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Investigation of the interplay between plasma lipids and macrophage polarization in small oral squamous cell carcinomas with different outcome: A pilot study of 17 cases☆,☆☆

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ABSTRACT

Introduction: Growing evidence suggests a correlation of alternative polarization of macrophages (M2) with a bad outcome of oral cancer. Macrophage polarization plays a significant role in the progression of hyperlipidemia and atherosclerosis, being influenced from plasma cholesterol. On the other hand plasma lipids have been studied epidemiologically as risk factors in carcinogenesis. Goal of our pilot study was the investigation of a possible association of plasma lipids with tumor outcome through their potential influence on macrophage polarization.
Materials and methods: 17 patients with small pN0 OSCC with different clinical outcome, treated operatively without postoperative R(C)T constituted our patient collective. Plasma lipids (total cholesterol and triglycerides) were studied in relation to macrophage polarization (determined through the expression of CD68, CD11c, CD163 and MRC1 antibodies) and tumor outcome.
Results: Patients with pathological chronic course of either plasma cholesterol or triglycerides demonstrated an increased infiltration with alternatively polarized macrophages in their specimens. Patients with pathological chronic course of plasma cholesterol showed moreover a bad tumor outcome.
Conclusion: A role of plasma lipids in the tumor outcome via alternative macrophage polarization could be assumed. A larger prospective study is needed to confirm our preliminary results.

1. Introduction

Tumor-associated macrophages originate from bone marrow-derived mononuclear cells and play a key role in the interaction of inflammation with immune response in cancer (Sica et al., 2002) as they infiltrate solid tumors. There are two main patterns of macrophage activation: the classic (M1) polarization, induced by Th-1 cytokines (interferon-γ and tumor necrosis factor-α), and the alternative (M2) polarization, induced by Th-2 cytokines (IL4 and IL13) (Ma et al., 2003). Although the M1 polarization is considered to be pro-inflammatory, M2 polarization promotes the progression of solid tumors such as those of the prostate, ovaries, pancreas, and breast (Comito et al., 2014; Lan et al., 2013; Liu et al., 2013; Tiainen et al., 2015).

Macrophage polarization has been identified as a potential independent prognostic factor for oral cancer (Metelmann et al., 2012). An increased macrophage infiltration with a simultaneous rise of alternative (M2) polarization was identified in patients with oral squamous cell carcinoma (OSCC) with primary lymphogenic metastasis (Weber et al., 2014). Moreover, a correlation of M2-macrophage polarization in the regional lymph nodes with increasing malignancy of OSCCs has been demonstrated (Wehrhan et al., 2014).

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Both M1 and M2 macrophages are also present in atherosclerotic lesions and play a crucial role in its development (Leitinger and Schulman, 2013). In the course of hyperlipidemia and atherosclerosis, macrophage polarization is affected by plasma cholesterol (Leitinger and Schulman, 2013). A shift to the direction of either classical or alternative macrophage activation seems to be important for the progression of those diseases. Another important observation is the fact that a number of studies over the years have associated disorders of plasma cholesterol, as well as of plasma triglycerides, with cancer (Goodwin et al., 1997; Yang et al., 2015).

The underlying hypothesis of our study was that plasma lipid levels affect the outcome in OSCC by influencing macrophage polarization. The primary goal of the present study was to compare plasma lipid levels in patients with small OSCCs and different tumor outcomes. We also looked into a possible association between the level of plasma lipids and the macrophage polarization in those tumor specimens.

2. Material and methods

For the purpose of the study, clinical records of tumor patients treated in our Department between 2006 and 2011 were retrospectively analyzed. Our inclusion criteria were the following: a) primary OSSCs with classification pT1/T2 pN0 G1/G2 R0; b) a minimum of 5 mm of clear resection margins; c) no postoperative radiotherapy/chemotherapy; d) a minimum follow up of 3 years; and e) no medical history of other oral malignancy or malignancy of the upper aerodigestive tract.

The initial review of the records detected 80 patients with pT1/T2 pN0 tumors without medical history of other malignancy (57 patients with good outcomes and 23 patients with poor outcomes, i.e., local or regional recurrence in the first 3 years after primary treatment). Of the patients, 34 had to be excluded, as they received postoperative radiotherapy because of increased invasion depth or G3 tumors. A total of 29 additional patients could not be included in the study because of incomplete records. Consequently, our collective included a final total of 17 patients. The patients were further classified in two groups. Group A included patients with no signs of local, regional, or distal recurrence (which we labeled as good outcomes), whereas Group B included patients with recurrence (which we labeled as poor outcomes) in a minimum follow up period of 3 years. Group A included 10 patients and Group B included 7 patients.

Routine biochemical analysis, including measurement of total plasma cholesterol and plasma triglycerides, was performed on admission day prior to biopsy. This initial measurement of plasma lipids was followed by a minimum of 4 measurements in the postoperative phase until discharge following completion of the surgical treatment.

All of the obtained measurements were collectively evaluated to assess the chronic course of plasma lipids. In case a minimum of 3 measurements with values over 200 mg/dl for either total cholesterol or triglycerides was present, the chronic course was defined as pathological; otherwise it was documented as normal.

The following variables were documented retrospectively for each patient in the cohort: a) total plasma cholesterol on admission day; b) chronic course of total plasma cholesterol; c) plasma triglycerides on admission day; and d) chronic course of plasma triglycerides.

For our study, we used samples from tissue specimens, which were harvested in the course of the routine histolopathological diagnostics and were collected by the Department of Pathology of the Friedrich Alexander University of Erlangen-Nürnberg. The ethical aspects of the study were approved by the ethical committee of the University (Ref.-Nr. 45_12 Bc).

Both specimens of the diagnostic biopsy and the definitive tumor resection were included in this study. A tissue micro-array (TMA) was performed for this material, followed by an immunohistochemichal (Peroxidase/DAB+, DAKO Autostainer) analysis of the following antibodies as macrophage polarization markers: CD68 (as general macrophage marker), CD11c (M1 polarization), and CD163 and MRC1 (M2 polarization).

A computer-assisted quantitative counting of macrophages per square millimeter (mm^2) was carried out on the "whole slide imaging" digitalized specimens with the use of the Biomas software (MSAB, Erlangen, Germany). Different tissue compartments (epithelial compartment and tumor stroma) were analyzed independently. The number of positively stained cells per square millimeter of specimen surface was counted.

Statistical analysis was performed with SPSS 22 for Mac OS (IBM Inc, Armonk, NY, USA) involving descriptive statistics along with analysis of variance and Pearson correlation. Results were expressed as median, minimum (Min), maximum (Max), and standard deviation (SD). Two-sided, adjusted p values ≤ 0.05 as well as r values >0.5 were considered to be significant and are presented in tables as well as in a box plot and scatterplot demonstration.

3. Results

The median age of our patient collective was 63 years (Min = 46, Max = 86, SD = 11.3) without any significant difference ($p = 0.483$) between group A (median 64 = years, SD = 10.32) and group B (median = 63 years, SD = 13.02). Group A consisted of 2 females and 8 males and group B included 3 females and 4 males.

With regard to group B, local recurrence occurred in 6 cases, and in 1 case a second tumor in a new oral site appeared. Regarding localization of the primary tumor, the floor of the mouth was the most common site (7 cases) followed by the soft palate (3 cases), mandibular mucosa (2 cases), buccal mucosa (3 cases), and tongue (2 cases).

Five measurements of cholesterol and lipid levels were obtained for the majority of the patients (n = 15) during their hospital stay

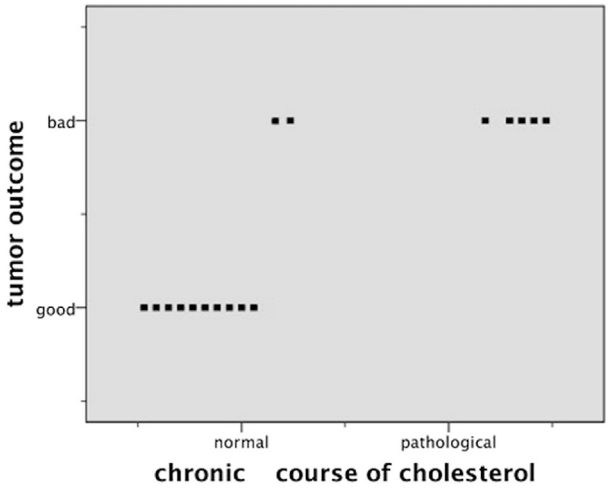

Diagram 1. Schematic representation of the patients in group A (patients with good outcomes) and group B (patients with poor outcomes), depending on the chronic course of cholesterol. All 10 patients from group A plus 2 of 7 patients from group B presented a normal course of plasma cholesterol. The remaining 5 patients from group B demonstrated a pathological course of plasma cholesterol.