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Original article

Erythrocyte membrane saturated fatty acids profile in newly diagnosed Basal Cell Carcinoma patients

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SUMMARY

Background: Skin cancers are the most prevalent malignancy worldwide and Basal Cell Carcinoma (BCC) include the major type of nonmelanoma skin cancers. Fatty acids (FA) have a structural role in cell membranes and play an important role for many physiological and pathological immunologic pathways. Several prospective studies have been conducted on circulating fatty acids and the risk of prostate, breast and other cancers. The present study aimed to determine the saturated fatty acid composition differences of red blood cells (RBCs) in BCC patients and healthy control.

Methods: A hospital-based case-control study was conducted on new cases diagnosed of BCC patients. All subjects completed dietary recalls for dietary assessment. After fatty acids extraction, purification and preparation, gas chromatography was performed. The results were expressed in relative values (percent).

Results: Cases had lower RBC levels of Caproic acid (6:0) ($P < 0.001$), Caprylic acid (8:0) ($P = 0.01$), Capric acid (10:0) ($P = 0.01$), Palmitic acid (16:0) ($P = 0.02$) and higher RBC level of Pentadecanoic acid (15:0) ($P = 0.04$) and Stearic acid (18:0) ($P = 0.01$) compared with controls but did not differ in the level of the other primary saturated fatty acids. Saturation Index as defined by Stearic to Oleic acid ratio was significantly lower in BCC patients in comparison with Control group ($P = 0.02$).

Conclusion: Here we showed that BCC patient had considerable differences in the SFA profiles in comparison with healthy subjects.

1. Introduction

Skin cancers are the most prevalent malignancy worldwide, with nonmelanoma skin cancers (NMSC) accounting for more than 30% of all cancers in the US. In Iran, skin cancer is the most common cancer with about 15% of all cancers. Basal cell (BCC) and squamous cell carcinomas (SCC) include the major types of NMSC. BCC is the common morphologic form of skin cancer in Iran. Although the mortality rate of BCC is remarkably low, NMSC represents a significant health care burden and can cause major morbidity particularly as most NMSCs occur on highly visible areas such as the head and neck and face [1,2].

The major constitutional risk factors for all skin cancers seem to be skin color and the skin response to strong sunlight. Particularly in BCC development several factors have been suggested such as age, male sex, skin phototype (I, II), frequent sun exposure and sunburn, severe actinic damage, history of radiotherapy and etc. [3].

Although several animal studies show that high level of dietary fat intake decrease the time between UV exposure and tumor appearance and influence the promotional stage of UV carcinogenesis, there have been few human studies [4]. An intervention study indicated that participants randomized to an isocaloric

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low-fat diet experience lower occurrence of actinic keratoses and nonmelanoma skin cancer [5]. Another human study also mentioned that participants consuming higher percentages of dietary energy from fat seemed at increased risk for skin SCC [6].

Fatty acids (FA) have a structural role in cell membranes, influencing their fluidity and membrane enzyme activities and play an important role for many physiological and pathological immunologic pathways [7]. The possible influence of lipids on neoplastic development could be attributable to influence on the metabolism of neoplastic cells (proliferation and incorporation in the membranes of neoplastic cells), the function of lipids as intercellular messengers, or as mediators of the inflammatory reaction [8]. Also In vitro and in vivo studies show different effects of individual fatty acids on expression of genes concerned in multiple biologic pathways, comprising inflammation, lipid metabolism, and oxidative stress [9].

Several prospective studies have been conducted on circulating fatty acids and the risk of prostate, breast and other cancers [10,11]. A meta-analysis published in 2004 [11] indicated that oleic acid and the saturated palmitic acid were associated with increased breast cancer risk. Also reduction of saturation index as defined by the ratio of stearic acid (the most common saturated fatty acid [SFA]) to oleic acid (the most common monounsaturated fatty acid [MUFA]) has been observed in colorectal cancer, bronchogenic carcinoma, lymphoma, leukemia, malignant liver neoplasms and gallbladder cancer [12].

Studies suggest that the composition of human erythrocyte membrane fatty acid, other than dietary macronutrient intake and the interactions between dietary intake and endocrine changes may reflect fatty acid composition in other body tissues and are suitable for exploring the relations of the patterns of fatty acid metabolism to skin cancer risk [4,9].

In the current study, we explore the fatty acid composition of red blood cells (RBCs) to address individual fatty acid concentrations and possible differences between BCC patients and healthy control.

2. Methods and materials

2.1. Study design, participants and data collection

A hospital-based case-control study was conducted on new cases diagnosed of BCC patients in Razi hospital, Tehran, Iran. Cases were eligible if they had a histopathologically confirmed BCC diagnosis in Razi hospital in contact time, without a previous history of any cancers. The control subjects were randomly selected from first-visit outpatients who visited Razi Hospital. Control subjects were eligible if they had no prior history of skin cancer and were within the age, gender, and ethnicity grouping. There was no relation between diseases of controls and exposures studied. No study participant took drugs known to affect lipid metabolism or hormone replacement therapy. Also other diseases like diabetes mellitus, cardio vascular diseases and hypertension were excluded. For controlling confounding factors, case and control groups were matched on age, and Body Mass Index. All patients gave their informed consent to participate in the study, which was approved by the ethical committee of TUMS and performed in accordance with the guidelines in the Helsinski Declaration. Trained interviewers systematically collect and check information from the questionnaire and contacted the participants to describe the study and invite them, and scheduled the interview for dietary recalls and other questions. Two 24-h dietary recalls by nutritionist help were completed in randomly selected days (week day) and then analyzed by Nutritionist IV software (First Databank, San Bruno, CA, USA) adjusted for Iranian foods. Body weight was measured without shoes and in a minimum clothes condition by the use of a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was calculated to the nearest 0.1 cm by using an inelastic tape measure (Seca, Hamburg, Germany). BMI was calculated as weight in kg divided by height in meters squared. Five millimeters of blood samples from vein were collected after 8–12 h fasting. We used serum for lipid profile (triglyceride, total cholesterol, HDL cholesterol and LDL cholesterol) (Pars Azmoon kit, Iran).

2.2. Sample size calculation

To measure the sample size, based on type one (α) and type two errors (β) as 0.05 and 0.20 (power = 80%), respectively and according to the previous study [9], we considered 1.04 as standard deviation (SD) and 0.78 as the difference in mean or effect size (d) of fatty acids in RBC membrane as the key variable. Therefore, we needed 28 subjects in each group. Eventually we recruited 40 patients per group to consider the possible dropouts. From April 2010 to February 2013 in 3 year-duration, 80 participants were interviewed for the study, 40 cases and 40 controls.

3. Reagents and standards

Analytical grade chloroform, methanol and n-hexane were obtained from Merck (Darmstadt, Germany). BF3 14%, sodium sulfates and sodium chloride standards were acquired from Sigma–Aldrich (St. Louis, USA). 37 FAME Mix standard, were purchased from Supelco, Pennsylvania, USA. Water (18.2 M, TOC < 5 ppm) was purified and filtered through a Milli-Q Plus system filter (Millipore, Bedford, USA).

3.1. Fatty acids extraction, purification and preparation of fatty acid methyl esters

Ten milliliters of fasting venous blood were collected from each patient in an EDTA bottle and centrifuged at 1500 Rpm for 5 min to separate the erythrocytes. The plasma was recorded for lipid profile analysis and the erythrocytes were washed three times with phosphate buffered saline (PBS) to exclude iron of hemoglobin for preventing oxidative degradation of fatty acids. All RBCs were reserved in 500 μL micro tubes in −80 °C for gas chromatography measurement. Each sample of red blood cells (RBC) was thawed to room temperature. The lipids were extracted from the erythrocytes with 2:1 (v/v) chloroform–methanol using the Folch et al. (1957) procedure [13] and centrifuged at 1500 × *g* for 10 min. Preparation of fatty acid methyl esters was carried out by of BF3·MeOH 14%. So, 200 μL of dried RBC samples (Samples dried by nitrogen gas in order to evaporate water for better fatty acid methylation), 2 μL BF3·MeOH 14% and 1 μL methanol incubated for 10 min at 60 °C [14]. To the samples were added 2 ml of N-hexane solution and they were stirred for 10 min by Vortex. The upper layer was recorded, drown off into a vial.

3.2. Gas chromatography and fatty acids quantification

Gas chromatography analyses were performed by a 6500GC YoungLin (Korea) instrument with auto-sampler, a split/splitless injector, FID detector and a hydrogen gas generator. Separation of FAME (fatty acids methyl ester) was carried-out on a TR-CN100 column (Teknokroma, 37 FAMEMIX-MS detector, 60 m × 0.25 mm × 0.20 μl, Standard: 1 μl FAME MIX in methylene chloride, Injection: 280 °C, split 20:1). Hydrogen was used as carrier gas, constant flow mode; the amount injected was 1 μL in splitless mode and 24 PSI. The temperature of the injector and the FID detector were 280 °C. Inlet temperature 240 °C, split 40:1, injection

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