

Infectious Diseases Journal of Pakistan
Official Organ of the
Medical Microbiology & Infectious Diseases Society of Pakistan

President Bushra Jamil
Internal Medicine, Infectious Diseases
Aga Khan University Hospital,
Karachi, Pakistan

Gen. Secretary Summiya Nizamuddin
Section of Microbiology
Shaukat Khanum Memorial Cancer Hospital
and Research Centre, Lahore, Pakistan.

Treasurer Sunil Dodani
Department of Infectious Diseases,
Sindh Institute of Urology & Transplantation
Karachi, Pakistan

Editorial Office

Editor: Ali Faisal Saleem

Associate Editors: Iffat Khanum
Sonia Qureshi
Muhammad Idris Mazhar
Sunil Dodani
Nosheen Nasir

Editorial Board: Aamer Ikram Naseem Salahuddin
Altaf Ahmed Ejaz A. Khan
Shehla Baqi Luqman Setti
M. Asim Beg Naila Baig Ansari
Rana Muzaffar

Manager MMIDSP: Luqman Mahmood

Rights:

No part of this issue or associated program may be reproduced, transmitted, transcribed, stored in a retrieval system or translated into language or computer language in any form or means, electronic, mechanical, magnetic, optical, chemical, manual or otherwise without the express permission of the editor/publisher and author(s) of IDJ.

Disclaimer:

Statements and opinions expressed in the articles, news, letters to the editors and any communications herein are those of the author(s), the editor and the publisher disclaim any responsibility or liability for such material. Neither the editor nor publisher guarantee, warrant, or endorse any product or service advertised in their publication, nor do they guarantee any claim made by the manufacturers of such product or service.

Submission:

Infectious Diseases Journal (IDJ) is published quarterly. Please submit manuscripts at pak_idj@yahoo.com. See author guidelines.

Designed & Published by:

Medishine Publications
A-452, Ground Floor, Block 7, K.A.E.C.H.S, Karachi.
Tel:34555263, E-mail:veterinaryguide@yahoo.com

Proprietor:

Medical Microbiology & Infectious Diseases Society of Pakistan
21 G /1, Block - 6, P.E.C.H.S., Shahrah-e-Faisal, Karachi. Ph: 0333-3977011
E-mail: info@mmidsp.com

CONTENTS

PAGE #

EDITORIAL

Misuse of Antibiotics in COVID-19 Era
Muniba Aslam, Sunil Kumar Dodani, Asma Naseem 68

ORIGINAL ARTICLES

Amphotericin B associated nephrotoxicity – A single center retrospective study
Muhammad Ammar Shafqat, Aun Raza, Junaid Iqbal, Amina Nawaz, Muhammad Abu Bakar, Faisal Sultan 70

Psychosocial Aspects of Covid-19 and Self-confidence: we can beat it.
Hamzullah Khan 75

Frequency of Chikungunya virus infection in samples tested concomitantly for dengue and malaria: experience from clinical laboratory, Karachi Pakistan.
Uzma Shahid, Joveria Farooqi, Faisal Riaz Mailk, Erum Khan 81

CASE REPORT

Rhinofacial Basidiobolus Misdiagnosed as Granulomatosis with Polyangiitis
Aneela Hussain, Aisha Ilyas, Kausar Jabeen, Syed Faisal Mahmood 85

INSTRUCTIONS FOR AUTHORS 88



15 Y/F, with Autoimmune Vasculitis admitted with sever left leg and foot pain. She also developed left foot ankle joint ulcer with discharge.

Description

Lucency within the left navicular bone representing infection. Lucency within the subcutaneous soft tissue along the medial aspect of left ankle joint likely representing ulcer.

No evidence of fracture or dislocation.

Underwent Wound debridement, tissue culture grew MRSA and Pseudomonas aeruginosa.

Diagnosis: Autoimmune Vasculitis with Secondary Bacterial Infection

Courtesy : Ali Faisal Saleem, Associate professor, Paediatric Infectious Diseases, Aga Khan University, Karachi, Pakistan

Misuse of Antibiotics in COVID-19 Era

The emergence of SARS-COV-2 pandemic in late December 2019 has taken the health care community around the world by storm. The race to medical breakthroughs for COVID treatment has shifted focus from other public health concerns that were at the forefront in the pre-COVID era. Among these are the alarming developments in antimicrobial resistance; a crisis in its own right which Larry Kerr, co-chair of the transatlantic task force on antimicrobial resistance describes as 'a multitude of small fires that are much less visible than the single massive firestorm that is the covid-19 pandemic.'¹ The first wave of the pandemic marked by diagnostic limitations and uncertainty about the pathophysiological nature of the virus saw a gigantic upsurge in the indiscriminate use of broad-spectrum antibiotics that has not declined even as we approach the end of a year. The scarcity of sufficient data detailing antibiotic prescription to Covid patients during the pandemic masks the probable long-term consequences of this lapse in antibiotic stewardship.

Based on limited data, we know that 70% of hospitalized COVID 19 patients receive antibiotics although only 8% had a bacterial co-infection and only 16% later developed a secondary bacterial infection.² The unpredictable course of the disease in addition to the high workloads in hospitals and increased admissions and antimicrobial prescriptions for respiratory tract infections has undoubtedly contributed to exacerbation of difficulties already present in implementation of antibiotic stewardship. Guidelines published by different authorities, especially during the initial period, contain varying recommendations thus creating a degree of uncertainty about best practice. These recommendations also lacked a solid evidence base with the use of antibiotics being recommended in severe disease by European and Italian guidelines and only for critical patients under Chinese guidelines.³

In Pakistan, as in other places around the globe, the documentation of the selection and course of antibiotic therapy has been largely ignored. Of more than 900,000 cases thus far confirmed in Pakistan, only 2.1% deaths and a 90% recovery rate showcase a wide mismatch between infectivity and serious disease in the country.⁴ Since many patients have mild disease, the majority must have only passed through Pakistan's weak primary care system. With already a pandemic of antibiotic usage in Pakistan, highly broad-spectrum antibiotics are being prophylactically prescribed to all patients with any respiratory tract symptoms in the last 1 year. It must be acknowledged that antibiotic prescription is often also a patient's demand, fuelled by the misinformation on social and mainstream media. Poor regulation of antibiotics and easy access to all kinds of information gives way to self-medication.

A retrospective observational study of COVID-19 patients from March 2020 to April 2020 in New York City shows that 70%

of all admitted patients received antibiotics among which were doxycycline, azithromycin, levofloxacin, ciprofloxacin, ceftriaxone, cefepime, intravenous vancomycin, and piperacillin/tazobactam. The use of triple therapy was also very common with 70% of patients receiving >3 classes of antibiotics together (β -lactams, glycopeptides, macrolides, or tetracyclines). The study also shows 100% of patients with MDR strains had previous history of broad spectrum antibiotic use within the same hospital stay. The same institute saw the susceptibility of *Klebsiella pneumoniae* to Cephalosporins, Ciprofloxacin and Meropenem fall by 10% from 2019. The number of *Enterobacteriaceae* isolates resistant to Carbapenems also increased in number compared to previous year.⁵ The ramifications of the liberal use of drugs classified as 'critically important antimicrobials' by WHO such as Azithromycin, Vancomycin, Carbapenems, Tigecycline, Ceftriaxone and Linezolid may well be felt during the pandemic. The development of resistance against these drugs reduces the survival of COVID patients with secondary bacterial infections due to prolonged hospital stays and immunosuppressants like Tocilizumab. The long-term implications of exacerbating one crisis while averting another will cost lives and resources, for example Azithromycin use in Covid pneumonia has not shown definite benefits yet but uninterrupted use of the last resort antibiotic for XDR salmonella can cause a major crises in the management of this highly resistant bug in the future.

The United Nations report for urgent action to avert the AMR crises, published before the COVID pandemic hit, declares that AMR could force up to 24 million people into extreme poverty by 2030 and drug-resistant diseases could cause 10 million deaths each year by 2050.⁶

The need for responsible antibiotic prescribing practices is greater now than ever. Strengthening antimicrobial stewardship programmes, especially in the midst of epidemics and pandemics when antibiotic misuse is rife, is extremely important in salvaging the hard work of decades.

Liew et al describe the Antibiotic Stewardship Program which was fully operational at Singapore General Hospital during the COVID pandemic. Daily electronic audits of selected broad-spectrum antibiotic prescriptions (Carbapenems, piperacillin-tazobactam, ciprofloxacin and levofloxacin) were performed to assess suitability in terms of indication, route, duration and choice by the pharmacy services in collaboration with Infectious Diseases team. The result was decreased duration of antibiotic therapy and shorter hospital stays where ASP intervention was accepted. This also alleviated the problem of overcrowded wards.⁷

It is difficult to distinguish severe Covid infection from sepsis due to secondary bacterial infections because the traditional

inflammatory markers and vital signs may already be deranged. In this scenario, empirical antibiotics are entirely justifiable. The need is for constant vigilance and quick de-escalation of therapy, conversion from IV to oral formulations and shortest possible duration of antibiotics. Real time antibiograms developed by institutions to guide empirical antibiotic therapy in their centre would be an added benefit.

It is important for the public health policy makers to reprioritize the fight against AMR. Prospective studies recording antibiotic use and misuse are an essential component required to guide recommendations. Clear, concise and identical guidelines for the management of COVID-19 should be made available at all levels of healthcare. Reinforcing AS programs at the hospital level and combatting disinformation at the community level with awareness campaigns such as Nigeria's 'Take Responsibility Campaign' launched by the Nigerian CDC may well forestall a pandemic of antimicrobial resistance.

References

1. Monnet DL and Harbarth S. Will coronavirus disease (COVID-19) have an impact on antimicrobial resistance? *Euro Surveill.* 2020 Nov 12; 25(45): 2001886.
2. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, *et al.* Bacterial and fungal co-infection in individuals with

3. coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis.* 2020; ciaa 530. doi:10.1093/cid/ciaa530
4. Dagens A, Sigfrid L, Cai E, *et al.* Scope, quality, and inclusivity of clinical guidelines produced early in the COVID-19 pandemic: rapid review. *BMJ* 2020;369. doi: 10.1136/bmj.m1936.
5. Worldometer. <https://www.worldometers.info/coronavirus/country/pakistan/>
6. Nori P, Cowman K, Chen V, Bartash R, Szymczak W, Madaline T, Punjabi Katiyar C, Jain R, Aldrich M, Weston G, Gialanella P, Corpuz M, Gendlina I, Guo Y. Bacterial and fungal coinfections in COVID-19 patients hospitalized during the New York City pandemic surge. *Infect Control Hosp Epidemiol.* 2021 Jan;42(1):84-88. doi: 10.1017/ice.2020.368.
7. Interagency coordination group on antimicrobial resistance (IACGs) 2019. No time to wait: securing the future from drug-resistant infections. Report to the Secretary-General of the United Nations. <https://www.who.int/antimicrobial-resistance/interagency-coordination-group/final-report/en/>
8. Liew Y, Lee WHL, Tan L, Kwa ALH, Thien SY, Cherng BPZ, Chung SJ. Antimicrobial stewardship programme: a vital resource for hospitals during the global outbreak of coronavirus disease 2019 (COVID-19). *Int J Antimicrob Agents.* 2020 Nov;56(5):106145. doi: 10.1016/j.ijantimicag.2020.106145.

Muniba Aslam, Sunil Kumar Dodani, Asma Naseem
Department of Infectious Diseases,
Sindh Institute of Urology and Transplantation,
Karachi, Pakistan.
Email: sdodani.siut@gmail.com

Amphotericin B associated nephrotoxicity – A single center retrospective study

Muhammad Ammar Shafqat*, Aun Raza*, Junaid Iqbal*, Amina Nawaz*, Muhammad Abu Bakar**, Faisal Sultan*

*Department of Internal Medicine, Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH & RC), Lahore, Pakistan

**Department of Cancer Registry and Clinical Data Management, SKMCH & RC, Lahore, Pakistan

Abstract

Objectives

To analyze frequency of acute kidney injury (AKI) and electrolyte derangements associated with the use of amphotericin B deoxycholate (conventional amphotericin B) and comparison of these adverse effects between children and adults.

Materials & Methods

This is a retrospective study. Medical records of patients who received amphotericin B deoxycholate during 12 months from 1st May 2016 to 30th April 2017 were reviewed to determine patient's baseline characteristics, and to identify use of amphotericin B and calculate incidence of associated nephrotoxicity.

Results

A total of 228 patients received amphotericin B deoxycholate in the study duration. Most of our patients were males (65.8%). 51.75% were adults (=18 years of age). Most patients had cancer and acute lymphoblastic leukemia was the most common primary diagnosis (34.2%). 6.6% patients had co morbid conditions at baseline.

Most of our patients developed amphotericin B related adverse effects. Out of 228 patients who received amphotericin B deoxycholate, 80% developed hypokalemia. Hypokalemia was observed more commonly amongst the pediatric population (86.4%) as compared to adults (74.6%). Similarly, 61% children and 45% adults developed hypomagnesemia. Moreover, 59.2% patients developed acute kidney injury (AKI) with amphotericin B therapy, 9 of which required renal replacement therapy. Development of acute kidney injury was seen more frequently among adults (63.6%), as compared to children (54.5%).

30 days survival among children was 70% as compared to 60% for adults.

*Corresponding Author: Muhammad Ammar Shafqat
Department of Internal Medicine,
Shaukat Khanum Memorial Cancer Hospital and Research
Centre,
Lahore, Pakistan.
Email: ammarshafqat@hotmail.com*

Conclusions

The results of the present study allow documentation of adverse effects associated with the use of conventional amphotericin B. This data shows that conventional amphotericin B is commonly associated with electrolyte abnormalities and acute kidney injury. This highlights the need for availability of liposomal or lipid-based formulations of amphotericin B in our country.

Key Words

Amphotericin B; AKI; Electrolyte Imbalance

Introduction

Invasive fungal infections carry high mortality and morbidity in immunocompromised patients.¹ Amphotericin B is used to treat such fungal infections. However, the use of conventional amphotericin B is associated with serious adverse effects like nephrotoxicity which occurs early during treatment.² Other adverse effects of amphotericin B include infusion reactions and electrolyte abnormalities like hypokalemia and hypomagnesemia.³

The nephrotoxicity is thought to be caused by the direct toxic effect of amphotericin B on renal tubular cells, resulting in acute tubular necrosis and vasoconstriction.^{4,5} Both these processes reduce glomerular filtration.^{4,5} This nephrotoxic effect of conventional amphotericin B has been observed to be dose dependent and duration dependent. Other risk factors for amphotericin B associated nephrotoxicity include patient weight, diuretic use during amphotericin therapy, abnormal baseline creatinine and concomitant use of nephrotoxins especially cyclosporine.⁶⁻⁷

Relatively limited data is available in Pakistan regarding the incidence of nephrotoxicity associated with the use of conventional amphotericin B. Previous international studies have reported a variable frequency of amphotericin B associated acute renal failure ranging from 15% to 80%.^{3,8} This high incidence of nephrotoxicity can be reduced by using lipid based or liposomal formulations of amphotericin B instead of conventional amphotericin B.⁹

Evaluation of the incidence of nephrotoxicity (acute kidney injury, electrolyte derangements) associated with use of

amphotericin could help us inform decisions about the need for newer, more costly but less nephrotoxic agents. Therefore, we performed a retrospective cross-sectional study that investigated the frequency of acute kidney injury and electrolyte derangements among patients who received conventional amphotericin B. In our study we also compared the incidence of these adverse effects between pediatric and adult populations.

Patients and Methods

Study setting

This study was conducted at Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC), a specialist charitable cancer hospital in Lahore, Pakistan. The hospital provides a full complement of cancer treatment to patients from all over the country.

Study Population

A total of 228 patients who received amphotericin B deoxycholate during the study period of 1 year from 1st May 2016 to 30th April 2017 were included in the study. This study was approved by the institutional review board of SKMCH&RC.

Data Extraction & Methods

The hospital's information system database was used to identify all patients who had received amphotericin B during the study period. The medical records were reviewed and retrospectively evaluated to collect the data. Baseline renal functions including baseline serum potassium, magnesium, creatinine, creatinine clearance and GFR were the last values before the start of amphotericin B & were noted. Following this, the peak value of serum creatinine in next 7 days & lowest values of serum potassium & magnesium during amphotericin B therapy were recorded. Incidence of acute kidney injury, hypokalemia & hypomagnesemia was recorded. Determinants including primary diagnosis, age, gender, indications for the use of amphotericin B, the dose of amphotericin B therapy, baseline absolute neutrophil count, days of neutropenia, co morbidities (Diabetes, Hypertension), need for renal replacement therapy, need for K & Mg replacement, hospital admission outcome, readmission, mortality & 30 days survival were also noted. Comparison of nephrotoxicity was made between children and adults.

Outcome Measures & Operational definitions

- § AKI was defined according **Criteria (KDIGO) of acute kidney injury** i.e., Increase in serum creatinine to =1.5 times baseline, which is known or presumed to have occurred within the prior seven days after the start of amphotericin B.
- § Hypokalemia was defined as serum potassium level of < 3.3 mmol/L. (Reference range 3.3-5.1 mmol/L)
- § Hypomagnesemia was defined as serum magnesium level < 1.5 mg/dl. (Reference range 1.5-2.5 mg/dl)

Statistical analysis

Statistical analysis was carried out using the Statistical Package

for the Social Sciences (SPSS) software (version 20.0; SPSS, Chicago, IL, USA). Continuous variables were stated as Mean \pm SD and categorical variables were computed as frequencies and percentages. Categorical variables were compared using chi square test or fisher's exact test (where necessary). Statistical significance was defined as a two-tailed p-value 0.05.

Results

Descriptive statistics

Altogether, there were 228 patients who received amphotericin B deoxycholate during the study period. Patients had a mean age of 21.03 \pm 19.30 years and most of the patients were male (65%). Patients were approximately equally distributed in adult and pediatric age groups (51% Vs 49%). Most of our study population comprised of cancer patients (97%) and acute lymphoblastic leukemia was the most common primary diagnosis (34.2%). Patients received a mean dose of 0.97 mg/kg of amphotericin B deoxycholate. (Table 1)

On assessment of patient's baseline status, we found that relatively few patients had co morbid conditions like diabetes mellitus, hypertension, and ischemic heart disease at baseline (6.6%). Similarly, only 5% patients had chronic kidney disease (CKD) and acute kidney injury (AKI) at baseline (Table 1).

We observed that most of our patients received amphotericin B empirically (78.5%) while 15% had positive fungal cultures and 6.5% had a positive fungal histopathology. Among the patients who had a positive fungal identification (either culture or histopathology), *Aspergillus* species were most common (42%) followed by *Candida* species (34%) and *Mucor* (14%). (Table 2)

On calculation of the incidence of adverse effects, we observed that a high percentage of our patients developed adverse effects with hypokalemia being the most common (80%). We also observed that the incidence of hypokalemia was higher in pediatric population (86%), as compared to adults (74.6%) (a statistically significant difference, p-value 0.02).

Furthermore, there was a statistically significant drop in mean serum potassium levels in both age groups as compared to baseline values with amphotericin B therapy. (1.22 mmol/L children, 0.82 mmol/L adults).

On analysis of hypomagnesemia, the incidence was found to be comparatively low as compared to hypokalemia (53.7% Vs 80%). Hypomagnesemia was also more common in pediatric population (61%) as compared to adults (45%) with a borderline statistical significance (p- value 0.09). Furthermore, a statistically significant drop in mean baseline Mg level was observed in both age groups with amphotericin B therapy (0.57 mmol/L children, 0.18 mmol/L adults). Table 2

Among our study population, 59% patients developed acute

Table 1: Description of study population.

Variables	Characteristics	Frequency N (%)
Age (years)	Mean ± standard deviation	21.03 ± 19.30
Age Groups	Pediatric	110 (48.2%)
	Adults	118 (51.8%)
Sex	Male	150 (65.8%)
	Female	78 (34.2%)
Comorbidity	None	213 (93.4%)
	Diabetes Mellitus (DM)	5 (2.2%)
	Hypertension (HTN)	3 (1.3%)
	Ischemic heart disease (IHD)	1 (0.4%)
	Multiple co-morbidities	6 (2.6%)
Chronic Kidney Disease (Baseline)	Yes	14 (6.1%)
Acute Kidney Injury (Baseline)	Yes	12 (5.3%)
Primary Diagnosis (Cancer patients)	Acute lymphoblastic leukemia	78 (34.2%)
	Non-Hodgkin's Lymphoma	59 (25.9%)
	Hodgkin's Lymphoma	16 (7.0%)
	Sarcoma	15 (6.5%)
	Breast Cancer	9 (3.9%)
	Others	45 (19.7%)
Infection as Primary Diagnosis (Non-Cancer patients)	Mucormycosis	3 (1.3%)
	Aspergillosis	3 (1.3%)
Baseline Absolute Neutrophil Count	Mean ± standard deviation	1.42 ± 3.64
Dose of Amphotericin B (mg/Kg*)	Mean ± standard deviation	0.97 ± 0.12

Table 2: Values of potassium, magnesium, and creatinine before and after therapy (Amphotericin B).

Variables	Before Therapy	With Therapy	Mean Difference	p-value
Pediatric Group				
Mean Potassium (mmol/L*)	4.06 ± 0.64	2.84 ± 0.67	1.22 ± 0.83	0.001
Mean Magnesium (mg/dl**)	1.98 ± 0.35	1.42 ± 0.40	0.57 ± 0.38	0.001
Mean Creatinine (mg/dl**)	0.19 ± 0.19	0.38 ± 0.33	0.18 ± 0.30	0.001
Adult Group				
Mean Potassium (mmol/L*)	3.84 ± 0.64	3.02 ± 0.70	0.82 ± 0.68	0.001
Mean Magnesium (mg/dl**)	1.71 ± 0.49	1.53 ± 0.50	0.18 ± 0.36	0.002
Mean Creatinine (mg/dl**)	0.70 ± 0.53	1.51 ± 1.09	0.81 ± 0.99	0.001

*millimole/liter, **milligram/deciliter

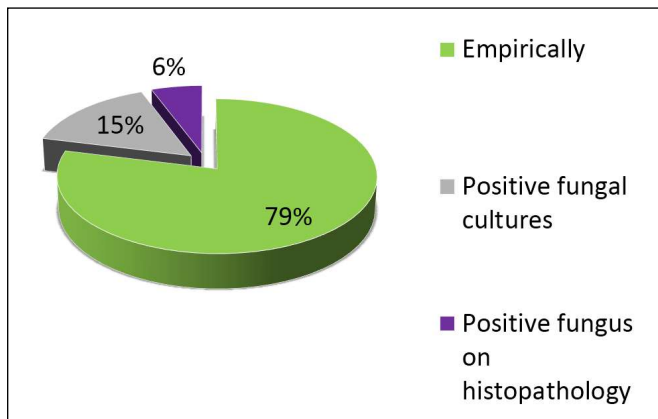


Fig 1. Indications to start amphotericin B

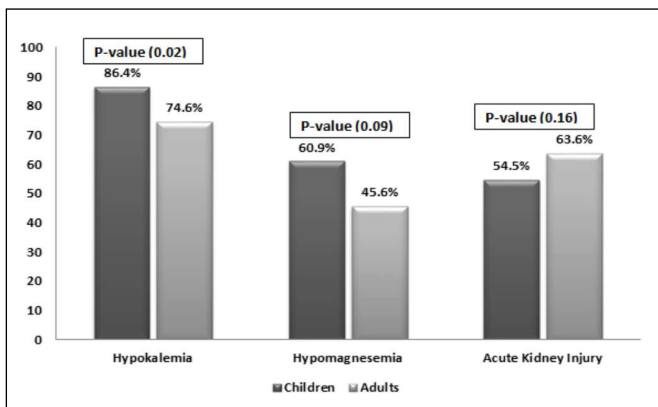


Fig 2. Incidence of side effects and comparison between children and adults

kidney injury with amphotericin B therapy. In contrast to electrolyte abnormalities, incidence of AKI was higher among adults (63.55%) as compared to children (54%), although this difference between age groups was not found to be statistically significant (p-value 0.16) Table 3. Furthermore, there was a statistically significant increase in mean serum creatinine levels in both age groups as compared to mean baseline values with amphotericin B therapy. (Increase of 0.18 mg/dl for children, 0.81 mg/dl for adults)

Evaluating mortality, we calculated that 30 days survival among children was 70% as compared to 60% for adults.

Discussion

Conventional amphotericin B has remained a cornerstone of therapy for systemic fungal infections for a long time, however its use has always been hampered by adverse effects like hypokalemia, hypomagnesemia and acute kidney injury.¹⁰ Therefore, a balance between toxicity and efficacy has to be maintained while prescribing amphotericin B.¹¹

The incidence of these undesirable effects can be reduced at the expense of cost with the use of newer forms of amphotericin

B.¹² Lipid based formulations or liposomal amphotericin B have replaced conventional amphotericin B in developed countries however conventional amphotericin B is still the main antifungal agent that is being used in resource limited countries.

Our data suggests that most of our patients who received amphotericin B developed adverse effects. Hypokalemia was most observed adverse effect (80%) followed by acute kidney injury (59%) and hypomagnesemia (53.7%) respectively. Prior work on this topic has reported a variable frequency of amphotericin B associated acute kidney injury and other adverse effects.^{3,13} The incidence of these adverse effects depends on many factors including dose of amphotericin B, baseline renal function status of patient and co morbidities.⁶⁻⁷

Gandhi et al. in 2005 reported incidence of renal adverse effects in up to 80% of the patients.¹³ This is like results of our study. Bates et al. and Harbarth et al. reported a frequency of 27% and 28% respectively.⁴⁻⁵

Pathak et al. conducted their study in a community hospital and reported a frequency of just 15% however they used a broader definition of acute kidney injury than the current definition.³ Additionally that study was conducted in a community hospital, and, therefore, the patients may have been less ill than the patients in the current study.

Our study documented a high incidence of amphotericin B associated nephrotoxicity with 59% of our patients developing acute kidney injury. In addition to the estimation of incidence of adverse effects our study also analyzed the quantitative difference in values of serum potassium, magnesium, and creatinine with amphotericin B therapy. We observed significant drops in mean baseline values of potassium and magnesium and a significant increase in mean baseline value of creatinine with amphotericin B therapy.

There has been no prior work on comparison of adverse effects between age groups. So, we also compared the incidence of adverse effects between the age groups. Our data suggests that the incidence of hypokalemia and hypomagnesemia was more common in pediatric population, though for hypomagnesemia the difference between age groups was found to have only borderline statistical significance (p- value 0.09). On analysis of acute kidney injury, the incidence was found to be higher in adult groups, though this difference between age groups was found to be statistically insignificant (p-value 0.16).

Our study has reported an alarming frequency of adverse effects related to conventional amphotericin B; however, this study has several limitations. This study is conducted in a specialized cancer-care institution, and the results may not be generalizable to other institutions. In addition, confounding by indication for initiating amphotericin therapy may exist. Most of the patients in current study received amphotericin B empirically (78.5%),

usually in the setting of fever and neutropenia, while only 21.5% received amphotericin B because of documented fungal infection. Also, confounding by severity of illness may exist. Similarly, we did not collect data about cyclosporine or hydration status at the time of acute kidney injury development, which might have been correlated with the presence of acute kidney injury.

Conclusions:

The results of the present study allow documentation of adverse effects associated with the use of conventional amphotericin B. This data shows that conventional amphotericin B is commonly associated with electrolyte abnormalities and acute kidney injury. This highlights the need for availability of liposomal or lipid-based formulations of amphotericin B in our country.

References

1. Rex JH, Bennett JE, Sugar AM, Pappas PG, Van Der Horst CM, Edwards JE, Washburn RG, Scheld WM, Karchmer AW, Dine AP, Levenstein MJ. A randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in patients without neutropenia. *New Eng J Medicine*. 1994 Nov 17;331(20):1325-30.
2. Fanos V, Cataldi L. Amphotericin B-induced nephrotoxicity: a review. *J Chemotherapy*. 2000 Jan 1;12(6):463-70
3. Pathak A, Pien FD, Carvalho L. Amphotericin B use in a community hospital, with special emphasis on side effects. *Clin Infec Dis*. 1998 Feb 1;26(2):334-8.
4. Bates DW, Su L, Donghui TY, Chertow GM, Seger DL, Gomes DR, Platt R. Correlates of acute renal failure in patients receiving parenteral amphotericin B. *Kidney International*. 2001 Oct 1;60(4):1452-9.
5. Harbarth S, Pestotnik SL, Lloyd JF, Burke JP, Samore MH. The epidemiology of nephrotoxicity associated with conventional amphotericin B therapy. *The Am J Medicine*. 2001 Nov 1;111(7):528-34.
6. Fisher MA, Talbot GH, Maislin G, McKeon BP, Tynan KP, Strom BL. Risk factors for amphotericin B-associated nephrotoxicity. *The Am J Medicine*. 1989 Nov 1;87(5):547-52.
7. Walsh TJ, Finberg RW, Arndt C, Hiemenz J, Schwartz C, Bodensteiner D, Pappas P, Seibel N, Greenberg RN, Dummer S, Schuster M. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. *New Eng J Medicine*. 1999 Mar 11;340(10):764-71.
8. Venkataraman R, Kellum JA. Prevention of acute renal failure. *Chest*. 2007 Jan 1;131(1):300-8.
9. Inselmann G, Volkman A, Heidemann HT. Comparison of the effects of liposomal amphotericin B and conventional amphotericin B on propafenone metabolism and hepatic cytochrome P-450 in rats. *Antimicro Agents and Chemotherapy*. 2000 Jan 1;44(1):131-3.
10. Bates DW, Su L, Yu DT, Chertow GM, Seger DL, Gomes DR, Dasbach EJ, Platt R. Mortality and costs of acute renal failure associated with amphotericin B therapy. *Clin Infect Dis*. 2001 Mar 1;32(5):686-93.
11. Dupont B. Overview of the lipid formulations of amphotericin B. *J Antimicro Chemo*. 2002 Jan 1;49(1):31-6.
12. Alexander BD, Wingard JR. Study of Renal Safety in Amphotericin B Lipid Complex-Treated Patients. *Clin Infec dis*. 2005 May 1;40(Supplement_6):S414-21.
13. Gandhi BV, Bahadur MM, Dodeja H, Aggrwal V, Thamba A, Mali M. Systemic fungal infections in renal diseases. *J Postgraduate Medic*. 2005 Oct 1;51(5):30.

Psychosocial Aspects of Covid-19 and Self-confidence: we can beat it.

Hamzullah Khan

Nowshera Medical College, Nowshera, Pakistan

Abstract

Background & Objectives

During the outbreak of the pandemic COVID-19, there is urgent need for intervention to protect the victims and their family members, high risk individuals, health care workers from the secondary mental health crises associated with the disease. In vague of the above-mentioned rationale we did a survey to assess the psychosocial aspects of the COVID-19 and level of self-confidence of the respondents in centre based study.

Material & Methods

A cross-sectional survey was conducted in March 2020. A total of 82 of the respondents were selected via convenient sampling, irrespective of age and gender for their knowledge of psychosocial impacts of COVID-19. Relevant information's regarding the impact of COVID-19 on mental health was collected on a pre-designed proforma prepared in accordance with the objectives of the study.

Results

The minimum age of the respondents was 20 years with a maximum of 50years. The mean with standard deviation of the respondents was 51+13 years. Thirty-Four (41.5%) were qualified practitioner with medical qualification and 48 (58.5%) were medical students and non medics. We observed a significantly higher difference in opinion of male gender (vs female gender) regarding their level of confidence in the fight against COVID-19 ($p=0.02$, $OR=4.5$), panic and anxiety they had ($p=0.001$, $OR=0.12$) and expectations from government in current situation ($p=0.01$, $OR=3.9$).

It was observed that male gender is more of favor of ban on videos related to COVID patients as compared to female gender ($p=0.005$). The fear regarding the shortage of food was not significantly higher in both genders ($p=0.724$). The level of confidence in both genders insignificantly differ (0.03). Furthermore, the fear regarding the shortage of food was more in the youngsters (age=20-30 years) ($p=0.03$). Majority of the youngster and adult age responders were confident based on their spiritual guidance of religion and adopting the precautionary measure that they can beat Covid-19 ($p=0.048$).

*Correspondence Author: Hamzullah Khan,
Professor of Hematology,
Nowshera Medical College,
Nowshera, Pakistan.
Email: hamzakmc@gmail.com*

Conclusion

We received a significant response of the respondents with higher level of self confidence, motivation and will to fight COVID-19. Although the respondents admitted that COVID-19 has disturbed their daily routine and income but they were committed that they will win the battle against COVID-19.

Key words

COVID-19, Confidence, Anxiety, and depression

Introduction

Corona Virus disease (COVID-19) is caused by a virus called SARS-COV2 and is a potentially fatal disease with associated social stigma.¹ Extensive measures have been taken by the authorities to reduce its spread, person to person transmission to control the current epidemic.

Since the first case of COVID-19 reported in China in early Dec 2019, and by February 2020 the cases crossed 17000 in China, the Chinese government ordered strict quarantine across the country.² Isolation and quarantine in one way to contain the virus but on other hand exposes the individual to psychosocial stress, being deprived from mental health counseling.³ During the outbreak of COVID-19 in China, more than half of the patients developed state of anxiety and depression.⁴ COVID-19 has caused serious physical (in term of morbidity and mortality) and psychosocial threat to the lives of the people as well. It has triggered a wide range of problems such as panic disorders, anxiety and depression.⁵

Psychologically it is a fact that when the living environment changes all sudden, people feel un-easy, unsafe and anxious.⁶ The etiology of the COVID-19 is yest not clear and this flourish the rumors that can create a stigma in public. The global times Newspaper, from China reports that the Innocent people of Wuhan were blamed unfairly at national and international levels, and the same the Chinese people experienced at international forums for a natural disaustor.⁷

People adherence to preventive and precautionary measures is essential, which largely depends on the knowledge attitude and practice of people toward COVID-19 in accordance with Knowledge attitude and practice (KAP) theory. Zhong BL *et al*⁸ observed that the majority (90.8%) of the participants in their study were optimistic to succeed in winning the war against

corona virus. Studies from China have reported that education level of clients increases the confidence 3- 5 times more to win the battle against COVID-19, (*OR: 3.13-5.04, P<0.001*).^{8,9}

Present study was designed to assess the psychosocial stress of COVID-19 on the respondents, and to determine their level of confidence in the wartime against 2019 nCoV.

Material & Methods

A cross-sectional research design was conducted in March 2020. A pre-validated questionnaire comprising 8 items was administered. A total of 82 of the respondents were assessed for their knowledge regarding the psychosocial aspects of (COVID-19). All the respondents irrespective of age and gender with minimum qualification of bachelor's degree were included. Respondents with qualification less than bachelor degree were excluded from the study with an exception for 4th year medical students of Nowshera Medical College.

The questionnaire had 2 parts. There were preliminary information's of the respondents regarding their name, age, email, qualification, and nature of employment. The second part containing information's about the knowledge of the respondents about COVID-19 virology, transmission, precautions, treatment options, impact of COVID-19 on social life, social distancing, and level of confidence to fight 2019-nCoV.

Ethical approval was taken from the institutional ethical review board of Nowshera Medical College before the execution of the survey. Prior informed consent was attained from all the contestants and they were assured of confidentiality. We distributed the questionnaire through email and in hard copies and the responders were given option to submit it via email or by hand.

Data was entered in SPSS version 25 for descriptive analysis of various variables. Frequency and percentage were calculated for different categorical variables. Binary logistic regression analysis was used to show the psychosocial impact of COVID-19. Chi-square test was used to determine the association of different psychosocial factors with categories of age and gender of the respondents.

Results

A total of 82 respondents completed the survey. Forty-one were females and 41 male responders. Out of total 45(54.9%) were in the age range 15-25 years of age followed by 31(37.8%) in age 26-35 years of range. out of total, 34 (41.5%) were qualified practitioner with medical qualification and 48 (58.5%) were medical students and non medics. (Table 1).

Using multiple regression analysis in gender group of the respondents for different 8 psycho-social variables related to COVID-19, we observed a significantly higher difference in

opinion of male gender (vs female gender) regarding their level of confidence in the fight against COVID-19(*p=0.02, OR=4.5*) panic and anxiety (*p=0.001, OR=0.12*) and expectations from government in current situation (*p=0.01, OR=3.9*). (Table 2).

Using Chi-square test we observed that there was a significant understanding in gender groups that the videos of Covid-19 are disturbing and must not be shown. Male gender is more of favor of ban on videos related to COVID patients as compared to female gender (*p=0.005*). The fear regarding the shortage of

Table 1. Demographic informations of the respondents

1. Gender of the Respondents	Frequency
Male	41 (50%)
Female	41(50%)
2. Age categories of the Respondents	Frequency
Age range	
15-25	45(54.9%)
25-35	31(37.8%)
36-50	6(7.3%)
3. Profession of the respondents	Frequency
Profession	
Doctors/GP/Specialist	34(41.5%)
Students/Bachelor/Master/technician	48(58.5%)
Total	82(100%)

Table 2. Gender Groups and regression analysis of variables.

S. No.	Questions/ Psychosocial stress factor	Significance (p-values)	Exp(B)- Odds Ratio
1	The videos of Covid-19 are disturbing and must not be shown	0.144	0.689
2	Reasons of Spread of disease	0.625	1.375
3	Has it disturbed your daily routine and income	0.126	2.786
4	Information on importance of isolation and social distancing	0.999	0
5	Are you panic/anxious	0.001	0.133
6	Do you afraid of food shortage in the prevalent situation	0.28	1.863
7	Your opinion on relief from Government in this situation	0.01	3.953
8	What keeps you confident	0.029	4.535
	Constant	0.999	510.479

food was not significantly higher in both genders (p-0.724). Male gender again is more in accordance with the observation that Isolation is an important Precautionary measure in covid-19 as compared to female gender (p-0.06). The level of confidence in both genders insignificantly differ (0.08). (Table 3)

Using Chi-square test on impact of Covid-19 on different age groups, we observed that there was insignificant difference in opinion of different age responders that the videos of Covid-19 are disturbing (p-0.516). The fear regarding the shortage of food unlikely gender group was more in the youngsters (age=20-30 years) genders (p-0.03). Majority of the youngster and adult age responders were confident based on their spiritual guidance of religion and adopting the precautionary measure that they can beat Covid-19 (p-0.048). (Table 4)

Discussion

We did this survey on respondents with age (Mean with SD of 51+13 years). There was a significant difference in opinion of male gender (vs female gender) regarding their level of confidence in the fight against COVID-19., the responders were panic and had anxiety. they had expectations from government in current situation in term of food and health issues during the expected lockdown. It was observed that male gender was more in favor of ban on videos related to COVID patients as compared to female gender. The fear regarding the shortage of food was more in the responders. Majority of the youngster and adult age responders were confident based on their spiritual guidance of religion and adopting the precautionary measure that they can beat Covid-19. World Health Organization Director General of Global hazard preparedness said that in every epidemic the fear and stigma goes side by side, when

Table 3. Chi-square test to show the impact of different psychosocial stress factor associated with COVID-19 on gender groups

Question-1	The videos of Covid-19 are disturbing and must not be shown					Total	p-value	
	SA	A	D	SD	No comments			
Gender of the respondents								
Male	8	2	23	5	3	1	0.005	
Female	10	10	20	0	1	41		
Total	15	12	43	6	6	82		
Question-2	Do you afraid of food shortage in lockdown							
	yes	no		no comments	Total		p-value	
Gender of the respondents								
Male	27	12		2	41		0.724	
Female	20	16		5	41			
Total	47	25		10	82			
Question-3	Isolation is important Precautionary measure in covid-19							
	SA	A	D	Total		p-value		
Gender of the respondents								
Male	34	3	4	41		0.06		
Female	40	1	0	41				
Total	74	4	4	82				
Question-4	Which think keep you confident in this situation							
	Government measures	Spiritual guidance of My religion ISLAM	Precautionary measures	awarenes+ Cleanliness	No comments	Total	p-value	
Gender of the respondents								
Male	1	15	17	7	1	41	0.034	
Female	1	25	9	5	1	41		
Total	2	37	26	17	2	82		

Table 4. Chi-square test to show the impact of different psychosocial stress factor associated with COVID-19 on different age groups

Question 1	The videos of Covid-19 are disturbing and must not be shown					Total	p-value
	Strongly agree	Agree	Disagree	Strongly Disagree	No comments		
Age of the responder							
20-30	5	8	27	3	2	45	0.516
31-40	8	3	14	3	3	31	
41-50	2	1	2	0	1	6	
Total	15	12	43	6	6	82	
Question 2	Do you afraid of food shortage in lockdown			Total	p-value		
	yes	no	no comments				
Age of the respondent							
20-30	31	9	5	45	0.036		
31-40	14	14	3	31			
41-50	2	2	2	6			
Total	47	25	10	82			
Question 3	Isolation is important Precautionary measure			Strongly	Total	p-value	
	Strongly Agree	Agree	Disagree	Disagree			
Age of the respondents							
20-30	40	1	2	2	45	0.217	
31-40	28	3	0	0	31		
41-50	6	0	0	0	6		
Total	74	4	2	2	82		
Question 4	Which think keep you confident in this situation				No	Total	p-value
	Government measures	Spiritual guidance of My religion ISLAM	Precautionary measures	Awarenes+ Cleanliness	comments		
Age of the respondents							
20-30	1	23	15	5	1	45	0.048
31-40	0	12	11	5	3	31	
41-50	1	2	0	2	1	6	
Total	2	37	26	12	6	82	

people get fear, stigma develops and that what healthcare worker do is to reduce the fear to reduce the stigma.¹⁰ The fear and stigma sometimes are more dangerous for a society, especially what we observed in Wuhan China, where they faced blaming for spread of virus, they were rejected, remained anxious, angry and even hunted.¹¹

A case report from the India revealed an Indian mistakenly thought to have been infected with COVID-19, committed suicide to protect his near and dears.¹² A section of the Chinese population had a misconception regarding Covid-19 and think it to be a part of a biological war.^{11,13} This misconception was rejected/denied by the respondent in present study. Female gender was more conscious, more worried, more panic,

with low confidence in this wartime, while the male gender confidence was 4.5 time higher and were committed to fight against COVID-19.

Zhong BL *et al*⁸ also have observed that (90.8%) of the participants in their study were optimistic to get succeeded in winning the war against corona virus, that strongly correlates our findings. Studies from China have reported that education level of clients increases the confidence 3- 5 times more to win the battle against COVID-19, that matches our findings.^{8,14}

Italy, the second mostly affected country, with 88% cases of SARS-CoV infection with history of travel to the epidemic area were initially reported in Lombardian and Veneto regions.

They attributed these infections with poor compliance of the people towards precautionary measures during the early stages of current epidemic in the country.¹⁵

The levels of understanding the importance of isolation and social distancing are the key to contain virus, besides of its adverse impact on social life. China did it very well. The National health commission of China issued protocols and guidelines with the slogan of “Big isolation and big disinfection” during the Chinese spring festival on 28th Jan 2020.¹⁶ We also noticed that male gender is more of opinion that isolation/quarantine is an important precautionary measure/strategy in Covid-19.

The advent of vaccines, public hygiene and precautions have largely controlled and eliminated infectious diseases. However, in COVID-19 the etiology and pathogenesis is not clear and the one thing that can help and improves the confidence is adopting precautionary measures, gaining more knowledge about the disease, its transmission and role of social distancing. Panic spread of the overwhelming information’s increases stigma and anxiety rather than what is caused by the disease itself. That is the same what the world experienced with Zika virus that was a mild viral disease without a great impact, that had vertical transmission and some cases of birth defects, had created a stigma around the globe for its related morbidities.¹⁷ And that’s how taking China as example again, where unprecedented measures were taken well in time to control the rapid spread of COVID-19. They succeeded to limit people to homes and was largely achieved by their improved Knowledge, attitude, and practices towards COVID-19 and by increasing their self-confidence about the disease.¹⁸

The limitation of this study was limited number of the respondents with one setting/Hospital/institution. Future studies should focus large population with representation of all sectors to have a better outcome to predict/suggest the findings for decision making by the competent authorities to prevent disastrous pandemic.

Conclusion

The respondents have sufficient knowledge about COVID-19 pandemic, its prevention, precautionary measures during the early outbreak across the country. Besides the challenges in term of limited resources, the severity of the disease, stigma, fear and anxiety associated with the disease, the enthusiastic approach of the respondents was a sign of confidence that as a nation we will win the war against virus.

There is need for multi-disciplinary approaches through advocacy, social mobilization for social distancing and distant communication through utilization of technologies is the need of time.

References

1. Rothan HA, Byrareddy SN. The epidemiology and

pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun.* 2020;102433. doi:10.1016/j.jaut.2020.102433. [Epub ahead of print].

2. Real-time situation of 2019 novel coronavirus in China. DXY.cn. Available at: <https://ncov.dxy.cn/ncovh5/view/pneumonia>. Accessed Feb 3, 2020.
3. C. A Novel Approach of Consultation on 2019 Novel Coronavirus (COVID-19)-a. 2020; 17(2): 175-6.
4. Wang C, Pan R, Wan X, Tan Y, Xu L, Ho CS. Immediate Psychological Responses and Associated Factors during the Initial Stage of the 2019 Coronavirus Disease (COVID 19) Epidemic among the General Population in China. *Int J Environ Res Public Health.* 2020; 6;17(5). pii: E1729. doi: 10.3390/ijerph17051729.
5. Qiu J, Shen B, Zhao M, Wang Z, Xie B, Xu Y. A nationwide survey of psychological distress among Chinese people in the COVID-19 epidemic: implications and policy recommendations. *Gen Psychiatr.* 2020;33(2):e100213. Published 2020 Mar 6. doi:10.1136/gpsych-2020-100213
6. Shigemura J, Ursano RJ, Morganstein JC, Kurosawa M, Benedek DM. Public responses to the novel 2019 coronavirus (2019-nCoV) in Japan: Mental health consequences and target populations. *Psychiatry Clin Neurosci.* 2020 Apr;74(4):281-282.
7. Rong X. Coronavirus a common foe the global village must fight together. *Global Times.* 6 Feb, 2020. Available from: <https://www.globaltimes.cn/content/1178729>.
8. Zhong BL, Luo W, Li HM, Zhang QQ, Liu XG, Li WT, Li Y. Knowledge, attitudes, and practices towards COVID-19 among Chinese residents during the rapid rise period of the COVID-19 outbreak: a quick online cross-sectional survey. *Int J Biol Sci* 2020; 16(10):1745-52. doi:10.7150/ijbs.45221. Available from <http://www.ijbs.com/v16p1745.htm>
9. Munster VJ, Koopmans M, van Doremalen N, van Riel D, de Wit E. A Novel Coronavirus Emerging in China - Key Questions for Impact Assessment. *N Engl J Med.* 2020;382:692-4.
10. China Daily. Responses to outbreak widely praised. Available from: <http://ex.chinadaily.com.cn/exchange/partners/45/rss/channel/www/columns/f8gszh/stories/WS5e3b7785a310128217275603.html>.
11. Rong X. Coronavirus a common foe the global village must fight together. *Global Times.* 6 Feb, 2020. Available from: <https://www.globaltimes.cn/content/1178729.html>
12. India Today. Andhra man wrongly thinks he has coronavirus, kills self to protect village. Available from: <https://www.indiatoday.in/india/story/andhra-pradesh-chittoor-man-wrongly-believes-coronavirus-kills-self-1645578-2020-02-12>.
13. NetEase. Novel coronavirus is a genetic weapon and a conspiracy of the United States. The wise will not listen to rumors. Available from: <https://c.m.163.com/news/a/F4H53T8H0517DRJS.html?spss=newsappspsw=1>.
14. Munster VJ, Koopmans M, van Doremalen N, van Riel D,

-
- de Wit E. A Novel Coronavirus Emerging in China - Key Questions for Impact Assessment. *N Engl J Med.* 2020;382:692-4
15. Porcheddu R, Serra C, Kelvin D, Kelvin N, Rubino S. Similarity in Case Fatality Rates (CFR) of COVID-19/SARS-COV-2 in Italy and China. *J Infect Dev Ctries.* 2020 Feb 29;14(2):125-128. doi: 10.3855/jidc.12600.
16. National Health Commission of People's Republic of China. Notice on printing and distributing the work plan for prevention and control of pneumonia caused by novel coronavirus infection in the near future. 2020. <http://www.nhc.gov.cn/tigs/s7848/202001/808bbf75e5ce415aa19f74c78ddc653f.shtml>. Accessed 31 Jan 2020.
17. Macciocchi D, Lanini S, Vairo F, Zumla A, Figueiredo LT, Lauria FN, *et al.* Short-term economic impact of the Zika virus outbreak. *New Microbiol.* 2016; 39(4):287-289.
18. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y. *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020;395:507-13
-

Frequency of Chikungunya virus infection in samples tested concomitantly for dengue and malaria: experience from clinical laboratory, Karachi Pakistan.

Uzma Shahid, Joveria Farooqi, Faisal Riaz Mailk, Erum Khan

Department of Pathology and Laboratory Medicine, Section Microbiology, Aga Khan University, Karachi, Pakistan.

Abstract

Background

Chikungunya virus (CHIKV) has recently emerged as cause of acute febrile illness. Exact burden of this viral infection in Pakistan, is not known. A cross-sectional study was performed to assess the frequency of CHIKV infection tested in a clinical laboratory as compared to that of dengue and malaria.

Methods

A descriptive cross-sectional study was conducted over a period of 6 months, at the section of microbiology, Aga Khan University Hospital Clinical Laboratories (AKU), in Karachi. Samples for detection of dengue Non-structural protein 1 antigen (NS1Ag) and/or Immunoglobulin M (IgM), Chikungunya IgM and Malaria Immuno-Chromatographic Testing (ICT) from patients of all ages and both genders were selected and enrolled.

Result

A total of 13,271 patient samples with acute febrile illness were received during the study period. Of which (N= 10160) were tested for malaria, (N=1484) were tested for dengue and (N= 595) for Chikungunya infection. Chikungunya IgM was detected in 8.2% (49 cases) samples. Most cases were diagnosed with malaria (256 cases, 2.5% of those tested) and dengue (251 cases, 16.9% of those tested).

Conclusion

We found CHIKV to be a common cause of febrile illness, its proportion of positive results second only to dengue. However, the burden of dengue and malaria experienced at AKU was much higher than CHIKV. Chikungunya is active in the region and should be considered as an important differential for febrile illnesses.

Key words

Chikungunya, Dengue, Malaria, frequency, Pakistan

Background

Communicable disease is a significant cause of morbidity and

mortality in Pakistan.¹ Amongst these, vector borne disease like Dengue (DENV), Chikungunya (CHIKV) and malaria, are endemic in the South Asian region^{2,3} and contribute to a significant burden of disease. Malaria is caused by a blood parasite of the genus *Plasmodium* transmitted by the bite of female anopheles' mosquito, four species of which are a cause of human disease, namely: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. In Pakistan *P. vivax* (approximately 64%) and *P. falciparum* (approximately 36%) are responsible for the disease.⁴ 500,000 malaria infections and 50,000 malaria-attributable deaths occur each year in Pakistan. *DENV and CHIKV are arboviruses transmitted by the bite of Aedes aegypti and Aedes albopictus* mosquitoes. *Dengue belongs to the family* whereas Chikungunya virus is a member of the *Togaviridae* family. *Coinfections have been reported with grave consequences if left undiagnosed.*⁵

Dengue is endemic since 2003⁶ with almost 71,649 cases reported in 2016 from Pakistan.⁷ Chikungunya, on the other hand, has recently been reported with an outbreak in Karachi during 2017.⁸ Little is known of the epidemiology of CHIKV in our population because of the lack of diagnostics in commercial laboratories before recent outbreak in 2016-17. Although several serological and molecular based diagnostic tools are available for both viruses, serological investigations are routinely used not only due to ease in performance but also for rapid turn-around time allowing prompt diagnosis and disease surveillance. Although CHIKV and DENV are both arboviruses however they belong to a diverse family of viruses. CHIKV is an RNA virus from the *Togaviridae* family, sequence diversity between this and family *flaviviridae* make them antigenically unrelated.^{9,10} Thus, diagnosis based on IgM antibody detection for either virus remains one of the common and rapid method as there is minimal chance of cross reactivity. A study on seroprevalence and cross-reactivity of Chikungunya Virus in arbovirus-infected patients showed a low level of cross-reactivity amongst serum samples from flaviviruses-infected patients, 6% particularly of DENV.¹¹

These pathogens cause acute febrile illness that may be difficult to differentiate in the early phase with high fever, myalgia, and rash. Diagnosis is essential as treatment with antimicrobial agent is important in cases with malaria whereas DENV and CHIKV require supportive therapy. CHIKV especially mimics

Correspondence Author: Erum Khan,
Professor, Section of Microbiology,
Department of Pathology and Laboratory Medicine,
Aga Khan University, Karachi, Pakistan.
Email: erum.khan@aku.edu

dengue syndrome in its clinical presentation and is often wrongly diagnosed as dengue even in the absence of positive dengue investigations. One reason can be due to the unavailability of tests identifying Chikungunya virus in commercial laboratories especially in Pakistan. Differentiation between the two viral illnesses is imperative due to different disease complications and outbreak investigations. Late debilitating sequelae of chronic joint disease is associated with the CHIKV disease where as DENV can result in a severe hemorrhagic fever. CDC recommends to refrain from using aspirin and other non-steroidal anti-inflammatory in treating CHIKV cases unless dengue has been ruled out to prevent risk of bleed.¹²

The aim of the study was to determine frequency of IgM antibody positive to Chikungunya virus in serum samples of patients suffering with acute febrile illness and compared to that for dengue and malaria. We determined the frequency of Chikungunya, malaria, and dengue amongst patients with acute febrile illness who submitted samples to AKUH for serodiagnosis of all three diseases.

Materials & Methods

A descriptive cross-sectional study was conducted from January-June 2018 at the clinical microbiology laboratory, Aga Khan University Hospital (AKUH). The study was approved from the Ethical Review Committee of the institution. Non-probability consecutive sampling was used. Samples received from patients for detection of malaria, dengue non-structural protein 1 antigen (NS1Ag) / Immunoglobulin M (IgM) and Chikungunya IgM were selected. Patient samples of all age groups both male and female presenting with acute febrile illness, as identified by the physicians, were included. These were tested for serological evidence for the 3 diseases. Repeat sample from same patient, hemolyzed samples on visual inspection were excluded from the study. Blood samples were collected according to the standard phlebotomy techniques and stored at -80°C after separation of serum for testing. Results were documented as detected/ positive and not detected / negative respectively in the study proforma and entered in Microsoft Excel 2010. Furthermore, samples investigating all three pathogens simultaneously in a patient with acute febrile illness were evaluated to see the possibility of co-infections.

Chikungunya Immunoglobulin M Detection

CHIKV immunoglobulin M (IgM) antibodies were tested using enzyme-linked immunosorbent assay (ELISA) (Inbios USA) according to standard manufacturer instructions. Immune status ratio (ISR) = 1.10 were considered "Reactive" and <0.90 were considered "Non-reactive". Any sample with range of 0.90 < ISR value <1.10 were retested and evaluated in duplicate to verify the sample status. According to manufacturer, the assay has a >90% sensitivity and specificity.

Dengue Immunoglobulin M Detection

DENV immunoglobulin M (IgM) antibodies were tested using

enzyme-linked immunosorbent assay (ELISA) (Panbio Diagnostics) according to standard manufacturer instructions. Results were interpreted according to Panbio units that were calculated by multiplying the index value by 10.>11 Panbio units were considered "Positive" and <9 Panbio units were considered "Negative". Any sample with range of 9-11 Panbio units was found equivocal and retested. The assay has a sensitivity of 94.7% and specificity of 100%, as claimed by manufacturer.

Dengue NS1 Antigen

This immunochromatographic assay was used for the qualitative detection of NS1 antigen in serum that can be detected for first day of fever onset up to day nine. Presence of two-colored bands (test and control) within the result window were considered as positive. The assay has >90% sensitivity and specificity.

Malaria Antigen Test

Presence of malaria infection was detected using immunochromatographic assay (SD bioline) for qualitative and differential detection of histidine-rich protein II (HRP-II) antigen of *Plasmodium falciparum* (*P.f*) and common *Plasmodium* lactate dehydrogenase (pLDH) of *Plasmodium* species (Pan) in human whole blood. Presence of two colored bands (test *P.f.* and control) or three colored bands (test *P.f.*, test Pan and control) within the result window were considered as positive for falciparum malaria, and two bands (test and Pan) were positive for other *Plasmodium* species. Only one band (test) was considered negative for malaria. According to manufacturer, sensitivity with >50 parasites / μ l is >98% and the test is 99.5% specific.

Data Analysis

SPSS 21 was used for analysis. Identifiers were kept anonymous and all cases given study identification numbers. Mean of the continuous variables i.e. age was calculated; frequency and percentage of the categorical variables i.e. gender, CHIKV /DENV/ malaria results were computed.

Result

From January 2018 to June 2018, a total of 12239 patient samples with acute febrile illness were enrolled. The number of samples tested for each disease type included; malaria antigen (N= 10160), dengue (N=1484: NS1Antigen n=773, IgM n= 696) and Chikungunya (N= 595) infection as requested by the physicians (table 1). The geographic distribution of sample received for each test type namely Malaria, Dengue and Chikungunya according to provinces is shown in table 1. Majority of samples were from Sindh. The mean age for malaria, dengue and Chikungunya positive cases was 29.7, 30.8 and 44.7 years respectively. Male: female ratio amongst infected cases was 1.9 (168/88) for malaria, 1.3 (143/108) for dengue and 0.6 (19/30) for Chikungunya infection. Malaria was seen in 2.5% cases (256/10160).

Amongst these, 0.3% were due to *P. falciparum* only and 2.2%

Table 1: Demographics and frequencies for samples tested individually for Chikungunya, Dengue and Malaria infections.

	Chikungunya N= 595 (%)	Dengue N=1484(%)	Malaria N= 10160(%)
Total Positive	49 (8%)	251(17%)	256 (3%)
Province-wise Positivity Rate			
Sindh	49 (8%)	251 (17%)	245 (2%)
Punjab	0 (0%)	0 (0%)	9 (3%)
Khyber Pakhtunkhwa	0 (0%)	0(0%)	0 (0%)
Baluchistan	0 (0%)	0 (0%)	2 (5%)
Mean Age of Positive Cases (Years)	44.7	30.8	29.7
Male (Positive Cases)	19 (39%)	143 (57%)	168 (66%)

N= total number of cases tested for the disease, (%) = percentage, IgM = Immunoglobulin M, NS1Ag= Non-structural protein 1 antigen

were positive for *Plasmodium* species other than *P. falciparum*, most likely *P. vivax*, as *P. malariae* or *P. ovale* are not seen in Pakistan.⁴ Of the 1484 patients tested for dengue, 16.9% (251) were positive by either NS1 antigen or IgM. Dengue NS1 antigen was positive in 4.8% cases and 11.9 % were reactive for dengue IgM. Chikungunya IgM was detected in 8.2% samples (49/595), indicating Chikungunya infection in these cases. Forty-seven samples were tested simultaneously for all three pathogens (table 2). Amongst these 27 were males and 20 females. Malaria was seen in 2.1%, dengue was identified as most common infection among patients tested 19.1% of samples concomitantly tested for three pathogens were positive for dengue (both NS1Ag and IgM). None of the concomitantly testes samples showed IgM antibodies to Chikungunya virus. No case of co-infection was observed. The overall positivity rate amongst the samples tested simultaneously for all three diseases, was 21.2%.

Discussion

Chikungunya is known for its epidemic potential worldwide.⁵ Recent reports from Pakistan confirm its presence in the country.⁶ This study was aimed to determine the frequency of CHIKV infection in patients with acute febrile illness which was found to be 8.2%. This is lower than the frequencies reported from India (25.37%)^{4,7} but slightly higher than those reported from

Sri Lanka (3.5%).¹⁴ One of the limitations of this study was that PCR was not performed for patients suspected of CHIKV infection, there is a possibility that early cases of CHIK might have been missed. Moreover, 95% of the total samples received at clinical laboratory from suspected acute febrile patients did not request for CHIKV testing, perhaps be due to lack of awareness by the physicians about CHIKV as emerging cause of febrile illness in Pakistan, therefore further studies are needed to investigate the exact burden of CHIKV.

Detection of dengue IgM in 21.1% cases and malaria in 2.5% cases highlight the fact that dengue is superseding endemicity of this infection over malaria in our tested population.

Patients with dengue infection had more cases detected by IgM (21.1%) than by NS1Ag (10%). This is most likely due to the fact that dengue IgM is detectable in 80% cases on serological testing around day ten of illness and remains elevated for up to 6 months or longer.¹⁵ We reported 2.2% cases for *Plasmodium* species and 0.3% *P. falciparum*. This finding is strengthened by the observations from other studies in countries reporting *P. species*, (most likely *P. vivax*) as the predominant cause of malaria.⁴

The three infections share similar presentations of acute fever but may be differentiated on basis of other disease characteristics. Malaria has a classic fever pattern of recurring at 12 hours (quotidian – *P. falciparum*), 48 h (tertian – *P. ovale* and *P. vivax*), or 72 h (quartan – *P. malariae*).¹⁶ Dengue can be suspected in presence of decreasing platelets indicating its hemorrhagic potential and disease severity whereas Chikungunya presents predominantly with arthralgia. Thus, clinical presentation can be useful in differentiating the disease but may be absent in the early phase of symptoms. Co-infection is rare¹⁷ and potentially fatal⁷, however, no co-infections were identified in this study. This may be due to the short study duration and

Table 2: Frequencies of infections for samples tested simultaneously for Chikungunya, Dengue and Malaria infections (N=47)

	Chikungunya	Dengue	Malaria
Positive result	0 (0%)	9 (19%)	1 (2%)
Negative results	47 (100%)	38 (81%)	46 (98%)
Co infections	None identified		

limited number of samples tested for all three diseases, and thus needs further evaluation.

One may consider review of this data as passive surveillance however the main objective was to analyze the spread of disease indifferent part of country as patient samples were received from all over country. The study results are generalizable to the population of Sindh only as most samples were received from there, but not the whole of Pakistan. Only forty-seven samples were tested simultaneously for the three diseases and were too few to provide an opportunity to detect co-infection, a rare possibility. Furthermore, clinical details were not recorded as analysis of patients' clinical presentation was out of the scope of this study.

Second limitation was inability to use additional confirmatory tests such as PCR for DENV and CHIKV and peripheral film for Malaria. However, since the intent of study was to see the burden of CHIK virus in the acute febrile patients presenting to clinical lab in comparison to dengue and malaria our study still provides useful information to the readers about the burden of this infection in clinical setup, using regular diagnostic testing methods. Since PCR was not used, early cases of CHIKV might have been missed; therefore, further studies using both diagnostic modalities are required to assess true burden of the disease.

In conclusion, CHIKV is emerging as a cause of acute febrile illness alongside dengue and malaria, and it should be considered in the differential diagnosis of all patients presenting with acute febrile illness in Pakistan. Factors like poor vector control and urbanization increased breeding sites for mosquitos play an important role in disease endemicity. Vector control is integral to limit the spread of these illnesses.

References

1. WHO. Communicable disease surveillance and response. 2018.
2. Raut CG, Rao NM, Sinha DP, Hanumaiah H, Manjunatha MJ. Chikungunya, dengue, and malaria co-infection after travel to Nigeria, India. *Emerg inf dis*. 2015;21(5):908-9. Epub 2015/04/22.
3. Kumar A, Chery L, Biswas C, Dubhashi N, Dutta P, Dua VK, *et al*. Malaria in South Asia: prevalence and control. *Acta tropica*. 2012;121(3):246-55. Epub 2012/01/18.
4. Khattak AA, Venkatesan M, Nadeem MF, Satti HS, Yaqoob A, Strauss K, *et al*. Prevalence and distribution of human Plasmodium infection in Pakistan. *Malaria j*. 2013;12:297. Epub 2013/08/30.
5. Salam N, Mustafa S, Hafiz A, Chaudhary AA, Deeba F, Parveen S. Global prevalence and distribution of coinfection of malaria, dengue and chikungunya: a systematic review. *BMC pub heal*. 2018;18(1):710. Epub 2018/06/09.
6. Khan E, Kisat M, Khan N, Nasir A, Ayub S, Hasan R. Demographic and clinical features of dengue fever in Pakistan from 2003-2007: a retrospective cross-sectional study. *PLoS one*. 2010;5(9):e12505. Epub 2010/09/22.
7. Saboor Ahmad MAA, Asad Aftab, Zia Ullah, Muhammad Irfan Ahmad and Abdul Mustan. Epidemiology of dengue in Pakistan, present prevalence and guidelines for future control. *Int J Mosq Res*. 2017;6(4):25-32.
8. Aamir UB, Badar N, Salman M, Ahmed M, Alam MM. Outbreaks of chikungunya in Pakistan. *The Lancet Infect dis*. 2017;17(5):483. Epub 2017/04/28.
9. Kikuti M, Tauro LB, Moreira PSS, Nascimento LCJ, Portilho MM, Soares GC, *et al*. Evaluation of two commercially available chikungunya virus IgM enzyme linked immunoassays (ELISA) in a setting of concomitant transmission of chikungunya, dengue and Zika viruses. *IJID*:2020;91:38-43. Epub 2019/11/11.
10. Strauss JH, Strauss EG. Virus evolution: how does an enveloped virus make a regular structure? *Cell*. 2001;105(1):5-8.
11. Kam YW, Pok KY, Eng KE, Tan LK, Kaur S, Lee WW, *et al*. Sero-prevalence and cross-reactivity of chikungunya virus specific anti-E2EP3 antibodies in arbovirus-infected patients. *PLoS negl trop dis*. 2015;9(1): e3445. Epub 2015/01/09.
12. <https://www.cdc.gov/chikungunya/symptoms/index.html>.
13. Ray P, Ratagiri VH, Kabra SK, Lodha R, Sharma S, Sharma BS, *et al*. Chikungunya infection in India: results of a prospective hospital based multi-centric study. *PLoS one*. 2012;7(2):e30025. Epub 2012/03/01.
14. Reller ME, Akoroda U, Nagahawatte A, Devasiri V, Kodikaarachchi W, Strouse JJ, *et al*. Chikungunya as a cause of acute febrile illness in southern Sri Lanka. *PLoS one*. 2013;8(12):e82259. Epub 2013/12/07.
15. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control: New Edition. Geneva 2009.
16. Ogoina D. Fever, fever patterns and diseases called 'fever' -a review. *J inf pub heal*. 2011;4(3):108-24. Epub 2011/08/17.
17. Chang SF, Su CL, Shu PY, Yang CF, Liao TL, Cheng CH, *et al*. Concurrent isolation of chikungunya virus and dengue virus from a patient with coinfection resulting from a trip to Singapore. *J Clin microbiol*. 2010;48(12):4586-9. Epub 2010/10/01.

Rhinofacial Basidiobolus Misdiagnosed as Granulomatosis with Polyangiitis

Aneela Hussain*, Aisha Ilyas*, Kausar Jabeen**, Syed Faisal Mahmood*

*Section of Infectious Diseases, Department of Medicine, Aga Khan University Hospital, Karachi, Pakistan

**Section of Microbiology, Department of Pathology and Microbiology, Aga Khan University Hospital, Karachi, Pakistan

Abstract

We report a case of a young female, initially misdiagnosed as Granulomatosis with Polyangiitis (Wegener's). Repeat histopathology and culture however revealed *Basidiobolus species*. She was managed successfully with antifungals, highlighting the need for a high index of suspicion for molds in unusual presentations.

Key Words

Basidiobolus; Granulomatosis with Polyangiitis.

Introduction

Basidiobolus species are saprophytic fungi of the order Entomophthorales; Class Zygomycetes found in the soil, decaying vegetation, and intestines of most animals. In humans, they cause chronic skin and subcutaneous infections usually following abrasions or insect bites.¹ While infections had been geographically restricted to tropical Asia and Africa, however recently its epidemiology has evolved about geographical location, host susceptibility and involvement of deeper tissues.^{2,3} Diagnosing *Basidiobolus* is difficult and can only be reliably made with both culture and histopathology. On histopathology, the infection is characterized by necrotizing granulomatous inflammation with predominant eosinophils and fungal element (hyphae).¹ Failure to detect hyphae due to presence of rare or degenerated fungal elements or inappropriate staining often lead to other diagnoses including soft tissue tumors, autoimmune disorders and tuberculosis.⁴

Here we present one such case of a 25-year-old female with Rhinofacial *Basidiobolus*, initially misdiagnosed as Granulomatosis with Polyangiitis (GPA).

Case History

A 25-year-old female from Iran, presented to outpatient clinic at the Aga Khan University, Karachi, Pakistan with complaints of right facial swelling for the past 3 months. On examination, the right side of her face was hyperemic, swollen with proptosis. Endoscopic nasal examination revealed necrotic tissue with

purulent discharge. Her extraocular movements and visual acuity were intact. The rest of the systemic examination was unremarkable.

Computed Tomography (CT) of Paranasal sinuses revealed an extensive 50 x 39 mm soft tissue mass in the right maxillary sinus with subcutaneous infiltration into the right orbit and the skin anteriorly. The mass extended into the ethmoid sinuses with bony erosion however, there was no intracranial extension.

She had undergone endoscopic sinus surgery 3 months ago in Iran with tissue histopathology report suggesting GPA (Wegener's granulomatosis), unfortunately the slides from that time were not available for reviewing. The patient had not undergone any therapy or work-up for the presumed GPA and travelled to Pakistan for further management.

Initial investigations showed a leucocyte count of 15.4×10^9 cells/liter, with neutrophilia, an ESR of 55 mm/hour and normal chest radiography. Given the scenario, a presumptive diagnosis



Figure showing CT Scan image of the patient.

Correspondence Author: Aneela Hussain,
Section of Infectious Diseases, Department of Medicine, Aga
Khan University Hospital,
Karachi, Pakistan
Email: doc.aneela@yahoo.com

of GPA with superimposed bacterial infection was made and Piperacillin-Tazobactam was empirically started. Later, her serum anti-nuclear cytoplasmic antibodies (ANCA) was reported negative. She had to undergo endoscopic debridement and hence tissue was resent for histopathology along with cultures. Potassium hydroxide (KOH) smear of the tissue showed broad hyphae with rare septation. Based on this Amphotericin B at 1mg/kg/day was added to cover Mucormycosis. Forty-eight hours later fungi had grown on all primary plates. A Lactophenol cotton blue preparation of the colonies showed broad hyphae with occasional septae and thick-walled intercalary zygosporangia with beak like projections; appearance typical of *Basidiobolus* species. Histopathological examination of the excised tissue showed mixed acute and chronic granulomatous inflammation with histiocytes and multinucleated giant cells with abscess formation with no evidence of vasculitis or necrosis to suggest a diagnosis of GPA. Periodic Acid Schiff stain also highlighted broad aseptate hyphae. Further clinical correlation of these findings with a negative ANCA helped exclude the possibility of GPA with secondary fungal infection.

A diagnosis of Rhinofacial *Basidiobolus* was made and Amphotericin B was changed to oral Itraconazole (400mg/day) and Potassium Iodide. The Potassium Iodide dose was gradually increased from 1 mg every eight hourly to 3 mg every eight hourly over a period of six days. She responded well to therapy and was discharged on Itraconazole and Potassium Iodide.

On follow-up her facial edema and erythema had substantially reduced, and she returned to Iran shortly thereafter. Unfortunately, due to non-availability of Potassium Iodide in Iran, Itraconazole monotherapy was continued. Periodic follow ups assured compliance and improvement. She received Itraconazole for six months with complete resolution of radiological findings and clinical symptoms. She remained symptoms free till her last follow i.e. six months after stopping Itraconazole.

Discussion

We describe a case of Rhinofacial *Basidiobolus* that was initially misdiagnosed as Granulomatosis with Polyangiitis (GPA). On review of literature search we found 85 cases of *Basidiobolus*. Most cases were reported from South Asia especially from India, Iran and Saudi Arabia with occasional cases from Bangladesh, Oman, Qatar, Thailand, Maldives and Pakistan.² The fungus is usually inoculated after trauma contaminated by soil which may also explain the male preponderance (M: F, 3:1).⁴ The disease is mostly seen in the younger population (22 out of 66 cases) with median age of less than 25 years.⁴ Visceral infections have more commonly been reported (54 of 85 cases) mostly involving GI tract (54.8%).² Infection of liver, lung and spleen have also been described. The skin and subcutaneous tissue infections are the second most common type of infection (22 out of 85 cases). Lower extremities are more commonly

affected.⁴ Unlike Mucormycosis, rhinocerebral involvement with *Basidiobolus* is rather uncommon (n=5). *Basidiobolus* skin lesion is described as firm, non-tender nodules; presentation that overlaps with many other inflammatory and infectious processes.⁴

While our patient denied any trauma, she did use homemade "Balochi Surma". This is a traditional mascara made by grinding lead sulfide and other ingredients (including herbs). As the fungus is endemic to the region and ubiquitous, we suspect this to be a possible source of inoculation.

Basidiobolus can easily be misconstrued, hence fungal cultures remain the gold standard for diagnosis. Our case was initially confused with PGA due to the similar clinical and histopathological presentation, but a high index of suspicion and tissue cultures led us to the diagnosis. Interestingly, *Basidiobolus* has been reported to cause superimposed infections in patients with PGA on steroid therapy, mimicking as disease relapse.⁵ However in our patient a negative ANCA, biopsy suggestive of *Basidiobolus*, improvement without steroids and response to antifungal therapy effectively rules out PGA.

While overall outcomes with *Basidiobolus* appear to be good, because of the rarity, treatment is not well defined. Surgical debridement with systemic antifungal has been the mainstay of therapy though the optimal antifungal; its dose and duration remain unclear. Azoles are most effective (Itraconazole, Posaconazole, Voriconazole); monotherapy with Amphotericin B was associated with poor outcomes.^{1,2} Combination of Azoles with Terbinafine, Amphotericin B, Potassium Iodide and Cotrimoxazole also seems effective, although with handful of cases generalization is difficult.² Our patient briefly received Amphotericin B initially suspecting Mucormycosis, but largely remained on Itraconazole monotherapy with good outcome.

To conclude, in contrast to Mucormycosis, *Basidiobolus* mainly causes visceral disease in children^{1,4} with rather good prognosis. As histopathological findings overlap in various chronic infectious and inflammatory processes, biopsy samples should be cultured in clinically relevant settings.

References

1. Gugnani HC. A review of zygomycosis due to *Basidiobolus ranarum*. *Eur j epidemiol* 1999; 15:923-9.
2. Khan ZU, Khoursheed M, Makar R, Al-Waheeb S, Al-Bader I, Al-Muzaini A, et al. *Basidiobolus ranarum* as an etiologic agent of gastrointestinal zygomycosis. *J Clin Microbiol*. 2001; 39(6):2360
3. Chetambath R, Deepa Sarma MS, Suraj KP, et al. *Basidiobolus*: An unusual cause of lung abscess. *Lung India*; 27:89-92.
4. Anand M, Deshmukh SD, Pande DP, Naik S, Ghadage DP. Subcutaneous Zygomycosis Due to *Basidiobolus ranarum*: A Case Report from Maharashtra, India. *J trop med*. 2010;950390.
5. Petrikos G, Drogari-Apiranthitou M. Zygomycosis in Immunocompromised non-Haematological Patients. *Mediterr J Hematol Infect Dis* 2011; 3:e2011012.

Instructions to Authors

Scope

The Infectious Diseases Society of Pakistan sponsors the Infectious Disease Journal of Pakistan (IDJ). The Journal accepts Original Articles, Review Articles, Brief Reports, Case Reports, Short Communications, Letter to the Editor and Notes and News in the fields of microbiology, infectious diseases, public health; with laboratory, clinical, or epidemiological aspects.

Criteria for publication

All articles are peer reviewed by the IDSP panel of reviewers. After that the article is submitted to the Editorial Board. Authors may submit names and contact information of 2 persons who potentially could serve as unbiased and expert reviewers for their manuscript, but IDSP reserves the right of final selection.

Submission of the Manuscript

Manuscripts must be formatted according to submission guidelines given below, which are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (originally published in *N Engl J Med* 1997;336:309-15). The complete document appears at www.icmje.org. Please submit one complete copy of the manuscript and all enclosures to **The Managing Editors, Infectious Diseases Journal of Pakistan, Department of Pediatrics & Child Health, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan**. An electronic copy of the manuscript must also be sent to pak_idj@yahoo.com. All manuscripts submitted to IDJP must be accompanied by an Authorship Declaration stating that '*The authors confirm that the manuscript, the title of which is given, is original and has not been submitted elsewhere. Each author acknowledges that he/she has contributed in a substantial way to the work described in the manuscript and its preparation*'. Upon submission a manuscript number will be assigned which should be used for all correspondence.

Manuscript Categories

I. Original Articles

Articles should report original work in the fields of microbiology, infectious disease or public health. The word limit for original articles is 2000.

Title page

This should list the (i) title of the article, (ii) the full names of each author with highest academic degree(s), institutional addresses and email addresses of all authors. (iii) The corresponding author should also be indicated with his/her name, address, telephone, fax number and e-mail address. (iv) A short running title of not more than 40 characters (count letters and spaces) placed at the foot end of the title page. (v) a conflict of interest statement should also be included in this section.

Abstract

Abstract should not exceed 250 words and must be structured in to separate sections headed *Background, Methods, Results and Conclusions*.

Please do not use abbreviations or cite references in the abstract. A short list of four to five key words should be provided to facilitate.

Background

The section must clearly state the background to the research and its aims. Controversies in the field should be mentioned. The key aspects of the literature should be reviewed focusing on why the study was necessary and what additional contribution will it make to the already existing knowledge in that field of study. The section should end with a very brief statement of the aims of the article.

Materials and Methods

Please provide details of subject selection (patients or experimental animals). Details must be sufficient to allow other workers to reproduce the results. The design of study and details of interventions used must be clearly described. Identify precisely all drugs and chemicals used, including generic name(s) and route(s) of administration. All research carried out on humans must be in compliance with the *Helsinki Declaration*, and animal studies must follow internationally recognized guidelines. The authors are expected to include a statement to this effect in the Methods section of the manuscript. A description of the sample size calculation and statistical analysis used should be provided.

Results

Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of 5 illustrations (in a combination of figures and tables) per article. The results should be in past tense and repetition of results presented in the tables should be avoided. Exact *P*-values should be reported along with reporting of OR and RR with their Confidence Intervals where applicable.

Discussion

Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat the details from the results section. Discuss the implications of the findings and the strengths and limitations of the study. Link the conclusions with the goals of the study but avoid unqualified statements and conclusion not completely supported by your data.

Acknowledgments

Acknowledge any sources of support, in the form of grants, equipment or technical assistance. The source of funding (if any) for the study should be stated in this section. Please see below for format of **References, Figures and Tables**.

II. Review Articles

Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. It should consist of critical overview of existing literature along with reference to new developments in that field. These should be comprehensive and fully referenced. Articles should contain an Abstract; Main Text divided into sections, Conclusions and References.

III. Brief Reports

Short clinical and laboratory observations are included as Brief Reports. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references.

IV. Case Reports

Instructive cases with a message are published as case reports. Routine syndromes or rare entities without unusual or new features are invariably rejected. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references. The authorship should not exceed 3-4 persons.

V. Letter to the Editor

These may relate to material published in the IDJP, topic of interest pertaining to infectious diseases, and/or unusual clinical observations. A letter should not be more than 300 words, one figure and 3-5 references.

VI. News and Views

Informative, breaking news updates in infectious diseases from around the world (approx. 200 words).

VII. Notices

Announcements of conferences, symposia or meetings may be sent for publication at least 12 weeks in advance of the meeting date. Details of programs should not be included.

References

Number references consecutively in the order in which they are first mentioned in the text. Identify references in text, tables and legends by Arabic numerals (in superscript). References cited only in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification of the particular table or illustration. Bibliography should be given in order. Authors, complete title, journal name (Abbr), year, vol, issue, page numbers. According to "Uniform

Requirements of Manuscripts submitted to Biomedical Journals", as cited in N Engl J Med 1997; 336:309-15.

Tables and Figures

Data reported either in a table or in a figure should be illustrative of information reported in the text, but should not be redundant with the text. Each table must be presented on a separate sheet of paper and numbered in order of appearance in the text. Table should be numbered consecutively in Arabic numerals. Tables and Figures legends should be self-explanatory with adequate headings and footnotes. Results which can be described as short statements within the text should not be presented as figures or tables.

Illustrations

Illustrations should be numbered, given suitable legends and marked lightly on the back with the author's name and the top edge indicated. Original drawings may be submitted although high quality glossy photographs are preferable. They should be kept separate from the text. If possible, figures should be submitted in electronic format as either a TIFF (tagged image file format) or JPEG format. Minimum resolution for scanned artwork is:

- √ Black & white line illustration (e.g. graphs): 600 dpi
- √ Black & white halftone illustrations (e.g. photographs): 300 dpi
- √ Color illustrations: 400 dpi (note that color images should be split CMYK not RGB)

Plagiarism

Authors should refrain from plagiarism and should double check their work before submitting it for publication. Adequate references should be provided for text from other sources.

Authorship criteria

Those who have contributed sufficiently to the conceptualization, design, collection and analysis of data and writing of the manuscript should be granted authorship. Ideally all authors should be from the same department except for studies that are multi center or multispecialty.

Instructions updated - April 2012.

Editor IDJ
