Expression of MicroRNA-34c in different grades of Oral squamous cell carcinoma versus normal oral mucosa

(Ex-Vivo Study)

التعبير النسيجي عن الميكرو الرنا المرسل34c في مختلف درجات سرطان الخلايا الحرشفية الفموي مقابل الغشاء المخاطي الطبيعي

(دراسة خارِج الجسم الحي)

Protocol

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By

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I. Administrative information:

1. Title:

Expression of MicroRNA-34c in different grades of Oral squamous cell carcinoma versus normal oral mucosa (Ex-vivo study).

2. Protocol registration

Site and registration number of the protocol should be reported before final approval of the protocol.

3. Protocol version:

6th April protocol version number: 1

4. Funding:

The trial is totally self-funded whether on a financial or a non-financial basis.

5. Roles and responsibilities:

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II. Introduction

a. Scientific background

Oral squamous cell carcinoma (OSCC) has a very poor prognosis because of its invasive nature, and despite advances in treatment and diagnostics, the five-year survival rate is below 50% and has not been improved in the last three decades**. (Ge et al., 2015)**.

Early detection and diagnosis of OSCC results in improved survival rates, on the other hand, delayed detection may result in a high OSCC mortality rate. Furthermore, high recurrence rate of OSCC has been found in many cases. Thus, the development of new methods to predict the prognosis of OSCC is essential **(Gao et al., 2018).**

Studying genetic changes improves understanding of the molecular basis of cancer in order to able to provide potential diagnostic or outcome markers, and therapeutic targets for cancer patients**. C**hanges of micro-RNAs (miRNA) expression levels in OSCC are related to tumor development, progression and they could be potentially used as biomarkers of OSCC **(Ge et al., 2015)**

The tumor suppressor miR-34c has been demonstrated to be downregulated in several malignancies, such as neuroblastoma (**Cole et al, 2008),** breast (**Yu et al, 2012**), lung cancer **(Liang, 2008; Liu et al, 2009),** colorectal cancer **(Toyota et al, 2008**) prostate cancer as mentioned by **(Hagman et al., 2013)** and in laryngeal SCC **(Hu & Liu, 2015**). In addition, a Previous study testing the expression of miR-34 members in plasma and tumor tissue of 196 non-small cell lung cancer (NSCLC) patients, revealed that the high expression of miR-34a and miR-34c in both plasma and tumor tissues was related to prolonged overall survival and disease-free survival compared with low expression, this might raise the possibility of regarding miR-34a and miR-34c as potential prognostic markers in NSCLC. **(Tu et al., 2019).** Far to our knowledge, studies concerning the dysregulation of miRNA-34c and its correlation with OSCC grades are still rarely reported.

6b. Specific objectives

This research will be conducted to evaluate the change of expression level of MicroRNA- 34c in different grades of OSCC versus normal oral mucosal tissues. This will be done through the use Quantitative real-time polymerase chain reaction (qRT- PCR).

PECO:

Population: Formalin Fixed for different grades of OSCC tissues embedded in Paraffin.

Exposure: Expression of Micro-RNA 34c

Comparator: Formalin Fixed Normal Oral Mucosal tissues

Outcome: The change of expression level of Micro-RNA 34C measured in fold change (fld)

	Outcome Name	Measuring device	Measuring unit
Primary outcome	Micro-RNA 34c expression level	qRT- PCR	Fold change (fld)

III. Methods:

7. Study design

An ex-vivo Study involving cases of oral squamous cell carcinomas retrieved from archival blocks from the Department of Oral and Maxillofacial Pathology, Faculty of Dentistry in Cairo University that are diagnosed according to histopathologic criteria set by World Health Organization for each lesion that will be compared to another control group of normal oral mucosal tissues, obtained from cases of gingivectomy covered with normal squamous epithelium from the Department of Periodontology, Faculty of dentistry Cairo university.

8. Settings

1. The dates of collection of oral squamous cell carcinoma lesions: 2016 to 2020.

2. Formalin fixed paraffin blocks will be retrieved from the archives of the Oral and Maxillofacial Pathology department, Faculty of Dentistry, Cairo University.

3. All the cases will be reviewed according to the latest World Health Organization (2017) classification of head and neck tumors.

A) Participants

9. Eligibility criteria and selection methods

Inclusion criteria:

- Patients of any age group and sex could be included in the study.
- All oral squamous cell carcinoma lesions that fit the histological criteria of the World Health Organization (2017) will be included in the study.
- All cases in the period between 2016 and 2020.

Exclusion criteria:

Cases not found in the period between 2016 and 2020

10. Matching criteria and allocation ratio

B) Variables

11. Details about variables

The primary outcome of this study would be the change of expression of mir-34c in OSCC lesions measured in Fold Change Versus its expression in normal oral mucosal tissues.

12. Data sources and management

The data will be collected from a period between 2016 to 2020 from the department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University. This data will be reviewed according to the histological classification of the World Health Organization (2017).

13. Addressing potential sources of bias

Selection Bias

The subjects will be selected from Cairo University teaching hospital, which is the same source population.

Information Bias

This study is hospital based, where the information will be collected from medical reports. Avoidance of information bias will be carried out by ensuring the completeness of the recorded information.

D) Study size

14. Study size

The study will be carried out on all oral squamous cell carcinoma lesions diagnosed according to the WHO guidelines, which is the total number of records in the past 5 years (2016 to 2020)

E) Statistical methods

16. Statistical methods

Quantitative variables. For parametric data, Independent sample t-test will be used to compare between two groups in non-related samples. For non-parametric data, Mann Whitney test will used to compare between two groups in non-related samples. The Spearman rank coefficient to evaluate the correlation between different variables.

Shapiro-Wilk test of normality is used to test normality hypothesis of all quantitative variables for further choice of appropriate parametric and non-parametric tests.

Significance level is considered at $P < 0.05$ (S); while $P < 0.01$ is considered highly significant (HS). Two Tailed tests are assumed throughout the analysis for all statistical tests

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