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Clinical study on thermography, as modern investigation method for *Candida*-associated denture stomatitis

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Abstract

Candida-associated denture stomatitis is an infectious inflammatory condition of the oral mucosa, with frequent recurrences. The aim of this study was to assess the use of infrared thermography as investigation method for *Candida*-associated denture stomatitis (as inflammatory disorder of the maxillary denture bearing area), by comparing disease and non-disease groups. An observational study was conducted on maxillary edentulous patients treated by acrylic dentures, with and without *Candida*-associated denture stomatitis. Diagnostic test methods used were clinical examination for denture stomatitis and conventional microbiological culture method for oral candidiasis. Thermography analysis of the maxillary denture bearing area was made using the ThermaCAM PM350 infrared camera (Inframetrics, Flir Systems) and ThermaGram Pro 95 software, data being acquired by usage of standard protocol of thermographic registrations. The sample included 52 patients, 21 with and 31 without *Candida*-associated denture stomatitis. The temperature of the maxillary mucosa corresponding to the denture bearing area was found to be statistically significantly higher in *Candida*-associated denture stomatitis (mean 36.20^{0}C), compared to healthy oral mucosa (mean 34.85^{0}C). The thermal threshold value of 35.44^{0}C was identified as best differentiating a pathological from normal state of the maxillary mucosa corresponding to the denture bearing area. In conclusion, infrared thermography, a rapid non-invasive investigation method, has the premises to bring valuable data in inflammatory disorders of the maxillary denture bearing area, as *Candida*-associated denture stomatitis that may be used for screening, diagnostic or monitoring purposes.

Keywords: inflammation, candidiasis, diagnosis, thermal analyses, removable prosthesis.

Introduction

Denture stomatitis is an inflammatory condition of the oral mucosa, related to denture irritation, which often associates an infection process with fungus of *Candida* genus. It is a condition with a relatively high prevalence of the mucosa corresponding to the denture- or over-denture-bearing area, with higher incidence in the elderly, with multifactorial etiology, different clinical patterns and poor symptomatology [1, 2]. It has a chronic evolution, sometimes with frequent recurrences, high severity conditions associating a negative impact on oral structures (*e.g.*, accelerating the rate of alveolar bone resorption) and on the prosthetic outcome (*e.g.*, may lead to poorly fitting dentures). Denture stomatitis prognosis depends mainly upon the maintenance of oral and denture hygiene, the early diagnosis and the correctness of the treatment, in accordance to the condition's etiology.

Thermography is used for detection, diagnosis, and/or monitoring for numerous medical conditions, including oral ones (*e.g.*, diagnosis of orofacial pain and temporo-mandibular disorders, dental caries), being particularly useful for the early diagnosis of inflammatory diseases, by detecting alterations before clinical signs appear [3–5]. Therefore, this method may be an appropriate investigation method for denture stomatitis, as an inflammatory infectious condition of the oral mucosa that associates to temperature changes [6, 7].

The aim of this study was to assess the use of infrared thermography as investigation method for *Candida*-associated denture stomatitis (as inflammatory disorder of the maxillary denture bearing area), by comparing disease and non-disease groups.

Patients and Methods

Participants

An observational study was conducted on patients from the Department of Prosthodontics, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. Enrolment was done prospectively, on a voluntary basis in accordance with investigators availableness, during years 2013–2014. The convenience sample included maxillary edentulous patients treated by acrylic dentures, made at least one year ago. Mainly patients with clinical signs of denture stomatitis were targeted, and age- and gender-matched controls, namely without clinical signs of denture stomatitis. The following participants were excluded: those with any other inflammatory condition of the oral mucosa (*e.g.*, allergic reactions); those with severe cognitive, mental and locomotor deficiencies; those with physiological or pathological conditions that associates changes in thermal homeostasis (*e.g.*, febrile state, oropharyngeal inflammation); those who refused study participation.

The study was implemented in compliance with the Helsinki Declaration on Human Rights. Patients were informed about the study coordinates and gave a written informed consent for study participation.

Test methods

Reference diagnostic standard for *Candida*-associated denture stomatitis consists of acknowledgement of clinical signs of stomatitis and positive results on microbiological culture [8].

Denture stomatitis was diagnosed based on the clinical criteria, as suggested by Newton [9]. All patients were examined and assessed by two dentists, namely Elena Preoteasa and Laura Iosif, firstly independently and secondly together, reaching an agreement upon the diagnosis. Both examiners are medical specialists in Prosthodontics.

Oral candidiasis was diagnosed based upon conventional microbiological culture method. Culture specimens were collected using prefabricated cotton swabs, from six regions of the maxillary denture bearing area (*i.e.*, anterior, middle and posterior palatal area; anterior, left and right ridge crest), in order to avoid false negative test results. The specimens were inoculated on Sabouraud dextrose agar (SDA) plates supplemented with 10% chloramphenicol and incubated at 37^0C for 24–72 hours. Number of *Candida* colonies was registered by counting colony forming units (CFU) on SDA plates, values above the limit of 20 CFU/cm^2 being considered positive for oral candidiasis, this lower value of the cutoff being considered appropriate for elderly [10, 11]. Microbiological analysis was performed by an external laboratory that did not have access to the information upon clinical diagnosis of denture stomatitis. Time needed for providing microbiology result was four days minimum.

Thermographic data was acquired using the Therma CAM PM350 infrared camera (Inframetrics, Flir Systems), images being stored on a PCMCIA memory card as *TIFF* file format, and processed using the ThermaGram Pro 95 software. The thermographic acquisition and processing was done by an expert in thermography that was not professionally related to the medical field, and who, for accuracy reasons, was not informed upon patients' clinical and microbiological status. The infrared camera was calibrated considering the emissivity parameters of the human body (ε=0.98, at λ>2 μm) and ambient conditions (humidity, temperature), with a thermal range from -10^0C to 65^0C, and adequate shade selection for an optimal color registration. Considering the healthy mucosa and skin homeothermy, with thermal differences between corresponding anatomical surfaces below 1^0C, but also the ambient and patient-related factors that influence human body temperature, a standard protocol for the thermographic registrations was used. Thermographic testing was made in the same dental office during 9 AM–1 PM time interval, in a room of about 14 m^2, with constant temperature of 20–24^0C, air humidity of 45–60%, the doors and windows being closed in order to reduce the effect of air currents. Due to heat release, electronic devices were positioned at least 2 m distance from the patient. Windows and reflecting surfaces were covered with opaque dark textiles. Artificial light sources were shut down. Patients were instructed to avoid acupuncture, ultrasound and anti-inflammatory treatments 24 hours before the thermographic testing, to avoid exercise, sun exposure and psychotropic substances (*e.g.*, nicotine, alcohol and coffee) in the day of testing, and to avoid food and beverage intake one hour prior to testing. Thermography was done after 30 minutes rest period in the dental office, for thermoregulation in the testing environment. Afterwards, patients were placed into a supine position, instructed to maintain a static posture (avoid movement) and use nasal breathing (*i.e.*, on both inspiration and expiration). Plastic retractors for the soft tissue (lips and cheeks) were used for a better visual access and image capture. Thermographic registration of the maxillary denture bearing area was made (Figure 1). Distance between the palatal area and the anterior lens of the thermographic camera was about 25–30 cm.

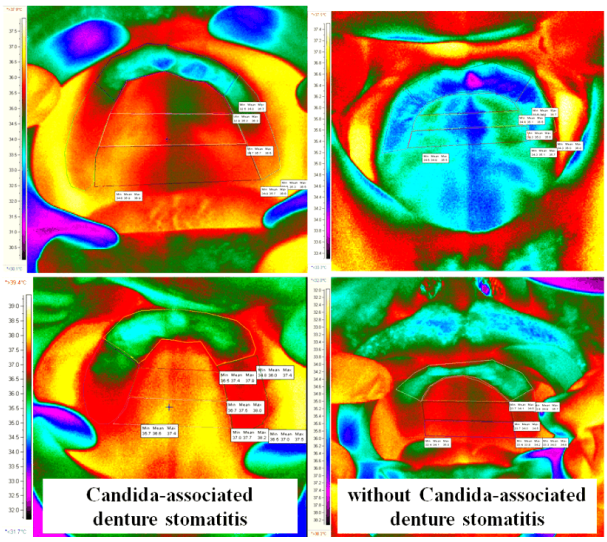

Figure 1 – ***Thermography images of the maxillary denture bearing area.***

Regarding the timeline of test methods used, clinical diagnosis of denture stomatitis, collecting specimens for microbiological analysis and thermographic testing were done in the same clinical appointment. Therefore, there was implemented a prospective data collection, this being planned before thermography and reference standard (clinical criteria and microbiological culture method).

Statistical methods

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 15. Mann–Whitney and *chi*-square tests were used to evaluate the statistically significant difference between two groups. The thermographic parameter recorded was the mean temperature of the maxillary denture bearing area. In order to better assess the use of thermography as investigation method, a diagnostic test evaluation was made, based on the analysis of receiver operating characteristic (ROC) curve and area under the curve (AUC), with the establishment of the cut-off point in accordance with the sensitivity and specificity of the thresholds. For the cut-off temperature established, in accordance with the prevalence of *Candida*-associated denture stomatitis, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-) were computed, at 95% confidence interval. The *p*-values ≤0.05 were considered to be statistically significant.

This study was performed according to STARD (Standards for Reporting Studies of Diagnostic Accuracy) reporting guidelines.

Results

Of the 56 maxillary edentulous patients, treated by acrylic dentures, included in the study, 31 were men, with a mean age of 71 years (ranged between 57 and 90 years). From these, 22 had clinical signs of denture stomatitis, and 24 had microbiologically confirmed candidiasis (Figure 2).

Considering that the number of patients without correspondence of the results of microbiological analysis and clinical assessment (*i.e.*, with clinical signs of denture stomatitis but without microbiologically confirmed candidiasis, and without clinical signs of denture stomatitis but with microbiologically confirmed candidiasis) was very low, and both conditions could have impact on temperature of the mucosa, these four patients were excluded from the forthcoming analysis.

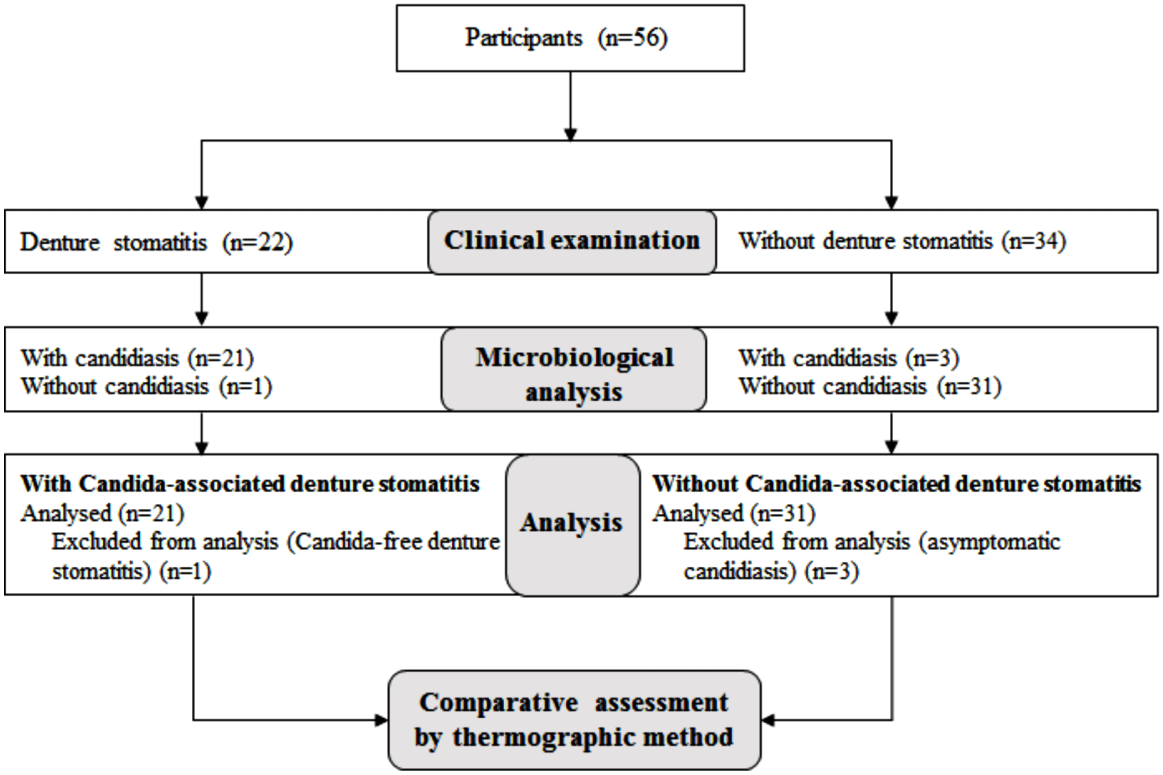

Figure 2 – ***The flowchart of the enrollment.***

Of the remained 52 patients, 28 were men, with a mean age of 71 years (ranged between 57 and 90 years), 21 with and 31 without *Candida*-associated denture stomatitis. According to the clinical presentation, seven patients had Newton type I stomatitis, nine patients Newton type II stomatitis and five patients Newton type III stomatitis. There was no statistically significant difference between patients with and without *Candida*-associated denture stomatitis regarding age and gender, but patients with *Candida*-associated denture stomatitis registered statistically significant higher temperature of the maxillary denture bearing area (Table 1).

Table 1 – ***Patients' characteristics among groups with or without Candida-associated denture stomatitis***

Variable		*Candida*-associated denture stomatitis	Without *Candida*-associated denture stomatitis	*p*
Gender	Men:Women	9:12	19:12	nss
Age [years]	Mean	71	72	nss
	Median	72	72	
	Range	57–87	57–90	
Temperature of the maxillary denture-bearing area [^{0}C]	Mean	36.20	34.85	<0.001
	Median	36.32	35.25	
	Range	34.53–37.42	33.43–35.58	

nss: Not statistically significant.

In order to better assess thermography as investigation method, the latter was evaluated as diagnostic test for *Candida*-associated denture stomatitis using ROC curves (Figure 3).

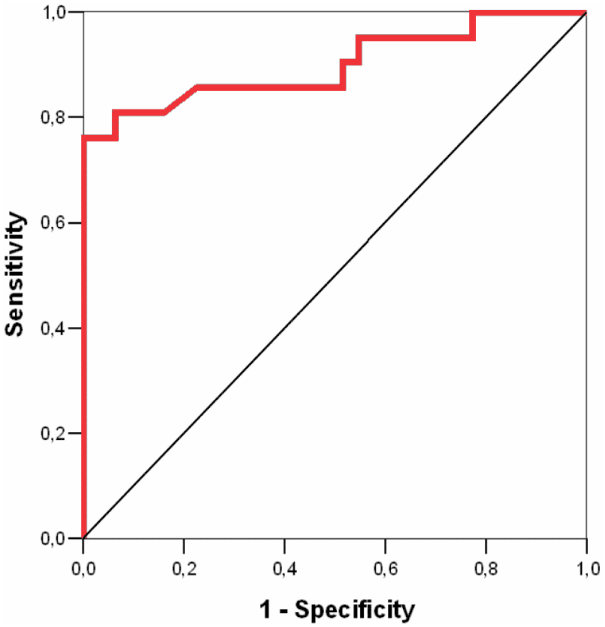

Figure 3 – ***ROC curve for Candida-associated denture stomatitis, for thermographic diagnostic test.***

The area under the curve (AUC) was 0.900; 95% CI: 0.802–0.998, $p<0.001$. The cut-off temperature of 35.44^0C provided an optimal balance between sensitivity (81%) and specificity (93.5%). For the cut-off temperature of 35.44^0C, the results of the thermographic diagnostic test can be observed in Table 2.

Table 2 – ***Thermographic test results (cut-off temperature of 35.44^{0}C) by diagnosis of Candida-associated denture stomatitis***

Results of thermographic test	*Candida*-associated denture stomatitis	Without *Candida*-associated denture stomatitis	Total
Positive	17	4	21
Negative	2	29	31
Total	19	33	52

Within these parameters, at a prevalence of 36.54% of *Candida*-associated denture stomatitis in this sample, thermography has an accuracy of 88.46%, sensitivity of 89.47% (95% CI: 66.82–98.39%), specificity of 87.88% (95% CI: 71.78–96.52%), PPV of 80.95% (95% CI: 58.08–94.44%), NPV of 93.55% (95% CI: 78.54–99.02%), LR+ of 7.38 (95% CI: 2.91–18.74) and LR- of 0.12 (95% CI: 0.03–0.45).

Discussion

According to the results of this study, considering its limitations, infrared thermography may be used as investigation method in oral disorders that may interfere on oral temperature, as *Candida*-associated denture stomatitis. The thermal threshold value of 35.44^0C was identified as best differentiating a pathological from normal state (with or without *Candida*-associated denture stomatitis) of the maxillary mucosa corresponding to the denture bearing area.

The temperature of the maxillary mucosa corresponding to the denture bearing area, was found to be statistically significantly higher in *Candida*-associated denture stomatitis in this sample (mean 36.20^0C), compared to healthy oral mucosa (mean 34.85^0C), with an average thermal difference of 1.35^0C, which, reported to the clinical context of the two investigated groups, can be interpreted in the pathological sense for cases with affected support maxillary mucosa. Thus, this thermal plus in the group with *Candida*-associated denture stomatitis, can be linked to both inflammatory and infectious factors that are interrelated, and act together in the specific biological parameters associated with wearing the acrylic denture. Wearing acrylic dentures involves morphological, histological and physiological changes of the oral mucosa (*e.g.*, changes of the palatal epithelium, of the metabolism of the soft tissue and blood vessels), as a response to the parameters implied by the denture-wearing process (*e.g.*, pressure exerted directly on the oral mucosa; coverage of the palatal area that associates disturbance of the natural airflow between the oral and nasal cavities; the polymethyl methacrylate denture base resins have low thermal diffusivity and increased surface porosity) [12, 13]. The previous can explain the increase in temperature of the oral mucosa covered by the denture-base, reported by some studies [14]. Additionally, most probably the temperature of the oral mucosa increases even more in denture stomatitis, in relation to the inflammation that characterizes this disorder. *Candida* species are normal commensals of the oral mucosa, the increased temperature under the dentures being a factor that is linked to the conversion of *Candida* into a pathogenic organism, by providing environmental conditions that trigger yeast-hyphae switch and promotes hyphae and pseudohyphae formation and growth [15–17]. Also, considering the ability of *Candida* to cause infection, its occurrence promotes inflammation of the oral mucosa, which associates an increase of mucosal temperature. This aspect should be especially considered in the elderly, where *Candida* colonization of the oral cavity increases, being suggested that the natural suppression of yeast carriage in the oral cavity breaks down in these patients [18]. Therefore, the higher temperature of the oral mucosa in *Candida*-associated denture stomatitis can be argued by the factors mentioned before, similar results being reported by White *et al.* [19, 20].

The usage of infrared technology to the clinical settings is currently accepted and considered relevant. According to the results of this study, it is suggested that thermography (as method of imaging of the heat emission) may be used as a screening test for inflammatory oral disorders of maxillary denture bearing area, as *Candida*-associated denture stomatitis. It is well known that for *Candida*-associated denture stomatitis the reference diagnostic standard is conjunctions of clinical assessment with traditional culture-based microbiological analysis. Compared to this technique, thermography has the advantage of providing an immediate noninvasive assessment, one recognized disadvantage of the culture analysis method being the delayed diagnosis, of at least 24 to 48 hours, which associate delaying of appropriate fungal treatment [21]. Additionally to being used as investigation method that brings additional important data for the diagnosis, thermography can be used for monitoring the evolution under treatment, changes of over/below 1^0C being considered as representing a clinical significant change, the method having the advantage of sensing temperature differences of 0.2^0C [22]. This is mostly appropriate in *Candida*-associated denture stomatitis, considering that relapse rate is rather high, even on competition of antifungal treatment. In these cases, using thermography bring valuable additional data for the diagnosis, that may help deciding on the clinical conduct. Due to its non-invasiveness, among other advantages there is the possibility to repeat as many times as desired the analysis, therefore technical errors can be rapidly noticed and addressed by repeating the thermography during the same appointment, and also comparative analysis of time-evolution can be assessed by comparing thermography images acquired in different moments in time.

The temperature values for the maxillary oral mucosa ranged between 33.43^0C and 37.42^0C, being partially similar to other studies, mostly depending on methods and instruments used for assessment. Other thermographic studies report higher temperatures for stomatitis compared to healthy mucosa (33.7^0C and 33.9^0C, compared to 31.9^0C), but with lower absolute values than those found in this study [19, 20]. In contrast, one recent study reports higher mean value of the maxillary oral mucosa, of 37.23^0C, but there was not considered the oral health status, such as the presence of denture stomatitis [14]. The most well known oral temperature reference is that

of 37^0C, respectively 36.3^0C in the elderly, measured through the usage of a thermometer placed sublingual [23]. Considering temperature differs according to the method of assessment used, future researches are needed in order to establish the normal thermal reference point for maxillary palatal area.

Study limitations are related to the small convenience sample, which may interfere with the validity of the results. Related to this, only clinical conditions of *Candida*-associated denture stomatitis and healthy oral mucosa (*i.e.*, without clinical signs of denture stomatitis or microbiologically confirmed candidiasis) were analyzed. Therefore, conditions defined by clinical signs of denture stomatitis without microbiologically confirmed candidiasis, and those without clinical signs of denture stomatitis but with microbiologically confirmed candidiasis, as well as other disorders that interfere with oral temperature were not analyzed, this being an important aspect to be assessed by future studies. Additionally, patients were investigated in one single moment in time, prospective evaluation, respectively after fungal treatment when appropriate, would ensure a more accurate evaluation. Therefore, further studies with larger samples, preferably prospective, are needed in order to assess if thermography could be used as a screening, diagnostic or monitoring test for inflammatory disorders in removable denture wearers, as *Candida*-associated denture stomatitis and other.

Conclusions

Patients with *Candida*-associated denture stomatitis have a higher temperature of the maxillary mucosa corresponding to the denture bearing area, compared to those with healthy oral mucosa. Infrared thermography, a rapid non-invasive investigation method, has the premises to bring valuable data in inflammatory disorders of the maxillary denture bearing area, as *Candida*-associated denture stomatitis, that may be used for screening, diagnostic or monitoring purposes.

Conflict of interests

The authors declare that they have no conflict of interests.

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