ORIGINAL ARTICLE

An Observational Study on The Effects of Cushing Syndrome in Pulmonary Nocardiosis

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ABSTRACT

Objective: Nocardia species is comprised of strict aerobic bacilli which forms the branched hyphae in culture and tissues. Nocardia infection is not well-known but its importance cannot be underestimated due to the mortality and morbidity inorgan transplant cases and immunocompromised cases. As Nocardia species is varying in the various regions, this research is targeted on the study an isolate Nocardia bacteria from immune-compromised cases which were referred to the specialists.

Material and Methods: The research was carried out in Mayo Hospital Lahorewith 142 participants observed with advancedsymptomatic pulmonary disease. Total sample 102 cases were assessed for sputum and Broncho alveolar lavage. In the remaining cases the collection of samples was not possible because of the accessibility issue. We prepared three thin smears to observed the samples through microscopic. Culturing of the samples was made in paraffin agar and sabouraud dextrose agar. To analyze the data, we also documented history of the patients through questionnaire. Asteroids of the Nocardiawere isolated from one case who was suffering from the syndrome of Cushing having bronchogenic carcinoma. Further in-vitro studies for isolate differentiation were carried out in order to confirm the growth of organism on primary media which was Nocardia steroids complex.

Conclusion: It is revealed through NaOH normal concentration 4%, which has been used for the Mycobacteria species identification, can stop the Nocardia growth. Therefore, collected samples were processed through decontamination procedure to isolate Nocardiawith the help of NaOH (1%).

Keywords: Pulmonary Nocardiosis, Cushing's Syndrome, Nocardia Asteroids Complex and Immunocompromised Host

INTRODUCTION

Nocardia is a gram positive acid-fastaerobic variable bacterium is an opportunistic pathogenin immune-compromised hosts. Nocardia species are available in nature like soil and water borne environments. Nocardia species in various varieties have been identified. In these diseases there are many potential pathogenic issues to humans including Nocardia steroids,N. otitidis caviarum, N. Brasiliense's and N. transvalensis. Current taxonomic Nocardia steroids taxon research studies have categorized this species with the N. nova and N. farcinica[1].

Pulmonary Nocardiosis impersonates pulmonary TB through clinical investigations and radiological features, it is also treated in a wrong way through anti TB medications. A systematic immuno-suppression, specifically an immunity mediated through cells disorders is counted as the vital Narcodial attack disposal factor which is harmful for the lungs occurring repeatedly in kidneys, liver, heart and lung graft recipients through the transplantation of the bone marrow[2]. In the patients of non-immunocompromised nature, the incidence of disease is also observed. It is demonstrated through all the previous research studies about the nocardiosis in different disorders which include systemic lupus erythematosus, chronic myelogenous leukemia combined with lung carcinoma, HIV and common pulmonaryinfection caused by the Nocardia steroids complex in the bronchiectasis patients[3].Our research was aimed at the Nocardia bacterium isolation from pulmonary infectious diseases patients, for the characterization of the isolated Nocardia on the level of the species; furthermore, it was also aimed at the isolation of bacteria from BAL samples and sputum. Various NaOH concentrations effects on the isolation ability of the organism was also under investigation.

MATERIAL AND METHODS

In this study 142 pulmonary infectiousdiseases patients in the time span of Jan, 2019to Aug, 2020in Mayo Hospital Lahore.The selected patients were suffering from the indexes of bronchoscopesuch as radiological anomalies, progressive pneumonia, atelectasis, pleural effusion and hemoptysis. Every patient was asked to fill a questionnaire before the start of the research. Research questionnaire included personalspecifications, disease and patient symptoms and medical history.Two samples of BAL and sputum were collected from every patient. The instructions were passed to every patient that the sample of sputum is to be taken from the deep part of the trachea. Since the procedures of decontamination may pose certain inhibitory effects Nocardia growth, no chemical treatment was given to the samples of the patients. To treat the BAL samples, we required centrifuge for lavage concentration. In order to achieve this ten minutes' centrifuge process was carried out at the rate of 1500 revolution per minute. We discarded supernatants and assessment was carried out in the deposited material.

We prepared 3 smears (thin) for every collected sample to carry out Kinyoun and gram staining. Processing of staining was carried out as described. Culturing of the samples was completed on paraffin agar and Sabouraud dextrose agar. Injected media were incubated atthe temperature of 37°C & 45°C. The recovery of the Nocardia species cabe achieved at 45°C. We also cultured the suspected colonies through blood agar for the assessment

and observation of the typical colonies.After micro &macroscopic assessment we observed that Nocardiaexistence was differential tests; substances hydrolysis such as Adenine, Esculin, Casein, Gelatin, Tyrosine, Hypoxanthine and Xanthineagar, along with ability tests of organisms for the utilization of the Arabinose, Inositol, Glucose, Rhamnose and Mannitol as the sources carbon.We also suspended standard Nocardia steroid organism in mixed and saline for a period of one minutewith vortex mixer. Organismsclumps wereadjusted to turbidity that was equal to the standard McFarland (0.5). We also treated every bacterial suspension for a time of fifteen minutes with (0.5, 1, 2,4, & 6 percent) NaOH concentrations containing red phenol. After the fifteen minutes suspension the neutralization process was carried out with HC – 1 and (0.1 ml) of each dilution that was subculture through Sabouraud dextroseagar plates and incubation of forty-eight hours on a temperature of 37°C was also carried out.

RESULTS

We studied 142 cases in the time of 7 months and patients' distribution is reflected in Table – I in age wise order.Both sputum and BAL samples and testing was carried out in 102 patients, remaining forty patients were not accessible for the collection of sputum sample. High risk disorders of immunocompromised have been shown in Table – II.

Table – I: Age Wise Distribution

High risk patients	Frequency Percentage	
Chronic Pulmonary Infection	5	4
Diabetics	22	18
Cancer-Leukemia	5	4
Other Infectious Diseases	9	7.2
Combined CPI & D	3	2.4
Unclassified	71	58
Corticosteroids treated	5	4
Organ transplantation	3	2.4
TOTAL	123	100

Table – II: High Risk Disorders of Immunocompromised

Age Grouping	Frequency	Percentage
20 - 20	5	3.5
21 - 30	9	6.3
31 - 40	15	10.6
41 - 50	33	23.2
51 - 60	40	28.2
61 - 70	21	14.8
> 70	19	13.4
Total	142	100

Table – III: Nocardia Asteroids as Primary Media

Character	Isolated Nocardia	Nocardia asteroids	Nocardia farcinica	Nocardia nova	Nocardia brasiliensis	Nocardia caviae	Nocardia transvalensis
Hydrolysis of: Adenine	-	-	-	-	-	-	v
Casein	-	-	-	-	+	-	-
Esculin	-	-	-	-	+	-	-
Gelatin	-	-	-	-	+	+	+
Hypoxanthine	-	-	-	-	+	-	-
Tyrosine	-	-	-	-	+	±	±
Xanthine	-	-	-	-	-	v	v
Utilization of: Arabinose	-	-	-	-	-	-	-
Citrate	+	+	-	-	+	-	+
Glucose	+	+	+	+	+	+	+
Inositol	-	-	-	-	+	+	+
Mannitol	-	-	-	-	+	v	v
Rhamnose	-	-	+	-	-	-	-
Growth at: 45°C	-	±	+	-	-	±	-

Diagnosis of microbial was made after the isolation of Nocardiain broncho-alveolar lavage(BAL) and in the samples of sputum. Single species isolation was possible through Nocardiain only single case who was affected by Cushing's syndrome having bronchogenic carcinoma (ACTH, ectopicsyndrome). The isolate was a gram positive that was also partially fast acid having rod elements which were finely branched. Through differential tests on Casein, Adenine, Esculin, Hypoxanthine, Gelatin, Xanthine and Tyrosine agar, the organism's ability for the Arabinose, Inositol, Glucose, Mannitol, Rhamnose as an alone source of carbon having observations of the microscope which were confirmed through the growth of organism through the Nocardiaasteroidesas primary media as shown in Table – III.Decontamination procedure with adverse effects having various NaOH concentrations is also studies in our research. It is controversial to use various substances of chemical as NaOH,L-Cysteine, N-acetyl and benzylkonium chloridein tri-sodium phosphate (Zephiran – TSP)to decontaminate the specimen except Nocardia. For the same purpose we also carried out NaOH serial dilution. The obtained results also revealed that Nocardia growth is inhibited through the NaOH concentrations, which makes NaOHan unreliable substance for the clinical specimen decontamination purpose.

Nocardia growthin NaOHconcentrationsabove (1%)were inhibited as we observed in this research. The dyspnea patient was at the age of forty-three years before being hospitalized felt weakness and fatigue. The patient also shared that severe weakness is observed by him from last one year, he also an incidence of DM.To observe the presence or absence of Cushing's syndrome we carried out Endocrine work. High and low dose dexamethasone suppression helped in the revelation of the plasma high levels with not suppressed cortisol when the dexamethasone dose was high.Through radiographic

observations we observed that entire left lung was involved when used nodular infiltration. Due to the normal levels of ACTH, there was a suspected hypercortisolism from ectopic ACTH tumor production. Through Pathological reports it was evident that patients were facing bronchogenic carcinoma. A deeper investigation also revealed the Ectopic ACTHsyndrome presence which was also confirm.

DISCUSSION

An important identification of the acid fast in organisms can be made through Ziehl – Neelsen (ZN) staining specially in the primary Mycobacterium tuberculosis. However, weaker acid decolorizationfor the concentration of acid (such as sulfuric acid 1%), can produce variety of the organisms appearing in the acid fast[4]. The fastness of the acid is the partial feature of Nocardioform family on mycolicacid which is presented in the bacteria cell wall. In case there is an invasion of the Nocardiabacterium in body a number of molecules of the carbon in the walls of the cell increases the protection against the immune system[5].In this state the myceliumof partial acid-fast stained Nocardiawill appear in the shape of filamentous or pink bacilli through light microscope. We have also considered the same feature in this research[6]. Which is also a differential feature that differentiatesNocardiafrom same natured morphologically organism likeActinomyces and Streptomycesspecies. After fewNocardiasub-culturing, the partial acidfastness features maydisappear and its identification is also difficult through smear for the identification of the mycelia or bacterial element[7].

We undertook the present research for the isolation of the Nocardiabacterium from pulmonary nocardial patients having historical chest symptoms. All negative sputum cases for ZN ondirect smear assessment for consecutive probe of the Nocardiathrough sputum assessment and in bronchoalveolar lavage liquid which is obtained through bronchoscopy[8]. The smears processing was carried out through Kinyounstaining method(Modified ZN). We observed a case of the positive nocardial infection also suffering from the Cushing's syndrome having bronchogeniccarcinoma[9].A similar research was carried out in Japan on the pulmonary nocardiosis patients that was hospitalized to be assessed through multiple nodules; these nodules were disclosed through chest roentgenogram[10]. After that it was identified as the adrenocorticotropic hormone(ACTH) which was also depending on the Cushing's syndrome.Bronchial secretion samples obtained throughfiberoptic bronchoscopy contained numerous bacteria's of Nocardiaasteroids [11].It is believed about the therapy of corticosteroids it is a pre-condition for the opportunistic Nocardia. We can conceive on the grounds of this idea that patient with the nocardiosisdevelopment in prolonged endogenous hypercortisolism, as in the absence of predisposing factors. Number of lymphocytes and eosinophils can be decreased through the high cortisol concentration in the blood[12]. The occurrence of this phenomenon can be obtained in very less time and worst shape can be obtained through the lengthy processing. Furthermore, in the large dose administration of cortisol results in the shape of severe atrophyin all body lymphoid organs, resultantly the outcome of the antibodies and T cells production decreased.It is therefore the humoral immunity cannot be that much strong that can fight and defend the agents like Nocardiabacterium[13].

We observed in this research that with the help of differential tests and microscopic observation through various substances, it was confirmed that all the growing organism primarilyon the media that was Nocardiaasteroids complex. Differentiation of the bacteria can be made through Nocardia nova and Nocardiafarcinica with the Rhamnose pattern, Citrate andgrowth at the temperature of 42°C as shown in Table – III[14].The research demonstrates Nocardia bacterium identification in bronchoalveolar lavage(BAL), which was successful. An expert was performed all the collections of the samples; whereas, the specimen of the sputum may be free of Nocardiabecause of the pulmonary infection which is also localized. We can also say that infection is not transferred through pulmonary bronchiole[15]. Bacteria trapping is not possible. In the collection of the samples of the sputum with BAL from every patient that will increase the isolating chances of the Nocardia, it is to be confirmed that sputum is sample is to be collected from depth of the chest through strong coughing. AlthoughNocardiaidentification also requires laboratory assessment to observe the partial acid fast in the helpful stained smears[16].

CONCLUSION

It is revealed through NaOH normal concentration 4%, which has been used for the Mycobacteria species identification, can stop the Nocardia growth. Therefore, collected samples were processed through decontamination procedure to isolate Nocardia with the help of NaOH (1%).

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