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Essential Fatty Acids - A Biomarker and Prognostic Factor for Breast Cancer Induction

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ABSTRACT

The mechanisms underlying the formation of different types of cancers and the different patterns of a single type of cancer are of clinical importance in diagnosing the disease and also for therapy. In epidemiological reports the intake of fatty diet has been ascribed to be an aetiological agent for cancer development in general and very specifically to certain types of carcinomas like breast (Mammary) adenocarcinoma. However, only a few studies have been made to contemplate the compositional differences in the fatty acids, towards cancer prognosis. Recently it has been substantiate that different fatty acids play a role either in enhancing or suppressing cancer formation. In the light of the above studies, it is delineated and construed that there exists a correlation between qualitative variation of essential fatty acids and the cancer manifestation. In the present study the fatty acid composition of breast cancer was analyzed by gas chromatography to substantiate their role in carcinogenesis. Metastatic breast cancer tissues were analyzed for the compositional difference in their fatty acids by gas chromatography. The causative roles of nB6 type essential fatty acids are discussed in the light of previous reports and evidences.

***Keywords**: Cancer, Metastatic breast cancer, Adenocarcinoma, Essential fatty acids*

INTRODUCTION

Various risk factors have been attributed to correlate the incidence of breast cancer among women. In India more than 2.5 million cases of cancer have been estimated and it is presumed to be one of the ten leading causes of death. More than 3 lakh deaths occur annually due to cancer. The national cancer registry programme revealed, out of the population based data that the leading types of cancer are the oral cavity, lung, oesophages and colonic among men while cervical and breast predominantly in women. These account for 50% of all cancer deaths in India. Cancer initiation, promotion and progression through metastasis is not only caused by the differential functioning of both the oncogenes and tumour suppressor genes but also by the consistent changes in the biochemical constituents of the cells which have undergone oncogenic transformation. Several enzymes were identified as diagnostic markers of cancer cells. e.g., alkaline phosphatase, Alanine amino transferase (ALT) / Glutamate pyruvate transaminase (GPT), Aspertate amino transferase (AST) / Glutamate oxaloacetate transaminase (GOT) (Transaminases) Lactate dehydrogenase etc.

In epidemiological reports the intake of fatty diet has been ascribed to be an etiological agent for cancer development in general and very specifically to certain types of carcinomas like breast (Mammary) adenocarcinoma. Recently it has been construed that different fatty acids play a role either in enhancing or suppressing cancer formation[5,10,11.]

In the light of the above studies, it is to be construed that there exists prognostic a link to the qualitative variation of essential fatty acids and the cancer manifestation. In the present study the fatty acids composition of breast cancer was analyzed by gas chromatography to substantiate their role in carcinogenesis. Metastatic breast cancer tissues were analyzed for the compositional difference in their fatty acids by gas chromatography. Since the control (Normal) breast tissue biopsy from healthy human subjects could not be carried out the diseased patients breast tissue composition of fatty acids was analysed and the probable role of certain types/ categories of fatty acids to attribute their etiological contribution to malignancy was analysed and discussed. The results are presented and the causative roles of nB6 type essential fatty acids are discussed in the light of previous reports and evidences. In addition the epidemiological data on the diet of cancer patients (vegetarian vs non-vegetarian) were also presented.

MATERIALS AND METHODS

The essential fatty acids in the breast cancer tissue samples (10nos) were analysed through gas liquid chromatography as described by previous authors[5]. One gram of breast cancer tissue was taken in a 250 ml round-bottomed flask with a round-glass neck fitted with a reflex condenser. 40 ml of anhydrous methanol R was added. Attached shook and heated the reflux condenser to boiling. When the solution was clear (usually after about 10 minutes of heating), was continued heating for a further 5 minutes. Methyl orange indicator followed by 2 % concentrated sulphuric acid in methanol was added to neutralize the reaction. The flask was cooled under running water and transferred the contents to a separating funnel. The flask was rinsed with 50 ml of Petroleum ether shooked and transferred to the separating funnel. The organic layer was passed through anhydrous sodium sulphate. The petroleum ether layer was evaporated and with sufficient acetone injected 1µl into chromatograph. The chromatogram consists of CHEMITO GC 8610 Flame ionization Detector, carrier gas as nitrogen and hydrogen, oxygen for ignition purpose. Column BPX-70 (50% cyanopropyl, 50% methylsiloxane)- injection port 250°, detector port 260°. Oven starting temperature 160° and increase by 7.5° per minute the final oven temperature is 240°. The data collection is done with winchrom software.

RESULTS

The malignant breast cancer tissues were analyzed for various essential fatty acid components. (Table 1-2 and Figures 1-10) The analysis showed about eleven different fatty acids namely: 1. Palmitic acid, 2. Steric acid, 3. Oleic acid, 4. Linoleic acid, 5. Linolenic acid, 6. Arachidic acid, 7. Behanic acid, 8. Lignoceric acid, 9. Myristic acid, 10. Lauric acid and 11. Richinoleic acid. Among these the three fatty acids namely palmitic acid ranged from 9.3528 to 29.0058 % with an average of 22.09 and ± 6.635, oleic acid ranged from 35.0152 to 44.1705% with an average of 39.56 and ± 4.699 and linoleic acid ranged from 15.8027 to 42.5028 % with an average of 24.53 and ± 10.01 found in higher percentages and other fatty acids steric acid (ave.5.03, ± 1.79), linolenic acid (ave 0.156, ± 0.072), arachidic acid (ave 0.738, ± 0.498), myristic acid (ave. 2.13 , ± 1.535), lauric acid (ave. 0.33, ± 0.414) and behanic acid (ave. 1.36, ± 1.28) recorded to be in low percentages. Among the eleven fatty acids found, richinoleic acid is seen only in one sample with 0.525 %.

Table : 1 Percentage of Fatty Acid Present in Breast Cancer Tissues

Name of the fatty acid	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
Palmitic acid	14.6222	9.3528	28.4704	29.0058	16.2523	23.2414	23.3963	23.7971	23.976	28.8806
Steric acid	3.3929	6.1902	3.9969	9.349	4.9883	5.0769	4.2048	3.2804	4.0435	5.7613
Oleic acid	37.2689	40.2682	43.0648	28.8201	35.0152	40.7414	41.3609	44.1705	43.4859	41.4076
Linoleic acid	37.1002	42.5028	20.3052	16.8025	35.9096	22.983	19.9392	16.3665	17.6288	15.8027
Linolenic acid	0.1789	0.2865	0.1185	-	0.1904	0.0759	0.155	-	0.091	-
Arachidic acid	1.7643	0.6793	0.9087	0.488	0.2241	-	0.6332	-	-	0.4749
Behanic acid	3.7384	0.1706	1.468	2.3575	0.6033	-	0.6169	-	-	0.5346
Lignoceric acid	0.9789	0.1016	0.5741	1.0528	-	-	0.0369	-	-	0.1952
Myristic acid	-	0.0111	0.5573	4.4099	1.595	4.0973		1.8679	2.4652	2.0684
Lauric acid	-	-	0.0874	0.9508	0.1228	-	0.1673	-	-	-
Richinoleic acid	-	-	-	0.525	-	-	-	-	-	-

Figure 1 -10: Gas Chromatographic Patterns of Breast Cancer Tissue Samples

Figure - 1

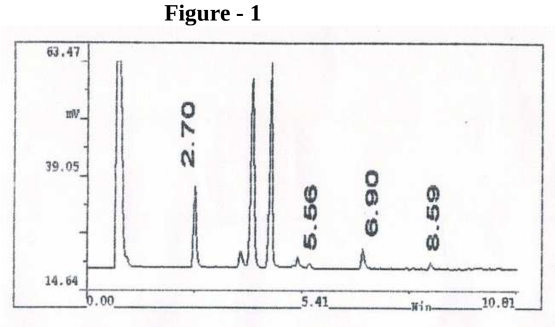


Figure - 2

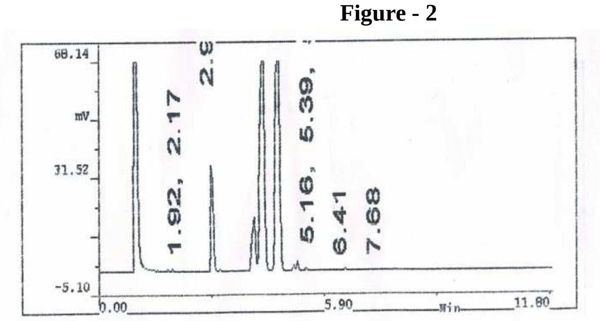


Figure - 3

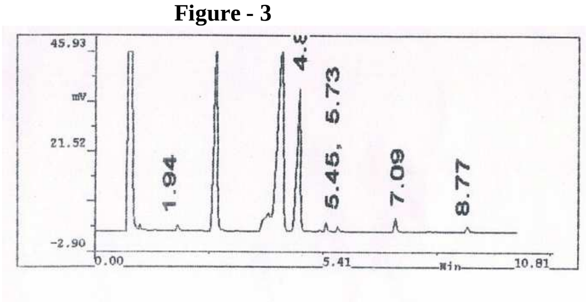


Figure - 4

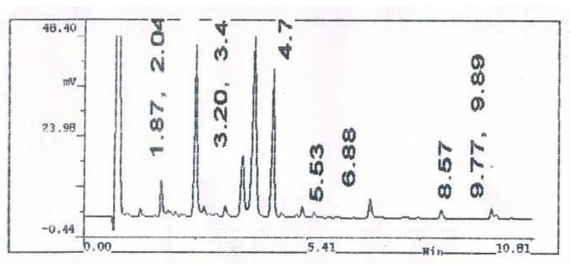
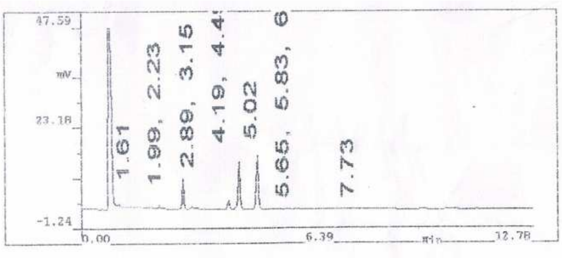


Figure – 5



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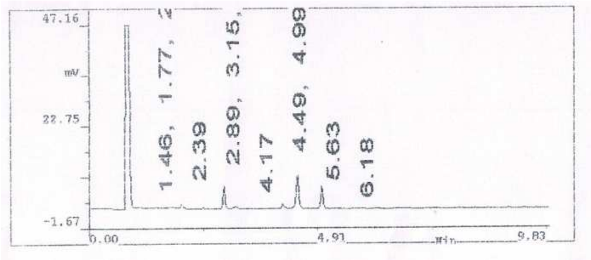


Figure – 7

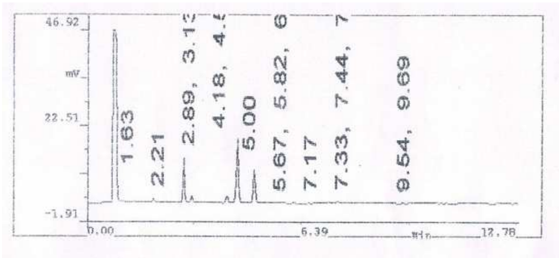


Figure - 8

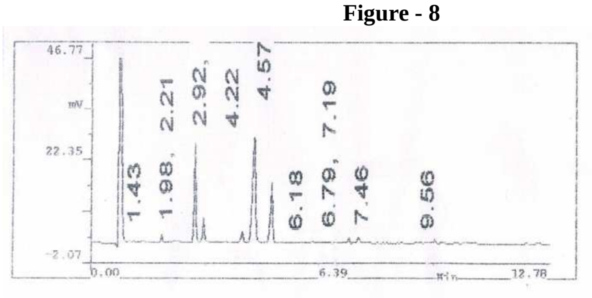


Figure – 9

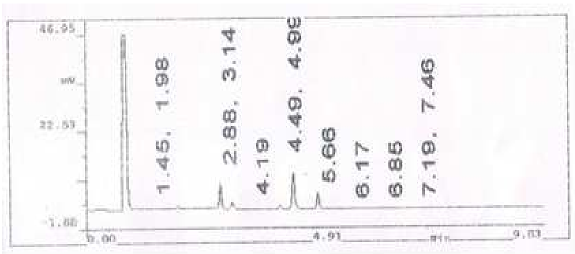


Figure - 10

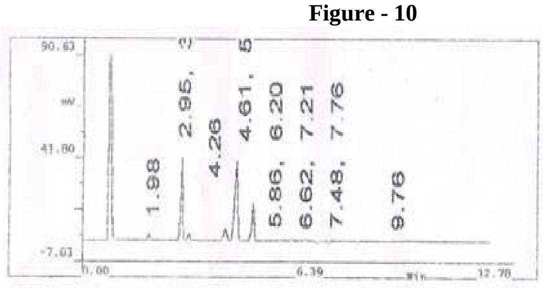


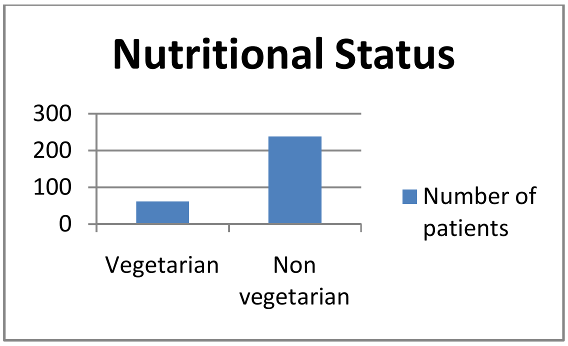
Table – 2 Essential Fatty Acid Composition in Breast Cancer Tissue Samples (Postoperative)

Name of the fatty acid	Mean %	± SD
Palmitic acid	22.099	6.635
Steric acid	5.028	1.795
Oleic acid	39.560	4.699
Linoleic acid	24.534	10.011
Linolenic acid	0.157	0.072
Arachidic acid	0.739	0.499
Behanic acid	1.356	1.283
Lignoceric acid	0.49	0.449
Myristic acid	2.134	1.535
Lauric acid	0.332	0.414
Richinoleic acid	0.525	

DISCUSSION

Metastatic mammary cancer tissues were analyzed for the compositional difference in their fatty acids. The results revealed significant variations in them. The dietary source of fatty acids in the patients cannot be ruled out, as the epidemiological data also revealed that both vegetarians and non vegetarians have got the cancer. Among the breast cancer patients, the non vegetarians showed a higher incidence of cancer manifestation. Out of 300 patients 238 were non-vegetarians while 62 were vegetarians (Fig 11).

Figure 11: Food Habits of 300 Breast Cancer Patients



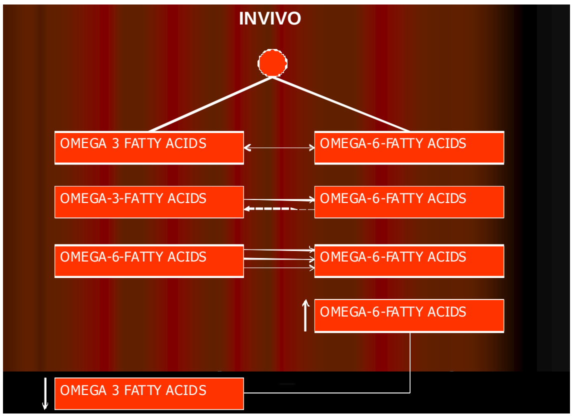
In view of the predominant incidence of cancers among non-vegetarians it may not rule out the contribution of fatty diet to the prevalence of higher proportions of certain essential fatty acids. However the internal metabolic changes in the post dietary lipid composition of tissues could also be ascribed to higher proportion of specific fatty acids like nB6. The changes in the fatty acid composition may also be due to hormonal imbalance in these patients. Studies have revealed previously that in the case of mammary glands, the musculo-epithelial cells retain fat globules and the neuro-hormonal reflex involving the posterior pituitary may influence the removal of the fat. However any disturbance or abnormality in the lipo- secretory reflexes may cause the accumulation of fatty inclusion which may become the precursors and / or mediators of the tumour micro environment to promote oncogenesis. A few recent studies have revealed the role of different fatty acids in enhancing or suppressing breast cancer formation. Moreover in the microenvironmental niche of developing cancer cells, several secretory factors like hormones, amines and prostaglandins (PGE) have been reported to enhance their growth[7]. Cancer initiation, promotion and progression is not only caused by the differential functioning of both the oncogenes and Tumour suppressor genes, but also by the consistent changes in the biochemical constituents of the cells which have undergone oncogenic transformation. In this context several biochemical substrates/metabolites were identified as diagnostic markers of cancer cells. The activity of alkaline phosphatase has been well established as a biomarker in clinical assessment of cancers. In epidemiological report the intake of fatty diet has been ascribed to be an etiological agent for cancer development in general and very specifically to certain types of carcinomas like breast adenocarcinoma. There is a close correlation between adipose tissue fatty acid composition and breast cancer patients.

Mamalakis *et al.*[5] very recently investigated the possible differences in adipose tissue fatty acid composition between breast cancer patients and healthy control women. The relationship between tumour promotion and adipose tissue fatty acid synthesis has also been investigated. Their results revealed that elevated adipose monounsaturated fatty acids and oleic acid are associated with reduced odds (risks) of breast cancer. On the contrary, adipose myristic acid is found to be associated with an increase in breast cancer risk. Punnomen *et al.* have also revealed the compositional difference in the phospholipids and neutral lipids of human breast cancer tissues. The relative amounts of unsaturated fatty acids increased in all the phospholipid subclasses. The differences were more marked in phosphatidyl ethanolamine than in other phospholipid fractions and further more the relative amount of the former increased in the cancerous tissues. Their study suggests that the lipid composition of cancerous breast tissues differs from that of the surrounding tissues and they may be involved in carcinogenesis.

In the present study the percentage of palmitic acid in the breast tissues was 22.09 compared to other free fatty acids. A recent study[9] has shown that the palmitic acid or its metabolic derivative is essential for the survival of breast cancer cells. In the above study it was also revealed that fatty acid synthesis especially the palmitic acid has a correlation with the genetic factors namely the BRCA1, inducing breast cancer, by silencing of ACCα or the fatty acid synthetase genes (FAS) in cancer cells resulting in the depletion of cellular pool of palmitic acid which in turn has induced the apoptosis of cancer cells concomitant with the formation of reactive oxygen species and mitochondrial impairment. Considering this it might be suggested that the level of palmitic acid in the breast cancer tissue would have promoted the death of cancer cells formed fortuitously along with the cancer suppressor genes such as the BRCA 1 in normal subjects. However in the present study the genetic modification of BRCA1 was not carried out but the DNA variations involving deletions, transitions and transversions in the D-loop of mitochondria have been noticed. The above mutations are established to alter the mitochondrial function through ROS and RNS production. Hence it may be suggested that the influence of palmitic acid is also determined by the mitochondrial genome alterations and polymorphisms. The cell line experiments using SP2O revealed that in the present study all the three compounds namely α keta glutarate, palmitic acid and sodium cholate have caused the 100% death of the cells. It remains contradictory to the report (personal observation) of Veronique[9] that lipogenesis is associated with cancer cell survival, However in their investigation they have also revealed that the palmitic acid along with vitamin E the anti oxidant only reduced the apoptosis of cells in which the ACCα and FAS genes were silenced.

Arachidonic acid alongside linoleic and linolenic acid constitute the polyunsaturated fatty acids. The higher ratio of poly unsaturated fatty acids to saturated fatty acids lowers blood cholesterol and considered beneficial to cardio vascular diseases. Of all fatty acids in human, 60% have chain lengths of ≥ 18 carbon atoms. The malonyl COA by donating carbon units, long chain fatty acids could be synthesized in cells in the endoplasmic reticulum and mitochondria. Fatty acids in diet give way to the synthesis of archdionic acid which in turn by enzyme pathways synthesize prostaglandins of D, E and F series and other eicosonoids. The eicosonoids synthesis has a correlation to cancer cell proliferation. Arachidonic acid also called eicosanoic acid is a saturated fatty acid; It is an omega 6 fatty acid and the counter part to the saturated arachidic acid. In the present study low levels of arachidic acid was observed in all samples tested. Linolenic is an omega 3 fatty acid while linoleic is an omega 6 fatty acid. These two EFA can create other omega 3 and 6 fatty acids and prostaglandins to carry out the various metabolic functions. But the balanced intake of these EFAs is important, since the tilt of balance may offset the regulatory cell functions and cause undesirable reactions[3]. The over abundance of linoleic as noticed in the cancer tissues in the present study may inhibit the absorption and utilization of linolenic acid which may have increased cancer risks. Oleic acid kills breast cancer cells. It is the chief fatty acid in olive oil which has been shown to dramatically cut the levels of a gene involved in the development of breast cancer. It cuts the level of oncogene HER2/neu also known as erb-B2. Higher levels of HER2/neu are associated with highly aggressive tumours that have a poor prognosis. Oleic acid not only suppressed over expression of the gene but boosts the effectiveness of the trastuzumab (Herceptin) the monoclonal antibody and helped to prolong the lives of many cancer patients. It significantly down regulates the expression of HER2/neu cutting it by up to 46 percent. However oleic acid percentage in the breast cancer tissue was found to be higher (39.56035%). The significance of this deviation especially in cancer tissue is to be contemplated. In the present observation the higher percentages linoleic acid (24.534%) as compared to linolenic (0.1566%) in the cancer tissues agrees to be the general hypothesis that nB6 fatty acids promote carcinogenesis.

Figure – 12 *In vivo* changes of essential fatty acids profile (nB3 and nB6) in tissues



In view of the previous reports and current observation the contention that intra-cellular fatty acid metabolism and their temporal composition and /or their reflex regulation abnormalities may become a predisposing prognosis factor for mammary adenocarcinoma seem to be supporting prognostic evidence alongside other causative factors. Some of the essential fatty acids of dietary origin by conversion in the post dietary metabolism may also play an inflammatory role to potentiate the tumorigenesis. This study and results also recommend that marine fish products having Omega fatty acids may have a protective role in the subjects than those consuming red meats.

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