INFECTIOUS DISEASES JOURNAL

IDJ

of Pakistan

Published by the Medical Microbiology & Infectious Diseases Society of Pakistan

ISSN 1027-0299

Recognised and registered with the Pakistan Medical & Dental Council
NO.PF.11-F-96 (Infectious Diseases) 2560
College of Physicians & Surgeons, Pakistan
Indexed - *WHO EMRO*

July - September 2019 Volume 28 Issue 03

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

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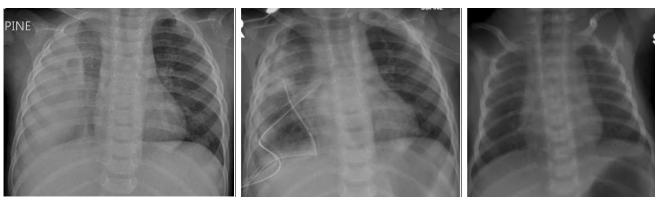
A 10 month old infant with Distress, tachypnea and hypoxia.

A B C



A: ill-defined airspace shadowing in the right lung with development of some areas of cavitation. This is suggestive of infective consolidation with cavitation.
B: There is interval placement of two right-sided chest tubes. There is suggestion of a kink in one of the chest tube. There is evidence of a right small pneumothorax and subcutaneous emphysema which could be secondary to recent intervention
C: Normal Chest X-ray after 6 weeks of therapy.

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

Cascades of Care as Monitoring Tool for Tracking Global Target of Hepatitis C Virus Elimination

Hepatitis C infection is caused by an RNA virus of the family *Flaviviridae and the genus* Hepacivirus, known as hepatitis C virus (HCV).[1] The HCV was first discovered in 1989. HCV is classified into seven phylogenetic clades also known as genotypes (HCV genotype 1,2,3,4,5,6, and 7). HCV is a blood-borne virus predominantly transmitted through parenteral route. However, in about 20% of HCV infections the route of transmission remains unknown.[2] While several studies have isolated HCV RNA from the saliva, semen, urine, sweat, and tears of the HCV infected patients, the risk of transmission associated with exposure to infected body fluids except serum is not clearly defined.[3-5]

Globally, HCV is one of the leading indication for liver transplanation.[6] According to a disease burden modelling study, in 2015, 71.1 million (range 62-79) which corresonds to 1% of global population had HCV infection.[7,8] In 2015, there were an estimated 1.75 million new HCV infections and 399,000 HCV-related deaths, while only 843,000 people with chronic HCV infection were cured.[8] Globally, a wide variation in the prevalence of HCV exists among different countries and regions. Almost half of the global burden of HCV is contributed by only 5 countries (China, Pakistan, India, Egype, Russia).[9] However, western countries account for only a small fraction of the overall disease burden.[10,9]

Some of the major risk factors for hepatitis C infection are; injecting drug use, recipient of blood or blood products transfusion (before 1990 in the developed world, and in the developing countries it may be a recent date depending on the availability of standard screening facilities and regulations for blood screening), received injections for medical treatment, underwent dental procedures or had a shave by barbers in a country with a high prevalence of HCV infection, had a haemodialysis, had a tattoo or body piercing from a poorly regulated facility, having a sexual partner with hepatitis C infection who is also HIV positive, having a mother with HCV infection, have been in prison and those who have had blood-to-blood contact with another person. Healthcare workers in the developing countries where personal protective equipment is not available and adherence to standard precautions is poor, are also at a higher risk of HCV infection.[1,10]

With the development of highly effective, well tolerable, oral direct acting antiviral (DAA) therapy during 2011, HCV is now treatable and curable. In 2016, World Health Assembly (WHA) endorsed global viral hepatitis strategy, setting the targets of 80% reduction in HCV new infections, and 65% reduction in mortality associated with HCV by 2030. More than 194 member countries of the World Health Organization (WHO) adopted this strategy. [11,12] With the availability of highly effective, interferon free DAA therapy, achieving this super ambitious goal is not impossible however, barriers related to screening, treatment access, and patients' retention in care need to be overcome. As of 2015 data, out of 71 million global HCV-infected people, only 20% (14 million) are diagnosed, and 8% (5.4 million) have ever initiated therapy.[8] According to the WHO, 90% of the total infected population has to be diagnosed and 80% of those diagnosed has to be treated so that to facilitate the elimination targets by 2030.[13] Therefore, close monitoring of the existing HCV elimination strategies through measuring the proportion of HCV infected people progressed through different stages from screening to the achievement of SVR is utmost important. Cascades of care or care continuum, first used for the monitoring of the treatment programs of human immunodeficiency virus (HIV) infections, is now adopted for the monitoring of elimination program of chronic HCV infection.[14] In the field of HIV, cascades of care with various stages such as diagnosis, linkage to care, retention in care, prescription of antiretroviral therapy and viral suppression is used as an effective tool in assessment of achieving the public health benefits of antiretroviral therapy.[15,16] Similarly, HCV cascade of care has a potential to assess the progress towards achieving WHO global targets of HCV elimination by 2030. It provides a useful framework for the monitoring of HCV treatment programs and HCV patients passing through different stages of the care continuum.

The HCV care cascades measure the effectiveness of public health response to HCV infection, by estimating the proportion of people with HCV infection in a specific population, measuring the proportion of those who are reached by the public health program and screened positive for HCV, proportion tested for HCV RNA, linked to care, proportion assessed for liver disease, proportion treated for HCV, and the proportion treated who achieve SVR.[17] The following figure (Fig. 1) adopted from WHO Health sector strategy on viral hepatitis 2016-21 clearly depicts the different stages of the HCV care cascades.[12]

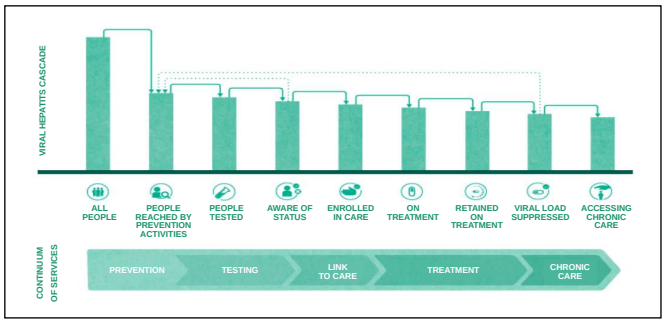


Fig. 1: HCV cascades of care. *Adopted from WHO Global health sector strategy on viral hepatitis 2016-21.*

To conclude, HCV infection is a global public health problem. With the recent development of highly effective DAA therapy,

global elimination of the disease is now possible. However, barriers such as screening, linkage to care and access to the current therapy needs to be addressed. Inequity in screening and treatment needs to be eliminated by giving special attention to the difficult to reach, high risk populations. Using the HCV cascades of care as monitoring tool for the assessment of HCV treatment program and identification of any barriers or facilitators is required.

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ORIGINAL ARTICLE

Quality Assurance (QA) of Qualitative serological Tests in the Clinical Microbiology Laboratory: Limitations and Solutions

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Abstract

In a clinical microbiology laboratory, accurate and reliable results can only be achieved by adhering to quality assurance and quality control protocols. This process starts from patient's sample collection, receiving, processing and final reporting. Method validation of each component is essential, and a breach may lead to faulty result. Due to high prevalence and rapid emergence of infectious diseases, the importance of rapid and reliable qualitative serological tests has increased. However, ignorance and noncompliance to quality assurance process by the laboratories, especially in low resource settings leads to inappropriate diagnosis which leads to wrong interpretation and hence inappropriate treatment and ultimately poor prognosis.

Key words
Method validation, quality assurance, quality control

The paradigm shift of laboratory medicine's role from specimen-centered clinical testing toward patient-centered clinical decision making has helped significantly in improving patient's outcome. The claim that laboratory diagnoses the contribute in clinical assessment can only be reliable if it is appropriately ordered, conducted, returned with results on a timely basis, correctly interpreted and affect a decision for further diagnosis and treatment. Medical laboratory Quality assurance (QA) plan ensures that the entire processes of any test are monitored at every step and the results generated are accurate, reliable, timely and reproducible. Results of In-vitro diagnostic (IVD) could be unreliable in the absence or deviance from QA plan. This could have negative consequences including unnecessary treatment, treatment complications, failure to provide the proper treatment, delay in correct diagnosis, additional and unnecessary diagnostic testing. As IVD cost has influence on health care expenditures, the issues consequently cause increased cost, time and personnel effort, and often in poor patient outcomes.

Communicable disease has emerged as significant cause of morbidity and mortality globally.[1] Research and development in IVD for reliable diagnosis of infectious diseases, has started its journey from conventional microbial culture along with serological techniques and now complex molecular methods.[2]

In diagnostic laboratories the infectious disease serological techniques are typically use for non-cultureable microorganism e.g. viruses, difficult to cultivate bacteria like *Treponema pallidum*, *Helicobacter pylori* and parasites. Some time it also helps in therapeutic monitoring. As a rule of thumb, serological methods also require vigilant quality assurance processes for generating reliable laboratory results. Those clinical laboratories which are perform infectious disease serological tests have questionable reliability due to their noncompliance with the quality practices. Quality assurance recommendations, e.g. College of American Pathologist and ISO 15189 are followed by few laboratories in Pakistan.[3,4]

The non-conformities with the quality standards are as follows:

1. Laboratories do not update the test methodologies in view with the updated recommended diagnostic guidelines.
2. The prefer to use low cost diagnostic kits which are generally not approved any regulatory authority (e.g. FDA, WHO, CE mark)
3. Knowledge gaps and limited application of total quality assurance process by technical staff.
4. Considering quality control as financial liability, generally the laboratory managements do not show commitment to put these extra monitory inputs.

Infectious diseases serological tests are qualitative and quantitative. Before introducing any qualitative tests in the laboratory, a vigorous quality assurance process allows a complete evaluation of the kit and the related method. The components of quality assurance process are as follows that must be followed prior to initiate qualitative serological tests.

Quality Assurance Process:[5-7]

A. **Pre-analytical component**

1. Sample collection: Appropriate container according to the required test is essential.
2. Sample transportation: It must be abided by recommended

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condition i.e. temperature. In case of delay, addition of preservative or refrigeration according to the manufacturer's recommendations.

3. Sample processing: Before performing the test, the quality of specimen must be assessed visually for hemolysis and turbidity.

B. **Analytical component**

Following are the analytical components

1. Method validation
2. Quality control
3. Equipment maintenance
4. Lot to lot verification
5. External quality assessment

1. Method validation

Validation and verification of process is key process in analytical component.

- **Validation**

Process of proving that a procedure, process, system, equipment, or method used works as expected and achieves the intended results. It includes calculation of accuracy, precision, specificity, sensitivity, positive and negative predictive value. These processes must repeat in case of change in method or manufacturer.

- **Verification**

Process of confirmation by examination and provision of objective evidence that specified requirements have been fulfilled.[8]

Following points must be considered before starting validation process:

- Thorough literature review and market search for authentic method and kit
- Select kits of reliable manufacturer approved by FDA or CE mark.
- Avoid kits which are intended for research purposes only.

Validation and verification process must start after reliable kit selection

Validation Process

It requires positive and negative control samples. In case of non-availability of controls, proficiency tests samples and well characterized positive patient sample can be used for this purpose. Well characterized samples are those that correlate with patients' clinical details and must be verified by reference laboratory. Twenty positive and negative samples can be used for FDA approved kits. However, for unapproved kits large sample size is required (e.g. > 100).[9]

2. Quality Control

Frequency and sample for quality control is very important. Commercial controls are available however positive patient sample can use as in-house control. These controls must run daily before start testing. Kits with internal controls should also

Summary of Quality assurance steps for laboratory bases tests (9)

Components	Definition	Calculation
Accuracy	How much is the given method accurate to generate the result	*A= No. of correct results/ total no. of results x 100*
Precision	Can we get the same result upon repeat testing on same sample numerous times at different days by different individual under the same operating conditions	*P= No. of repeated results in agreement/ total no. of results x 100*
Specificity	Ability of method to detect only the analyte in the presence of other factors	*Sp = No. of true negative results/ (No. of true negative results + No. of false positive results) x 100*
Sensitivity	Ability of method to detect smallest quantity of analyte	Se = No. of true positive results / (No. of true positive results + No. of false negative results) x 100
Positive Predictive Value	Check probability of the presence of an analyte in a specimen	*PPV=No. of true positive results / (No. of true positive results + No. of false positive results) x 100*
Negative Predictive Value	Check probability of the absence of an analyte in a specimen.	*NPV=No. of true negative results / (No. of true negative results + No. of false negative results) x 100*

be monitored vigilantly with each test.

Results should be documented in designated forms and must review daily by bench in charges and monthly by laboratory manager.

In case of erroneous quality control results, do not hold patient testing. Repeat with alternate kit (same manufacturer and lot or different lot). If problem persists, perform root cause analysis. Check the temperature and conditions of the storage area. The storage condition of the associated reagents must also be evaluated.

3. Equipment maintenance

Equipment maintenance schedule as daily, monthly, annually. Check manufacture's recommendation also. Yearly maintenance schedule for each instrument with frequency and designated person must maintain by manager.

Instrument calibration should perform before initiating clinical sample testing.

Coordinate with biomedical department for any erroneous instruments. For placed equipment, there should be clear documentation in contract regarding maintenance responsibilities.

4. Lot-to-Lot verification

New lot of kit must be verified before testing patient sample. New shipment requires verification

5. External assessment

External Quality assurance scheme is an important quality assessment tool.[10]

C. Post-analytical

1. Review of results

Results must review and correlate with other tests before final result.

2. Audits

Internal and external audits are performed at regular intervals to ensure compliance

3. Reference ranges

Authentic and recent reference ranges must be provided for result interpretation.

Conclusion

Adherence to pre analytical, analytical and post analytical component is essential for reliable result. Laboratories must develop their quality assurance plan; collaborate with laboratories that follow authentic quality assurance protocol

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Extensively Drug Resistant Typhoid Fever Seen at Tertiary Care Hospital in Lahore

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Abstract

Background
Typhoid fever is endemic in Pakistan. Multi-drug resistance is commonly observed in Salmonella Typhi (*S.Typhi*) isolates, due to which first line of drugs, ampicillin, trimethoprim-sulphamethoxazole and chloramphenicol become ineffective for treatment. Towards the end of 2016 these MDR strains also developed resistance to ceftriaxone and quinolones and were called extensively drug resistant (XDR). Since first reported outbreak of XDR *S.Typhi*, it continues to increase in number in Sindh.

Objectives
Present study was planned to see the sensitivity pattern of *S. Typhi* isolated in a tertiary care hospital, Lahore.

Study Design
Retrospective analysis

Setting and Duration
The study was done at Microbiology Department of Services Institute of Medical sciences from January 2018 to April 2019.

Materials & Methods
All 16288 blood culture samples received from indoor and outpatient department were analyzed for *S. Typhi* by standard microbiological techniques. Sensitivity of *S. Typhi* to ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, ciprofloxacillin, ceftriaxone, azithromycin and imipenem was assessed Kirby-Bauer method according to CLSI guidelines 2018.

Results
During Jan-Dec 2018, number of *S. Typhi* isolated were 29 out of these 13 were MDR and 14 XDR. In the first four months of 2019, 69 *S. Typhi* were isolated; of these 6 were MDR and 58-XDR. Theses findings are significant *p*-value <0.00017. Another alarming finding was the presence of one azithromycin resistant XDR. Most of *S. Typhi* were isolated from children less than 15 years old.

Conclusions
With increasing XDR *S. Typhi* being isolated in Lahore, coordinated efforts are needed to control it. This will include vaccination and awareness campaign for better hygiene, safe water and improved sanitary conditions. Antibiotic stewardship should be taken seriously at Government level and over the counter sale of antibiotics must be stopped.

Key Words
Typhoid, Salmonella typhi, extensively drug resistant, XDR

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Background
The Indo-Pak subcontinent has seen a decline in prevalence of typhoid fever from the turn of the century to 2015. In a multi -center study conducted in Pakistan on blood culture isolates; the percentage of *Salmonella Typhi* (*S. Typhi*) was 1.32% in 2015, a decrease from 6.42% in 1992.[1] In India this decline has been accompanied with a decreased incidence of multi-drug resistant strains (MDR) of *S. Typhi*.[2] However, in Pakistan research has shown an increase in MDR *S. Typhi* over the years.[1,3,4] The estimated incidence of *S. Typhi* infections is more than 500/100000 both in Pakistan and India.[5]

Salmonella enterica serotype Typhi is a Gram negative bacterium that causes typhoid fever. It causes fever, malaise, headache and rash and can in severe cases progress to life threatening complications including encephalitis and intestinal hemorrhage.[6,7,8] *S. Typhi* spreads via food and water contaminated with human feces. Diagnosis of typhoid fever is by blood & bone marrow culture in first week followed by stool/urine culture in second week of illness. Sensitivity of blood cultures is 40-80% but this drops to 30% if tested after the first week of infection.[9,10,11,12] Diagnosis of typhoid in low to middle income countries (LMIC) is mostly done by serological tests, Widal and Typhi dot, which are of low sensitivity and specificity and are not recommended for diagnosis of typhoid fever.[19]

In Pakistan we are increasingly seeing MDR strains of *S. Typhi* which are resistant to ampicillin, trimethoprim sulphamethoxazole and chloramphenicol. Some cases of extensively drug resistant (XDR) *S. Typhi* have also been reported. XDR strains are defined as MDR strains exhibiting additional resistance to quinolone and ceftriaxone.[13,14] All risk factors associated with transmission of *S. Typhi* are present in Pakistan; there is often sewage mixing with water pipes, wide

spread poor hygiene, and a lack of awareness of basic preventative measures.[1,15,16,17,18]

Since reports of sporadic cases of one to two XDR *S. Typhi* over the years[3,20,21,22] an outbreak started on 30 Nov 2016 to March 2017. In this 101 cases of XDR *S. Typhi* were reported from Latifabad and Qasimabad, Sindh.[15] XDR salmonella has now spread with travel to USA, UK and Canada.[13,23,24]

This paper intends to add to the growing field of research in this area by measuring the prevalence of *S. Typhi* and especially drug resistant strains in a 1450 bed tertiary care hospital in Lahore.

Materials & Methods

This retrospective cross-sectional study of blood culture isolates was done at Microbiology Department of Services Institute of Medical Sciences, Lahore.

All blood cultures received and processed from January 2018 to April 2019 were analyzed for *S. Typhi*. A total of 16288 blood cultures were received in aerobic, tryptic soya broth from admitted patients and outpatient department.

Blind subcultures were made on blood agar and MacConkey agar plates after incubation of blood culture bottles at 35^0C for 24 hours. Inoculated plates were examined after overnight incubation at 35^0C. Identification of isolated growth was made on colony morphology and Gram stain reaction. All non -lactose fermenter on MacConkey agar were tested for oxidase production. Further biochemical tests done on oxidase negative colonies were urease, citrate utilization, motility ant triple sugar iron (TSI) tests.[25] If results were ambiguous API20E was set up. In 2019 second subculture was done on day 7 before discarding blood culture bottle as sterile. Procedure for second subculture was the same as the first subculture.

Sensitivity of *S. Typhi* was carried out on Mueller Hinton agar by Kirby–Bauer method according to CLSI guidelines 2018 (26). Antibiotic discs used were ampicillin (AMP 10ug, oxoid), trimethoprim sulphamethoxazole, chloramphenicol (C 30ug), iprofloxacillin (CIP5ug), ceftriaxone (CRO 30ug), azithromycin (AZM 15ug) and imipenem (IPM 10ug).

Statistical Analysis was done by percentage and chi square. Numbers and percentages are reported in this study along with year wise trends.

Results

Most of the typhoid cases were seen in children under 15 years old as seen in Fig.1. More males, 58%, were seen than females as shown in Fig 2. The *p*-value of this result is 0.10604 hence it is not significant at $p<0.05$

In Jan-Dec 2018, 29 laboratories confirmed cases of *S. Typhi* were seen in 14020 blood cultures received. In comparison the

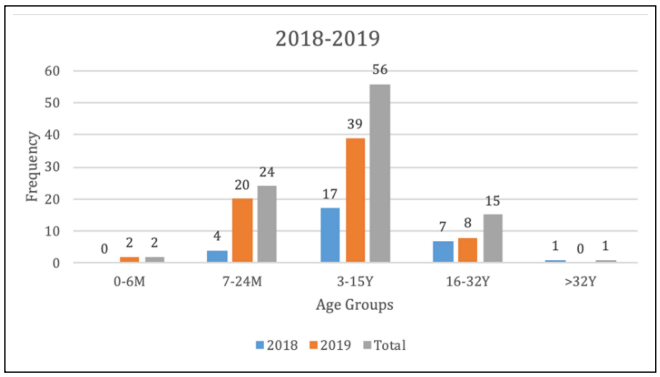

Fig 1. Distribution of *Salmonella* Typhi according to Age N=98

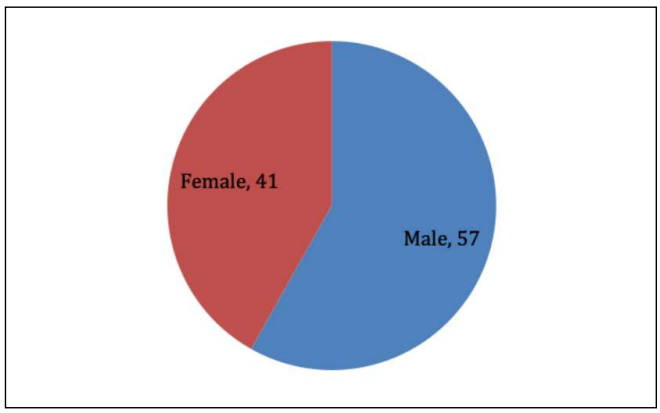

Fig 2. Distribution of *Salmonella* Typhi according to Gender. N=98

period between Jan-April 2019 saw 69 *S. Typhi* cases isolated from 2268 blood culture specimens. Sensitivity of these isolates to antibiotics is shown in Fig.3. Blue bars indicate the sensitive strains isolated in 2018 whilst red bars represent sensitive strains isolated in 2019.

The ratio of MDR to XDR cases is shown in Table1. There was a significant increase in XDR in 2019; 58 cases isolated in comparison to 14 the year before. The number of MDR isolated conversely decreased with only 6 isolated in 2019, down from 13 in 2018. The difference came back as significant with a *p*-value of 0.00017.

Month wise trend of XDR *S.Typhi* isolated is shown in Fig 4. It shows not only an increase incidence of XDR *S.Typhi* in 2019 but also shows the increasing number of cases seen each month.

Discussion

Typhoid is a systemic illness associated with serious complications.[6] Morbidity and mortality increase significantly in the presence of MDR and XDR strains. This is associated with an increased financial burden disproportionately affecting low to middle income countries.[8,27,28]

Table1. Ratio of Non-Resistant, MDR TO XDR *Salmonella* Typhi

	Non –Resistant	MDR	XDR
2018 (N29)	2	13	14
2019 (N69)	5	6	58
2018-19 (N 98)	07	19	72

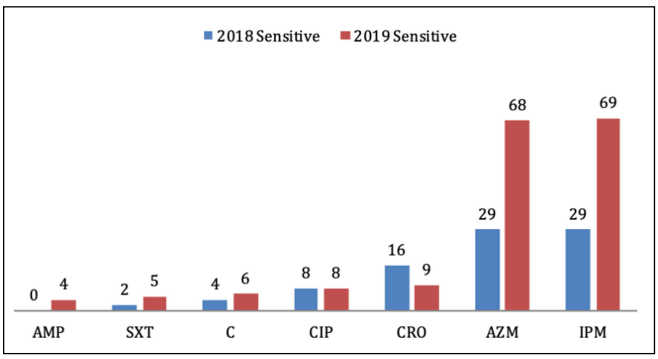

Fig 3. Sensitivity Pattern of *Salmonella* Typhi

AMP Ampicillin, SXT trimethoprim sulphamethoxazole, C chloramphenicol, CIP ciprofloxacillin, CRO ceftriaxone, AZM azithromycin, IPM imipenem.

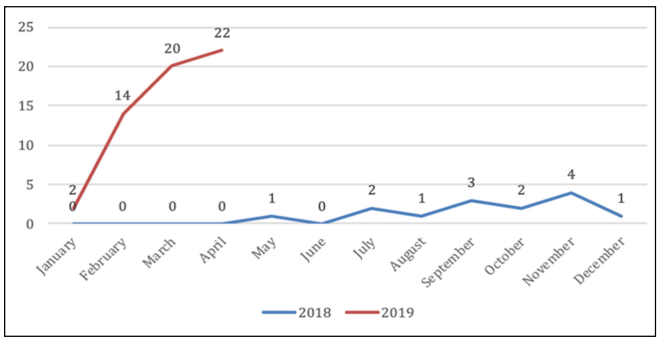

Fig 4. Month wise trend of XDR *Salmonella* Typhi isolated (JAN2018-APRIL 2019)

One common theme in the majority of the studies carried out has been the high rate of infection in children less than 15 years old[3,6,15, 29, 30] as seen in our study. What is even more concerning are the numerous reports of cases seen in 2-year old and even in under 6-month-old babies.[15,31] In a systematic population based study conducted in in Karachi the incidence of infections seen in infants under 12 months age was 506.4/100000. Low rates of exclusive breast feeding were pointed out as a possible explanation for the high rates seen.[30] In the present study 2 cases were seen in under 6-month-old infants, with one of them being a 12-day old neonate. The young age of patients seen at presentation also raises questions about vaccination and prevention strategies. Currently vaccinations in Pakistan can only be given to children over two years old yet, as highlighted above, a significant number of cases got infected before they reached that age. Furthermore, vaccination against typhoid is not part of the routine program of immunization in Pakistan in contrast to countries like China, India and Vietnam.[17]

Typhoid fever was seen more in male, 58%, as compared to female children. (Fig 2). This gender distribution of typhoid cases is similar to 56.4% reported by Yousafzai *et al* [15] and has been reported to be as high as 65.7% in another study.[22] This could be due to outdoor activities of male children making them more susceptible to getting the infection and/or male children being more likely to be brought for treatment.

The ratio of non-resistant to MDR and XDR *S. Typhi* is shown in Table 1. It reveals a significant increase in XDR *S. Typhi* and decrease in MDR *S. Typhi* in 2019 as compared to 2018. In Pakistan the most concerning finding has been the increase in cases of XDR *S. Typhi* seen in recent years and, in particular, 2019 since the outbreak of XDR typhoid fever in Sindh.[29] The first case of XDR *S. Typhi* in our microbiology laboratory was detected in Feb. 2018 and till 31 December 2018 a total of 11 XDR strains were isolated from 29 laboratory confirmed typhoid cases. However, there has been a significant increase in XDR *S. Typhi* isolated in 2019. Since 1st Jan to 30th April 2019 there have been 58 XDR *S. Typhi* isolated out of 69 laboratory confirmed typhoid fever cases as shown in Fig 3 & Fig 4. So far there is no published data from Punjab on XDR S Typhi after the outbreak reported in Sindh. In Sindh the burden of XDR *S. Typhi* is continuing to increase despite preventive measures having been adopted. According to latest report, there are 7646 laboratories confirmed *S. Typhi* cases of which 4763 are of XDR *S. Typhi*.[32]

There is an urgent need to conduct further studies in other hospitals to determine if this increase is an isolated finding at a particular hospital or part of a regional trend across Punjab. An alarming finding is the detection of one azithromycin resistant XDR strain of *S. Typhi* seen in 2019, which indicates the evolving drug resistance in this microorganism. Although azithromycin resistance has been reported previously[33], in presence of XDR resistance it gains added significance.

Conclusion

Considering the increase in incidence of MDR and XDR *S. Typhi* there is a need to form and implement basic guidelines to prevent this from becoming an epidemic.[34] Typhoid can be prevented by good sanitation, safe water and proper hygiene. People need to be educated on the modes of spread of typhoid helping increase public awareness.[9,18] Vaccination drive is the immediate solution to reduce the impending typhoid outbreak in monsoon season and attention should be given in increasing

uptake of the typhoid vaccine.[29] With judicious use of antibiotics in hospitals and community, resistance can be controlled and reverted as has been shown in studies in Pakistan and India.[2,6,35]

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Japanese Encephalitis IgM among Patients with Acute Encephalitis in Karachi, Pakistan –Implications of Laboratory Diagnostic Results

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Abstract

Japanese encephalitis (JE) is a mosquito-borne illness and a major cause of viral encephalitis in southern Asia and Southeast Asia. Infection is symptomatic in less than 1% of all infected cases. Past studies from the 1980s identified JE as an infrequent cause of viral encephalitis in Pakistan. We conducted Enzyme-linked immunosorbent assay (ELISA) based laboratory testing for Japanese encephalitis IgM in cerebrospinal fluid (CSF) and serum samples among patients with acute encephalitis presenting to a tertiary care hospital in Karachi, Pakistan. Among 117 patients hospitalized with encephalitis of unknown etiology at the Aga Khan University Hospital whose sera or CSF were tested, 4 (n=4; 3.4%) were positive for JE IgM. Further testing of the samples against dengue and West Nile IgM is warranted to ensure whether these are true positive cases of JE or cross-reactive with other flaviviruses.

Introduction

Japanese encephalitis (JE), a mosquito-borne illness, is a major cause of viral encephalitis worldwide. JE virus (JEV) is a positive sense single-strandedÊ virus belonging to family Flaviviridae, which is maintained in a cycle involving mosquitoes and vertebrate hosts, mainly pigs and wading birds. Infection is symptomatic in less than 1% of all infected cases,[1] however, post-infectious neuropsychiatric sequelae are observed in 30-50% of patients and a high mortality of up to 25% has been reported.[2] The disease is epidemic in temperate regions of Asia and is endemic in many tropical countries in Southeast Asia, with an estimated rate of 67,900 cases reported annually from the region.[3]

According to the WHO, borders of the region of endemicity extend from Western Pacific islands in the east to the Pakistan border in the west. Pakistan itself, however, is considered a 'negligible-risk' region for acquisition of JE.[4, 5] This risk categorization is based on infrequent reports published in 1985 and 1994. In 1983, Sugamata *et al* undertook a seroepidemiological study of West Nile virus (WN) encephalitis (caused by another mosquito-borne flavivirus that is closely related to JE virus) in Karachi and found high JEV antibody titers in 2 out of 156 patients.[6] In 1992, Igarashi *et al* found JE viral genome sequences in 1 of 24 patients with encephalitis from Karachi.[7] High antibody titers have also been reported form healthy volunteers.[8] Indirect evidences of possible JEV activity has been reported very recently by Khan *et al* where 16.1% of cases with suspected West Nile Virus infection showed some neutralizing antibodies for JEV on Plaque Reduction Neutralization Assay (PRNT) therefore it is important to have focused study on JEV as the current situation on the prevalence in southern Pakistan is uncertain.[9]

To detect the presence of JE antibodies among patients with encephalitis hospitalized at Aga Khan University Hospital Karachi, we tested serum and cerebrospinal fluid (CSF) samples for JE IgM.

Materials and Methods

Study Design, Study Setting, Study Period

This was a hospital-based surveillance of acute encephalitis cases, conducted at The Aga Khan University Hospital (AKUH) Karachi during the period of May 2015-August 2017. Laboratory testing was done at the Infectious Disease Research laboratory (IDRL) at the AKUH.

Sample Size

According to a study published in northern India in 2011,[10] Japanese encephalitis viruses (JEV) has been the major cause of outbreaks in the Uttar Pradesh State, accounting for 10% to 15% of total AES cases annually. Assuming an average of 12.5% as the estimated prevalence, a sample size of 117 patients with encephalitis was required determine the presence of JE in our sample, with expected 6% desired level of absolute precision (d), 95% confidence interval, and 5% level of significance.

$$n = \frac{1.96^2\, p\,(1\text{-}p)}{d^2} = n = \frac{1.96^2 \text{x} (0.125)(1\text{-}0.125)}{(0.06)^2} = 117 \text{ Total number of samples}$$

Selection Criteria

Inclusion Criteria

Patients with acute encephalitis fulfilling the following criteria were included: patients of any age, any gender, at any time of year, with the acute onset of fever and a change in mental status

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(including symptoms such as confusion, disorientation, coma, or inability to talk) and/ or new onset of seizures (excluding simple febrile seizures).[11]

Exclusion criteria

Patients with acute uncorrected dehydration, cirrhosis and suspected hepatic encephalopathy or hepatorenal syndrome, uremic encephalopathy, prolonged (> 3 months) undiagnosed systemic illness, known cerebrovascular etiology/ stroke, recent rash (e.g. measles or varicella), and brain or spinal tumors causing neurological deficits were excluded.

Informed Consent

Patients were enrolled after they (or their legal guardians) provided written informed consent. The study protocol was reviewed and approved by the Ethics Review Committee at Aga Khan University.

Laboratory Testing and Diagnostic Criteria

All patients satisfying the inclusion criteria were included in the study by non-probability consecutive sampling. CSF samples were collected in universal containers by the primary physician through lumbar puncture; serum samples were collected, where lumbar puncture was not possible, by phlebotomists through venipuncture. All samples were transported immediately at room temperature to the diagnostic laboratory. All CSF / serum samples were tested for JE IgM antibodies using InBios test JE IgM Capture ELISA as recommended by the manufacturer.

Results

Clinical Cases

Patient presenting to the study hospital from May 2015- August 2017 were recruited and maximum recruitment of cases was seen from August to September. Total 117 patients were recruited according to the clinical criteria and the patients were predominantly male (n=70; 59.8%). Median age of the patients was 19 years (Q3-Q1=37). Majority of the cases belonged to different areas of Sindh (n=110), followed by Baluchistan (n= 4) and Punjab (n= 3). The most common clinical diagnosis encountered upon discharge was meningitis (aseptic and viral both) (n=42; 35.9%) followed by encephalitis/ meningoencephalitis (n=36; 30.8%). Hypertension was a commonly encountered comorbid seen in 23 patients (19.7%) and diabetes in 17 patients (14.5%). Neurological symptoms were accompanied by fever in 71% of patients (n=83), followed by headache in 52% (n=61), and drowsiness in 36% (n=42).

Laboratory Testing

Among the 117 recruited patients, 100 CSF samples and 17 serum samples were obtained and tested. Four (n=4) of these samples (3 CSF and 1 serum) tested positive for JE IgM antibody. CSF culture was negative in all recruited patients and CSF was tested and was negative for Herpes Simplex Virus (1 and 2) nucleic acid in 101 of these patients (86.3%). All the positive cases were residents of Sindh. Median age of the patients with positive results was 56.5 years (Q3-Q1=42.25). All patients with positive results for JE IgM presented to the hospital between August and October. Further patient details are provided in table 1.

Discussion

Our results have established the presence of JE IgM in patients presenting with acute encephalitis in Pakistan. However, it is important to remember that significant antibody cross-reactivity exists between JE and other flaviviruses to warrant additional testing and confirmation of the etiological agent in such cases. Testing should especially be directed toward prevalent infectious agents in Pakistan such as dengue and West Nile virus, which can both cause acute encephalitis. Data available from studies performed in the recent past on patients with acute febrile illnesses in Pakistan reveal other cross-reacting flaviviruses such as the West Nile virus as a common cause.[9]

Similarity in the clinical symptoms along with serological cross-reactivity makes definitive diagnosis of JE difficult. Pakistan

Table 1. Summary of patient characteristics, clinical presentation and outcomes of cases tested positive for JE IgM

	Case 1	Case 2	Case 3	Case 4
Age	70 years	53 years	60 years	16 years
Gender	Male	Female	Female	Male
Presenting complaint	Fever, drowsiness, headache	Fever, drowsiness, headache, neck rigidity	Fever, drowsiness, seizure	Tonic clonic seizures
Comorbid	Hypertension	None	Hypertension	None
Outcome	Discharged	Discharged	Discharged	Discharged
Type of specimen tested	CSF	CSF	CSF	Serum

is endemic for dengue virus, and with increasingly reported WNV, the diagnosis of JE has become challenging due to coexistence of these flaviviruses. With increasing climatic changes, frequent human migration and global warming, various arboviral diseases are on the rise globally, and in Pakistan. It is therefore important to have adequate surveillance for all arboviral illnesses, including the ability to diagnose and differentiate between flaviviral infections in Pakistan.

Methods available for the definitive diagnosis of JE are limited. RT-PCR is not valuable in diagnosis of JE as viremia is usually low-level and transient. Therefore, confirmation of these preliminary results requires a testing algorithm including testing for Dengue virus IgM, Westnile virus IgM, to exclude cross reactivity, and the reference Plaque Reduction Neutralization Test (PRNT) for these flaviviruses.[12] We plan on performing further confirmatory testing in the near future.

Given the lack of peripheral and intermediate level laboratory diagnostic services for extensive serological and virological testing, JE and other arboviral encephalitides remain difficult diagnoses to establish as a cause of infectious syndromes. To inform the true prevalence and risk posed by arboviruses and flaviviruses, active human and animal surveillance along with mosquito-burden studies should be undertaken.

Conclusion

The current prevalence and burden of JE in Pakistan remains uncertain due to significant cross-reactivity of JE IgM with other flaviviruses. Laboratory diagnostics for flavivirus encephalitis are complex and need confirmation by more sophisticated procedures such as PRNT. Further testing with these laboratory methods is planned.

Funding

This study was funded by Program for Appropriate Technology in health (PATH) Diagnostics.

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ORIGINAL ARTICLE

Fungal Infections of the Central Nervous System in the Seemingly Immunocompetent – common or unusual

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Abstract

Objective
To identify and describe characteristics of invasive Central Nervous System [CNS] fungal infections including their clinical presentation, diagnosis, treatment and outcome at our center.

Methods
This is a retrospective study design using secondary data analysis. Medical records of patients with CNS fungal infections presenting during an eight-year period, from June 2011 to June 2018 were reviewed to determine patients' baseline characteristics (age, gender, comorbidities), site of infection in brain, clinical presentation, imaging findings, medications used and response to treatment including mortality.

Results
Twenty-one patients with invasive CNS fungal infection were identified and reviewed. A majority of patients were men (81%). The clinical presentation was variable and most patients presented with more than one feature. Headache was the commonest symptom and was seen in 67% of the patients. Response to treatment was better in patients with Aspergillosis (71.4%) as compared to other organisms (43%). The overall response rate of CNS fungal infections was 62%. Overall mortality in these patients was 24%. Voriconazole is better tolerated while Amphotericin B deoxycholate use was associated with expected kidney injury. Surgical excision at any time during the treatment was associated with better response.

Conclusion
The results of present study show that CNS fungal infections cause significant mortality. The index of suspicion should be high even in immunocompetent patients presenting with headache, facial swelling or neurological involvement. Prompt and accurate diagnosis and early treatment should be instituted to avoid disease progression and mortality. There is a need for better tolerated drugs (Liposomal Amphotericin B and Posaconazole) to be available at low cost. Surgery should be considered as a treatment option wherever feasible.

Key words
CNS – Central nervous system

Introduction

Fungal infections contribute to high disease burden including Pakistan. These infections are under diagnosed due to insufficient diagnosing modalities and lack of index of suspicion.[1,2] Invasive fungal infections and candidemia has poor outcomes. Invasive fungal infections are common in diabetics and in immunocompromised patients but also reported to occur in substantial numbers in overtly immunocompetent hosts. Involvement of CNS in invasive fungal infection is less common but is a diagnostic and treatment challenge and carries poor outcomes.[1,2,3]

Common fungi that can cause disease in CNS include Aspergillus, Zygomycetes, Cryptococcus and Candida species. Such infections may be more commonly seen in tropical climates.[4] They can present as meningitis or can form brain abscesses.[5] Cerebral fungal infections occur due to extension of infection from contiguous structures (paranasal sinuses, mastoid, middle ear cells), hematogenous spread or via direct invasion during neurosurgical procedures or trauma. Sino-orbital Aspergillosis is emerging infection in Asia, Africa and Middle East making it a common cause of contiguous spread to CNS. Infections from paranasal sinuses usually extend to frontal lobe of brain and those from middle ear and mastoid air cells involve the temporal lobes.[2,4]

There are no characteristic clinical or laboratory parameters to diagnose cerebral fungal infection. Diagnosis of CNS fungal infections relies mainly on radiological findings, histopathology and culture. CSF examination has limited yield in diagnosing cerebral aspergillosis however it can help in identifying fungal

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meningitis.[5,6] Findings of CNS fungal infections on MRI and CT scan are variable and can present as a intra/extra parenchymal solid mass lesion (with or without mass effect, infarct, haemorrhage), abscess, meningeal enhancement or mucosal thickening of sinuses and as bone erosions.[7]

Serological tests (galactomannan antigen and Beta D glucan) on blood or CSF can support the diagnosis of CNS fungal infection. Galactomannan is used for invasive aspergillosis and Beta D glucan is detected in patients with mold or yeast infections. Tissue sampling for histopathology and culture is ultimately required in many cases for exact diagnosis and identification of fungal species.[8]

Various drugs are used to treat invasive fungal infections including amphotericin B, triazoles, echinocandins and 5-flucytosine. Echinocandins have little penetration in brain. The course of treatment is often complicated due to inappropriate diagnostic tests, side effects of treatment, resistance and cost issues.[8,9,10,11] Outcome is better when surgery is combined with medical treatment.[12]

To date there are very few studies to evaluate presentation, treatment and outcome of CNS fungal infections in Pakistan. Invasive fungal infection remains challenging due to diagnostic delays and inappropriate treatment. The rationale of this study is to highlight the clinical and radiological characteristics and outcome of this serious disease in our country.

Patient and Methods

Study design and settings

A retrospective review was performed of all the patients, who were diagnosed with CNS fungal infections from June, 2011 to June, 2018, after approval from institutional review board. A total of 21 patients were identified and included in this study using the keyword CNS fungal infection to search in the hospital's electronic database. The demographics, age, risk factors, clinical presentation, investigations, drugs and surgical options were assessed in addition to the associated outcome and mortality.

Inclusion / Exclusion criteria

We included patients with the diagnosis of CNS fungal infections, from June 2011 to June 2018, based on biopsy result or radiological findings suggestive of CNS fungal infections in with supportive clinical evidence. Patients with meningitis are excluded from this study.

Patient characteristics

The baseline characteristics of the patients including age and treatment duration were recorded as quantitative variables. The gender, associated comorbidities, clinical findings, biopsy results, type of surgical intervention, response to treatment and side effects were recorded as qualitative variables.

Definitions

Confirmed CNS fungal infection is defined as diagnostic histopathology or culture results.
Suspected fungal infection is defined as mass lesion on MRI that was treated as fungal infection but biopsy not done.

Response was defined as improvement in both symptoms and radiological findings in clinical follow up over 6 months.

No response was defined as no improvement in either symptoms or radiological findings in clinical follow up over 6 months.

Disease progression was defined as progression in either baseline symptoms or radiological signs in clinic follow up over 6 months.

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 ± SD and categorical variables were computed as frequencies and percentages.

Results

Descriptive statistics

Out of 21 patients, 20 (87.5%) patients were adults and one was of pediatric age group; mean age was 34.88 ± 19.49 years. Seventeen (81%) patients were men. Comorbidities were present in 12 (57%) patients while 9 (43%) patients had no comorbid illness. Four patients had cancer (Hodgkin's lymphoma in three and nasopharyngeal cancer in one patient). Four patients were diabetics and two of them had chronic sinusitis as well. Chronic sinusitis was also present in two other patients without diabetes. One patient had ischemic heart disease and another was using long term steroids for dermatitis. Location of home cities of patients is illustrated in figure 1.

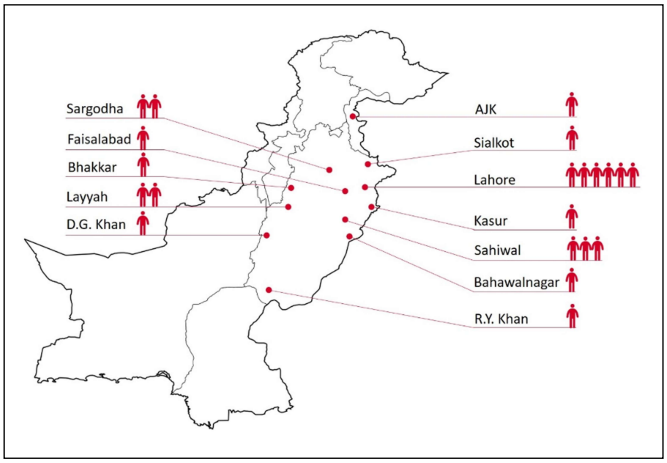

Fig.1 Demographic presentation of patients with CNS fungal infection

Clinical presentation (represented in table 1) was variable and most of our patients had more than one presenting symptoms. Biopsy was done in 19 (90%) patients; surgical excision was done in 10 (47.6%) patients. Surgical procedures are detailed in table 2. MRI (Magnetic resonance imagining) findings also remained variable and are elaborated in table 3.

Table 1: Clinical presentation of patients with CNS Fungal infections

Clinical Presentation	No. of patients
Headache/facial pain	14
Focal deficit	
Facial Numbness	2
Decreased vision/ cranial nerve palsy	8
Limb weakness	5
Slurred speech	1
Orbital swelling/proptosis	6
Nasal blockade	5
Altered mental status/memory loss	4
Seizures	3
Fever	3

Table 2: Details of surgical procedures

Procedure	No. of patients
Excision	5
Biopsy	7
Trans-sphenoidal biopsy/ Nasal sinus biopsy / debridement	6
Biopsy of cervical node/ neck sinus	1
None	2

Table 3: MRI Brain findings

Findings	No. of patients
Mass lesion in brain	
Mass lesion with no complications	3
Mass lesion in brain with complications (mass effect/ infarcts)	4
Ring enhancing lesion with meningeal enhancement/mass effect	3
Sinuses/ mastoid/orbit infection with intracranial extension	8
Skull based osteomyelitis with intracranial extension	1
Gliomatosis cerebri with mass effect	2

MR spectroscopy was done in 4 patients suggestive of glial tumor in two, glioma in one and meningioma in another patient. Cultures were available in only 6 patients. Three of them showed no growth, one patient had mixed growth of Mucormycosis and Aspergillus, one had Aspergillus and another had Fonsecaea species grown in culture. Histopathology result and diagnosis made, is shown in table 4. Site of fungal infection within the brain is described in figure 2.

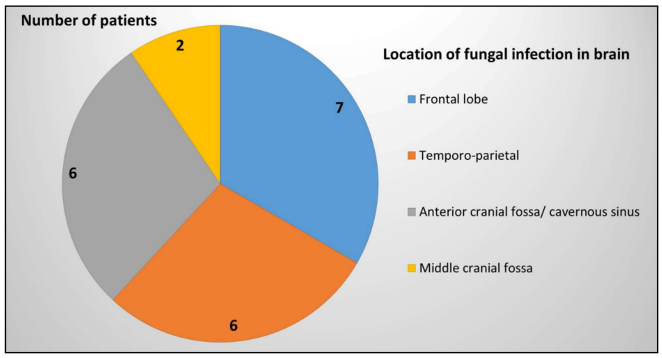

Fig 2. Location of CNS fungal infections

Response to treatment

Response was described by analyzing patients in two groups. The first group was CNS aspergillosis who received voriconazole and the second group was CNS fungal infections other than aspergillosis who were treated with Amphotericin B deoxycholate or Posaconazole. Response was determined after clinical and radiological review. Mean duration of treatment was calculated in both groups. The number of patients who had undergone surgical excision was also documented.

Fourteen patients were treated as CNS Aspergillosis. Twelve patients had confirmed while two had suspected CNS Aspergillosis. All patients were treated with voriconazole. Details about response in patients with CNS aspergillosis and patients who had fungal infections other than aspergillus is given in table 5.

Side effects of medical therapy

In patients treated with voriconazole, adverse events were seen in 3 out of 14 (21.4%) patients. One patient developed drug associated rash and visual symptoms occurred in 2 patients requiring stopping of voriconazole at 4 months in one of them.

Out of seven patients who were given Amphotericin B, 5 (71.4%) developed acute kidney injury.

Discussion

In this retrospective analysis, we have presented the clinico-radiological features, treatment and outcome of 21 patients with CNS fungal infection. In our study 14 patients belong from southern Punjab, which may be a noteworthy point when patients present with CNS complaints from these districts.

Table 4: Histopathology of patients with CNS fungal infections

Histopathology	Number of patients	Diagnosis n
Septate fungal hyphae with necrotizing granulom atous inflammation	7 (33%)	Aspergillosis 7
Septate fungal hyphae with non necrotizing granulomatous inflammation	4 (19%)	Aspergillosis 3 Fonsecaea spp. 1
Septate fungal hyphae with inflammation / no granuloma	2 (9.5%)	Aspergillosis 2
Non septate fungal hyphae with inflammation	4 (19%)	Mucormycosis 1
Non septate hyphae with necrotizing granuloma	1 (4.7)	Mucormycosis 1
Necrotizing granuloma with mixed infection	1 (4.7%)	Polymicrobial infection 1 (Aspergillus and Mucor)
Not identified	2 (9.5%)	Aspergillosis 2

Table 5: Response in patients with CNS aspergillosis and CNS fungal infections other than Aspergillosis

Diagnosis	Mean Duration of Voriconazole treatment in days	Surgical excision	Clinical response	Radiological response	Over all response	Disease Progression	Mortality
Aspergillosis (14 patients)	227 ± 138 days	7 (50%)	10 (71.4%)	10 (71.4%)	10 (71.4%)	1 (7%)	3 (21.4%)
CNS fungal infections other than Aspergillosis							
Mucormycosis (5 patients)	126 ± 56.17	2	2	2	2	2	1
Fonsecaea (1 patient)	90	0	0	0	0	0	1
Mixed infection (1 patient)	335	1	1	1	1	0	0
Overall (7 patients)	151 ± 94.18	3	3	5	3 (42.8%)	2 (28.5%)	2 (28.5%)

However, this effect may be due to institutional referral bias.

To date most of the research suggested certain risk factors (cancer, diabetes, HIV, steroids, transplant, neurosurgery etc.) for invasive fungal infections[2,,10,13,14]. Our study highlights the occurrence of invasive fungal infections in previously healthy patients as 43% of studied patients had no conventional risk factor for CNS fungal infections. In the remainder, diabetes, chronic sinusitis and cancer were identified as risk factors for invasive fungal disease.

Patients of CNS fungal infections usually present with headache, fever, focal neurological deficit (including cranial neve palsies, visual loss), change in mental status, nasal blockade and ear

discharge.[16] Dubey *et al*. pointed out that patients of CNS fungal infections usually have more than one clinical complaint and headache was the most common presenting feature. Also, patients can present with neurological disturbances (including visual impairment and limb weakness), nasal blockade, and eye swelling, though fever and fits remained less common presentation. Similar to this study, our patients also present with more than one clinical feature and most common presenting feature was headache while fever and seizures were not common, consistent with the afore mentioned study.[12]

Most of the CNS fungal infections had predilection for frontal lobe followed by temporo-parietal lobes, anterior cranial fossa and middle cranial fossa however none of the patients had infection in posterior cranial fossa. [12]

Similar to Dubey *et al*., the most performed surgical procedure was craniotomy for excision/biopsy (12 out of 21 patients). However, 6 patients in our study required trans-sphenoidal biopsy, with or without debridement, twice the number mentioned by Dubey *et al*, where only 3 patients underwent this procedure.

As published in literature, MRI findings of CNS fungal infections in our study remained non-specific.[16,17] Radiological findings included mass lesion and ring enhancing lesion that can be complicated with mass effect and infarcts. MRI remained a helpful tool in revealing possible source of infection as 8 (38%) patients showed mucosal thickening of sinuses, orbital wall or mastoid as well, suggesting contiguous cause of intracranial disease. MRI of 2 patients showed gliomatosis cerebri, one had disease of both frontal lobes and the other had disease in tempro-parietal lobes.

Four patients of aspergillus granuloma had undergone MR spectroscopy (MRS), which failed to differentiate aspergilloma from possible glial tumor in two, glioma in one and meningioma in another patient. These results are consistent with the study of MRS that showed its inability to differentiate Aspergilloma from brain tumors and other infections.[18]

Sundaram *et al*. reviewed pathology of 130 cases of CNS fungal infections reported in Southern India.[4] He reported most common fungal infection in CNS was Aspergillus (56%) followed by Mucormycosis (30%). Our study revealed similar findings with 12 (57%) out of 21 patients showed thin branching septate fungal hyphae, with positive GMS and PAS stain, suggestive of aspergillosis. Out of these 12 patients, granuloma was found in 10 patients while 2 patients had only chronic inflammation without granuloma formation. In high TB burden countries like Pakistan its crucial to differentiate fungal granuloma from tuberculous. The second most common diagnosis based on histopathology was zygomycosis in 5 (23.8%) patients. One of the patients showed mixed infection with Aspergillus and Mucor, and another revealed Fonsecaea spp. The above mentioned study showed availability of cultures in only 29% patients, similar to our study where cultures were available in only 6 patients (28.5%). The reason of low numbers of culture requests was mainly biopsy done before referral to the ID physicians.[4]

Our study exhibited that fungal infections invade frontal lobe of brain in 33% of patients followed by temporo-parietal lobe in 28% of patients, anterior cranial fossa and cavernous sinus in 28% of patients and middle cranial fossa in 9.5%. None of the patient had fungal granuloma of posterior lobe and these findings are same as existing data.[12]

Schwartz *et al*. conducted study on 81 patients of probable or confirmed CNS Aspergillosis who were treated with voriconazole.[19] In this study response (complete, partial and stable disease) was seen in 50.6% and failure to therapy was reported in 49.3% of patients. Mortality due to CNS aspergillosis was 46%. Neurosurgical intervention was associated with better outcome.[19] In our study treatment for aspergillosis was given in 14 patients. All patients received voriconazole with mean duration of 227 +/- 138 days. The duration was determined by clinical judgement of response (clinical resolution of symptoms and radiologic improvement). Clinical and radiological response was seen in 10 (71.4%) patients that was better than the aforementioned study. Failure of therapy was seen in 3 (28.6%) patients. Disease progression was seen in one patient who had gliomatosis cerebri of both frontal lobes. He was advised re-biopsy but patient was lost to follow up. Three patients (21.4%) died in this group, that is lower than that reported by Schwartz *et al*. The reason of this difference could be the fact that majority of patients included in aforementioned study were immunocompromised and had undergone organ/stem cell transplant while in our study most of the patients were immunocompetent. In our study surgical excision of diseased tissue was done in 7 patients and all of these 7 patients survived, highlighting that surgical intervention at any time during the course of illness may cause better survival.

A study done on mucormycosis showed response in only 37% of patients with mucormycosis and out of 41 patients who died, 41 (87%) died due to mucormycosis.[20] In our study of CNS fungal infection other than aspergillosis, combined clinical and radiological response remained low i.e. 3 out of 7 (42.8%) patients and failure of treatment was observed in remaining 4 (57.2%) patients. Three patients with treatment failure had mucormycosis, one of whom died and the other two had disease progression till their last follow up. Spellberg *et al*. showed surgical debridement in patients who had mucormycosis is related with good outcome[21]. In our study two patients of mucormycosis had surgical debridement, and both showed disease progression. Possible reason of disease progression could be the treatment interruption in both patients due to Amphotericin B intolerability, non-access to Posaconazole, unavailability of liposomal Amphotericin B and additionally superadded bacterial infection leading to skull-base osteomyelitis

in one of them. Both of these patients were also diabetics.

Voriconazole was well-tolerated in our population with side effects seen in only 3 (21.4%) patients, better than existing literature that reports adverse events in 39.5% of patients treated with voriconazole.[19] In our study Amphotericin B deoxycholate was poorly tolerated with 5 out of 7 (71.4%) patients developing acute kidney injury at some point requiring intermittent discontinuation of drug. This is higher than reported nephrotoxicity of Amphotericin B i.e. 50%.[22] Studies showed that liposomal Amphotericin B has better tolerability than non liposomal Amphotericin B and former is associated with survival rate of 67% as compared to only 39% with later one.[21,22]

Conclusion

The results of our study showed that mortality in CNS fungal infections especially those other than aspergillosis is high. Consideration to this diagnosis should be made even in immunocompetent patients presenting with headache, facial swelling or neurological involvement. Prompt and accurate diagnosis with tissue biopsy for histopathology and culture should be attempted as all septate hyphae should not be considered as a confirmed diagnosis of aspergillosis and voriconazole may not be the best option in such cases. Early treatment should be done to avoid disease progression and mortality. Drugs with better tolerability (Liposomal Amphotericin B and Posaconazole) are required to be available at low cost. Surgery should be opted as a treatment option, of CNS fungal infections, wherever feasible.

Limitations

This study has various limitations including use of retrospective data and lack of availability of culture data since in many instances the patients were referred to us after surgery and only histopathology was available.

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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.

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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.

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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.

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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.

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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**.

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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.

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