INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Comparative Evaluation of Lipid Profiles of Nose and Blood in Leprosy Patients using Thin Layer Chromatography

MC. Prabhakar[1], D. Santhi Krupa[2*] and N. Manasa[3]

[1]Shri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh, India.

[2]Aditya College of Pharmaceutical Sciences and Research, Surampalem, Andhra Pradesh,India.

[3]Anurag College of Pharmacy, Ananthagiri, Andhra Pradesh, India.

ABSTRACT

Leprosy has long latent, induction period which is often difficult to diagnose in the early stages. Our present study is aimed to compare lipid profiles of nasal flushings and plasma samples of MB Patients using Thin Layer Chromatography (TLC). Nasal flushings collected by jalaneti technique and blood samples collected by double syringe method was processed to extract the lipid contents. The concentrate was reconstituted by adding 0.4 ml of chloroform and 0.2 ml of methanol on vortex mixer. TLC was run on a readymade silica gel plate in a solvent system of chloroform: methanol: water (65:25:4). The plates were dried and the spots were marked after spraying with the detector reagents and the number of spots were noted. In our present study we had compared the nose and plasma lipid profiles of MB patients (n=36). Some MB patients showed more lipid spots in nostrils and less number of lipid spots in blood. Some MB patients showed 4–8 lipid spots in blood and nearly equal number of lipid spots in the nostrils. The patients having more nasal involvement had observed to produce more number of lipid spots in TLC. Determination of lipid Profiles based on TLC can be used as simple diagnostic tool to distinguish between PB and MB leprosy patients in early cases. Moreover this is a cost effective, less time consuming, reproducible and reliable method.

Keywords: Autacoids, Lipid, Reconstituted, Diagnostic, Spraying.

INTRODUCTION

Leprosy is often difficult to diagnose in the first place, with stigma leading to reluctance to self report in many societies. It has struck fear into human beings for thousands of years, and was well recognized in the oldest civilizations of China, Egypt and India. Leprosy is a chronic infectious disease caused by *Mycobacterium leprae,* an acid-fast, rod-shaped bacilli. *M. leprae* was discovered by Norwegian physician, Gerhard Henrik Armauer Hansen in 1873, it was the first bacterium to be identified as causing disease in man. Leprosy has long latent, induction period, usually three to six years in the tuberculoid form of disease and three to ten years in the multi bacillary form[1]. Its transmission rate greatly exceeds the clinical attack rate. The disease mainly affects mucosa of the upper respiratory tract, the eyes, the skin and also the peripheral nerves. The first symptom normally noticed in the advanced cases of Leprosy is the nasal stuffiness as in them the nostrils may be packed with the plugs, clots of debris and *M. leprae*[2]. Such patients find it difficult to breath. *M. leprae* that lodge in the nose releases a variety of autacoids which are responsible for the emission of intolerable foul smell, upper palate perforation, and inflammation of the nose. During inflammation, the blood vessels get dilated so the *M. leprae,* depending upon their size, can penetrate beneath the skin to reach different parts of the body from the nose to various peripheral sites[3]. *M. leprae* are nonmotile organisms but are thrusted forward from the site of higher density that is the nose to different parts of the body, will reach the peripheral sites like ear pinnae, fingers, toes and the scrotum in the male patients.

MATERIALS AND METHODS

MB patients (n=36) from Leprosy centre and Leprosy colony in Bhimavaram subjects were

involved in this study. All the methods are prior approved by institutional ethical committee.

TLC Plates

Readymade marketed silica gel-G-coated on aluminum plates with thickness of the layer 0.2 mm were used (E.MERK). The solution of total lipids prepared in chloroform: methanol (1:2) was applied on to plates at a distance of 1 cm from lower edge of the plate.

Solvent system

Chloroform: Methanol: Water = 65:25:4.

Spray reagent used

Iodine vapours

This was used as a general purpose detector for all lipids, since both saturated and unsaturated lipids absorb iodine by simple dissolution.

Preparation

Iodine crystals were packed between cotton plugs in a Pasteur pipette and this was blown on to the TLC plates by using aerator. Lipid spots turned yellow on exposure to iodine vapors. The spots were marked with a pencil soon after exposure to iodine vapors as they were disappearing with time.

Extraction of lipids from nasal flushing

After Collection of sample by "JALANETI" method, it was processed to get concentrated mucous[3]. 0.8 g of mucous was transferred to a stoppered 8 ml glass tube. To this 1 ml chloroform, 2 ml methanol were added and mixed on vortex mixer. These tubes were set aside for one hour. During this tubes were occasionally vortexed. Care was taken to see that the mixture in the tube was homogenous. The mixture was filtered using fluted filter paper into bigger test-tube. To this filtrate added 1 ml of chloroform and about 10 to 15ml of distilled water with occasional mixing till bottom chloroform and top aqueous–methanol layers were distinctly separate. The bottom layer was transferred to glass tube , clarified by adding few drops of methanol and traces of water was removed from chloroform phase by azeotropic distillation with benzene during concentration of extract in vacuum at 35° C. The concentrate was reconstituted by adding 0.4 ml of chloroform and 0.2 ml methanol on vortex mixer. TLC was run on readymade silica gel plate in a solvent system of chloroform: methanol: water (65:25:4). The plates were dried and spots were marked after spraying with detector reagents and the number of spots was noted.[4,5]

Extraction of lipids from plasma

Double syringe method was used for the blood collection from the patients,[6] which prevents the entry of *M. leprae* into the blood through the needle while pricking the skin[7]. This method follows withdrawal of 0.1 ml of blood and changing the syringe to a fresh second one but using the same needle. The blood was added to a test tube and mixed thoroughly and centrifuged at 2000 rpm for 5 minutes. Plasma was separated, 0.8 ml of plasma was added to 1 ml of chloroform and 2 ml of methanol and mixed on the vortex mixer and set aside for one hour. Care was taken to see that the mixture in the tube was homogenous. The mixture was filtered using fluted filter paper into bigger test tube. To filtrate was added 1 ml of chloroform and about 10 to 15 ml of distilled water with continuous stirring till chloroform (bottom) and aqueous–methanol (top) layers were distinctly separated. Bottom layer was transferred to a glass tube, clarified by adding few drops of methanol and traces of water was removed from chloroform phase by azeotropic distillation with benzene during concentration of extract in vacuum at 35° C. The concentrate was reconstituted by adding 0.4 ml of chloroform and 0.2 ml of methanol on vortex mixer. TLC was run on a readymade silica gel plate in a solvent system of chloroform: methanol: water (65:25:4). The plates were dried and the spots were marked after spraying with the detector reagents and the number of spots was noted.

RESULTS AND DISCUSSION

One of the most essential components of effective control of leprosy is its detection in early stage. Nasal symptoms in leprosy cause considerable distress both psychologically and physically and hence it has been emphasized that regular care of nose is of great of importance in the overall management of leprosy[8,9]. There has been a constant search for a simple and convenient test by which leprosy patient s belonging to a particular type could be identified and treated accordingly at the earliest[10]. In our present study we had compared the nose and plasma lipid profiles of MB patients (n=36). Some MB patients showed more lipid spots in nostrils and less number of lipid spots in blood. Some MB patients showed 4-8 lipid spots in blood and nearly equal number of lipid spots in the nostrils. The number of lipid spots may also depends on spraying agent used and also the presence of autocoids during inflammation[11]. The patients having more nasal involvement had observed to produce more number of lipid spots in TLC. The patients with no nasal involvement had also shown to develop more lipid spots; this may be due to recent increase in the live *M. leprae* in their nose. From TLC studies it is clear that in such patients whose nasal spine is collapsed there is a free access and exchange of the constituents of the blood and the nostrils, mainly the lipids[12]. These studies revealed that number of lipid spots seem to depend on the extent of nasal damage thus greater the damage more were the lipid spots. This

technique can differentiate PB and MB patients within a very short period and assist in starting the right kind of therapeutic regimen soon after diagnosis.

CONCLUSION

This can be laudably proposed as a simple diagnostic tool to distinguish between PB and MB leprosy patients. The nasal flushings of MB patients had shown more number of lipid spots than the blood sample of the same patients, which is due to more break down of lipids in the nose. The existence of various autacoids to cause the inflammation of nose can also be studied using this technique. This TLC characterization can be useful to early diagnosis and proper assessment of treatment based on the type of patient.

ACKNOWLEDGEMENTS

We are very thankful to the staff of franscogna leprosy rehabilitation centre sisters and to the staff of chemistry department of Shri Vishnu College of Pharmacy, Bhimavaram for their cooperation during our study.

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