Protease activity on dysplastic leukoplakia sites

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Ethische beoordeling	Niet van toepassing
Status	Werving nog niet gestart
Type aandoening	-
Onderzoekstype	Observationeel onderzoek, zonder invasieve metingen

Samenvatting

ID

NL-OMON23952

Bron
NTR

Verkorte titel
Protease activity on dysplastic leukoplakia sites

Aandoening

Oral leukoplakia

Ondersteuning

Primaire sponsor: Amsterdam UMC
Overige ondersteuning: None

Onderzoeksproduct en/of interventie

Uitkomstmaten

Primaire uitkomstmaten

Protease activity in saliva and saliva biofilm

Toelichting onderzoek

Achtergrond van het onderzoek

Oral leukoplakia (OL) is defined as a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer [1]. It is the most common oral potentially malignant disorder (OPMD), with an estimated worldwide prevalence between 1.5 - 4.1% [2, 3]. The malignant transformation (MT) rate of OL into an oral squamous cell carcinoma (OSCC) varies between 0.13% to 36.4%, depending on the used definition for OL, composition of the study population (e.g. population or hospital-based), prospective or retrospective analysis, etiological factors, duration of follow-up and worldwide geographical location [4]. The reported annual MT rate of OL varies between 1.36% - 2.9% [2, 5-10].
Several clinical, histopathological and molecular factors have been reported to be related with an increased risk for MT, among others age, gender, smoking habits, alcohol consumption, clinical presentation, size of the lesion, oral subsite, presence and degree of epithelial dysplasia, cytokeratin expression, DNA methylation, loss of heterozygosity (LOH), survivin positivity, matrix metalloproteinase 9 positivity and DNA aneuploidy [6, 7, 11-18]. Although 3p and/or 9p LOH are the most significant predictors of progression, in most studies the presence and degree of epithelial dysplasia is the most common routinely assessed risk factor for MT that has clinical implications [19]. Since OL is a clinical diagnosis, a biopsy is taken first to exclude an OSCC and second to determine the possible presence and degree of epithelial dysplasia [4, 5]. We have recently described the importance of recognizing differentiated dysplasia as a separate morphological entity in OL in addition to the 3-grade grading classification of epithelial dysplasia of the World Health Organization (WHO), further referred to as classic dysplasia [20, 21]. In this study, we showed that differentiated dysplasia markedly adds to the prediction of MT of OL [20]. Proteolysis is an irreversible protein modification involved in different cellular processes under physiological conditions [22]. However, increased proteolytic activity is implicated in numerous diseases including cancer pathogenesis, as demonstrated by the prominent role of proteases in tumor growth, angiogenesis, invasion, and metastasis [23]. Consequently, proteases have been investigated for diagnosis, prognosis and therapeutic purposes in cancer [24, 25, 26
Until now, no studies are performed on protease activity in the diagnosis and prognosis of oral leukoplakia. Recently, a panel of specific protease substrates have been developed. Therefore, the analysis of protease activity from saliva and the salivary biofilm, using these substrates, may provide a new layer of information useful in the discovery of prognostic markers with respect and related to the histopathological features of oral leukoplakia
Aim: To investigate the proteolytic events, with possible prognostic utility, taking place in saliva and the salivary biofilm of oral leukoplakia patients compared to oral squamous cell carcinoma (OSCC) and compared to healthy controls

Doel van het onderzoek

The analysis of protease activity from saliva and the salivary biofilm, using these substrates, may provide a new layer of information useful in the discovery of prognostic markers with

respect and related to the histopathological features of oral leukoplakia

Onderzoeksopzet

During 1 visit the following outcome measures will be determined:
1. Whole mouth stimulated saliva (SWS) will be collected and stored for further analysis according this protocol:
To collect SWS, patients will be asked to chew a 5x5 cm sheet of paraffin (ParafilmM, Pechiney, Chicago, USA) and expectorate into a pre-weighed tube every 30 seconds during a 5-minute period. The collection tube is placed in a container with ice.
Immediately after collection, the collection tube is closed and placed in the refrigerator with an anonymous patient code (1 to 16, based on the order of application and with the addition of a letter (A, B, or C) depending on the study group) and date. Saliva will be transferred to Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and centrifuged for 5 min at 10,000 g at 4°C as described by Silletti et al. (2007) [27]. After centrifuging, the saliva supernatant will be decanted and stored at -80°C in plastic containers (Cryogenic Vials Nalgene tubes).

2. Salivary biofilm will be harvested according this protocol: A sterile Periotron paper point will be placed on surface of the oral leukoplakia lesion or the oral squamous cell carcinoma for 10 seconds. This will be followed by a second paper point at the same location. Subsequently, salivary biofilm will be collected similarly at the healthy contralateral location with two consecutive periotron paper points. The paper points will be transferred to prelabeled Eppendorf vials containing buffer and will be stored at -80°C until biochemical analysis.

To quantify the protease activity in saliva, the collected saliva and Periotron paper points of each participant will be retrieved from − 80 °C storage and thawed on ice. The paper points will be centrifuged to retrieve the salivary biofilm. The protease activity will be determined using black 96-wells microplates (F Bottom, Greiner Bio- One GmbH, Frickenhausen, Germany). Each microwell will be filled with 70 ml of PBS, and 8 µM protease substrate PEK-054 ([FITC]-NleKKKKVLPIQLNAATDK-[KDbc]), a substrate for total protease activity. As a positive control, trypsin from bovine pancreas will be added in duplicate in two-fold serial dilutions, and sterile PBS will be used as a negative control. 30 µl of saliva will added to each microwell. The increase in fluorescence will be monitored over 60 min using a fluorescence microplate reader (Fluostar Galaxy, BMG Laboratories, Offenburg, Germany) with an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Relative fluorescence (RF) values were obtained for periodontitis patients and controls. The total protease activity will defined in RF per Unit.

Onderzoeksproduct en/of interventie

Saliva and saliva biofilm collection

Contactpersonen

Publiek

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Wetenschappelijk

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Deelname eisen

Belangrijkste voorwaarden om deel te mogen nemen (Inclusiecriteria)

OL and OSCC groups (group A and B): all new patients (age 18 years) diagnosed with oral leukoplakia or OSCC at the Amsterdam UMC location VUmc
Control group (group C): Volunteers in the control group will be age and sex matched compared to the OL and OSCC groups. Healthy is defined as an American Society of Anesthetists score I and without medication use except for oral contraception and no OL and/or OSCC

Belangrijkste redenen om niet deel te kunnen nemen (Exclusiecriteria)

Age < 18 years

Onderzoeksopzet

Opzet

Type:	Observationeel onderzoek, zonder invasieve metingen
Onderzoeksmodel:	Parallel
Toewijzing:	Niet-gerandomiseerd
Blindering:	Open / niet geblindeerd
Controle:	N.v.t. / onbekend

Deelname

Nederland

Status:	Werving nog niet gestart
(Verwachte) startdatum:	01-04-2021
Aantal proefpersonen:	48
Type:	Verwachte startdatum

Voornemen beschikbaar stellen Individuele Patiënten Data (IPD)

Wordt de data na het onderzoek gedeeld: Ja

Toelichting
All collected data will be deposited in an open access digital repository in an anonymous form.

Ethische beoordeling

Niet van toepassing

Soort:	Niet van toepassing

Registraties

Opgevolgd door onderstaande (mogelijk meer actuele) registratie

Geen registraties gevonden.

Andere (mogelijk minder actuele) registraties in dit register

Geen registraties gevonden.

In overige registers

Register	ID
NTR-new	NL9288
Ander register	METC VUmc : METc VUmc 2021.0087

Resultaten