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RESEARCH ARTICLE

HPLC Analysis of Water-Soluble Vitamins (B_1, B_2,B_3, B_6, B_7 and B_9) in Milk, Human Urine and Pharmaceutical Formulations

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Abstract

A HPLC method was developed for the simultaneous determination of six water-soluble vitamins, thiamine (B_1), riboflavin (B_2), nicotinic acid (B_3), pyridoxine (B_6), biotin (B_7) and folic acid (B_9) in milk, human urine and multivitamin pharmaceutical formulations. Experimental designs have been applied for the optimization of the method in order to determine the experimental conditions for maximized resolution and minimized retention time. The water-soluble vitamins were analyzed by HPLC on a Dionex Acclaim 120 C_{18} column (4.6 × 250 mm; 5 µm) with Dionex UVD170U detector operated at a wavelength 270 nm. Isocratic elution was performed using solvents methanol: phosphate buffer (0.0025 M, pH 3.5) in the ratio of 35:65 (v/v) at a flow rate of 0.8 mL/min. All six water-soluble vitamins were separated in a single HPLC run within less than 11 min. Detection limits were in the range of 6.29-13.65 ng/mL. Accuracy, intra-day repeatability (n =6), and inter-day precision (n =6) were found to be satisfactory. Sample preparation of biological fluids was performed by SPE (Solid Phase Extraction) and MEPS (Micro Extraction by Phase Sorbent) using C_{18} as packing material with methanol (50:50) as eluent adhering to green chemistry principles.

Introduction

Vitamins are a broad group of fundamental constituents of nourishment required for the normal growth, self-maintenance and functioning of human and animal bodies [1]. Vitamins are grouped by their biological and chemical activity, not by their structure. Generally, these compounds can be classified in two main groups: water-soluble and fat-soluble vitamins. Among water-soluble vitamins, the B group including thiamin (Vitamin B1), pyridoxine (Vitamin B6) and cyanocobalamin (Vitamin B12) are the most critical needed for good vision, red blood cell formation, healthy skin, nervous system and normal appetite. Each member of the B-complex has a unique structure and performs vital functions in metabolism, and their lack or excess produces specific diseases [2-5]. Undoubtedly vitamin examination is ending up noticeably more across the board, and subsequently a quick, simple and dependable technique for vitamin investigation is required by the food and pharmaceutical industry. Determination of vitamin B in a complex matrix is cumbersome due to the presence of multiple biologically active forms [6]. Vitamins could not be determined directly as they are present in phosphorylated or glycosylated

Examination of the eye on diffuse illumination revealed grayish white /slate blue pigmentation of the right lower lid and the sclera (Figure 1) while, on fundoscopy dark retina (Figure 2) as compared to fellow eye was seen. Ocular tension was normal.

Figure 1: Depicting gray/slate white macules affecting the right lower eye lid and sclera of eye.

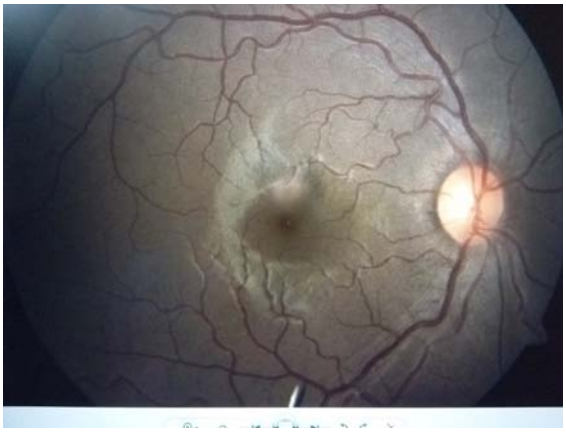
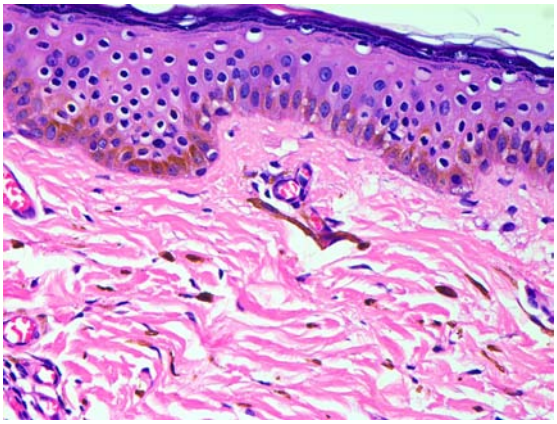


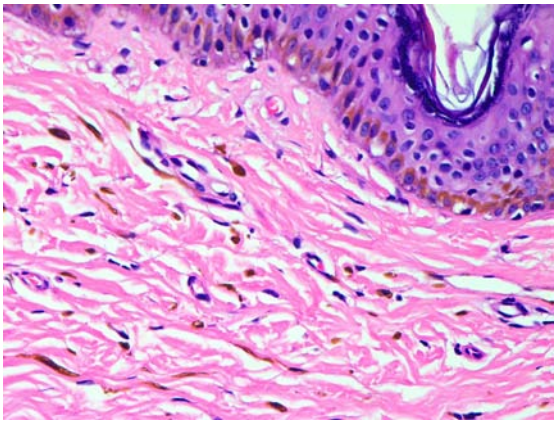
Figure 2: Fundoscopy, showing darker retina of the right eye.

Histopathology

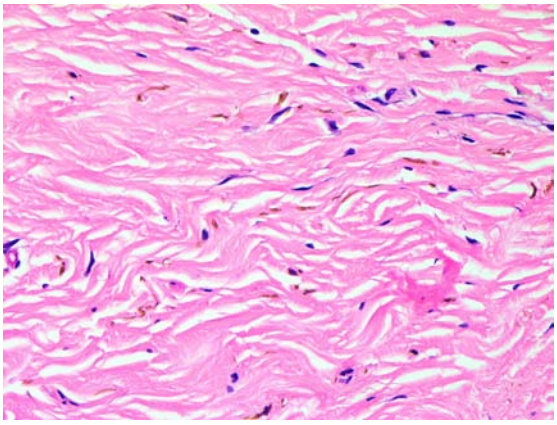
Haematoxylin and eosin stained sections prepared from the representative lesion, showed an apparently unremarkable epidermis, and dermis with scattered pigment visible throughout the dermis. Single, scattered, bipolar, slender spindle shaped, melanocytes intermixed with collagen bundles of the dermis was a prominent feature. A few melanophages (plump cells) were also visible (Figure 3 a,b,c).



(A)



(B)



(C)

Figure 3a,b,c: Showing Single, scattered, bipolar, slender spindle shaped, melanocytes intermixed with collagen bundles of the dermis. (H&Ex100; H&Ex400; H&Ex400).

Discussion

Nevus of OTA also known as nevus fusco caeruleus opthalmomaxillaris [1] / congenital melanosis bulbi [5], oculodermal melanocytosis Its presence on the face is

cardinal, embarrassing the distribution of ophthalmic and maxillary branches of the trigeminal nerve [6,7] impelling the patient to seek advised for cosmetic abrasion, despite it being asymptomatic.

Nevus of OTA histopathology is imperative to perform in order to identify its characteristics features, wherein, epidermal involvement is conspicuous by absence, implying that it is abrasion of the dermis and its adenexa, evidence as prominent sebaceous lobules of the facial skin in addition to many slender, dendritic, pigmented melanocytes arranged horizontally between / entrapped amongst the collagen bundles in the mid-dermis. Melanophages are either few or none. Melanocytic proliferation extending deep into the sub-cutis. The preceding histopathology was characteristics enough to prompt an endeavor to define its plausible classification [8], envisaging the following, the advantage of which may ultimately percolate in the management[6] of nevus of OTA.

- Superficial (type S),
- Superficial dominant (type SD),
- Diffuse (type Di),
- Deep dominant (type DD),
- Deep (type De)

The case under review conformed to superficial (type S), warranting cognizance of application of this classification in the future deliberations on the subject. Periodic follow-up of the eyes [9] to take stalk of definition of clinical features and subsequent changes is recommended to be carried in order to prevent complication arising out of affliction of the eye. Currently, the QYAG5 [10] Q-switched lasers has become and acceptable treatment modality, and is being recommended as a feasible option for the purpose [11,12]. Q-switched lasers are better than "cosmetic abration" [13].

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