ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Association Of Alpha 1 Antitrypsin And Fatty Acid Desaturase Activity In Women With Breast Cancer: A Comparative Study.

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ABSTRACT

The relationship between plasma fatty desaturase activity and inflammatory markers is unclear. We investigated the association of plasma fatty acid desaturase activity with Alpha 1 antitrypsin(A1AT) in breast cancer women. 60 breast cancer women and 60 control women were recruited for the study. A1AT was measured via Roche autoanalyzer and fatty acids by gas chromatography. The student t-test was used to compare differences and Spearman correlation was used to measure the linear correlation between the variables. A1At was higher in breast cancer cases than the control group (P=0.03) where 18% of the breast cancer women had A1AT values above the upper reference range. Delta 5 desaturase and delta 9 desaturase activities were higher in cancer cases. A1AT was positively correlated with delta 6 desaturase activity in cancer cases (P=0.05) whereas, A1AT was negatively correlated in control group. A high level of n-6 fatty acids seems to be a contributor to the inflammatory profile. Plasma fatty acids depend on the diet intake and data suggest a possible association of A1AT and the type of fatty acids in inflammation-related breast cancer.

Keywords: A1AT, breast cancer, fatty acids

https://doi.org/10.33887/rjpbcs/2019.10.5.7

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ISSN: 0975-8585

INTRODUCTION

Breast cancer is a major cause of death among women all over the world. Breast cancer development, invasion, and metastasis are promoted by inflammation and proteolytic enzymes are shown to have a significant role in carcinogenesis. Lifestyle and nutrition influence inflammation.

A1AT is a glycoprotein and an inhibitor of serine proteases. It is a 52 kDa anti-inflammatory glycoprotein exerts its result on immune allied pathologies[1]. Increase in the serum level of A1AT has been documented in malignant diseases, including gastrointestinal cancer [2], lung cancer[3], biliary tract cancer [4], pancreatic adenocarcinoma [5] and breast cancer[6].

Delta 5 desaturase (D5D) and delta 6 desaturases (D6D) are the rate-limiting steps in the conversion of linoleic acid (LA) and gamma-linolenic acid (GLA) into long chain n-6 and n-3 polyunsaturated fatty acids. The delta 9 desaturase (D9D) converts saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA). Desaturase enzymes are overexpressed in cancer cells and inhibition of these enzymes are of interest [7]. Lipid classes and the molecular species studies support tumor growth and metastatic dissemination envisioning new therapeutic targets and improve apoptosis signaling [8].

Depending on the interaction with lipid moieties, the biological actions of A1AT can be modified. Binding of A1AT with polyunsaturated fatty acids like Alpha-linolenic acid and oleic acid was recently reported. The expression is increased in the bound form of A1AT and release of Angiopoietin-like protein 4 (Angptl4) which targets the gene of PPAR-γ. Fatty acid (FA) regulates the Angptl4 depending on the type of fatty acid binds to it [9]. Our study aimed at finding the relationship between acute phase protein A1AT and plasma fatty acid desaturase activity and its association with breast cancer.

MATERIALS AND METHODS

Subjects for this observational study were recruited from K S Hegde Charitable Hospital, Deralakatte, Mangaluru. After getting approval from Institutional ethics board of NITTE deemed University, participants within the age group 25-60 years were enrolled for the study by taking their written informed consent. The study was conducted between July 2017 and December 2018. A non-fasting blood sample was collected based on the inclusion criteria from all the recruited subjects. Samples were immediately separated after blood draw and stored as plasma aliquots at -20°C.

Selection of breast cancer cases and controls

60 women who had been diagnosed with breast cancer and proven with the pathology reports were included in the study. Subjects those who have undergone breast implants, concurrent malignancy, and chronic use of omega fatty acids was excluded. We selected 60 control participants among the women who attended for health check-up without previous history of any tumors and will provide a blood sample.

Laboratory analysis

Alpha-1 antitrypsin was measured in Cobas ® e411(Roche Diagnostics GmbH, Mannheim, Germany) by the immunoturbidimetric method. The concentration of A1AT was expressed as mg/dl (Reference values 90-200 mg/dl). Appropriate quality control and calibrators were used for the assay.

Fatty acids composition in plasma was estimated as their corresponding fatty acid methyl esters. Preparation of fatty acid methyl esters (FAMES) was carried out according to the protocol of Metcalfe et al [10]. FAMEs were analyzed on a 7820A Agilent gas chromatography-flame ionization detector (FID system) equipped with Agilent J&W DB-23 column. Based on the peak retention time of the FAMES of the known standard fatty acids individual fatty acids were measured. Individual plasma fatty acids are expressed as a percentage of total fatty acids present. Triheptadecanoin (C:17) was used as an internal standard.

Calculation of desaturase activity:

D5D activity=Arachidonic acid/Dihomo gamma-linolenic acid

ISSN: 0975-8585

D6D activity = Gamma-linolenic acid/ Linoleic acid
D9D-18= Oleic acid/Stearic acid
D9D-16 = Palmitoleic acid/Palmitic acid

Statistics

In this observational comparative study, the results obtained are analyzed and expressed as the mean±SD. The student t-test was used to compare differences between the two groups. Spearman correlation was used to measure the linear correlation between the variables. All P values were 2 sided at a significance of P <0.05. The data were fared using Excel 2010 (Microsoft Corp., Redmond, WA, U.S.A.) and SPSS software (SPSS V.16.0; IBM Corp) was used to analyze all the outcome data.

RESULTS

The mean value of the protein alpha-1 antitrypsin levels was significantly higher in cancer cases and control women well-understood in figure 1 (P=0.035). Breast cancer women had A1AT levels between 105-265mg/dl and the A1AT levels in control women were between 98-180mg/dl. 18% of the breast cancer women (11 of 60) had A1AT levels above the upper reference range (>200mg/dl).

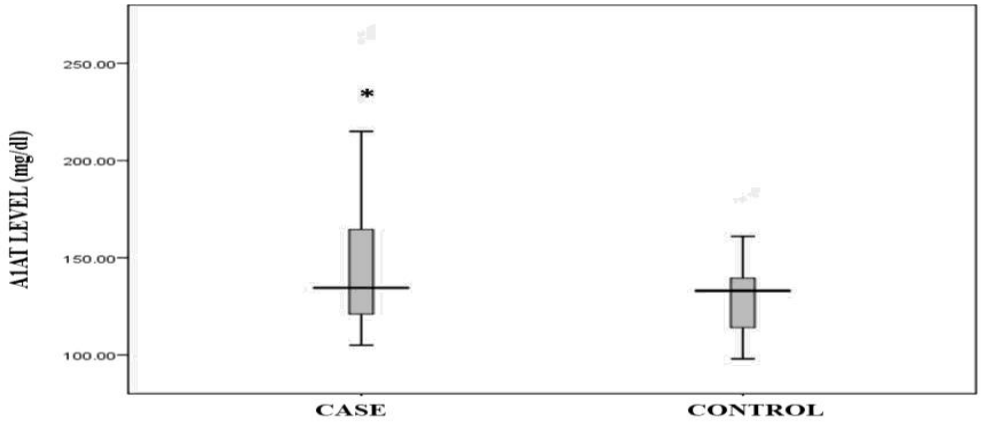

Figure 1: Boxplot showing A1AT levels (mg/dl) in case and control subjects. Results shown are median with percentiles. Statistical significance is indicated as *P<0.05.

Figure 2 shows delta desaturase activity in breast cancer women and control women. D5D and D9D-18 activity were higher in cancer cases whereas control subjects had higher D6D and D9D-16 desaturase activity. No statistically significant difference was observed in both groups.

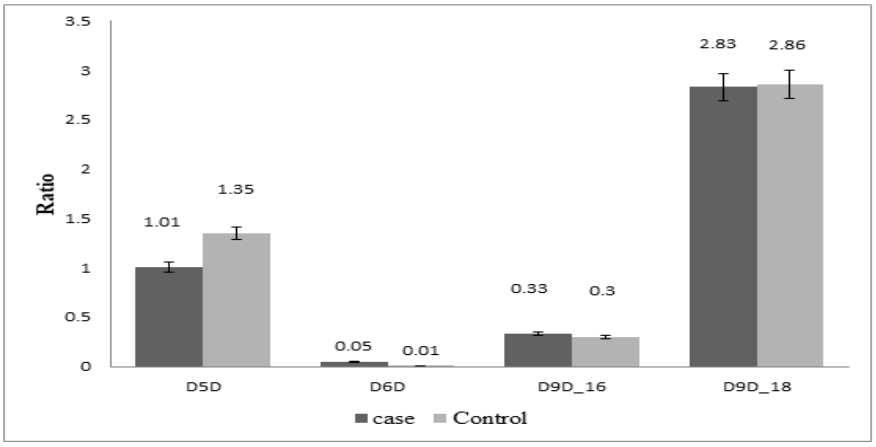

Figure 2: Delta desaturase activity in breast cancer and control women. Delta desaturase activity is expressed as the ratio. (n=60 breast cancer women;n=60 control women)

Correlation between A1AT and delta desaturase activity were calculated (table 1). Among the case group, A1AT was inversely related to the ratios of D5D and D9D resulting from its desaturation and elongation; levels of A1AT was positively correlated with D6D activity with a P value of 0.05. No statistically significant correlation was observed among the control group but, A1AT was inversely correlated with all the delta desaturases.

Table1: Correlation between plasma A1AT levels and delta desaturase activities in case and control groups

		D5D	D6D	D9D-18	D9D-16
Case (n=60)	A1AT	-0.215	0.376	-0.177	-0.107
	P value	0.281	0.05*	0.36	0.588
Control(n=60)	A1AT	-0.201	-0.148	-0.027	-0.131
	P value	0.315	0.470	0.893	0.514

With this sample size, *P≤0.05 is statistically significant using spearman coefficient correlation

DISCUSSION

Elevated serum/plasma proteins are reliable observation associated with different types of tumors [3,4,5,6]There are limited data on the association between plasma fatty acids and inflammatory status. In this comparative study, we have evaluated the association between plasma inflammatory marker and plasma fatty desaturases. In our study, mean A1AT levels were higher in breast cancer cases than the control subjects. Our findings agree with the other investigators who have reported increased A1AT in breast cancer [11]. Huang H et al [14] reported increased levels of A1AT with early stages and advanced stages of breast cancer.

The increase in the protein is due to systemic inflammation and the exact mechanism of effect on tumor progression is still unclear. Few studies have documented the association of A1AT and TGF-β which regulates differentiation, development, and homeostasis in all tissues [15]. Limited angiogenesis studies have shown increased VEGF expression is caused by A1AT [16].

Neutrophil-released elastase (NE) and proteinase 3 (PR3) is rapidly inhibited by A1AT is well-understood function. A1AT accelerates the parallel inhibition of NE and PR3 inside and outside the cell thereby confirming its role in controlling and resolution of inflammation. A1AT has been shown to have independent anti-inflammatory/immunomodulatory properties which might be helping tumor cells from apoptosis [17]. A small cohort study on nipple aspiration fluids conducted by Zhou et al discovered C-terminal peptide of A1AT as a biomarker for breast cancer [18].

Our data indicate that D5D and D9D activities were higher in breast cancer women. Lipid modification caused by increased fatty acid desaturation is an important process in cancer cells. Cellular content of monounsaturated fatty acids (MUFA) will be increased with the enhanced D5D activity. Increased D6D activity shows increased production pro-inflammatory n-6 fatty acids. The increased conversion of precursor fatty acids to products, associated with increased desaturase activity, is a common feature of tumors [19].

The underlying mechanism could be the competition between n-3 and n-6 long chain fatty acids for eicosanoid production. This increased ratio of fatty acids could be involved in the breast cancer carcinogenesis by varying the homeostasis of eicosanoids leading to the production by the n-6 derived pro-inflammatory 2-series prostaglandins and 4-series leukotriene [20,21]. The precursor fatty acids of n-3 and n-6 fatty acids are essential fatty acids, where diet also plays an important role in desaturase activity.

To our knowledge, it is the first time to show the association of delta desaturase activity and A1AT in breast cancer women . A1AT binding with other biomolecules exerts many biological functions. Pulldown assays confirmed that A1AT, like human serum albumin, binds with unsaturated FAs, linoleic and oleic acid,

and that only FA-bound forms of A1AT induce the expression of Angiopoietin-like protein 4 (Angptl4) [22]. Fatty acids are recognized as the best inducers of Angptl4 [23] which targets PPAR-γ [9]. Angptl4 plays an important role in lipid metabolism and homeostasis [22].

Up-regulation of angptl4 by FA bounded A1AT suggests the role in tumor regulation and requires further research. Decreasing the pro-inflammatory n-6 fatty acids and increasing anti-inflammatory n-3 fatty acids could be an approach for altering the desaturase activity, inflammatory response, and associated tumor growth**.**

Variation in the A1AT and delta desaturases may provide new insights for diagnostic and therapeutic strategies for breast cancer women and help women from cancer. So further Research with larger sample size is required for the anti-inflammatory/immuno-modulatory activity of A1AT and fatty acids. The current study supports the hypothesis that omega fatty acids may contribute to tumor development and progression by aggravating the underlying inflammatory processes. Potentials of dietary fatty acids to reduce or increase alpha-1 antitrypsin levels warrant further investigation.A1AT levels and delta desaturase activities based on the types of breast cancer was not evaluated. This can be evaluated in a large sample size on different age groups and molecular subtypes.

CONCLUSION

Effect of A1AT (stimulating/inhibiting) depends on the molecular form A1AT along with a binding partner. The mechanism of action still remains unclear. The preliminary findings suggest that A1AT and omega fatty acids can be used for the management of breast cancer and point en route for comprehensive investigations into the interactions between the concentration and function of A1AT and desaturase activity.

ACKNOWLEDGMENT

We would like to thank Dr. Jayaram Shetty, Radiooncologist and Dr. Vijith Shetty for helping in recruiting the participants for the study.

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