Review Article

Hypericin Over Aminolevulinic Acid - A Photosensitizer for Diagnosis of Potential Malignant Disorders and Early Oral Cancer: A Review

Parita K Chitroda[1], Girish Katti[2], Bhuvaneshwari Kalmath[3], Irfan Baba[3] Ashutoshkumar Singh[3]

[1]Reader, Department of Oral Medicine and Radiology, Al-Badar Rural Dental College and Hospital, Gulbarga, Karnataka, India, [2]Principal & Head, Department of Oral Medicine and Radiology, Al-Badar Rural Dental College and Hospital, Gulbarga, Karnataka, India, [3]Postgraduate Student, Department of Oral Medicine and Radiology, Al-Badar Rural Dental College and Hospital, Gulbarga, Karnataka, India

Corresponding Author: *Dr. Bhuvaneshwari Kalmath, Department of Oral Medicine and Radiology, Al-Badar Rural Dental College and Hospital, Opposite Koranti Hanuman Temple, Naganhalli Road, Gulbarga - 583 105, Karnataka, India. Phone: +91-8197633109. E-mail: bhuvaneshwarikalmath@gmail.com*

Abstract

It is important to develop a rapid and accurate means for the detection of early oral cancer. More recently, a new technique is introduced which uses hypericin (HY), a plant based photosensitizer that accumulates selectively in abnormal cells, including tumor cells and emits red fluorescence indicative of selective uptake when compared to normal tissue that enables to diagnose oral cancers. This review tends to highlight the sensitivity and specificity of HY over aminolevulinic acid and the application of HY in diagnosis of oral potential malignant disorders and early oral cancer as compared to standard techniques in day today practice.

Keywords: Diagnosis of oral cancer, Hypericin, Photosensitizer

INTRODUCTION

The most common cancer of the oral cavity is squamous cell carcinomas (SCC) and South Asia has relatively very high incidence rates. The main high-risk groups are adult men and women[1] who use tobacco, alcohol, and betel nut, which lead to many other potentially malignant disorders (PMD). SCC is usually preceded by dysplasia presenting as white, red or mixed red, and white epithelial lesions on the oral mucosa (leukoplakia, erythroplakia). Malignant transformations occur in 1-40% of patients with leukoplakia and 1-90% patients of erythroplakia over 5 year.[2] Thus, routine oral and dental examinations can help in the detection of oral PMD at an early stage.

The early diagnosis of PMD can reduce mortality,[3,4] thereby it becomes important to develop a rapid and accurate means for the detection of early oral cancer.[1]

The paper aims to review and highlight the non-invasive diagnosis of oral potential malignant disorders and early oral cancers using hypericin (HY), a photosensitizer that would enable monitoring and detection of these oral lesions at routine dental and oral examination.[5]

Conventional methods of diagnosis of oral PMD and malignancy are mainly done by visual examination and biopsy. It is the standard method of revealing PMD and SCC. The current approach to detecting the transformation of PMD to carcinoma is regular surveillance combined with biopsy or surgical excision. However, visual examination provides very poor diagnostic accuracy while biopsy techniques are invasive and unsuitable for regular screening and also time consuming slow process, requiring several days.[1,6]

Several studies have investigated the use of vital staining with agents such as lugol iodine, toluidine blue, and tolonium chloride for detection of oral malignancy. Sensitivity of these agents is in the hands of experts that provides approximates 90% and rapidly decreases when used by non-experts such as screeners. The specificity of these agents is poor.[7-9]

Photosensitizers

A photosensitizer is defined as a light triggered fluorescent compounds, which upon absorption of light induces a chemical or physical alteration of another chemical entity. The photosensitizer may be synthesized or induced endogenously by an intermediate in heme synthesis, 5-aminolevulinic acid (5-ALA). The photosensitizer commonly used in clinical practice has porphyrin ring structure. These photosensitizers generally have higher affinity and accumulate in the fast proliferating cancer cells than normal cells.[10]

Studies showed that these photosensitizers were used in the photodynamic therapy (PDT) to eradicate cancerous cells due to its high affinity and preferential uptake by tumor cells. The same property of photosensitizers could be used as a promising biomarker in the diagnosis of PMD and early oral cancer. Such diagnoses are commonly referred to as photodynamic diagnosis (PDD). PDD of PMD and early cancer has found its way into clinical use and has been extensively studied.[11-14]

Main properties of photosensitizer used in PDD:

- Homogeneity.
- High affinity to lesions.
- High-fluorescence quantum yield.[15]
- Rapid pharmacokinetic elimination.
- Low levels of dark-toxicity.[16]

HY, which is excited at 590 nm is a plant-based photosensitizer,[5] and has evolved in fluorescence diagnostic imaging of oral lesions. *Hypericum perforatum* (St. Johns Wort) is a plant found in the regions of moderate climate of Europe, South America and in India.[17]

Constituents of HY[18]

- HY
- Pseudohypericin
- Flavonoids
- Biflavones
- Hyperforin
- Tannins
- Procyanidines
- Xanthones
- Other constituents: Chloregenic acid, caffeoylquinic, and p-coumaroylquinic acids.

Topical or systemic application of photosensitizers can render pathologic tissues fluorescent when exposed to specific wavelengths of light.[19] Fluorescence diagnosis of oral cancer utilizes the characteristic fluorescence signatures, either from endogenous or exogenous fluorophores, to distinguish abnormal tissues from surrounding normal tissues. If the fluorescence signatures use endogenous biomolecules then, the diagnostic technique is termed as auto fluorescence diagnosis and if exogenous fluorescence are used in the diagnosis, then its exogenous fluorophores.[20]

Endogenous Fluorescence

Most of these endogenous fluorophores are weak fluorescence when excited optimally by ultraviolet (UV) blue region (300-450 nm). In addition, high-tissue scattering and strong absorption of hemoglobin in 400-500 nm region often limit the penetration depth of UV-blue light in tissue.[21] The epithelial thickening, which typically occurs in tumors, can further reduce the amount of excitation light reaching the inner sub-mucosa where most endogenous fluorophores are located.[13,22] Collagen and reduced nicotinamide adenine dinucleotide are the best examples.

Exogenous Fluorescence Diagnosis

These are synthetic fluorophores, which are excitable at longer wavelength and are used to visualize neoplastic lesions that cannot be seen with white-light imaging and auto-fluorescence.[23] HY and ALA are evaluated for its clinical application.

HY and its Photodynamic Properties

HY consistently exhibits fluorescence maxima at around 590 and 640 nm. With absorption maxima of HY being at longer wavelength, allow excitation light to reach HY in deeper tumors.[13]

HY-induced PDD has been first used in detecting flat carcinoma *in situ* lesions of the bladder.[24] Thereafter, the applications of HY were also extended to diagnose oral cancer and stomach cancer.[1,25]

The most important defining property of HY as photosensitizer in PDD is its photo stability and its fluorescence that could be detected for up to 16 h after administration as compared to 5-ALA that has fast photo-bleaching action, less tissue penetration due to low lipophilicity and lower specificity.[26]

HY exhibits tumor selectivity because of its mechanical properties such as diffusion and endocytosis.[27] Study has showed that HY has nearly 3 times higher intensity for Grade 3 cancerous cells than of Grade 1 cells.[28]

Sensitivity and Specificity between HY and 5-ALA

The diagnosis with 5-ALA has sensitivity between 78% and 100% when compared to HY, which is between 82% and 94%.[29] ALA was shown to yield low specificity ranging from 41% to 66% and so many give false positive results when compared with HY.[23] On the other hand, HY has superior specificity ranging from 91% to 98% and this greatly reduces the incidence of false positive results.[24,29]

Advantages of Fluorescence HY over Standard White Light Endoscopy (WLE) (Figure 1)

- Facilitating guided biopsies and reducing the number of biopsies.
- Providing visualization of tumor margins during surgical procedures.
- PDD allows noticing very small foci of early cancer lesion and PMD, which are visible as the red fluorescence dots on the green oral mucosa background.
- Same-day diagnosis and cancer staging in a clinical setting.[1]

Fluorescence Endoscopy (FE)

FE is the use fluorescence photosensitizer in endoscopy as it can easily overcome the shortcomings of WLE.[30] The mean value of the sensitivity for FE is 93% compared to only 73% for WLE.[23] The FE system uses excitation filter chosen to exactly match its absorption peak, and the resulting fluorescence emission could be specifically filtered for sole detection of photosensitizer fluorescence. This filtered fluorescence photosensitizer is finally interfaced to a PC for image acquisition and final analysis. The acquired fluorescence images are first pre-processed to extract the region-of-interest (ROI). The ROIs, are then extracted and expressed in terms of the color intensity as with red (IR), green (IG), and blue (IB) as well as the hue and saturation (HS) values.[1,31]

Clinical Study of Oral Disease with PDD

Photosensitizer application to PMD was studied by K jurczy sz *et al*-where in the series of photographs were taken with white light, UV light, and Blue light using hoya filters. The images with blue and UV light using the orange filter were taken and in both cases total areas of the lesion appeared to be larger than it has been clinically observed.[15]

In a study conducted by PSP Thong *et al*., 23 patients were examined by FE using HY solution (100 ml) that was topically administered to each patient by oral rinsing over 30 min. The entire oral mucosa was HY photosensitized, however, the oral mucosa with lesions showed to retain HY for a longer period of time compared to the normal oral mucosa. At 2 h after topical application, most normal tissues tend to have cleared HY, whereas abnormal lesions still show fluorescence.[1]

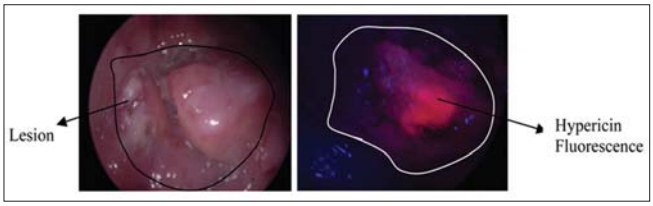

Figure 1: White light (left) and hypericin image (right) of the oral lesion acquired using a fluorescence endoscopy system

The IR/IB and IR/IG ratios were calculated for each image. These mean image parameters were used to distinguish the tissue type at three levels:

- Normal versus hyperplastic tissue
- Normal versus SCC tissue; and
- Hyperplastic versus SCC lesions.

Discussion of Image Colors (Figure 2)

- Red fluorescence was observed in lesions, indicating a selective uptake of HY in lesions compared with normal tissue. SCC tissue is most likely to be ulcerated and inflamed resulting in brighter fluorescence compared with normal tissue.
- The IR/IB ratio is a good image parameter for distinguishing between normal and hyperplastic tissues with the sensitivity and specificity of 100 and 96%, respectively; normal from SCC tissues with sensitivity and specificity of 100%, respectively; and hyperplastic from SCC with 92 and 90%, respectively.[1]
- The IG/IB ratio is not a good image parameter for distinguishing tissue types.
- The IR/IG ratio, which is the ratio of tissue fluorescence to auto fluorescence, could distinguish between normal and hyperplastic tissue and normal to SCC tissue.[1]

False positive results are observed in hyperplasia of the tongue (histologically confirmed presence of Candida) that is known to take up fluorescent dyes.[32] al-Bagieh, 1991 another condition like benign neoplasia of salivary gland, pleomorphic adenoma of the palate there may be an accumulation of higher level of photosensitizer, than usual.[1]

The use of HY as with PDD and PDT, is now applied in association with nanotechnology for therapeutic purpose and also in multimodality imaging.[20] The technology uses measured optical signals that could be essentially correlated with the pathological status of the tissues that are considered superior to WLE for the diagnosis of various types of cancer, including those in the oral cavity.[28,33,34]

CONCLUSION

HY is the strongest photosensitizer; it can be used along with WLE that helps is identification of early and flat lesions. HY when used in histopathological observation of tissues, it reduces the time required for examination. The other added advantages include reducing the number of biopsies required by guiding the biopsy procedure and aid in the delineation of tumor margins during clinical procedures. All these properties of HY in PDD could someday make it a promising applicator in the chair side

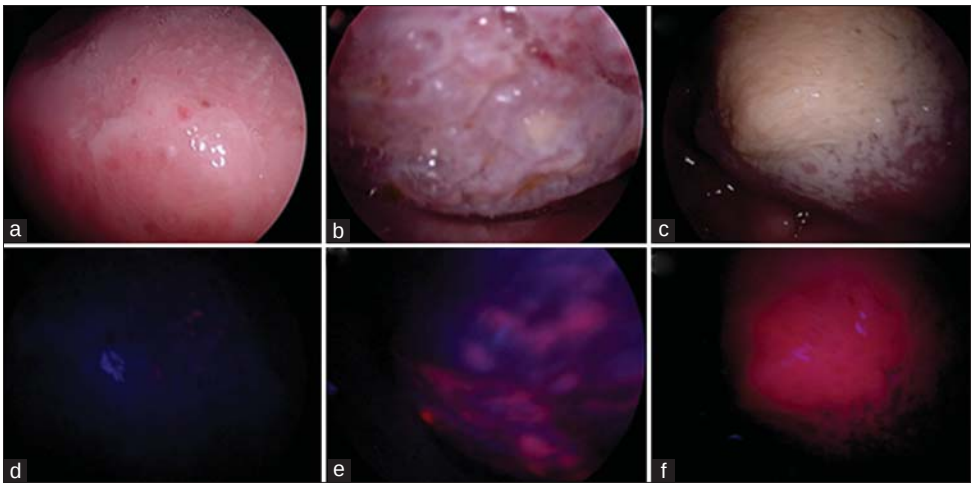

Figure 2: Images of tongue. White light images from (a-c) and hypericin fluorescence images (d-f), endoscopic images of normal tongue mucosa (a and d), images of hyperplastic tongue (b and e), images of squamous cell carcinoma of the tongue (c and f). Red fluorescence indicates pathology tissue. Blue fluorescence indicates normal mucosa. The fluorescence images show a progressive increase in the red-to-blue intensity (IR/IB) ratios from normal (a and d) (R/B ratio=0.3) to hyperplastic (b and e) (R/B ratio=1.0) to SCC (c and f) (R/B ratio=2.0) tissue

detection, conformation, and monitoring of the oral potential malignant disorders and early oral cancers as a rapid and accurate means with no much time consumption.

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