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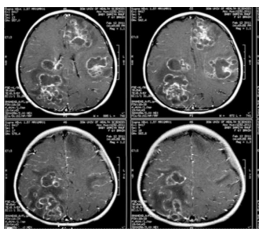

Courtesy: Dr Ali Faisal Saleem, Aga Khan University, Karachi.

Awareness: the Best Defense against Rabies

Rabies is one of the most horrific forms of death because of its slow, painful and torturous termination, with the victim consciously anticipating the end of life. More than 99% of all human rabies deaths occur in the developing world, yet rabies remains a neglected disease throughout most of these countries. Although effective and economical control strategies are available for both human and animal rabies, developing countries do not apply them, as other infectious and non-infectious diseases beg priority over rabies.

Among Asian countries, India takes the lead in having the highest burden of rabies. Most South- East Asian countries have pledged to eliminate the disease by 2020. Pakistan has an estimated 2,000-5,000 annual deaths due to rabies. Ironically, the Federal Health Services have turned a blind eye to public pleas for protection from rabies; the provincial governments, too, have no plans for its elimination. We are one of the last two remaining countries in the world that still produce and provide nerve tissue vaccine to government -run hospitals and health units because of its presumed low production-cost. Consequently, health care workers (HCWs) in these institutions are unaware of current changes in rabies prevention with newer vaccines, nor their administration for post exposure prophylaxis (PEP).

Several studies in Pakistan have revealed abysmal gaps in knowledge of rabies PEP among Emergency Room HCWs. In one study it was found that there were serious gaps in classifying wound severity and correct application of PEP with vaccine and RIG.[1, 2] Another study revealed that over 97% of HCWs knew how to inject vaccine, but only a third had ever used Equine Rabies immunoglobulin (ERIG). Most had never used ERIG because it was not provided in their ERs due to its "high expense", while others were concerned about anaphylactic reactions following ERIG.[3] Thus, an important lifesaving biological was disregarded by most HCWs for a serious animal bite. This issue continues to be responsible for human rabies deaths. Almost similar conclusions were drawn from a study in India. There, too, deficiencies in knowledge, attitudes and practices were apparent.[4]

Cell culture vaccines derived from purified verocell, chick embryo and duck embryo have been in use for several decades now, and their use is largely universal. These can be used by intramuscular route for individual dog bite victims, or by intradermal route in resource-constrained countries where several patients can share a vial with low dose vaccine, and still receive full protection, provided the course is completed. Rabies immunoglobulin (RIG), which gives immediate protection lasting 10-14 days when injected into the wound/s, is absolutely essential following a serious bite. RIG is available as Human RIG (HRIG) and ERIG. Both are equally effective. HRIG is nearly ten times as expensive as ERIG, and is hardly ever used in most developing countries. The fear of serious adverse events is unfounded as modern ERIGs are purified and do not cause anaphylaxis as the previously unpurified ERIG did. This lack of understanding continues to deprive patients from receiving correct PEP, and exposes them to the hazard of rabies.[5]

A new development is the production of monoclonal antibody cocktails against rabies, which is expected to ultimately replace RIG. Cost will be an ultimate issue in it's implementation.[6]

In Pakistan, there is a great need to develop adequately designed awareness programs for all physicians dealing with rabies exposures to follow current WHO guidelines. There is a dire need to open referral centers in small towns, which should not only have trained HCWs, but also reasonable stocks of vaccine and ERIG. Rabies prevention in dogs should also be taken up seriously by veterinarians. Only a "one health", comprehensive approach can overcome this deadly, ancient disease.

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ORIGINAL ARTICLE

Non-tuberculous Mycobacteria in Extra-pulmonary Specimens: Role of Nosocomial Transmission

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Abstract

Background
Non-tuberculous mycobacteria (NTM) cause infections not only in immuno-compromised but also in immuno-competent individuals. Healthcare associated NTM infections have been reported. Assessment of clinical significance of an NTM isolate is vital before embarking on the rapy. Treatment of NTM infections requires identification of isolate and susceptibility. There is a lack of data on healthcare associated NTM infections from Pakistan.

Objective
This study was performed to determine NTM isolates and their clinical significance from extra-pulmonary clinical specimens submitted at a tertiary care hospital laboratory.

Methods
Extra-pulmonary NTM isolated from 25955 specimens over 24 months (January 2010-December 2011) were included. All isolates were identified using conventional tests. Clinical details were extracted from laboratory data. Drug susceptibility testing was performed by broth microdilution.

Results
A total of 12 NTM were isolated from extra-pulmonary specimens over the study period. Clinical significance could be determined in 9/12 specimens. Seven NTM isolates from 3 patients were post-surgical and were likely to be healthcare associated.

Conclusion
This is the first study reporting healthcare associated NTM infections from extra-pulmonary clinical specimens from Pakistan. Isolation of NTM from clinical specimens should prompt evaluation of their clinical significance. Healthcare associated NTM infections should be suspected in non-healing post-surgical wounds.

Key words
Non-tuberculous mycobacteria, extra-pulmonary, Pakistan, healthcare associated infection

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Introduction

Non-tuberculous mycobacteria (NTM)are a group of environmental mycobacteria which are widely distributed in nature. They are found in natural water bodies, soil, water-damaged walls, etc. Also known as mycobacteria other than tuberculosis (MOTT), they are opportunistic pathogens and most infections caused by NTM are documented in immuno-compromised patients including HIV/AIDS (human immunodeficiency virus/acquired immunodeficiency syndrome) patients[1], however, immuno-competent individuals are not considered immune from becoming infected.[2] Human to human transmission of NTM is not known to occur, a feature that separates them from *Mycobacterium tuberculosis* complex. Interest in infections caused by NTM is highlighted by an increasing frequency of their isolation coupled with geographic diversity in NTM species demonstrated in a multi-country study involving 14 countries.[3] An increasing trend in NTM isolation and species variation has also been documented from India[4] and Taiwan.[5]

Pulmonary and extra-pulmonary NTM infections both have been documented. Since these organisms are ubiquitous in nature, and are also reported to be found in hospital water supplies[6], NTM represent a potential threat as nosocomial infectious agents.[7] NTM have the ability to resist certain disinfectants e.g. quarternery ammonium compounds, increasing their ability to survive hospital environments.[8] Data regarding NTM infections from Pakistan is lacking. This work was performed with an objective to study NTM isolated from extra-pulmonary specimens with an emphasis on healthcare acquisition of these infections.

Materials & Methods

Extra-pulmonary NTM isolates were prospectively collected from Clinical Laboratory, Aga Khan University Hospital (AKUH), Karachi, Pakistan, from 2010 to 2011. Strains were saved at -80ºC and revived when required for identification and susceptibility testing. Clinical information was obtained from laboratory data (patient's age, gender, specimen anatomical source, site of infection and its chronicity, co-morbids, surgical procedures (if any), treatment and its response). Significance assessment of NTM isolation was carried out in cases where clinical information was available. Diagnosis was assessed on patient history and by isolation of NTM from aspirated pus, tissue biopsies or sterile body fluids.[9]

Cultures were performed using Lowenstein Jensen (LJ), Mycobacterium Growth Indicator Tube (MGIT) and Middlebrook 7H10 agar media for all the specimens by standard microbiological procedures and NTM were identified using standard biochemical tests.[10] Susceptibility testing was performed by broth microdilution using 96 well sensititre plates (TREK Diagnostic Systems Ltd, UK) as per manufacturer's recommendations. Susceptibility data thus obtained was interpreted according to the Clinical and Laboratory Standards Institute's criteria (Susceptibility testing of Mycobacteria, Nocardia and other aerobic Actinomycetes: Approved standard—second edition. CLSI document M24-A2. 2011).[11]

Results

A total of 12 NTM were isolated from extra-pulmonary specimens over the study period. Rapidly growing mycobacteria (RGM) constituted 66% (8/12) of the isolates and the rest were slow growing mycobacteria (4/12). Clinical significance could be determined in 9/12 specimens obtained from 5 patients (table 1). Six NTM were isolated from aspirated pus specimens and 3 were isolated from tissue biopsies and sterile body fluid. Three patients (patient 1, 2 and 3) developed NTM infections with a prior history of a surgical procedure. Patient 1 developed multiple discharging non-healing sinuses over anterior abdominal wall following liposuction operation. Two aspirated pus specimens grew *Mycobacterium mucogenicum* and the patient responded to antibiotics following susceptibility results. Patient 2 was a female and underwent an abdominal wall hernia repair. Two months later she developed tender induration over surgical site which did not respond to debridement and antibiotic therapy. Pus specimens from the surgical site yielded a rapidly growing mycobacterium (*Mycobacterium chelonae/abscessus* group). Patient 3 had a total knee replacement and developed gradually progressive knee dysfunction with pain, swelling, erythema, warmth that culminated in discharging sinuses. Multiple specimens from her right knee grew same rapidly growing mycobacterium (*Mycobacterium fortuitum*). Patient 4, with generalized lymphadenopathy, had*Mycobacterium avium* complex (MAC) isolated from lymph node aspirate.

Discussion

Non-tuberculous mycobacteria are becoming more clinically relevant not only in immuno-compromised patients but also in immuno-competent individuals. Both pulmonary and extra-pulmonary infections may be caused by these mycobacteria. These infections are both difficult to diagnose and treat. Current guidelines recommend speciation of all clinically significant NTM isolates because therapy depends on the involved organism, disease site and its susceptibility profile.[9] To our knowledge this is the first study from Pakistan describing extra-pulmonary NTM infections and their possible association with healthcare derived infections.

Table 1: Patient characteristics for clinically significant extra-pulmonary NTM isolates

Patient number	Sample/ number of specimens	Age/Gender of subject	Sample AFB smear	NTM isolated	Clinical Information
1	Pus/2	43/M	negative	*M. mucogenicum*	Multiple discharging wounds with sinus tracts on anterior abdominal wall following liposuction. Routine cultures negative.
2	Pus/2	45/F	negative	*M. chelonae/ abscessus* group	Subcutaneous collection after abdominal wall hernia repair. Routine cultures negative.
3	Synovial fluid, synovium & granulation tissue/3	70/F	negative	*M. fortuitum*	Right knee discharging sinus, pain & swelling one year post total knee replacement. Routine cultures negative.
4	Lymph node pus/1	10/F	Numerous AFB	MAC	No co-morbids, not investigated for immunodeficiency, generalized lymphadenopathy for 6 months, no response to ATT. Biopsy of lymph node showed foamy histiocytes with acute & chronic inflammation, multinucleated giant cells (no granuloma), numerous intracytoplasmic AFB
5	Pus/1	26/F	negative	MAC	Non-healing ulcer on arm post burn. Multiple antibiotics used without any improvement

M, male; F, female; NTM, non-tuberculous mycobacteria; MAC, *Mycobacterium avium* complex; AFB, acid fast bacilli; ATT, Anti-tuberculous treatment

During the study period 12 extra-pulmonary NTM were recorded and 9/12 isolates from 5 patients deemed clinically relevant. Three cases developed NTM infections following a surgical procedure and represent a high probability of being nosocomial. Such healthcare associated NTM infections are well documented in literature.[7,12] NTM are environmental organisms and survive in natural and man-made bodies of water and other damp areas. NTM have also been isolated from tap water[13] and hospital water supplies.[6] This may represent a source of contamination of medical/surgical equipments if proper sterilization practices are not adhered to and appropriate mycobactericidal disinfectants are not used. In our patients exact source of NTM acquisition could not be determined, however, prior history of a surgical procedure points towards healthcare associated infection. Although not postsurgical, one young patient developed MAC lymphadenitis which is an uncommon presentation in a tuberculosis endemic area. It would be interesting to find out its true incidence in our setting.

It is important for doctors and clinical microbiologists alike to assess clinical significance of an NTM isolate and maintain a high index of suspicion in non-healing surgical wounds.

Study limitations include small sample size, lack of clinical outcome data for all except one patient and unavailability of proven source of infection for post-surgical infections. Further, larger studies are required looking at these issues.

Conflict of interest

All authors declare no conflict of interest.

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ORIGINAL ARTICLE

Detection of *metallo-beta lactamases (IMP, VIM, NDM)* and *KPC carbapenemases* in Carbapenem Resistant Enterobacteriaceae: Report from Clinical Laboratory Aga Khan University Karachi, Pakistan.

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ABSTRACT

Background
Carbapenem resistance in enterobacteriaceae due to New Delhi metallobetalactamase (*NDM-1*) has recently been reported as a major global health problem with potential spread from Indian subcontinent. The purpose of this study was to assess presence of different carbapenemases: metallobetalactamases*(NDM-1, IMP-1, and VIM-1)* and *KPC (Klebsiella pneumoniae carbapenemases)* enzymes among the carbapenem resistant enterobacteriaceae (CRE) isolated from different centers of Pakistan.

Methods
114 carbapenem resistant enterobacteriaceae (CRE) isolated from different clinical samples were prospectively collected from June 2009 to July 2010. MICs for meropenem were determined by E-test. Presence of gene for metallobetalactamase *(NDM-1, IMP-1, VIM-1)* and *KPC* enzymes were detected using PCR.

Results
114 CRE isolates yielded from clinical samples were included in the study. Among the bacterial pathogens 63.11% were *Klebsiella pneumoniae* (n=72) followed by *E.coli* 31.5% (n=36).MIC of meropenem tested by E-test strip on isolates positive for NDM-1 detected 33 isolates to be highly resistant, with MIC (>32µgm/ml). Gene for NDM-1 enzymes was detected in 93.8% (n=107) of clinical isolates. None of the clinical isolates were found positive for bla_{IMP}, bla_{VIM} and genes for KPC enzymes.

Conclusion
NDM-1 was found to be the commonest underlying gene among carbapenem resistant Enterobacteriaceae in this study. Its very high prevalence suggests that NDM-1 may be the most common enzyme responsible for carbapenem resistance among Enterobacteriaceae in Pakistan.

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Key Words
metallo-beta lactamase, carbapenemase, Pakistan

Introduction
Enterobacteriaceae are among the most common pathogens in humans, affecting all age groups and causing syndromes ranging in severity from simple cystitis to pneumonia, peritonitis, bacteremia, and meningitis.[1] Historically, these bacteria were susceptible to a wide range of antibiotics. During the past 25 years, however, multidrug resistance has emerged and become widespread leading to resistance to all common antibiotics such as ampicillins, ureidopenicillin, carboxypenicillin, cephalosporins, aminoglycosides, fluoroquinolones and trimethoprim-sulphamethoxazole. Resistance to penicillins and cephalosporins is mediated primarily via acquired genes that code for enzymes known as beta-lactamases (extended spectrum beta-lactamases, ESBLs).[2]

In view of resistance spectrum mentioned above, carbapenem group of antibiotics have become first line in treatment of infections caused by multi drug resistant (i.e. resistant to more than two classes of antimicrobials) *Klebsiella pneumoniae* and *E-coli*. Subsequently, carbapenem resistance among Enterobacteriaceae (CRE) has emerged, and increasingly being reported from different countries.[3,4,5] An emerging pathogen may be defined as a newly appearing infectious organism and includes both new and old, reappearing pathogens which have been identified by CDC. The emergence and dissemination of carbapenem resistance among Enterobacteriaceae represent a serious threat to public health. These organisms are associated with high mortality rates and have the potential to spread widely.[6]

Resistance to carbapenems may be conferred through different mechanisms including modifications to outer membrane permeability (OMP), up-regulation of efflux systems, hyper production of AmpC β lactamases (cephalosporinases) and production of specific carbapenem-hydrolyzing β lactamases (carbapenemases).[5]

Metallo-beta-lactamases (MBLs) and serine carbapenemases such as *KPC* constitute the most important group of carbapenemases rendering carbapenem resistance. Infections

caused by CRE are associated with high mortality.[7] This emergence and rapid spread of MBLs has been attributed to plasmid mediated spread of resistance genes.

Approximately thirty different types of MBLs have been reported from different countries. Since their initial discoveries, MBL abbreviated as *SPM, GIM,* and *SIM* have not spread beyond their countries of origin and remained confined to certain class of bacteria such as *Pseudomonas spp.s* and were not effectively transferred into other genera.[7] In contrast, *IMP* and *VIM* continue to be detected worldwide. These two metallo-beta lactamases have been reported to be moving beyond *Pseudomonas aeruginosa* and non-fermenters like *Acinetobacter spp*. to *Enterobacteriaceae*, spreading worldwide.[7] More recently, *NDM-1* (*New Delhi Metallo-betalactamase*) was identified in *Klebsiella pneumoniae* and *E. coli* recovered from a Swedish patient who was admitted to a hospital in New Delhi, India.[8] MBL-producing strains exhibit resistance to almost all currently available antibiotics; therefore treatment of infections caused by MBL-producing strains constitutes a challenge.[3,7,8] Moreover cross transmission of plasmids carrying resistant gene to gram-negative bacteria endemic in many developing countries such *as Salmonella spp., Shigella spp.* and *Vibrio spp.* is an impending threat.

In Pakistan, carbapenem resistant strains of *E. coli* and *Klebsiella pneumoniae* are emerging. Continued surveillance of antibiotic resistance by means of disc diffusion done half yearly shows an increasing trend of carbapenem resistance. Carbapenem resistance in *Klebsiella pneumonia* was reported at 8% in outpatients and 23% in hospitalized patients in 2011.[9] These findings are alarming and alert towards immediate attention to proper surveillance and to ascertain the mechanism of carbapenem resistance in such isolates in Pakistan.[10]

In current study we analyzed the carbapenem resistant isolates for detection of three most common types of MBLs (*IMP, VIM, NDM*) and *KPC* (*Klebsiella pneumoniaecarbapenemase*) which is a class A serine carbapenemase. These enzymes have been reported in Enterobacteriaceae globally. However, their frequency from Pakistan has not been reported yet.

This study was conducted to determine the type and frequency of carbapenemase mediated resistance in Enterobacteriaceae at a tertiary care hospital laboratory in Karachi Pakistan, using PCR assay.

Material and Methods

This study was conducted at the clinical Laboratory, Section of Microbiology Aga Khan University Hospital, Karachi during the period of 2009- 2010. Enterobacteriaceae cultured from clinical samples of blood, pus, urine and other body fluids received for routine bacteriological culture were included in the study. This study was exempted from review by ethical review committee of Aga Khan University. To avoid duplication caution was taken to exclude repeat sample from same patient. Isolates were identified to genus-level through routine biochemical tests. Antimicrobial susceptibilities were determined by Kirby-Bauer disk diffusion method, in accordance with CLSI 2011 standards.[11]

All carbapenem non- susceptible isolates (i.e. zone size ≤23 mm of meropenem/imipenem on Kirby-Bauer disk diffusion method) were then evaluated for MIC (minimum inhibitory concentration) of meropenem by Epsilometer strip (AB BIODISK Solna, Sweden). However MIC of other carbapenems was not determined due to resource limitation as meropenem and imipenem MIC are cross reportable as per CLSI.

PCR detection of carbapenemases

PCR was performed on all screened carbapenem non-susceptible isolates for detection of genes for metallobetalactamases *(IMP, VIM, NDM),* and *KPC* (a class A serine carbapenemase). Detection of resistance genes was performed as described by Kumarasamey *et al* for *NDM*, Woodford *et al* for *IMP, VIM* and Poirel *et al* for *KPC*.[8,12,13] ATCC strain BAA-1705 *Klebsiella pneumoniae* was used as positive control for KPC. Known strains of *NDM-1* positive *E-Coli* from a previous study were used as positive control for *NDM-1*primers.[8] Strains of *IMP-1* and *VIM-1* positive *Acinetobacter baumanii* strains were used as positive control.[12,13] Amplification products were visualised on 2% ethidium bromide gel by electrophoresis (figure 1).

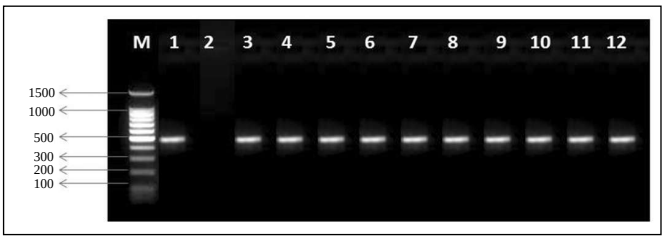

Fig 1: 2% agarose gel picture shows 100bp ladder with positive & negative controls (lane1,2) and bands of the expected amplicon (size=475 base pairs) for NDM-1in the positive samples (lanes 3-12) respectively.

Results

During one year study period (2009-2010) a total of 7192 enterobacteriaceae were obtained from different clinical samples. Majority of clinical isolates (57%) were obtained from blood cultures (n=65), followed by urine (28%, n=32) and pus aspirates (7.9%, n=9). In addition two isolates each from tracheal aspirate and peritoneal fluid were also included in the study.

One hundred and fourteen (1.7%) isolates of Enterobacteriaceae were found to be non-susceptible to meropenem by Kirby-Bauer disk diffusion method with zone sizes ≤23 mm to Meropenem/ Imipenem.

Antibiotic susceptibility

All the initially screened carbapenem non-susceptible isolates were ESBL positive and hence resistant to, ampicillin,

cephalosporins and aztreonam. Among the aminoglycosides tested, gentamicin resistance was identified in 100% while only 8% (n=10) of these isolates were found to be sensitive to amikacin. Fosfomycin was checked in 29 urinary isolates and was found sensitive in 25 of them.

Majority of these isolates were pan resistant to all major groups of antimicrobials and sensitive only to polymyxin B. Polymyxin susceptibility was determined by automated MIC using Vitek-2 (NE-card) and interpreted as per CLSI 2011 breakpoints for *Acinetobacter baumanii* with MIC <2µg/ml as sensitive.

On MIC testing by E-strip for meropenem, 18 isolates were found to have MIC < 2 µg/ml(with 8 being susceptible MIC<1µg/ml, and10 intermediate MIC >1 µg/ml). Additionally 12 isolates with MIC in the (>2 to <4µg/ml) range were considered intermediate and 84 were found to be resistant (MIC ≥4 µg/ml) as shown in Table 1.

Table 1: Distribution of Type of organism and Minimum Inhibitory Concentration (MIC) distribution of Meropenem (10µg) disk resistant Enterobactereaciae studied at Aga Khan University Hospital Karachi, 2009-2010. Total (n=114)

Organism	*Klebsiella pneumoniae*	72(63.1%)
	E coli	36(31.6%)
	Citrobacter spp.	2(1.8%)
	Enterobacter spp.	3(2.6%)
	Serratia spp.	1(.9%)
MIC Meropenem (µg/ml)	<2µg/ml	18(15.7%)
	2 µg/ml	6(5.3%)
	3 µg/ml	6(5.3%)
	4 µg/ml	10(8.8%)
	6 µg/ml	14(12.3%)
	8 µg/ml	5(4.4%)
	12 µg/ml	13(11.4%)
	16 µg/ml	6(5.3%)
	24 µg/ml	3(2.6%)
	>32 µg/ml	33(28.9%)

PCR typing of MBL and KPC genes

Gene for NDM-1 (bla_{NDM}) enzymes was detected in 94% (n=107) of clinical isolates as shown in Figure 1. None of the clinical isolates were found positive for genes of bla_{IMP}, bla_{VIM} and bla_{KPC} enzymes by PCR. The remaining 6% (n=7) of the tested carbapenem non-susceptible isolates were negative for all the carbapenemases genes tested in this study by PCR.

Discussion

NDM-1 was found to be the most prevalent carbapenemase in CRE (carbapenem resistant enterobacteriaceae) in this study. Although other metallo-beta lactamases *IMP* and *VIM* have been reported in non-enterobacteriaceae, like *Pseudomonas aeruginosa* and *Acinetobacter species* from Pakistan[12], these were not detected in CRE isolates tested in the current study. Serine carbapenemases such as *KPC* which is reported to be prevalent in enterobacteriaceae in USA, UK, Sweden and other parts of Europe was not found in any of the isolates tested in this study.[2,3,8] Even though globally *KPC, OXA-48, VIM* and *IMP* producers are currently the most widespread types of carbapenemase in enterobacteriaceae, *NDM-1* producers are likely to become highly prevalent.[14,15] Kumarasamy *et al* in 2010 showed *NDM-1* emergence as a new antibiotic resistance mechanism in India, Pakistan, and the UK in 48, 25 and 37 isolates respectively from these countries.[8] This molecular, biological, and epidemiological study was the first to investigate the prevalence of *NDM-1*, in multidrug-resistant enterobacteriaceae in these countries. This study shows that frequency of infections caused by *NDM-1* positive CRE in Pakistan is higher than previously reported as it was found to be the most prevalent gene responsible for carbapenem resistance among the four different carbapenemases considered.

Treatment options for infections due to NDM-1 positive isolates are limited. In our study polymyxin B was the only antibiotic that all the NDM-1 carbapenemase producing isolates were susceptible to, however the use of this nephrotoxic antibiotic requires careful dosing, as well as meticulous management of fluid and electrolyte abnormalities along with avoidance of concurrent administration of other nephrotoxic and/or neurotoxic drugs.[16,17] Urinary isolates were found to be susceptible to fosfomycin, however its use is limited for treatment of urinary tract infections only.

Emergence and rapid spread of *NDM-1* as the predominant metallo-beta lactamase worldwide has been attributed to various factors such as inadequate or delayed infection control measures in hospitals, contamination of community water supply.[18,19] There are reports of *NDM-1*-positive bacteria recovered from gut flora, community water supply systems and sewage water from Pakistan and India.[14,15] An environmental point prevalence study conducted in New Delhi and Chennai, have shown that tap water and sewage water of these cities is contaminated with *NDM-1* harbouring bacteria[20]. It is difficult to predict the rate of spread of the gene encoding NDM-1 in the faecal flora of patients in the Indian subcontinent. Exchanges of the *blaNDM-1* gene between unrelated bacterial isolates and species have been identified already in enterobacteriaceae and *Acinetobacter baumannii*.[20,21] An Indian report indicates rapid spread of carbapenem-resistant enterobacterial species in a hospital in Mumbai.[22] There is now an urgent need to evaluate the spread of NDM-1 among hospital-acquired and community-acquired pathogens in the Indian subcontinent. Community spread of NDM-carrying isolates is a real threat due to global travel and indicates an important avenue for implementing control strategies.

It is also imperative that the spread of NDM-1 harbouring CRE among hospital environments is curtailed through early identification of resistance, strict implementation of standard hygiene measures and contact isolation of patients with these strains, and stringent antibiotic control policy. This should prevent the development of outbreaks, and help optimize antibiotic therapy in carriers who go on to develop infection with these highly resistant bacteria.[13]

The spread of NDM-1 constitutes a study in real time of how resistance can become global. Critically, it warns us that antibiotic resistance can become a global problem requiring a bold and decisive global action. It is essential that such recommendations are no longer ignored but fully implemented in a transparent and accountable manner.

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Conflict of interests

None to declare.

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ORIGINAL ARTICLE

Seroprevalence of Hepatitis B and C Virus in Diabetic Patients and its Association with Increased Alanine Aminotransferase (ALT) at Bahawal Victoria Hospital, Bahawalpur

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Abstract

Background
Diabetes mellitus type II (DM-2) and hepatitis C virus (HCV) infection often co-exist while DM also causes increase in levels of serum alanine aminotransferase (ALT).We aim to determine the frequency of HCV and hepatitis B virus (HBV) infections in DM-2 patients and its association with ALT.

Methodology
A cross-sectional study was conducted at the diabetic clinic of Bahawal Victoria Hospital, Quaid-e-Azam Medical College, Bahawalpur during Dec. 2006-June 2007. A total of 439 consecutive diabetic patients of either gender were evaluated for HCV and HBV infection by using Enzyme Linked Immunosorbant Assay (ELISA-3) along with serum ALT levels. Frequencies of HBV and HCV infection were noted. Patients with high ALT with underlying HCV/HBV infection were compared with patients with normal ALT by using chi square test.

Results
Among 439 DM2 patients, 221(50%) were males. Age ranged from 18-95 years. Most of them were married (n=432, 98%), belonged to rural areas (n=309, 70 %), had DM2 (99.8%), and majority were and non-smokers (79 %). Seroprevalence for HCV, HBV and both were 25%, 3% and 2% respectively. Sixty percent (n=264) had a normal ALT. Patients with raised ALT were more commonly had an underlying HCV infection compared to those with a normal ALT.

Conclusion
HBV and HCV infections are frequent in patients with DM. Patients with raised ALT level were more likely to have an underlying HCV infection than those with a normal ALT.

Key Words
Diabetes Mellitus, Hepatitis C, Hepatitis B, Alanine Aminotransferase.

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Introduction

Diabetes Mellitus (DM) hepatitis B (HBV) and hepatitis C virus (HCV) infection are very common worldwide. About 346 million people are suffering from DM worldwide.[1] The estimated prevalence of DM in Pakistan is 9.5-11.47 % of adult population placing it at number 07 in DM in the world.[2] WHO estimates that approximately two billion people in the world have been infected with HBV while approximately 350 million people are living with chronic HBV infection.[3] Seroprevalence of HBsAg in Pakistan is 2.5%.[4,5] Worldwide, 3-4 million people get infected with HCV, each year whereas 130-170 million people are living with chronic HCV infection.[1,3,6] The estimated prevalence of HCV infection in Pakistan is around 5-6% in the general population.[4,5] As evidenced by the above mentioned epidemiological data, DM and chronic hepatitis (HCV/HBV) are quite common in Pakistan and are associated with significant morbidity and mortality.

DM has been found to be more prevalent among patients suffering from chronic liver disease (CLD) and cirrhosis.[7,8] Further, diabetics have a high prevalence of HCV infection compared to the general population.[8] Whether DM predisposes an individual to HCV infection or HCV infection leads to the development of DM in the infected individual is not yet known.[8] Moreover DM and HCV infection is an ominous association as it is severe and leads to early cirrhosis, HCV infection is resistant to treatment with interferon and ribavirin combination therapy, and increased risk of hepatocellular carcinoma (HCC),[9,10] therefore early diagnosis and treatment of underlying HCV infection is prudent in diabetics.

A raised level of alanine aminotransferase (ALT) is one of the several biochemical abnormalities commonly found in DM patients.[11] This may be due to several reasons like nonalcoholic fatty liver disease (NAFLD), alcohol ingestion or obesity.[12,14] Due to these reasons, many times raised ALT levels in diabetic are not further investigated by the physicians.[15] A raised ALT level is also a feature of HCV and or HBV infection. Data suggests that diabetic patients with raised ALT have a high prevalence of HBV and HCV infection compared to patients who do not have these infections.[15,17] We performed this study to determine the frequency of HCV and HBV infection in diabetics in Bahawalpur, and to determine if raised ALT in

diabetics is associated with underlying HCV and or HBV infection.

Materials and Methods

This was a cross sectional study. A total of 439 consecutive diabetic patients who visited the medical outpatient facility of Bahawal-Victoria Hospital (BVH), Bahawalpur from 30th December 2006 to 30th June 2007 for the control of their diabetes were evaluated for HCV and HBV infection by using Enzyme Linked Immunosorbant Assay (ELISA-3) along with serum ALT levels. Complete history and physical examination was performed. The heights and weights were measured and BMI of each patient was calculated. Written informed consents were obtained from participating patients. ELISA for HBV, HCV and serum ALT levels was performed at the pathology laboratory of Quaid-e-Azam Medical College (QAMC). Normal ranges for alanine aminotransferase levels in our laboratory were 10-40 for both men and women. Data was analyzed using SPSS version 10.00. The frequency of Hepatitis C and Hepatitis B was calculated and the patients were then grouped into two, depending on their level of ALT (Group 1 patients with raised ALT and group 2 patients with normal ALT). We applied chi square test to determine the association of raised ALT among diabetics with underlying Hepatitis C or B infection. A p value of <0.05 was taken as statistically significant.

Results

Out of 439 patients 221 (50%) were males. Age ranged from 18 to 95 (median = 50). Most of them were married 432 (98%), belonged to rural area 309 (70%), had type II DM (99.8%), normal ALT 264(60.1%) and were non-smokers 345 (79%). Majority of the patients 288 (65%) had diabetes for more than 1 year.

In our study seroprevalence of HCV, HBV and both were 27%, 3% and 2% respectively. Chi square test showed a significant association between ALT & HCV/HBV(P<.0001). The odds of underlying HCV/HBV infection in diabetics with raised ALT were 3 times compared to those with normal ALT (OR=3.06, CI 1.99 -4.69, P= <0.0001).

Table 1 shows that 125 (28%) out of 439 were seropositive. HCV infection was present in 117 (94%) out of 125 seropositive patients. Among seropositive, HBsAg was present in just 15 (12%) patients. Raised ALT was present in 175 (40%) patients. Table 2 shows association of raised ALT with seropositivity

Table 1: ALT and Seroprevalence of HBV and HCV in diabetic patients

ALT (U/l)	<40	264 (60%)
	≥40	175 (40%)
HBsAg	Yes	15 (3%)
	No	424 (97%)
Anti HCV	Yes	117 (27%)
	No	322 (73%)
Seropositivity	Yes	125 (29%)
	No	314 (72%)
Both HCV and HBV positive*		7

ALT, Alanine Aminotransferase; *HBsAg*, Hepatitis B Surface Antigen; *Anti HCV*, Anti Hepatitis C virus antibodies

*Seven patients were positive both for HBV and HCV. We included them in each group (HBsAg and Anti HCV positive groups) that is 7 patients out of 15 who are positive for HBsAg are also positive for HCV also 07 patients out of 117 who are positive for HCV were also positive for HBV. Similarly 7 patients out of 125 are those who are positive both for HCV and HBV.

Table 2: Association of raised ALT with seropositivity in diabetic patients

	≥40 ALT	<40 ALT	OR (95% CI)
Seropositive*	74(42%)	51(19%)	3.06(1.99 -4.69)
Seronegative**	101(58%)	213(81%)	

**Seropositive*, HBsAg and/or Anti HCV antibody positive by ELISA.

***Seronegative*, HBs Ag and Anti HCV antibody negative by ELISA.

of DM or impaired glucose tolerance (IGT) or with chronic liver disease/cirrhosis especially due to HCV infection.[7,8]

Whether diabetes mellitus or insulin resistance predisposes a patient to HCV infection or HCV infection itself causes insulin resistance or DM2, yet needs to be established.[8] Currently there are two hypotheses; one supporting the fact that DM2 predisposes to HCV infection while the second hypothesis suggests that HCV infection induces insulin resistance/DM in infected individuals. We are unable to refute either hypothesis and accept the alternate on the basis of available evidence.[8] The hypothesis that DM predisposes to HCV infection is evidenced by studies showing that HCV is associated with DM only in those patients who already have risk factors for the development of diabetes.[18,19] As liver has a pivotal role in carbohydrate metabolism many studies show HCV and diabetes only in patients with cirrhosis not in chronic hepatitis due to HCV.[7,20-22] Prevalence of diabetes in cirrhosis increased with increasing child-Pugh score and

Discussion

In this study HCV infection was found to be 5.4 times high among diabetics compared to general population of Pakistan and almost four times to that of general population of Punjab where HCV prevalence is approximately 6.7%.[4] Also prevalence of HBV is 3.6% in our study whereas in general population it is 2.5%.[4,5] There is some relationship between DM2 and HCV documented in medial literature. There is a high coexistence

increasing age.[7] Also diabetes is equally frequent in HBV and HCV related cirrhosis.[21,22] In a nutshell it is the advanced fibrosis of the liver and classical risk factors for the development of type 2 diabetes that are responsible for increased prevalence of type 2 diabetes in these patients. On the other hand the notion that HCV infection induces insulin resistance and DM is evidenced by studies showing a high prevalence of DM/insulin resistance in patients with chronic hepatitis due to HCV compared to HBV,[7] Similarly diabetes is more common in cirrhosis due to HCV not HBV.[23] Recent data suggests that HCV core proteins impair insulin downstream signaling and regulatory role of IGFBP-1 expression which translates as insulin resistance and impaired glucose tolerance.[24]

Liver injury is common among diabetic patients due to several reasons. In this study we found that 32% diabetics who were seronegative also have raised ALT indicating that liver injury is common in diabetics for reasons other than HCV/HBV infection. One reason for this damage is probably DM itself because cryptogenic cirrhosis is common among diabetics.[25] DM causes liver injury by several mechanisms. It is now increasingly being recognized as a risk factor for liver disease. NAFLD is probably responsible for the diabetes associated liver injury. NAFLD once thought to be a trivial disorder is now considered hepatic manifestation of metabolic syndrome. The spectrum of NAFLD varies from hepatic steatosis to non-alcoholic steatohepatitis which can progress to cirrhosis. Estimated prevalence of NAFLD among diabetics is approximately 70 % although the precise prevalence is unknown.[26] Insulin resistance is a universal phenomenon in NAFLD.

We found that patients with a raised ALT were three times more likely to have an underlying HCV/HBV infection compared to those with a normal ALT (OR=3.06, CI 1.99 -4.69, P= <0.0001). Both DM2 and HCV infection are potential causes for liver cell injury and raised ALT.

Both cause liver cell injury by some dependent and some independent mechanisms. For example hyper-insulenemia in type 2 diabetics causes excessive activation of hepatic stellate cells which leads to increase liver fibrosis.[27] Also, hyperglycemia itself leads to increased hepatic fibrosis in HCV infected individuals.[28] Insulin resistance induces hepatic steatosis and hepatic steatosis is associated with more severe fibrosis in chronic HCV.[29] HCV itself induces insulin resistance and also impairs the lipid oxidation as well as export of lipids as very low density lipoproteins (VLDL) causing lipid accumulation in hepatocytes and leading to hepatic steatosis.[24,30] Histologically the pattern of fibrosis (sub sinusoidal and around the central vein) in chronic HCV infection is similar to that seen in NAFLD suggesting that both may share some common mechanisms.[31] So a diabetic patient with an HCV infection is likely to have more chances of raised ALT than one who have either of them.

As asymptomatic HCV infection is fairly common it is possible that we may continue to treat a patient conventionally who has DM or IGT or metabolic syndrome attributing his/her raised ALT due to NAFLD or alcohol or drugs whereas he/she might be having an underlying HCV infection.[15]Considering the high prevalence of HCV among diabetics presenting with raised ALT it is reasonable to check for an underlying HCV infection in these patients. The results of our study are consistent with other studies conducted locally and internationally.[15,16] There are several limitations to our study. It was a cross sectional analytic study and does not imply any causal relationship between raised ALT level in diabetics and HCV infection. Moreover this is a hospital based study in Bahawalpur and not a population based study so caution must be observed in generalizing the findings of this study.

Conclusion

HBV and HCV infections are more prevalent in diabetic patients. Diabetics with raised ALT should be investigated for underlying HCV or HBV infection

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Frequency of *Tinea Capitis* in Children 5-15 Years of Age Presenting to Primary Health Care Centre in Karachi, Pakistan

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ABSTRACT

Objective
Primary Objective: To determine the frequency of *Tinea capitis* in children 5- 15 years of age at a primary care center in Karachi. Secondary Objective: To determine the risk factors for *Tinea capitis* in children 5- 15 years of age at a primary care center in Karachi.

Methods
A cross sectional study was conducted at a primary health care center in Sikanderabad during 1st October 2012 - 31st May 2013. Children aged 5-15 years were included in the study. Clinical examination of the scalp was done for the diagnosis of *Tinea capitis* and to confirm the diagnosis, hair scrapings were collected for mycological analysis and a proforma containing demographic and laboratory information was filled. Mean and standard deviation were calculated for numerical variables. Frequencies and percentages were computed and fisher's exact test was used to determine the association between socio-demographic factors and *Tinea capitis.*

Results
A total of 176 children aged 5-15 years were included in the study. The estimated frequency of *Tinea capitis* was 24/176 (13.6%). Mean age of the children was 9.1+3 years. The clinical features included itching n=18(10%), alopecia n=42 (24%), scaling n= 58(33%), tender occipital and cervical lymphadenopathy n= 18 (10%), erythema n= 17 (10%) and discharging lesions n=7(4%) were found.Factors including poorsocio-economic status, overcrowded household , poor hygiene, habit of sharing combs and low level of parental education were significantly associated with *Tinea capitis* infection.

Conclusion
A high frequency of *Tinea capitis*is was seen in our population as compared to previous study[1]. Factors like low socio-economic status, poor hygienic care and overcrowding are major concerns that lead to increased frequency of *Tinea capitis* in children.

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Keywords
Tinea capitis; dermatophytosis; alopecia; tender lymphadenopathy.

Introduction
Tinea capitis is a superficial fungal infection of the scalp and associated hairs caused by a variety of species of the genera *Trichophyton* and *Microsporum*. Nowadays, there is an increasing trend in its incidence worldwide.[2] According to their host preference and natural habitat, *dermatophytes* are also classified as anthropophilic, geophilic and zoophilic. Moreover, the predominant dermatophytosis causing *Tinea capitis* in a given geographic region can also change over time.[3] The disease is more prevalent in tropical countries owing to high temperatures and humidity.

Tinea capitis is characterized by irregular or well-demarcated alopecia and scaling. When swollen hairs fracture a few millimeters from the scalp, "black dot" alopecia is produced. *Tinea* scalp infection also may result in a cell-mediated immune response termed a "kerion," which is a bogie, sterile, inflammatory scalp mass. Cervical and occipital lymphadenopathy may be prominent.[4] *Tinea capitis* is a common infection in young children. Low socioeconomic status, infrequent bathing, sharing beds and combs and similar illness in the family are a few predisposing factors.[7] Dermatophytes infections can be readily diagnosed based on the history, physical examination and potassium hydroxide (KOH) microscopy. Diagnosis occasionally requires Wood's lamp examination and fungal culture or histologic examination.[4]

Tinea capitis has neither been the focus of intensive study nor of active control programs in Karachi and this neglect is likely because fungal diseases of healthy humans tend to be relatively benign. Consequently, there is paucity of information on the epidemiology of *Tinea capitis* in Karachi and this dearth of scientific information has affected adequate patient management, diagnosis, control programs and identification of risk factors of *Tinea capitis*. In this study we can report the underlying factors of *Tinea capitis* in our society. If considerable quantum of children are seen to be affected by *Tinea capitis* then we should reinforce our primary care providers to educate themselves and provide awareness in the society to eradicate

the factors leading to it, i.e., community education to avoiding sharing of personal items like hats, combs etc. in people susceptible to *Tinea capitis.*

We aim to determine the frequency and risk factors for *Tinea capitis* in children 5- 15 years of age at a primary care center in Karachi.

Methods

A cross sectional study was carried out at a primary health care center in Sikanderabad, Karachi from October 2012 - May 2013. All children from 5-15 years of age coming to primary health care center coming for any complaint were enrolled in the study. *Tinea capitis* was diagnosed if two or more of the following signs and symptoms of fungal infection were seen on the scalp of the children: Alopecia, erythema, scaling and pruritus of the scalp and tender cervical and occipital lymphadenopathy.[1] Scalp of the children was examined by the primary investigator in bright sunlight wearing gloves. Each lesion examined thoroughly and cervical and occipital lymph nodes were palpated. Kerions were specially looked for, lesions were then cleaned with alcohol swabs to remove any ointments or local applications and scrapings (skin scales, crusts, hair pieces) were taken from the active edge of lesion using a blunt sterile scalpel blade. The scrapings were mounted on clean slides in a drop of 20% KOH solution then covered with cover slip and taken to the laboratory of Ziauddin University Hospital for microscopic examination. Presence of hyphae and spores invading the scales and hair was labeled as *Tinea capitis*.

Those children who did not prove to have *Tinea capitis* by microscopy were labeled as Tinea free group. Hence two groups were made, those having *Tinea capitis* and those without *Tinea capitis.*Informed consent was taken from the parents of all the children included in the study. Children with congenital anomalies, development delays and neurological deficits were excluded from the study. Data on demographic and other risk factors was collected for both children with *Tinea capitis* and without, to see effect of various factors on *Tinea capitis* and recorded in the proforma. The details of characteristics of the lesions e.g. size of the biggest lesion and discharging status was recorded. Questions were asked regarding sharing of combs, hats and beds, poor hygiene, family history of dermatophytosis in the preceding one month, pets in the house, overcrowding, low socioeconomic status and low level of education in parents.

Overcrowding in the house was considered in our study if there were more than 3 people inhabiting one room no matter how many rooms were there in the house. Low socioeconomic status was considered in our study if monthly combined income of all the earning members of the house was less than Rs. 10000. Low level of education in the parents was considered if either of the parents had less than 10 years of continuous school education.

Poor hygiene was assessed by asking bathing frequency, bathing <twice a week was considered as living in poor hygienic conditions. If the same comb, hat and bed were shared by two or more persons for more than 1 week then it was labeled as sharing. If similar lesions of the scalp were found in any of the family member living in the same house in the preceding 1 month or diagnosed by doctor as having *Tinea capitis*, then family history was considered positive. Pets such as goats, dogs and cats etc. were considered positive if living in the house in the past one month and the subjects were in physical contact with the pet.

Data was analyzed by using SPSS version 20. Mean and Standard deviation were taken for numerical variables. The numerical variables were age, number of lesions and size of biggest lesion in cm. Frequency and percentage were taken for categorical variables. Categorical variables were gender, sharing of combs, hats and beds, keeping bad hygiene, family history of dermatophytosis, and pets in the house, overcrowding, low socioeconomic status and low level of education in parents. Fisher's exact test was applied in order to prove the association of risk factors with *Tinea Capitis*. P-value <0.05 was considered as significant.

Results

Out of the 176 children 92 were males (52%) and 84 were females (47.7 %). The estimated frequency of *Tinea capitis* in these children was 13.6%. Among males 18 (10.2%) and among females 6 (3.4%) had *Tinea capitis*. Mean age of presentation was 9.1 yrs. Table 1 shows the comparison of characteristics of children with and without *Tinea capitis***.** Table 2 shows the frequencies, p-value and fisher's exact test. Low socioeconomic conditions, overcrowding, keeping bad hygiene, sharing of comb and low level of parental education came out to be significant risk factors of *Tinea capitis* in the present study. Sharing of bed and hat, keeping pets in the house and family history of dermatophytosis did not come out to be a significant risk factor in the present study.

Discussion

Tinea capitis is a dermatosis of the scalp due to dermatophytes that can cause hair loss. It is a common infection among school

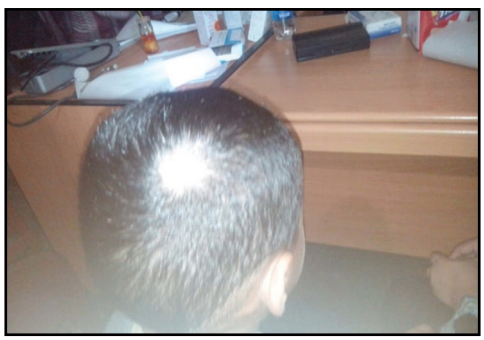

Fig 1. A child at PHC having grey patch *Tinea capitis***. Also post-auricular lymphadenopathy is prominent.**

children in developing countries.[3] The estimated prevalence of *Tinea capitis* in the present study came out to be 13.6%, higher than the study done in Karachi in 2006[1] and lower than the study done in Riyadh Saudi Arabia in 2008[5] and Gabon (16.3%) in 2011.[6]

In our study, mean age of presentation of *Tinea capitis* came was 9.1+2.79 years, consistent with the age of presentation in a study in 2006 in Nepal i.e. 9.2 yrs.[7] Similar age of presentation was seen in a study in Karachi in 2006.[1] The low incidence after puberty is believed to be due to the sebum containing fungistatic free fatty acids. The increased incidence in males is due to the presence of short hair in males and spores; therefore, reach the scalp easily.[8] In our study males were more infected with *Tinea capitis* as compared to females similar to the results by Fathi *et al*.[9]

Table 1: Comparison of characteristics of children with and without *Tinea Capitis*

	Tinea Capitis n (%)	*No Tinea Capitis n(%)*
Gender (Male)	18 (10)	6 (3.4)
(Female)	86 (49)	66(37.5)
Mean age (yrs.)	9.1± 3	9.3 ± 3
Clinical Features		
Scalp lesions	24 (100)	74 (49)
Average number of lesions	2.9 ± 2.0	1.0± 0.5
Size of biggest lesion (cm)	2.0±1.3	1.0± 0.4
Discharging lesions of scalp	7 (4)	1(0.6)
Itching of the scalp	18 (10)	12 (7)
Alopecia	42 (24)	13(7)
Scaling of the scalp	58 (33)	19 (11)
Erythema of the scalp	17 (7)	7(4)
Tender cervical/occipital lymphadenopathy	18 (10)	2(1)

Table 2: Association of common factors with "*Tinea Capitis***".**

	Tinea Capitis n (%)	*No Tinea Capitis n(%)*	*p-value*
Low Socioeconomic status	18 (75)	*42(28)*	*0.002*
Overcrowding	17 (71)	*41(27)*	*0.002*
Low level of mother education	6 (25)	*27(18)*	*<0.001*
Low level of father education*	4 (17)	*39(26)*	*<0.001*
Poor hygiene	16 (67)	*31(20)*	*0.029*
Sharing of comb*	16 (67)	*4(3)*	*0.007*
Sharing of bed*	1 (4)	*4(3)*	*0.180*
Sharing of hat10	(42)	*5(3)*	*0.197*
Family history of dermatophytosis preceding 1 month*	6 (25)	*1(1)*	*0.059*
Keeping pets in the house (goats, cats ,dogs)	14 (58)	*11(7)*	*0.549*

*Fischer exact test applied.

After improvements in socio economic conditions in the developed countries, the incidence of *Tinea capitis* has significantly reduced. In our study 18(75%) children suffering from *Tinea capitis* belonged to low socioeconomic background which were lower than the figures in the previous study.[7] Hence low socioeconomic conditions proved to be a significant risk factor for *Tinea capitis*. Several international studies[10,11,12,3] have documented differences in occupation, parent income as a significant risk factors for *T. capitis* as shown in this study.

Our study showed 17(70.4%) children living in overcrowded living conditions, lower than the result in previous study.[13] Overcrowding also came out to be a significant risk factor for *Tinea capitis*. The community around Sikanderabad is characterized by large families occupying a single house where the number of family members may reach up to 15-20 people in one small house. This results in frequent close contact between family members and hence spreads the disease.

The frequency and severity of *Tinea capitis* is likely to be linked to personal cleanliness. Poor personal hygiene is a reflection of low standard of living. Poor hygiene 'bathing twice or less in a week' turned out to be a significant risk factor for *Tinea capitis*. In our study 16 (66.6%) children had poor hygiene, almost consistent with figures in the previous study 17%.[7] This might be due to the problem of poor water supply in this area.

Sharing of personal items such as combs and hats were seen in 16(66.6%) and 10(41.7%) respectively in contrast to 90%.[7] and 25%[9] respectively previously. Sharing combs came out to be significant risk factors for *Tinea capitis*. Transmission of the scalp infection is fostered by poor hygiene and overcrowding, and can occur through contaminated hats, brushes, pillowcases, and other inanimate objects. After being shed, affected hairs can harbor viable organisms for more than one year.[4]

Majority of the children with *T. capitis* had significant history of animal contact which was 58.3% compared to N and Jha *et al*.[7] The predominant animal contact was with goats. Abdulkadir[14] had earlier confirmed that enzootic ringworm of horses, dogs, and livestock as a common source of sporadic infection among owners or their care takers which might include the owner's children. Animal type ringworm was also viewed by Macura as an occupational hazard for farmers and pet keepers.[15,16] In contrast, in our study, keeping pets in the house did not seem to be a significant risk factor for *Tinea capitis*. In this area goats

are mostly kept for getting milk and also for commercial purposes.

Parental education was significantly related to *Tinea capitis* in our study. Our study showed low level of mother's and father's education in 6(25%) and 4 (16.7%) children respectively. Previous study showed low level of father's education in 18.5% cases.[9] Family history of dermatophytosis in the preceding one month was not a significant risk factor for *Tinea capitis*.

Limitations

The limitations in our study were that sample size was relatively samll. Population studied was uniform in terms of people with low socioeconomic status and overcrowded household. Further studies are required having population with different socio-demographic characteristics so that the results could be generalized. Secondly fungal culture which is more sensitive than microscopy was not done due to resource limitation and may take upto 4 weeks to become available, so we were not able to identifiy the specie causing the disease and there could be chances that some non-dermatophytes could have been diagnosed erroneously as *Tinea capitis*.

Conclusion

Our study has shown that *Tinea capitis* has been associated with low socio-economic background, overcrowding, poor hygiene, sharing of combs and low level of parental education.

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CASE REPORT

Tuberculous Pericarditis in Children - Case Series

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Introduction

World Health Organization (WHO) reported a total of 8.8 million new cases of tuberculosis (TB) world-wide in 2010 alone.[1] Deliberating on the pediatric population, Tuberculosis ranks among the top ten causes of death in children globally, constituting 20% to 40% of all TB cases in high burden countries.[2]

Extra pulmonary TB contributes to 15% of all TB cases.[3] One form of extra pulmonary TB is pericarditis, presenting either as pericardial effusion or constrictive pericarditis.[4] Constrictive pericarditis entails a process of chronic inflammation culminating in fibrous thickening of pericardium, interfering with diastolic filling of the heart, decreasing venous return and cardiac output.[5] TB remains the major culprit behind pericarditis in the developing world, unlike the developed world where TB is the causative agent of acute pericarditis in less than 4% of the cases.[6] Systemic abnormalities secondary to this disease present with the clinical spectrum of dyspnea, orthopnea, fatigue, cough, peripheral edema, raised jugular venous pressure, ascites, and hepatomegaly, while systemic clinical signs and symptoms of night sweats, weight loss and low grade fever may co-exist.[3,7] Diagnosing cases of childhood TB continues to be a challenge, since involved techniques do not have a high yield for diagnosis in this population.[8] In order to diagnose, TB scoring systems comprising of an amalgamation of clinical spectrum, history of TB contact, Tuberculin Skin Test (TST) results and radiologic evaluations have been devised and availed.[9] For pericardial TB, pericardial tap, positive culture of the effusion fluid, caseous granuloma on pericardial biopsy, and echocardiography have been used as diagnostic modalities.[5] Pericardiectomy along with anti tuberculous chemotherapy and concomitant corticosteroids is the treatment of choice for constrictive pericarditis secondary to TB.[5,7]

To minimize mortality associated with TB pericarditis, rapid and early diagnosis and prompt management are of utmost significance. This is of particular importance in high burden countries where TB is endemic; hence a high clinical suspicion should be taken into consideration during diagnostic interventions. We present a case series of pediatric patients with acute pericarditis secondary to TB, concentrating on their presentation, clinical course, management and outcome. This study strives to lay emphasis on importance of vigilantly treating TB as a disease without neglecting the possibility of fatal complications like pericardial TB secondary to this debilitating illness.

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Methodology

This is a retrospective case series based on review of records of pediatric patients diagnosed with TB pericarditis. From 1985-2011, all pediatric cases (up till 18 years of age), admitted at The Aga Khan University Hospital, Karachi, with a confirmed or suspected diagnosis of TB pericarditis were studied. Aga Khan University Hospital operates with 577 in-patient beds and provides high quality of patient care to over 50,000 hospitalized patients and to approximately 600,000 outpatients annually. Diagnosis of TB was made in accordance with the National guidelines for diagnosis and management of TB in children (Table 1). Scoring chart of the Pakistan Pediatric Association was used to screen and score children for TB.[8] They were then classified into four groups based on the scoring points; TB unlikely (0-2 points), possible TB to be kept under observation for three months (3-4 points), TB possible, investigations justifying therapy (5-6 points), and TB probable and needs confirmation (≥7).

A total of 9 cases were included in our final report. No patient had any evidence of human immunodeficiency virus infection or primary immune deficiency. For each patient, a complete clinical history, physical examination, chest X-ray, and 2-dimensional Doppler echocardiography were performed. All nine patients underwent pericardiocentesis and/or pericardiectomy.

Cases

TB unquestionable (Score ≥7)

Case1

An 8 years old boy, presented with low-grade fever and difficulty in breathing for 4 days. It was associated with chest pain which was relieved on leaning forward and mild non-productive cough. There was no history of TB contact and the child had received BCG at birth. On chest examination, there were signs of respiratory distress, CVS exam revealed heart rate of 144 beats/min with muffled heart sounds. Pericardial rub was present. Peripheral pulses were weak and perfusion was poor. TST (tuberculin Skin test) was positive with significant (> 15 mm) induration. Chest x-ray revealed an enlarged cardiac silhouette, indicating pericardial effusion. Initial lab work indicated a

Table 1: Modified Kenneth Jones scoring criteria / Pakistan Paediatrics Association scoring chart for diagnosis of TB in children.

Features	1	2	3	4	5	Score
HISTORY						
Age	<2 years	-	-		-	
Closed contact in last 2 years	with TB patient		With sputum+ve TB patient			
BCG Scar	Absent	-				
History of measles and whooping cough	Between 3-6 months	< 3 months				
Immunocompromise/ immunosuppressant*	Yes	-				
PCM Grade 3	Yes	-	Not improving			
EXAMINATION AND INVESTIGATION						
Physical examinations	-	Suggestive of TB		Strongly suggestive		
Radiological findings	Non specific	Suggestive of TB				
Tuberculin skin test	5-10 mm		>10mm			
Granuloma	Nonspecific				Specific for TB	
AFB					Positive	

TOTAL SCORE

0-2 TB unlikely, 3-4 Keep under observation, 5-6 Tuberculosis probable (Investigations may justify therapy) 7 or more TB unquestionable

leucocyte count of 22.2/mm^3 (N 78.2 L 11.2) while ESR came out to be 107 mm in 1st hr. and auto immune work up was negative. ECG showed low voltage tracing with T wave inversion, also suggestive of pericarditis. 2-Dimensional Echo revealed mild to moderate pericardial effusion. Pericardiocentesis was done along with pericardial window which revealed thick and highly inflamed pericardium. Histopathology reports showed nonspecific inflammation with granulation tissue. AFB culture of pericardium and blood culture both came out to be negative.

However, on high clinical suspicion of TB pericarditis, the patient was started on ATT (anti tuberculous treatment) for 9 months with corticosteroids for 8 weeks. In the follow up visits, patient showed improvement. Based on criteria listed in Table 1, his TB score was 9.

Case 2

A 4 year old girl, presented with low grade fever and respiratory distress for one day. There was also on going periorbital puffiness since last 2 months. There was positive history of TB contact. BCG vaccination was done at birth and a scar was identified. On chest examination there were signs of respiratory distress with basal crepitations. There were no significant findings on CVS exam except tachycardia. Abdominal exam demonstrated ascites with positive shifting dullness. TST revealed significant induration of > 15 mm. Initial blood workup showed a leucocyte count of 18.4/mm^3(*L* 33, *N* 55). A 2D Echo showed a large pericardial effusion, with right ventricular tamponade. Pericardiocentesis was done which revealed straw colored fluid. AFB culture of pericardial fluid and blood culture were both negative. Auto immune disorders and nephrotic syndrome were also ruled out.

However, on high clinical suspicion of TB pericarditis (TB Score 9), the patient was started on ATT with corticosteroids.

In the follow up visits the patient showed improvement on ATT.

Case 3

A 14 year old boy presented to the pediatric clinic with complains

of low grade fever and productive cough for the past one year. He was a known case of pulmonary miliary tuberculosis and was on anti-tuberculous therapy (ATT) for ten months. Associated symptoms included intermittent generalized edema, shortness of breath and paroxysmal nocturnal dyspnea. At presentation his weight and height were 32 kg and 145 cm respectively. On examination his heart rate was 98 / min, respiratory rate 28/min. Systemic pertinent findings included crepitations in lower left lung, pericardial rub and ascites. Chest X-ray revealed atelectasis in the right lower lung field, silhouetting of the right heart border with right-sided mild pleural effusion. CT scan results followed, showing dense pericardial calcification encircling the heart with mediastinal and hilar lymphadenopathy features suggestive of TB. He underwent elective pericardiectomy. Intraoperative findings revealed pericardium adherent to heart and great vessels with calcification extending laterally into pericardial tissue. His TB score was 7. As no active inflammation was seen on histopathology ATT was stopped and he was followed up as a case of constrictive pericarditis secondary to healed TB.

Case 4

An 8 year old male reported with cough, fever and shortness of breath for 3 months. On physical examination he weighed 18 kg and was found to have raised JVP, gallop rhythm and enlarged liver. Other systemic examinations were unremarkable. Further laboratory tests revealed an ESR of 43 with a WBC of 11.9/mm^3. Chest X-ray showed an enlarged cardiac shadow. Echocardiography was done revealing myocarditis, pericardial calcification, and pulmonary hypertension. Pericardiectomy was subsequently scheduled. Intraoperative findings revealed thickening and calcification along the right heart border. Dense inflammatory adhesions between chest wall and pericardium, between pericardium and myocardium and between pericardium and pleura were visualized. Histopathology of the pericardial tissue was suggestive of TB pericarditis. TB score was 7. On high clinical suspicion, ATT was given for 9 months.

TB possible, investigations may justify therapy (Score 5-6)

Case 5

A 7 month old male infant presented with respiratory distress for 6 weeks. This was accompanied with fever, loose motions and vomiting for the same duration. On physical examination he had tachycardia and tachypnea with peripheral cyanosis and clubbing. He was noted to have a BCG scar. His height and weight were on 10^{th} percentile for age. Systemic examination was unremarkable. Laboratory findings showed an ESR 3 mm in 1^{st} hr. and a leucocyte count of 9.4/mm^3. Chest X-ray showed an enlarged cardiomediastinal silhouette. Work up for autoimmune conditions was negative. 2D echocardiography showed patent foramen ovale, pulmonary artery hypertension and moderate to severe pericardial effusion with a hyper contractile heart. Pericardial tap was done which showed glucose of 69 mg/dl, protein of 4300 mg/dl, WBC of 100 with 20 neutrophils and 80 lymphocytes. Pericardial window was performed along with drainage of 60-65 ml of straw colored fluid. Histopathology of pericardial tissue had no significant abnormality. He was started on ATT for 9 months with complete resolution of symptoms at the end of treatment. His TB score was 5.

Case 6

A10 year old boy presented with chest pain and shortness of breath since 20 days. At the time of presentation he had an abscess on the left tibia. On physical examination he appeared pale and emaciated. He was tachycardiac and tachypnoeic on admission. He had a raised JVP, muffled heart sounds, poor perfusion and bilateral pedal edema. Respiratory exam revealed decreased air entry bilaterally with stony dull percussion over right hemi thorax. He was found to have a BCG scar. Laboratory investigations revealed an ESR of 35 and WBC of 25.3 mm^3. His blood cultures and autoimmune work up was negative. Chest X-ray showed right sided effusion with increased cardiac silhouette in transverse diameter. 2D echocardiography showed massive pericardial effusion. Pericardial tap was performed which revealed glucose of 39 mg/dl, proteins of 4000 mg/dl, and. Histopathology of the tissue showed acute and chronic nonspecific inflammation. His TB score was 6. He was started on ATT for 9 months on high clinical suspicion with resolution at the end of treatment.

Case 7

A10 year old girl, presented with low grade fever and cough for 4 months, shortness of breath for 2 months. She was a known case of TB and on ATT since last 2 months. On persistence of shortness of breath, she was referred for evaluation. There was no history of close TB contact and BCG scar was not visualized. On examination, chest expansion was decreased bilaterally and respiratory rate was 22 breaths/min. CVS examination was unremarkable with a heart rate of 120 beats/min. ECG was normal. Initial blood work up revealed a WBC count of 13.5/mm^3 and ESR of 55 mm 1^{st} hr. Initial Chest X-ray findings showed bilateral pleural effusion. Pleural tap was done which showed protein 3.2 gm/dl TLC 19/mm^3 (N90 L 10) glucose 20mg/dl. A 2-D echocardiography showed mild to moderate pericardial effusion with no evidence of tamponade. Pericardiocentesis was done along with pericardial window which revealed thick pus and fibrous adhesions surrounding the heart. Pericardial tap failed. Histopathology demonstrated no evidence of granuloma or malignancy. On high clinical suspicion of TB pericarditis (TB score 6), the patient was continued on ATT and corticosteroids. In the follow up visits, patient showed improvement on ATT which were continued till 12 months while corticosteroids were tapered and stopped after 8 weeks.

Case 8

A 4 year old girl, presented with low grade fever and dry cough for the last one and a half month and respiratory distress for the last ten days. She had received BCG vaccination at birth

and there was no history of close contact with a TB case. Respiratory exam revealed bilateral crepitations with decreased air entry on the right side. Respiratory rate was 35 breath/min. CVS exam showed muffled heart sounds and a pericardial rub. Heart rate was 150 beats/min. Initial blood work up revealed a WBC of 18.1/mm^3(L 25, N 68) and ESR of 28mm1st hr. Chest X-ray findings showed bilateral pleural effusion and cardiomegaly. A 2-D echocardiography demonstrated moderate pericardial effusion with thick strands, right sided pleural effusion, mildly dilated left ventricle (LV) and moderate systolic LV dysfunction. Echo findings were suggestive of mild constrictive pericarditis. Pericardiocentesis along with pericardial window, thoracotomy and pleural fluid drainage was carried out. Histopathology revealed marked acute inflammation with abscess formation. Pleural fluid drainage revealed large turbid pleural fluid of around 200cc. Pleural tap showed glucose of 106 mg/dl, protein 4300 mg/dl and TLC 7300 mm^3 (N 5, L 95). AFB culture of biopsy and fluid was negative. Based on the above findings her TB Score was 6. She was started on ATT for 9 months with steroids for 2 months with improvement at 2 month follow up. Treatment was continued for 9 months.

Keep under observation for three months (3-4)

Case 9

A 1 year 8 months old male child presented with complains of fever since 3 days, poor feeding and irritability since 1 day. The fever had a maximum spike of 103^0F. He had been on ATT for 4 months at presentation. On physical examination he had mild subcostal recessions. His weight and height were 9.3kg and 78cm respectively. He had received BCG vaccine at birth. Other systemic examination was unremarkable. A TST was done, which was negative. Further investigations revealed WBC of 16.8/mm3 and ESR of 31 in 1st hour. Chest X-ray showed cardiomegaly and atelectasis. Echocardiography showed moderate pericardial effusion. Pericardial tap was done which revealed glucose of 90 mg/dl, protein of 4500 mg/dl; RBCs were positive, leucocytes of 20% and lymphocytes of 80%. Although the TB score was 4 in this case but ATT was continued on the suspicion of TB pericarditis secondary to pulmonary TB. He received 9 months of ATT along with 2 months of steroid.

TB unlikely (0-2): No cases were found in this group.

Discussion

Tuberculosis remains the main culprit behind pericarditis in a high percentage of cases in the developing world and may lead to a mortality rate of 80-85% in a 6 month follow up in all stages of the diseases.[10, 11] However, with early recognition, anti-tuberculosis medication and timely surgical intervention, the mortality can be brought down to as low as 3-5%.[10] All patients in our case series underwent surgical intervention and antituberculous treatment and showed good response to treatment.

Tuberculosis is also the commonest cause of constrictive pericarditis (most serious complication of TB pericarditis) in TB endemic countries. Occasionally, children may present to the hospital with cardiac tamponade as seen in some of our cases with a high TB score. Despite proper treatment with anti tuberculous drugs and steroids, almost 30-50% will go on to develop constrictive pericarditis [12].

Tuberculosis Pericarditis (TP) usually develops in four stages:
i). Dry,
ii). Exudative,
iii). Absorptive or early fibrosis, and
iv). Restrictive or late fibrosis.

The source of the mycobacterium remains ambiguous. Less than half of the patients manifest signs and symptoms of pulmonary TB. However, it is believed that it most commonly gains access through mediastinal lymph nodes. Mediastinal lymph node inflammation with central necrosis is considered to be characteristic of TP. Concomitant active pulmonary TB with TP can be seen in 1-2% of the cases indicating Tuberculosis pericarditis to be a late complication of TB.

The main criteria for diagnosis of TB pericarditis depends upon the following:
(i) positive *M. tuberculosis* culture from pericardial fluid or tissue biopsy specimen,
(ii) positive acid-fast stain or typical caseous granuloma on histology of pericardial biopsy specimen, or
(iii) positive tuberculosis polymerase chain reaction in the pericardial biopsy specimen. [5, 13]

However, despite the advances in microbiology and laboratory techniques, diagnosing TB pericarditis in children continues to be a challenge. The yield of culture and biopsy of pericardial tissue or pericardial fluid in children can be very low ranging from 0-18%.[12,14,15] A two-dimensional echocardiogram is diagnostic of pericardial effusion.[14] Also, the value of TST in the diagnosis of tuberculosis in children in endemic area is limited and unreliable. Almost 50% of children with extra pulmonary TB may not show a positive TST (>10mm) at initial presentation. More advanced techniques such as T-SPOT. *TB* and Quanti FERON-TB which are based on detection of IFN-γ released by T cells in response to *M. tuberculosis* antigens offer an improvement on other diagnostic modalities but are not easily available and carried out in the developing world, further their utility is still challenged.[16]

In developing countries, where TB and related complications are endemic, cost, technical difficulties, and lack of resources make diagnosis of TB even more challenging. Thus, the diagnosis of any form of tuberculosis in children becomes more of a clinical diagnosis. [15, 17] Several clinical scoring systems have been proposed and used in clinical practice depending on available laboratory techniques. Most systems consider history of contact with an adult with pulmonary TB: presence of

symptoms, especially chronic cough; results from a tuberculin skin test, chest radiograph and response to anti-TB therapy as important clues in diagnostics.

The cornerstone of treatment remains anti-tuberculous therapy along with corticosteroids for TB pericarditis. Treatment can be started empirically on clinical suspicion especially in children in endemic areas, as culture yield is very low. Early treatment can stop progression of the disease to constrictive pericarditis. Adjuvant use of corticosteroids for duration of 6 -8 weeks (with gradual tapering off after four weeks) can suppress inflammation and can cause resorption of pericardial fluid. It can be of assistance in both early effusive and late constrictive phases of the disease.[18] Furthermore it can also prevent the progression to development of constrictive pericarditis and hence reduce the overall mortality from the condition. However, some schools of thought consider immunosuppressive effect of steroids to be detrimental.[18] Thus, use of steroids as an adjuvant therapy remains controversial, and further evaluation in form of large RCTs are required to assess its benefits.

Time of surgical intervention and the kind of procedure to be carried out also remains controversial in the literature. Strang JI *et al* proposed that pericardiocentesis is the standard treatment for pericardial effusion while cardiac tamponade being an absolute indication for drainage.[11] Moreover, in pediatric patients of pericarditis, pericardial window and biopsy is recommended both as a diagnostic and therapeutic modality. Pericardiectomy may be necessary in more advanced cases of the disease with myocardial thickening and fibrosis.

Conclusion

Tuberculous pericarditis is not a rare entity in children and diagnosis is based on clinical criteria as bacteriological confirmation in children is rare. Most children show complete resolution with early pericardiectomy / pericardiocentesis along with anti tuberculous chemotherapy and concomitant corticosteroids.

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CASE REPORT

Purulent Pericarditis due to *Pseudomonas Species* in an Immunocompetent Infant

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Abstract

Purulent pericarditis is an important and devastating morbidity in children. *Pseudomonas* infection causing purulent pericarditis is a rare entity in children with a very few case reports. It is mostly associated with primary or secondary immunodeficiency and concomitant cystic fibrosis. We are reporting a case of an immunocompetent infant with cardiac tamponade secondary to pericarditis due to *pseudomonas species*. A pericardiocentesis was done and he was treated with intravenous antibiotics for 6 weeks.

Key Words

Pseudomonas species, pericarditis; pericardiocentesis; pardiac tamponade

Introduction

Purulent pericarditis is pus collection localized to the pericardial space and it is a serious, life threatening and fatal infection if left untreated. *Streptococcus pneumoniae, Staphylococcus aureus, Meningococcus*, and *Haemophilus influenza* are the predominant microorganisms causing purulent pericarditis in children. [1,3] Most cases are a result of hematogenous seeding of the pericardium from various sources i.e., pneumonia, acute osteomyelitis, or soft tissue abscess. The incidence of purulent pericarditis has been considerably low because of immunizations against these common organisms and use of antibiotics for management. A high index of suspicion for the diagnosis is needed as the infection is extremely uncommon. Feldman[1] reported that 8% of cases of pericarditis in children were caused by *pseudomonas aeruginosa*. *Psuedomonal* pericarditis is extremely rare in immunocompetent neonates and infants,[4,5] however its association with cystic fibrosis (CF)[6] and with immunodeficiency either primary or secondary i.e., human immunodeficiency virus[7], is established. We are reporting a case of an immunocompetent infant with acute purulent pericarditisdue to *pseudomonas species*.

Case History

An 11 month old infant was admitted with high grade, continuous fever for the past one month, along with respiratory distress for two days. His fever intermittently responded to antipyretics. He received seven day courses of amox-clav and cefixime and also an antimalarial in the past one month, but without any relief. He had no associated otalgia, rhinorrhea, seizures, dysuria or arthralgia or swelling of joints. Multiple visits at various clinics were done where he was assessed and laboratory work up was performed. Complete blood count, malaria parasite smear and serology for typhoid fever were all unremarkable. Because of continuous fever spikes and sick condition chest x-ray was done, which showed cardiomegaly (figure 1). Therefore he was referred for further clinical and echocardiographic evaluation.At the time of presentation he was pale and a febrile, sick looking. He was tachycardiac and tachypneic without any obvious distress. His precordium was quiet and the heart sounds were muffled, there was no friction rub. Electrocardiogram showed ST segment changes, Echocardiography revealeda structurally normal heart with moderate to large circumferential pericardial effusion around 20-22mm with strands, it was with early echocardiographic signs of cardiac tamponade (with mild compression at atrial and ventricular level and significant respiratory variability at mitral and tricuspid valve), no vegetations were seen (figure 2). An initial impression of pyogenic or tuberculous effusive pericarditis was made. Laboratory investigations showed hemoglobin of 8.3g/dl, WBC of 16000/mm3 with 63% neutrophils and 34 % lymphocytes, absolute neutrophil count was normal, thrombocytosis of

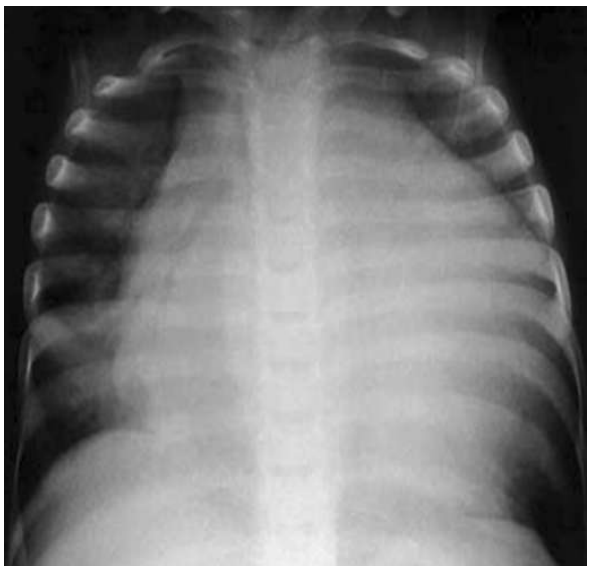

Fig 1: Frontal projection chest X-Ray showing cardiomegaly with clear lung fields.

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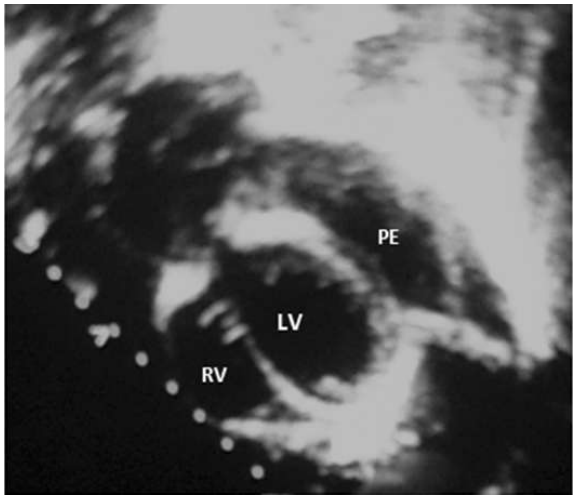

Fig 2: Echocardiography - subcostal four chamber view showing large pericardial effusion (PE), mostly around left atrium and left ventricle (LV)

1038/mm^3 was seen. ESR was 114mm in1st hour. A Montoux test was placed, that later read negative. Pericardiocentesis was performed and 70 ml of pus-like fluid was drained, followed by placement of a pericardial drain. The patient was managed at the cardiac intensive care unit. He was empirically started on intravenous ceftriaxone and cloxacillin. Pericardial fluid analysis revealed exudative picture with high WBC counts of 9500/mm^3 with predominant polymorphs(80%), RBC were moderate, with protein concentration of 5100 mg/dl and a very low glucose of 6 mg/dl. Child had an uneventful and clinically stable hospital course; there was 20-30 ml of pus like fluid from the pericardial drain over the next 24 hours. His blood and pericardial fluid culture grew *Pseudomonas species*. Antibiotics were changed to Ceftazidime and co-triamoxazole according to sensitivities. Further speciation of the *pseudomonas species* could not be performed. The child was investigated for primary immunodeficiency and the CD4, CD8 and Nitroblue tetrazolium test were all normal. Further workup for CF (including sweat chloride) was also negative. Pericardial drain was removed in 72 hours. His follow-up echocardiography on 7th day (prior to discharge) did not show any pus recollection. He was discharged on the 8th admission day with six weeks of ceftazidime and co-triamoxazole. His condition remained stable during the therapy. He remained well till his last (six months post procedure) follow-up.

Discussion

Purulent pericarditis is a rare disease in children. The pericardium is seldom the primary site and spread usually occurs via five routes: direct extension from intrathoracic disease, hematogenous spread, local extension from myocarditis, myocardial abscess or endocarditis, perforating injuries or spread from sub-diaphragmatic suppurative disease. [8] In infancy it is mostly hematogenous, as was probably in our patient. Cardiac tamponade, septicemia and constrictive pericarditis are the major life threatening complications with this disease. Untreated purulent pericarditis is rapidly fatal, an early diagnosis and aggressive management are important. Cardiac tamponade usually occurs early in the course of infection and is due to rapid accumulation of pus and requires urgent treatment. [9,10]

Pseudomonas pericarditis is very infrequently documented in medical literature. Risk factors mentioned in medical literature for *Pseudomonas* infections are mainly in adults including immunosuppressed states, use of prolonged antibiotic treatment, cytotoxic or steroid therapy, nosocomial acquisition following long hospitalizations with central lines and indwelling catheters, male gender, genitourinary tract instrumentation and mechanical ventilation.[10] Infection with *Pseudomonas* has been reported in two case reports in neonatal sepsis.[4,5] Other literature reports pseudomonas infection with associated with HIV infection and concomitant cystic fibrosis. [6,7] Infections with the above risk factors have a high morbidity and mortality ranging from 27-48% mainly due to late diagnosis and early occurrence of potentially fatal complications. Therefore a high index of suspicion is essential for diagnosis and treatment in such a potentially fatal medical condition.

Purulent pericarditis requires both appropriate antibiotics therapy and early pericardial drainage.[1,3,5] Adult purulent pericarditis guidelines from the European Society of Cardiology suggest that pericardiectomy or a pericardial window is required in the treatment of purulent pericarditis.[11] However, literature concerning the need for these surgical procedures in children is inconclusive. Mode of therapy is a highly variable outcome predictor. A combination antibiotic therapy (two drugs) along with intervention has a high success rate and is lifesaving in majority of the cases. [5] With the use of appropriate antibiotics and early surgical drainage, mortality though declined from previous data; is still reported as 10-30%.[1,3,12] Our patient showed clinical and echocardiographic improvement following pericardiocentesis and appropriate antibiotic treatment. Follow up to date has shown resolution of pericardial fluid with no evidence of development of constrictive pericarditis.

Conclusion

Pericarditis from *pseudomonas* infection is rare in immunocompetent children. However physicians must observe a high index of suspicion when dealing with pericarditis. Early recognition and timely management yields good prognosis in otherwise healthy infants and children, as was in our case.

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CASE REPORT

Griscelli Syndrome- A rare disorder

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Abstract
A two and a half month old male child presented with fever, progressive pallor, hepatosplenomegaly, partial albinism, and pancytopenia. A diagnosis of Griscelli syndrome was based on clinical features and suggestive findings on bone marrow biopsy.

Key words
Partial albinism, hemophagocytosis, pancytopenia

Introduction
Griscelli Syndrome (GS) is a rare autosomal recessive disorder characterized by immunodeficiency and partial albinism[1]. It results in pigmentary dilution of the skin and hair (silver hair), with large clumps of melanosomes on microscopy of hair shafts.[1] One variant of this fatal disease presents with hepatospenomegaly, lymphohistiocytosis, and a combined T and B-cell immunodeficiency. The disease seems to be invariably lethal without bone marrow transplantation.[2] It was first described by Griscelli in 1978, and since then only around 101 cases have been reported.[3] To the best of our knowledge this is the first case report from Pakistan. We present an infant with classic clinical features and confirmatory findings of hemophagocytosis on bone marrow biopsy.

Case report
A two and a half month old male infant presented to us with complaints of fever and progressive pallor for the last 15 days. He was admitted and intravenous antibiotics were given, he was found to have silver gray hair, hepatosplenomegaly and progressive pallor with pancytopenia on blood counts. This infant was the third born of consanguineous parents with an uneventful antenatal and neonatal period. There was a history of death of a second male sibling at the age of two hours of life, with the cause of death being unknown. The physical appearance of that child was also similar to the index case with silver hair. On physical examination the child was found to be febrile with a temperature of 38^0C. His anthropometric measures showed FOC at 25^{th} centile while weight was on 10^{th} centile and height on 5^{th} centile. He was very pale, having sparse silvery grey hair, eyebrows and eyelashes. There was seborrheic dermatitis, rest of the skin and iris had normal pigmentation. There was no dysmorphism or lymphadenopathy. Abdomen was distended, liver was palpable 7cm below right coastal margin and it was soft and non-tender. Spleen was palpable 6cm below left coastal margin. A soft grade 2/6 systolic murmur was audible at the upper left sternal border. Rest of the physical examination was unremarkable.

Investigations revealed hemoglobin of 6.6g/dl (12-14.5g/dl), a total leukocyte count of $5.9x10^9$/mm3 (5-15) with lymphocyte 23% (45-65%), a platelet count of $31x10^9$ /mm3 (150-400) and a reticulocyte count of 1.7% (0.3-1). Peripheral smear showed target cells and nucleated RBC's, but there were no giant cytoplasmic granules in leucocytes which are seen in Chediak Higashi syndrome. Serum LDH was 1294 U/l (115-257), total proteins were 4.1g/dl (2.5-4.5) with albumin 2.2g/dl, globulins 1.9g/dl, and ALT 81 IU/L (<45), Prothrombin time and APTT (partial thromboplastin time) were normal, plasma fibrinogen titers was 1:40 (control 1:32), serum ferritin was 2132ng/ml (50-200), serum triglyceride level 369mg/dl (296). HbSAg, and antibodies for HIV and HCV were negative. CD4 counts were $623/mm^3$ (900-2300). Bone marrow aspiration and biopsy revealed hemophagocytosis suggestive of HLH (Hemophagocytic lymphohistiocytosis), see figure 1.

In view of clinical features, laboratory findings, bone marrow findings, a final diagnosis of Griscelli Syndrome (partial albinism and immunodeficiency) was made. The parents were counseled about the nature of the disease, its prognosis, recurrence risk and the therapeutic options (immunosuppressive therapy or bone marrow transplant). Grave prognosis due to the fact that the infant was in accelerated phase of the disease and financial constraints, precluded any form of treatment.

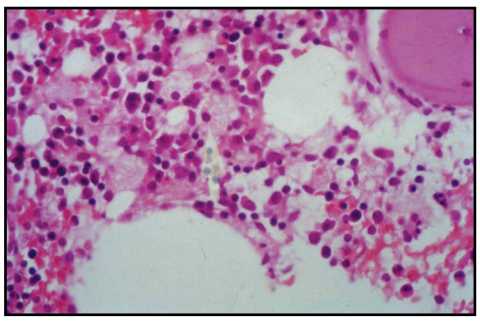

Fig.1 Showing lymphohistocytes on the bone marrow examination.

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Discussion

Griscelli syndrome (GS) is a rare and fatal disorder.[1] This syndrome, associating pigmentry dilution and immunodeficiency was described by Griscelli et al.[2] almost 100 cases have been reported till the last decade.[3] It is characterized by partial oculocutaneous albinism, variable cellular and humoral immunodeficiency and the occurrence of "accelerated phases"consisting of haemophagocytosis, pancytopenia, elevation of serum triglycerides levels, hypofibrinogenemia with associated bleeding diathesis, and hypoproteinemia.[4] It has a fatal outcome, caused by uncontrolled T lymphocyte and macrophage activation[4]. The clinical features and course resemble those of the Chediak-Higashi-like syndrome; however the two syndromes can be differentiated histologically: presence of granulocytic giant lysosomes in the leucocytes and absence of abnormal membrane bound lysosome like organelles detected in hair, skin of patients with Chediak-Higashi syndrome[5].

Griscelli Syndrome is caused by mutations in 1 of 2 genes located at band 15q21: RAB27A and MYO5A.[9] GS that occurs due to a defect in the RAB27A gene will result in an uncontrolled T-lymphocyte production and macrophage activation syndrome known as hemophagocytic syndrome (HS) or hemophagocytic lymphohistiocytosis (HLH).[6] Hemophagocytic syndrome usually results in death unless the child receives a bone marrow transplant. Children with a defect in the MYO5A gene develop neurologic problems but no immunologic problems.[7] However immunologic and hematologic manifestations together are seen in patients with RAB27A defect and these include pancytopenia and lack of natural killer cell function, with fever, jaundice, hepatosplenomegaly, lymphadenopathy, pancytopenia and generalized lymphohistiocytic infiltrates of various organs including CNS with the development of an accelerated phase of disease.[8, 9] The onset of GS (accelerated phase) is associated with a viral (eg, Epstein-Barr virus, hepatitis A virus, herpes virus[6]) or sometimes a bacterial infection.[9] When a remission is obtained, recurrent, accelerated phases of increasing severity are seen. Dermatologic findings may be limited to hair, with skin and retinal pigmentation being occasionally affected. Microscopic examination of hair reveals uneven clusters of aggregated melanin pigment, accumulated mainly in the medullary area of the shaft.[10] The differential diagnosis includes Chediak-Higashi syndrome (CHS) and Elejalde syndrome. CHS differs from GS by presence of abnormal giant cytoplasmic granules in leucocytes, more frequent cutaneous involvement, smaller, more evenly distributed pigment clumps in hair shafts and more consistent defective granulocyte activity.[11]

The prognosis for the long term survival of patients with GS is very poor. Curative hope is offered only by bone marrow or stem cell transplantation, which is more successful when, performed early in the course of the disease. Palliative management includes treatment of associated infections, and immunomodulatory therapy during accelerated phases (high dose systemic methylprednisolone, etoposide, intrathecal methotrexate, cytosine arabino-side and prednisone, or ATG, cyclosporine and steroids).[12]

Prenatal diagnosis of Griscelli syndrome was first accomplished by light microscopy examination of the hair shaft by Durandy et al in 1993.[13] Nowadays prenatal diagnosis is possible in families with defined gene.[14]

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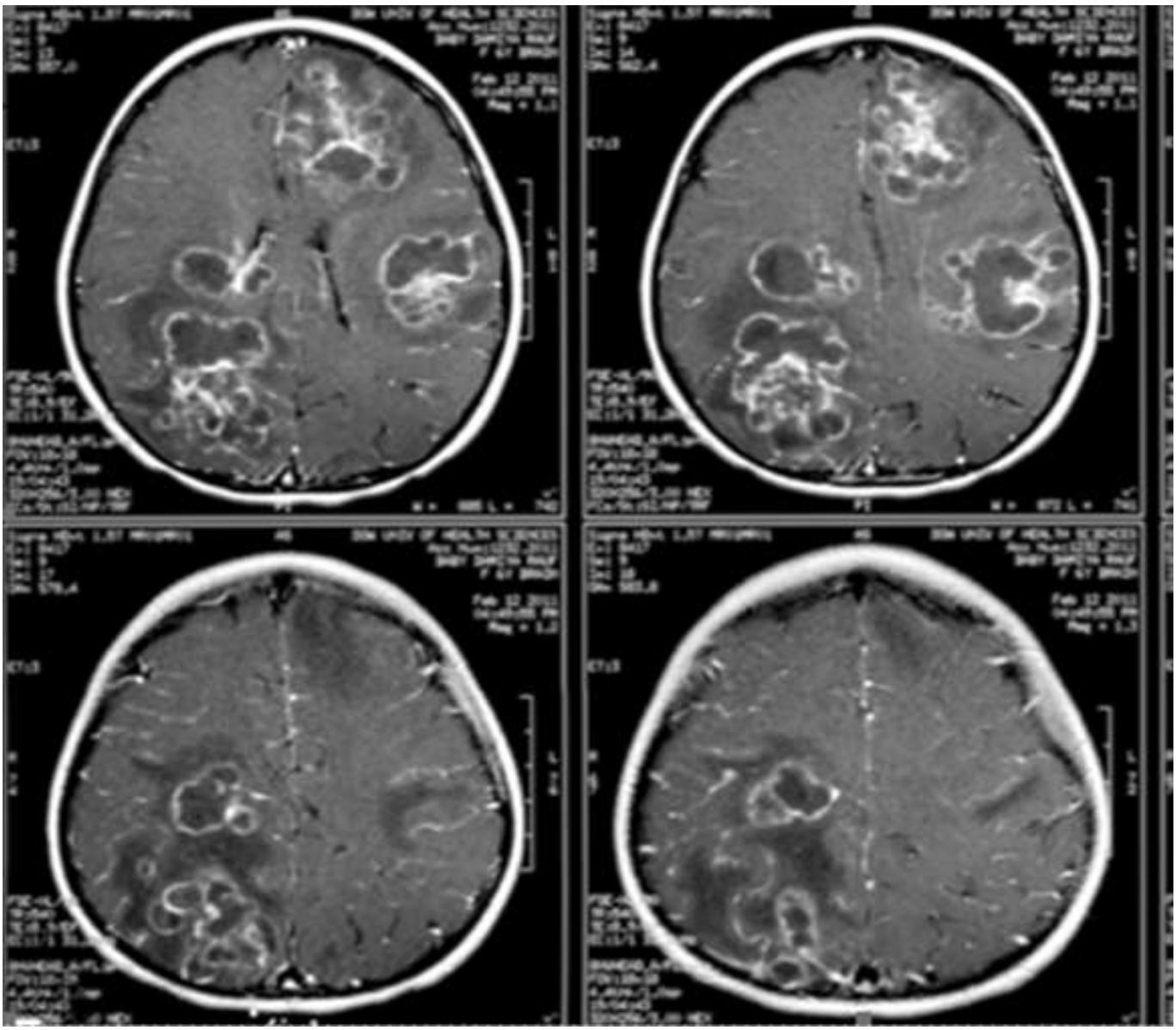

This is the MRI brain of a 7 years old girl who presented with headache, fever and seizures for 5 months. Headache was generalized, moderate-severe in intensity with low grade fever. She was treated by local physician with analgesics and antipyretics without improvement. Three months later she had a generalized tonic–clonic seizure that, lasted for about 1 hour, workup was done to rule out metabolic seizures. She was discharged on steroids and cefixime. Her seizures fever and headache continued, work up for lymphoma, tuberculosis and autoimmune disease, was unremarkable. Suspecting fungal infection fluconazole was started along with steroids and antiepileptic. MRI brain was done and a brain biopsy was performed. Histopathology report revealed the diagnosis.

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Q1. Describe the MRI findings?
Q2. What are the differential diagnoses?
Q3. What is the most likely diagnosis and the likely causative agents?

Answers on page 607

Medical Microbiology & Infectious Diseases Society of Pakistan (MMIDSP) website changes

In the last IDSP Conference General Bodymeetingheld at Shaukat Khanum Memorial Cancer Hospital (SKMCH), Lahore in February 2013, it was decided to revive society website and responsibility was voluntarily taken over by Dr. Altaf Ahmed (Indus Hospital, Karachi).

With the assistance of Dr Ayesha Almas (Aga Khan University, Karachi), a qualified web designer company was approached and a contract was signed. The task was to update society website and incorporate required changes within six months. During this period a number of updates were incorporated making the website active and useful. The important changes were MMIDSP logo change, list of upcoming Infectious Diseases conferences, animated banners,latest issue of Pakistan Infectious Diseases Society Journal, latest news, abstracts of 10th Annual Conference of IDSP 2013, updating IDSP executive committee members list, Journals updated from 2007 to 2010, contact addresses updated, Pakistan Biological Safety Association (PBSA) logo added and all unnecessary banners and old information was removed.

Please visit MMIDSP website and give your feedback. **www.idspak.org**

Answers of Picture Quiz

Answer 1: MRI brain contrast showing multiple ring enhancing lesions, conglomerate masses predominantly in left frontal & right parietal lobe posteriorly

Answer 2: Lymphoma
Tuberculosis
Brain abscess (bacterial /fungal) - *Aspergillus/ Fusarium/ Acremonium*

Answer 3: **Brain Abscess**

Histopathology of brain tissue revealed Acute & Chronic inflammation with necrosis. Numerous septate fungal hyphae. No evidence of malignancy

Fungal culture showed: Colonies of *Acremonium* species

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 andFigures 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

MEMBERSHIP APPLICATION FORM

MEDICAL MICROBIOLOGY & INFECTIOUS DISEASES SOCIETY OF PAKISTAN

No. _____

Name

Mailing Address

Institute/ Organization

Department & Division

Field of Interest

Designation | PMDC No.

Phone No. Resdence | Office

Cell | E-mail

Degree/ Diploma:

☐ MBBS ☐ MD ☐ MSc Biological Scinec ☐ BSc Nursing
☐ MRCP ☐ MCPS ☐ FRCS ☐ FCPS
☐ MRCPath ☐ Ph. D ☐ M. Phil ☐ Pharma. D
☐ DCH ☐ Diplomat American Board of
Other

Application for member as

☐ Full Member (Annual/ Life) Rs.500 for 1 yr, Rs.3000/- for life
☐ Overseas Member US$.100/- for Life
☐ Associate Member Rs.500 for 1 yr, Rs.3000/- for life

Signature Date

For Office Use Only

Approved/ Not Approved

Membership No: _____ Reference No: _____

Comments: _____

Signature General Secretary: _____

FULL MEMBERSHIP:

Should be at least medical graduates registered with PMDC and having postgraduate qualification in any field.
Full member may be

1. Life: with payment of Rs.3000/-
2. Annual: with 1 year fee of Rs.500/-

ASSOCIATE MEMBERSHIP:

Ph. D, Master degree & M. Phill in biological sciences, BSc in Nursing & allied medical science with 1 yearly fee of Rs.500/-

PRIVILEGES OF MEMBERSHIP:

FULL MEMBER:

All the members shall have the right to:
1. Participate in all activities of the society.
2. Receive all publication including quarterly ID Journal free of cost.
3. Vote according to constitution of the society.

ASSOCIATE MEMBERS:

All the members shall have the right to:
1. Participate in programs of the society.
2. Receive all publication including quarterly ID Journal free of cost.

Please send your Application form by hand or by mail only.
Membership fee will only be received in cash/ cross cheque/ pay order or bank draft made out to Infectious Disease Society of Pakistan.

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