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# Erythrocyte membrane fatty acid profile and saturation index in gallbladder carcinogenesis: a case-control study

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## Abstract

**Background:** Gallbladder cancer is a common neoplasm of biliary tract, with an unknown etiology.

**Patients and methods:** This study was carried out to evaluate the changes in the membrane fatty acid profile and saturation index in patients with gallbladder cancer. The study sample consisted of 50 newly diagnosed cases of gallbladder cancer and 50 patients undergoing cholecystectomy for cholelithiasis were recruited as controls. The fatty acid estimation was carried out by high performance liquid chromatography (HPLC). Statistical analysis was carried out by student 't' test and one-way ANOVA. Pearson's correlation coefficient was also obtained.

**Results:** A significant lowering of erythrocyte membrane stearic acid ($p = 0.000$), arachidonic acid ($p = 0.001$), and saturation index ($p = 0.001$) was observed in patients with gallbladder cancer compared to controls. A significant inverse relation was observed between stearic and oleic acid ($r = -0.269$ $p = 0.007$).

**Conclusion:** Results of the present study suggest an increase in the delta 9 desaturation in cancer patients compared to controls however, a possible role of biliary stasis due to altered gallbladder motility or derangements of signal transduction secondary to altered membrane lipid bilayer cannot be ruled out.

## Introduction

Fatty acids are long chain organic acids and form a major component of membrane lipids. A membrane fatty acid profile is thought to reflect the body composition as well as dietary fat intake [1]. Of these, polyunsaturated fatty acids (PUFA) are prone to free radical attack and hydrogen abstraction, the oxidative damage being termed as lipid peroxidation [2]. This causes a reduction in the membrane fluidity and permeability [2]. These changes in the lipid bilayer are also thought to interfere with the electron transport chain and signal transduction across the cellular membrane.

**Table 1: Erythrocyte fatty acid concentration (mean and Standard deviation) in cholelithiasis and carcinoma of the gallbladder (figure in parenthesis is range)**

| Fatty acid | Cancer (n = 50) | Gallstones (n = 50) | Test value | respective P value |
|---|---|---|---|---|
| C 12:0 Lauric Acid | 5.0 ± 6.7 (0–18.4) | 6.8 ± 7.3 (0–20.4) | t = 1.27 F = 1.62 | 0.20, 0.21 |
| C 14:0 Myristic Acid | 3.5 ± 3.0 (0–10.1) | 3.8 ± 3.6 (0–11.1) | t = 0.45 F = 0.21 | 0.64, 0.65 |
| C 16:0 Palmitic Acid | 8.6 ± 5.0 (0–16.4) | 9.4 ± 5.8 (0–19.6) | t = 0.77 F = 0.59 | 0.44, 0.44 |
| C 16:1 Palmitoleic Acid | 2.05 ± 2.7 (0–8.4) | 3.6 ± 9.0 (0–47.0) | t = 1.22 F = 1.49 | 0. 22, 0. 22 |
| C 18:0 Stearic Acid | 23.6 ± 23.0 (0–76.0) | 45.57 ± 24.1 (0–90.8) | t = 4.65 F = 21.6 | 0. 000, 0. 0000 |
| C 18:1 Oleic Acid | 24.2 ± 21.6 (0–92.8) | 19.8 ± 14.1 (0–35.2) | t = 1.2 F = 1.4 | 0. 22, 0. 22 |
| C 20:4 Arachidonic Acid | 9.08 ± 16.6 (0–80.0) | 24.3 ± 28.2 (0–82.12) | t = 3.2 F = 10.74 | 0. 001, 0. 0014 |
| Saturation Index | 0.08 ± 1.2 (0–4.4) | 2.0 ± 1.8 (0–8.5) | t = 3.45 F = 11.9 | 0. 001, 0. 0009 |

F = one-way Anova value; t = student 't' test value; p = probability value.

Stearic acid is an 18C saturated fatty acid, which is converted into delta 9 monounsaturated 18C oleic acid by the liver microsomal desaturase system [3]. An increase in delta 9 desaturation and lowering of saturation index has been observed in colorectal cancer [4], bronchogenic carcinoma [5], lymphoma [6], leukemia [7], malignant liver neoplasms [8,9] as well as in gallbladder cancer [10]. Hence on one hand the saturation index might prove to be a diagnostic marker of malignancy, the fatty acid profile changes might provide a clue to carcinogenesis. We carried out this case-control study to evaluate the fatty acid profile and saturation index in patients with carcinoma of the gallbladder and cholelithiasis.

## Material and Methods

This case-control study was carried out at University Hospital, Varanasi, cases constituted 50 newly diagnosed gallbladder cancer, while another 50 patients undergoing cholecystectomy for cholelithiasis constituted controls. Five milliliter of venous blood was collected in heparinized vials and was centrifuged at 10,000 rpm for 5 minutes to separate erythrocytes. These were washed twice with phosphate buffer saline and centrifuged, the supernatant was decanted and erythrocytes were stored at -20°C till further analysis.

Erythrocytes were thawed and 2 ml of distilled water was added to hemolyze the erythrocytes. The hemolyzed solution was saponified by refluxing with N/2 alcoholic KOH in water bath and the fatty acids were extracted in ether. Remaining saponified matter was acidified with 10% HCl and extracted with ether. The ether extract was washed with distilled water till acid free, dried under anhydrous sodium sulphate to obtain free fatty acids. These were then converted to fatty acid methyl esters (FAME) by methylating with methanol sulphuric acid. The mixture was heated in water bath for 4 hours, cooled to room temperature and then extracted with ether. The ether soluble layer was washed with 1% KOH and then by distilled water till alkali free. The ether was evaporated and FAME were dissolved in benzene and stored at 4°C till analysis.

For chromatographic analysis 100 μg of FAME, 10 ml of Phenacyl bromide solution and 10 μl of triethylamine solution were combined and allowed to stand overnight at room temperature. An aliquot of this solution was injected directly into liquid chromatograph. High performance liquid chromatography was carried out using Waters 680 AGC chromatograph equipped with Water's 501 pump solvent programmer and Waters UV detector. Absorbance was measured at 254 nm. A 90 cm × 0.64 cm : bondapak C-18 column was used with acetonitrite (8:20 v/v) as eluent at a flow rate of 0.50 ml / minute.

Statistical analysis was carried out by student 't' test and one-way ANOVA (F). Pearson's correlation coefficients (r) were also calculated.

## Results

The mean age of the patients with cholelithiasis was 41.8 ± 10.0 years (range 24–60) and 49.8 ± 9.5 years (range 34–67) for cancer patients, majority of the patients in both groups were females (88 and 90% of cancer and stones respectively).

A significant lowering of the erythrocyte membrane stearic acid and arachidonic acid levels was observed in patients with carcinoma of the gallbladder compared to controls (p = 0.000 and p = 0.001 respectively) (Table 1). Lowering was also observed in the levels of lauric acid, palmitic acid and palmitoleic acid, however, this was statistically not significant. On the other hand an increase in the concentration of oleic acid (C 18:1) was observed in patients with carcinoma of the gallbladder, however, this too was statistically not significant (p = 0.22).

A significant lowering in the saturation index (SI), which is defined as stearic to oleic acid ratio, was observed in pat-

**Table 2: Correlation between erythrocyte fatty acid levels, age and sex in cholelithiasis and gallbladder carcinoma (figure in parenthesis is probability)**

| | Age | Sex | Lauric acid C12:0 | Myristic acid C14:0 | Palmitic A C16:0 | Palmitoleic A C16:1 | Stearic A C18:0 | Oleic A C18:1 | Arachidonic A C20:4 |
|---|---|---|---|---|---|---|---|---|---|
| Age | 1.0 | | | | | | | | |
| Sex | -0.255* (0.01) | 1.0 | | | | | | | |
| C 12:0 | -0.239 (0.01)* | 0.064 (0.52) | 1.0 | | | | | | |
| C 14:0 | -0.039 (0.7) | -0.09 (0.37) | -0.028 (0.77) | 1.0 | | | | | |
| C 16:0 | 0.04 (0.69) | 0.063 (0.53) | -0.143 (0.15) | 0.437 (0.000)* | 1.0 | | | | |
| C 16:1 | 0.094 (0.35) | 0.03 (0.74) | -0.117 (0.246) | -0.18 (0.07) | -0.017 (0.86) | 1.0 | | | |
| C 18:0 | -0.10 (0.32) | 0.05 (0.60) | 0.27 (0.007)* | -0.08 (0.42) | 0.117 (0.24) | -0.225 (0.025)* | 1.0 | | |
| C 18:1 | -0.22 (0.82) | 0.026 (0.79) | -0.11 (0.274) | -0.08 (0.42) | -0.182 (0.07) | 0.011 (0.91) | -0.269 (0.007)* | 1.0 | |
| C 20:4 | -0.256 (0.01)* | 0.085 (0.40) | -0.08 (0.41) | 0.087 (0.38) | 0.127 (0.207) | 0.081 (0.42) | 0.005 (0.95) | 0.069 (0.49) | 1.0 |

* Significant correlation. A = Acid

ents with carcinoma of the gallbladder compared to patients with cholelithiasis (t = 3.45 p = 0.001; F = 11.9, p = 0.0009).

Pearson's correlation analysis showed lauric acid (r = -0.239, p = 0.01) and arachidonic acid (r = -0.256 p = 0.01) to be negatively correlated with age. The stearic acid levels significantly correlated with lauric acid (r = -0.27, p = 0.007), palmitoleic acid (r = -0.225 p = 0.025) and oleic acid (r = -0.269 p = 0.007) (Table 2).

## Discussion

The results of the present study show a reduction in Saturation Index (SI) in patents with gallbladder cancer compared to controls. However, in preliminary study [9], this fall in SI was primarily due to fall in the content of stearic acid and increase in oleic acid in patients with carcinoma of the gallbladder. We did not observe a significant rise in the level of oleic acid in carcinoma patients, however, fall in the levels of stearic acid were significant in this study. This change in SI was similar to earlier observations by other authors at various cancer sites [4–8]. The Kelly's concept that "cancer is a systemic disease and that the decrease in SI is not type specific but is associated with neoplasia" is a supported by the present results as well. [4,10]

Similar to the results of our earlier study [11], the present study too show a fall in concentration of arachidonic acid in patients with gallbladder cancer, compared to patients with cholelithiasis, however, absence of a normal control group preclude the exact interpretation of this observation. Although not much delta 9 desaturation was seen in the present study when compared to the results of our earlier study [11], however this may be due to less number of patients in our earlier study and high variance thus observed. We had earlier proposed a role of free radical mediated lipid peroxidation in gallbladder carcinogenesis, however, our attention was subsequently drawn to the relationship of lipid peroxidation product 4-hydroxynonenal (HNE) and electrolytes suggesting that increase in lipid peroxidation products was probably due to increased stasis rather than increased peroxidation [12]. Even though the concentration of biliary lipid peroxides were not measured in the present study the fatty acid profile changes observed here probably indicate mechanism other than lipid peroxidation in gallbladder carcinogenesis. This could either be due to altered motility and concentration of carcinogens in gallbladder as proposed by us earlier [13] or by altered signal transduction brought about by a change in lipid bilayer. We are presently carrying out the lipid peroxide product maelonaldehyde (MDA) estimation in the frozen gallbladder tissue of the same patients reported here, and presume that will not only throw additional light but might be able to answer the question of probable role of lipid peroxidation in gallbladder carcinogenesis.

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