n=15)**	**High(n=53)**	**Low(n=5)**	**High(n=63)**	**Low(n=31)**	**High(n=37)**	**Low(n=12)**	**High(n=56)**	**Low(n=27)**	**High(n=41)**
Myristic Acid (C14:0)	2.26 [1.94-2.90]	1.96 [1.61-2.48]	2.25 [1.90-2.90]	2.00[1.65-2.49]	1.90[1.80-2.33]	2.01 [1.66-2.51]	2.09 [1.80-2.44]	1.90 [1.65-2.72]	2.095 [1.90-2.21]	1.98[1.62-2.64]	1.97 [1.70-2.44]	2.01[1.65-2.72]
P value	0.07		**0.02***		**0.004****		0.064		**0.017***		**0.106**	
Palmitic acid (C16:0)	14.84[12.64-17.03]	14.05[11.96-16.1]	14.56[12.85-16.87]	14.05 [11.96-16.76]	15.08[0.44-16.09]	14.05 [12.50-16.87]	14.73[13.09-17.48]	13.50[11.57-15.02]	14.39[12.86-16.09]	14.21[12.02-16.87]	14.73[12.55-17.48]	14.05[11.95-16.15]
P value	**0.04***		**0.013***		**0.003****		**0.039***		**0.034***		**0.05**	
Stearic Acid (C18:0)	5.2[4.19-6.31]	5.25[4.04-5.95]	5.25 [4.20-6.25]	5.23 [4.12-5.95]	4.17[2.05-5.58]	5.26[4.15-6.17]	5.27[4.17-6.25]	5.21[3.73-5.95]	4.1[2.49-5.76]	5.27[4.20-6.25]	4.20[3.24-5.95]	5.27[4.29-6.25]
P value	0.595		0.492		0.124		0.214		**0.025***		**0.05***	
Mono-Unsaturated fatty acids (MUFAs)												
Myristoleic Acid Acid (C14:1)	0.51 [0.38-0.66]	0.49 [0.37-0.68]	0.56 [0.39-0.66]	0.49[0.36-0.67]	0.35[0.33-1.94]	0.49[0.39-0.66]	0.49 [0.39-0.65]	0.47[0.35-0.68]	0.49[0.35-0.57]	0.51[0.39-0.68]	0.56 [0.39-0.65]	0.47[0.36-0.68]
P value	0.210		0.079		**0.004*****		**0.039***		**0.031***		**0.049***	
Palmitolate (C16:1)	1.57 [1.43-1.98]	1.50 [1.20-1.90]	1.40[1.15-1.97]	1.57 [1.23-1.92]	1.81[1.57-3.45]	1.57 [1.20-1.88]	1.46[1.15-1.97]	1.58[1.27-1.98]	1.56[1.23-2.86]	1.58[1.25-1.97]	1.44 [1.14-1.78]	1.63[1.25-1.98]
P value	0.544		0.912		0.258		0.177		0.723		0.05	
Oleic Acid (C18:1)	12.88[12.29-16.10]	12.49[10.89-13.95]	12.43 [10.86-13.90]	12.73 [11.05-14.06]	14.26[11.48-18.05]	12.71 [10.90-13.30]	12.43 [10.90-13.90]	12.75[11.48-14.05]	12.11[10.38-15.9]	12.72[11.19-13.71]	12.43 [11.19-14.26]	12.75 [11.03-13.87]]
P value	0.231		0.739		0.346		0.721		0.791		0.05	
ω3- Fatty acids												
α-Llinolenic acid(C18:3)	0.027[0.012-0.422]	0.023[0.021-0.032]	0.32 [0.01-0.42]	0.23 [0.023-0.032]	0.023[0.017-0.027]	0.032 [0.012-0.42]	0.023[0.023-0.32]	0.032[0.012-0.42]	0.023[0.023-0.032]	0.032[0.012-0.42]	0.023[0.017-0.032]	0.023[0.014-0.422]
P value	0.421		0.093		**0.000*****		0.129		0.086		0.984	
Eicosapentaenoic acid (EPA) (C20:5)	0.41 [0.30-0.48]	0.38 [0.28-0.49]	0.43 [0.30-0.48]	0.37 [0.27-0.49]	0.27 [0.17-0.45]	0.39 [0.28-0.49]	0.47[0.29-0.49]	0.32[0.28-0.50]	0.32[0.27-0.49]	0.41[0.28-0.48]	0.47 [0.28-0.49]	0.32[0.28-0.48]
P value	0.05		**0.024***		0.863		0.05		**0.022****		0.130	

Variables	FATP1		FATP2		FATP3		FATP4		FATP5		FATP6	
ω6- Fatty Acids	**Low(n=14)**	**High(n=54)**	**Low(n=15)**	**High(n=53)**	**Low(n=5)**	**High(n=63)**	**Low(n=31)**	**High(n=37)**	**Low(n=12)**	**High(n=56)**	**Low(n=27)**	**High(n=41)**
Linoleic acid(C18:2)	15.78 [8.82-17.35]	15.63 [11.98-17.0]	16.13 [13.66-17.42]	15.54 [11.98-16.75]	16.15 [6.31-17.52]	15.59 [11.98-17.32]	15.54[11.98-18.01]	15.56[11.98-16.58]	14.52[9.36-18.21]	15.63[11.99-17.22]	1.97 [1.70-2.44]	2.01 [1.65-2.72]
P value	0.401		0.208		**0.018***		0.05		0.43		0.302	
Dihomo-GLA (C20:3)	0.23 [0.19-0.32]	0.21 [0.14-0.26]	0.22 [0.20-0.25]	0.21 [0.14-0.27]	0.17 [0.11-0.56]	0.23 [0.18-0.54]	0.21 [0.14-0.31]	0.21 [0.18-0.25]	016[0.12-0.24]	0.21 [0.19-0.27]	0.22 [0.20-0.37]	0.21[0.14-0.24]
P value	**0.041***		**0.011***		**0.000*****		**0.037***		**0.036***		0.394	
Gamma-Linolenic acid (C18:3)	0.73 [0.62-0.83]	0.8 [0.64-0.98]	0.68 [0.60-0.75]	0.81 [0.66-1.03]	0.81 [0.74-0.88]	0.78 [0.62-0.97]	0.82 [0.68-1.03]	0.75[0.60-0.83]	0.88[0.71-0.96]	0.77 [0.62-0.94]	0.79 [0.64-1.03]	0.79 [0.63-0.86]
P value	**0.048***		**0.013***		**0.015***		0.05		**0.027***		0.08	
Arachidonic acid (C20:4)	5.25 [4.01-5.62]	5.23 [3.24-5.95]	5.22 [3.56-5.56]	5.23 [3.28-5.96]	5.22 [3.55-8.05]	5.23 [3.30-5.95]	5.27 [4.25-5.95]	5.19[2.63-5.75]	5.95[4.36-7.99]	5.22 [3.27-5.54]	5.48 [4.27-6.90]	5.17 [3.06-5.32]
P value	0.958		0.589		**0.041***		0.174		0.05		**0.05***	
Trans- Fatty acids												
Linoelaidic Acid (C18:2)	1.41 [0.39-1.69]	0.71 [0.43-1.48]	0.95 [0.40-1.69]	0.73 [0.43-1.51]	1.51 [0.96-2.28]	0.71 [0.40-1.47]	0.48 [0.40-1.56]	0.95[0.44-1.46]	0.54[0.35-1.32]	0.8 [0.44-1.54]	0.6 [0.44-1.51]	0.78 [0.39-1.51]
P value	0.05		**0.027***		0.863		**0.037***		0.05		0.083	
Omega Ratio												
Omega 6/3 ratio	23.56 [19.14-55.52]	37.86 [21.60-50.60]	24.53 [19.67-43.00]	38.60[21.53-53.46]	38.60[24.04-52.55]	32.23[21.25-51.85]	38.60[22.59-43.83]	30.51[18.47-53.46]	42.7[31.73-55.37]	30.79[19.67-51.40]	32.23[22.56-43.60]	38.60[19.67-55.53]
P value	0.05		**0.03***		0.97		0.094		**0.05***		0.088	

Values are expressed as median (Interquartile range). p value derived from the student t test for FATP mRNA level. P values ≤ 0.05 were considered to indicate statistical significance. ***p<0.001, & p<0.05*.

Correlation between Relative mRNA expression of FATPs and percentage of serum LCFAs in BC subjects

When the relative mRNA expression of FATPs were correlated with the percentage of serum LCFAs in BC subjects, a statistically significant negative correlation was observed between the relative mRNA expression of FATP1, FATP2, FATP3, FATP5 and FATP6 and myristic acid (C14:0), ω3-fatty acids (i.e., α-linolenic acid (C18:2), Eicosapentaenoic acid (EPA) (C20:5), Dihomo GLA (C20:3), and gamma linolenic acid (C18:3). A moderately significant negative correlation was observed between the relative mRNA expression of FATP4 and FATP6 and that of α-linoleic acid (C18:3), and a moderately significant correlation was also observed between FATP4 and Eicosapentaenoic acid (EPA) (C20:5). A moderately significant negative correlation was observed between the relative mRNA expression of FATP2 and FATP6 and linoleic acid (C18:2). FATP4 showed a moderately significant negative correlation with Dihomo GLA (C20:3). A significant positive correlation was detected between the relative mRNA expression of serum FATP1and palmitic acid (C16:0), palmitolate (C14:1), oleic acid (C18:1), linoleic acid (C18:2), Linoelaidic acid (C18:2), and the omega 6/3 ratio. FATP6 relative mRNA expression also showed a moderately significant positive correlation with palmitic acid. The mRNA expression of FATP2 and FATP6 showed a moderately significant positive correlation with palmitic acid (C14:1). In addition, the level of Omega 6/3 ratio showed a moderately significant positive correlation with the relative mRNA expression of FATP4. Linoleic acid (C18:2) was moderately significantly correlated with the relative mRNA expression of FATP6. The results obtained are represented in **Table 8**.

Table 8: Correlation of relative mRNA expression of FATPs with the percentage of serum LCFAs in BC subjects

Variables		*FATP1*	*FATP2*	*FATP3*	*FATP4*	*FATP5*	*FATP6*
Saturated fatty acids (SFA's)							
Myristic Acid (C14:0)	r	-0.480	-0.478	-0.532	-0.282	-0.415	-0.427
	Sig	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **
Palmitic acid (C16:0)	r	0.417	0.325	0.465	0.032	0.518	0.201
	Sig	**<0.001*** **	**<0.001*** **	**<0.001*** **	0.712	**<0.001*** **	**0.019 ***
Mono-Unsaturated fatty acids (MUFAs)							
MyristoleicAcid (C14:1)	r	-0.143	-0.150	-0.165	-0.083	-0.150	-0.089
	Sig	0.097	0.081	**0.05***	0.339	0.081	0.303
Palmitolate (C16:1)	r	0.227	0.244	0.065	0.198	0.091	0.125
	Sig	0.05	**0.04***	0.599	0.106	0.460	0.310
Oleic Acid (C18:1)	r	0 .201	0.105	0.292	0.100	0.319	0.212
	Sig	**0.016 ***	0.195	**0.039***	0.248	**0.024 ***	**0.013 ***
n3- Fatty acids							
α - Llinolenic acid(C18:3)	r	-0.482	-0.402	-0.498	-0.186	-0.375	0.251
	Sig	**<0.001*** **	**<0.001*** **	**<0.001*** **	**0.030***	**<0.001*** **	**<0.01** **
Eicosapentaenoic acid (EPA)(C20:5)	r	-0.631	-0.521	-0.565	-0.271	-0.612	-0.422
	Sig	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.01** **	**<0.001*** **	**<0.001*** **
n6- Fatty acids							
Linoleic acid (C18:2)	r	0.389	0.291	0.362	0.069	0.381	0.190
	Sig	**<0.001*** **	**0.001** **	**<0.001*** **	0.424	**<0.001*** **	**0.027***
Dihomo-GLA (C20:3)	r	-0.711	-0.573	-0.655	-0.277	-0.641	-0.495
	Sig	**<0.001*** **	**<0.001*** **	**<0.001*** **	**0.001** **	**<0.001*** **	**<0.001*** **
Gamma-Linolenic acid (C18:3)	r	-0.433	-0.429	-0.476	-0.315	-0.464	-0.386
	Sig	**0.000** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **
Arachidonic acid (C20:4)	r	0.109	0.099	0.113	0.083	0.186	0.098
	Sig	0.206	0.252	0.190	0.336	**0.030***	0.257
Trans-Fatty acid							
Lino-elaidic Acid(C18:2)	r	0.674	0.556	0.632	0.247	0.615	0.416
	Sig	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.01** **	**<0.001*** **	**<0.001*** **
Omega Ratio							
Omega 6/3 ratio	r	0.674	0.556	0.632	0.247	0.615	0.416
	Sig	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.01** **	**<0.001*** **	**<0.001*** **

Spearman correlation (r) was done for all non-parametric variables. p<0.05 was considered significant. P<0.001*, p<0.01**, p<0.05*.**

Risk estimates of breast cancer based on the relative mRNA expression of FATPs in BC subjects:

We assessed the risk estimates of BC associated with the relative mRNA expression of FATPs in our study cohort. We found that elevated relative mRNA expression of FATPs were significantly associated with an increased risk of breast cancer (FATP1: OR = 7.714, 95% CI ,(3.785-15.722); FATP2: OR = 3.786, 95% CI (2.334-6.140); FATP3: OR = 10.500, 95% CI (4.87-22.60); FATP4: OR = 1.321,95% CI (0.924-1.890);FATP5: OR = 3.786,95% CI (02.334-6.140); FATP6: OR= 1.952, 95% CI, (1.303-2.926); Whereas lower relative mRNA expression of FATPs were associated reduced risk of breast cancer. Results are shown in **Table 9.** The results obtained were statistically significant.

Table 9: Risk estimates of breast cancer based on the relative mRNA expression of fatty acid transport proteins in BC subjects:

FATPs		Control (n=68)	Case (n=68)	Odds Ratio (95% CI)	P-value
FATP1	Low	61	14	0.230 (0.143-0.369)	<0.001***
	High	7	54	7.714(3.785-5.722)	
FATP2	Low	54	15	0.278(0.175-0.441)	<0.001***
	High	14	53	3.786(2.334-6.140)	
FATP3	Low	62	5	0.810(0.035-0.188)	<0.001***
	High	6	63	10.500(4.87-22.60)	
FATP4	Low	40	31	0.775(0.559-1.075)	0.05*
	High	28	37	1.321(0.924-1.890)	
FATP5	Low	55	12	0.218(0.129-0.369)	<0.001***
	High	13	56	4.308(2.610-7.110)	
FATP6	Low	47	27	0.574(0.412-0.802)	<0.001***
	High	21	41	1.952(1.303-2.926)	

P values ≤ 0.05 were considered to indicate statistical significance. The cutoff value of each FATPs were determined by the ROC curve with an area under the curve (FATP1=0.968, FATP2=0.894, FATP3=0.949, FATP4:0.684, FATP5=0.958, FATP6=0.812; p<0.001*, considered to indicate significance. The obtained cutoff value of FATP1 is 2.3, FATP2 is 1.99, FATP3 is 1.8, FATP4 is 1.1, FATP5 is 1.6 and FATP6 is 1.8 respectively, which was further divided into lower and higher value groups, p-value derived from the chi-square test for FATP relative mRNA expression.**

Discussion:

FATP proteins are a set of six integral membrane proteins (FATPs) that differ in tissue expression and intracellular localization [43,44]. The varied expression and location of FATPs emphasizes their role in maintaining lipid homeostasis in different tissues. It has been observed that the patterns of FATPs expression in BC differ from those in normal breast tissue. Research has demonstrated that specific FATPs, such FATP1 and FATP4, are elevated in BC cells, which may have an impact on the development and progression of the tumor [8]. Furthermore, these dysregulated FATPs are linked to alterations in fatty acid metabolism and lipid droplet production, both of which are essential for cancer cell survival and development [8]. As a result, we hypothesized that knowing the role of FATPs in BC might offer valuable insights into the progress of targeted therapies for this disease. In this context, the present study aimed to determine the serum levels of LCFAs, protein expression of FATPs and its relative mRNA expression in subjects with BC and to find correlation and association between serum LCFA levels and FATPs mRNA in women with BC. According to epidemiological research, anthropometric parameters and the risk of BC have a complicated relationship. Significant variation in BMI between the case and control groups in the current study. This result is consistent with prior study [45]. Tumor cell growth and proliferation depend on the intricate modifications in carbohydrate, protein, and lipid metabolism. The metabolism, regulation, and modification of lipids influence cell division, apoptosis in both healthy and malignant cells [46]. It is interesting to note the significance of SFAs and MUFAs in the synthesis of lipids. Our study showed, significantly higher levels of Saturated FAs such as myristic acid (C14:0), palmitic acid (C16:0); in case subjects compared to control subjects. These FAs are known to play important role in the progression of BC, suggesting a potential link between elevated SFAs levels and increased risk of BC [47]. Additionally, case subjects also showed higher level of Mono unsaturated fatty acids, specifically oleic acid, which has been connected with cell proliferation and metastasis in BC cells [48]. These results suggest an imbalance in these FAs level might contribute to the pathogenesis of BC. Along with this omega 3 FAs such as ALA and EPA have been shown to exhibit strong anticancer effects [49]. Our results showed lower levels of these FAs in case subjects compared to control, these findings are consistent with existing research [46]. Findings of this study indicate that insufficiency of omega-3 fatty acids might also play crucial role in the BC progression. Earlier research has revealed that supplementation of Omega 3 FAs can hinder tumor growth in BC, indicating their potential as a therapeutic intervention in BC [50]. Omega 6 FAs, LA, DGLA, GLA, and omega 6/3 ratio varied significantly between groups. These findings imply that the progression of BC might be significantly influenced by the ratio of omega 6 to

omega 3 fatty acids. Specifically, the case group had significantly higher Ratio of omega6/omega3 FAs ratio than the control group. These results support the possible contribution of omega6 FAs in developing BC. These results are consistent with the previous findings that found a significant association of higher omega6 ratio and a greater risk of BC [51,52]. Imbalance between these FAs may lead to chronic inflammation, which further contributes to cancer. It is important for individuals to maintain a balanced rich in omega 3 FAs, such as those found in fatty fish, simultaneously minimizing the intake of omega6 FAs, which are usually found in the processed foods. This can reduce the risk of BC and improve overall health [53-55]. Furthermore, we evaluated the differences in FAs profiles in relation to BC receptor status. Here we found that PA, MA, OA, LA levels differed significantly across BC subtypes. Comparison of the FATPs mRNA levels in individuals with BC and control was our primary focus of the study. This was crucial for understanding the possible function of these proteins in the development of BC. Studies on the involvement of FATP1& 4 in BC tumor tissues are being investigated; however, studies on other FATPs ,have focused only on their involvement in other cancers, except BC [8]. Hence it is crucial to explore the precise role of FATPs in the development and progression of BC. Understanding the contribution of these proteins in BC could provide valuable insights for the development of targeted therapies. While comparing the mRNA level of FATPs between the BC receptor status, we found significant difference in the level of FATP 1-3 &FATP5 among the receptor status of BC. Further analysis showed increase in the mRNA level of FATP1,4&6 in TNBC subjects, Whereas, FATP2,3&5 level was high in ER+ BC. Our results were consistent with previous results [16]. These results suggest that different BC subtypes might have distinct metabolic signatures and lipid uptake mechanisms. Differences in the pattern of FATPs gene expression among the receptor status in BC suggests potential implications for targeted therapy. For instance, Targeting FATP1 in ER-ve BC could disrupt the lipid uptake and hinder the tumor growth. Further studies are required understand the metabolic patterns of different BC subtypes to provide novel biomarkers, that can be used for early detection and prognosis of BC. Current study showed increased level of relative mRNA expression of FATP 1-6 in the case subjects compared to control, indicating a potential connection to BC. In order to support this data, we employed IHC analysis to determine the expression of FATPs in BC Tissues. The findings of the study demonstrated that, BC tissues had higher FATP1-6 expression compared to tumor adjacent tissue. The noted rise in FATP expression confirmed the role for FATPs in BC. Our findings supported the earlier findings, which reported elevated expression of these proteins in different cancer subtypes including BC [8]. Upregulation of these proteins in in cancer cells may be connected to their higher demand for FAs to support rapid proliferation. Further, we evaluated the association between the LCFAs and FATP gene expression. These results allowed us to

understand the complex interaction between the protein and the LCFAs in BC. These results indicate a potential regulatory mechanism of FATPs in transporting the FAs and metabolism of FAs in BC, also highlighting their importance as biomarkers and therapeutic targets in BC [56]. Simultaneously we correlated the FATPs gene expression with LCFAs in BC subjects. There was a significant positive correlation among the levels of FATP1-6 and the levels of SFAs, including myristic and palmitic acid, these findings imply that the regulation of LCFAs may be highly influenced by the FATPs expression in BC. Along with this, MUFAs such as palmitoleic and OA also demonstrated substantial positive correlation with FATPs. This indicates that, BC cells are Likely to use both SFAs and MUFAs trough the involvement of FATPs. These findings emphasize the importants of understanding the accurate role of different fatty acid types and their interaction with FATPs in BC. Additionally, omega ratio and trans fatty acids showed significant positive corelation with FATPs, these results imply that a comprehensive analysis of these fatty acids are required for elucidating their impact on the development of BC. While correlating omega6 FAs with FATPs, we obtained significant positive correlation between LA and FATP, indicating higher level of these FAs are associated with the increase expression of FATPs in BC. Furthermore, potential impact of the trans-FAs as must be important to consider, as they have been associated with the adverse health effects in previous research [54]. Most importantly, omega 3 fatty acids such as ALA and EPA showed significant negative correlations with FATPs, suggesting that they may have a protective effect against BC development and this result also indicates that lower levels of omega-3 fatty acids are related with higher expression of FATPs in BC. Further research should be conducted to explore the potential benefits of omega-3 fatty acids in preventing or treating BC. Additionally, it would be valuable to investigate the mechanisms through which these fatty acids interact with FATPs and their impact on tumor growth and progression. However, precise mechanisms behind the role of each FATP in BC are not fully understood, but few studies reported the involvement of FATP2 in cancer progression via PI3K/ Akt /mTOR pathway [57]. Our prior publication also reported that FATPs might promote the BC by enhancing the PI3K pathway [58]. Our study also examined the association between the relative mRNA expression levels of serum FATPs and the risk of BC. We used ROC curve analysis to identify the cutoff values for FATPs expression levels, and our findings indicate that elevated expression levels of FATPs are significantly associated with an increased risk of BC. Overall, in our study, we observed increased expression of FATPs along with the increased level of palmitic acid, linoleic acid, arachidonic acid, and the omega 6/3 ratio in case compared to control subjects. Conversely, lower levels of α-linolenic acid (ALA) and Eicosapentaenoic acid (EPA) were observed in the case subjects. This observation speculates a potential link between FATPs and LCFAs in BC women. This also suggests that, increase in fatty acid level could lead

to increased uptake of these LCFAs by FATPs, which further leads to elevation of FATPs mRNA level in the case subjects. Whereas, Lower levels of ALA & EPA in BC women indicates an imbalance in the level of LCFAs causing an increase in the above-mentioned LCFAs, this might also contribute in the elevation of FATPs. Increased level of FATPs might be due to dysregulated fatty acid metabolism in BC and the increased supply of LCFAs via fatty acid transporters (FATPs) to meet the energy demand required for the cancer cell. These findings suggest that the balance between SFA (PA), MUFA (OA), omega-6 (LA, AA) and omega-3 (ALA, EPA) fatty acids may be an important factor in the prevention of BC, this might also maintain the expression of FATPs in BC. These findings highlight the differential role of various LCFAs in BC metabolism and the critical function of FATPs in mediating their effects. Our results reveal substantial relationship between LCFAs and FATP expression, suggesting that these biomolecules may play important role in the BC pathogenesis. This novel understanding may pave the way for new therapeutic targets and diagnostic markers in BC. However, statistical strength of our analysis may have been limited by comparatively small number of cases and unequal distribution across molecular subtypes, grades and stages. Which may have impacted the ability to draw conclusions specifically for subtype specific observations.

Conclusion

Our findings provide novel perspectives into the role of FATPs in BC risk, specifically in relation to pathological subtypes. The observed significant variations in the FATPs among different BC subtypes might help to identify possible biomarkers for the early detection and monitoring BC progression. Our results implies that upregulation of FATPs play key role in BC pathogenesis. Study also emphasized the complex interplay between fatty acids and FATPs in BC pathogenesis. The level of FATPs among BC subtypes such as TNBC highlights the importance of these proteins as therapeutic targets. This study is notable because understanding the relationship between FATPs and serum LCFAs in BC patients could provide insights into the development of new therapeutic strategies. Our findings highlight that understanding relationship between LCFAs and FATPs might provide valuable insights into potential therapeutic strategies targeting FATP or altering LCFAs level to decrease the risk of BC. Further investigations are needed to elucidate the specific mechanisms by which FATPs contribute to BC development and progression and also validating these findings in lager cohorts by Targeting FATPs or the pathways regulated by them, could lead to novel therapeutic approaches in BC.

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AK, RPG- Formal Analysis, writing original draft
KPHL- Data curation and validation of pathological slides with Breast cancer
ASS, SSS and SKN- Interpretation of the manuscript, Final approval of the manuscript

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Data availability
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