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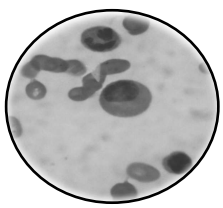

Plasmacytoid cell under 100 x

Courtesy: Dr Ayesha Junaid, Shifa International Hospital, Islamabad.

Lessons and News from Pediatric Infectious Diseases

Today is a typical day again! My new patient is a 3-year-old autistic child presenting with high-grade fever off and on for last 2 months. The history warmed my heart as I wanted to know from the parents about the sequence of admissions, antibiotics received and antimalarials. I requested them not to share their previous workup yet. Examination revealed a pale child with hepatosplenomegaly and palpable small inguinal lymph nodes. My residents were now eager to see the old labs and blurting out some differentials. Eventually, we observed the labs already done showing bicytopenia (anemia and thrombocytopenia), raised erythrocyte sedimentation rate and vague infiltrates on chest X-ray while rest of the previously done workup was unrevealing. The child was as yet undiagnosed. We deliberated on further workup keeping in mind the differential diagnosis, and minimizing workup in reducing the cost. Our gut feeling was that the etiology was probably non-infectious. After a normal bone marrow and positive coombs test, a diagnosis of autoimmune hemolytic anemia was made and therapy started. The take home message here is that non-ID related causes of fever should always be considered.

The importance of pediatric infections cannot be overemphasized. The burden of pediatric infections is huge with significant "pain and loss". Of the estimated 8.8 million worldwide deaths in children < 5 years in 2008, infectious diseases were responsible for 68%; with largest burden due to pneumonia (18%), diarrhea (15%), and malaria (8%). It is important to note that 49% (4.3 million) of child deaths occurred in five countries; India, Nigeria, Congo, Pakistan and China. Other pediatric infections such as tuberculosis, typhoid, common childhood communicable diseases and parasitic infections are also common in our region contributing to morbidity and mortality. Over last many years lot of diagnostic, therapeutic and preventive innovations and research have made it possible to "conquer" these infections more readily.

Some of the news highlights of last few years included crisis due to antibiotic resistance that has reached alarming proportions. The good news is that a consensus has been reached regarding its importance and physicians have agreed to support in the effort to maintain activity of the currently available drugs through antibiotic conservation, consistent with good practice with emphasis on developing new drugs.[1,2]

There has been a surge of substantial progress in the field of microbiology in providing results that are rapid and accurate to facilitate clinical decision-making and antibiotic selection. Particularly attractive are molecular methods and point-of-care tests.[3] Examples to detect specific microbes include polymerase chain reaction (PCR) for detection of *Clostridium difficile*, *Staph aureus*, methicillin-resistant *Staph aureus* (MRSA), *Mycobacterium tuberculosis* (MTB), *Strept agalactiae*, and influenza. The rapid HIV test; highly accurate, rapid (minutes to 60 seconds) and inexpensive, has revolutionized HIV care.[4] Perhaps the most dramatic recent breakthrough in this technology has been the ability to identify MTB and multidrug-resistant TB within 1 hour and 45 minutes of specimen inoculation.[5] It is anticipated that increasing use of this technology will substantially revolutionize microbiology as we know it.

Vaccine development and use has been an important chapter in medical accomplishments, best demonstrated by the eradication of smallpox. While close to polio eradication, the most profound message in vaccine success has been the story of PCV vaccine (Prevnar-7) which is credited with a 45% reduction in the incidence of invasive pneumococcal infection.[6] The newer PCV vaccines and their widespread use will definitely trample the major killers of childhood illnesses. Similarly the availability of rotavirus vaccine in developing countries will have a huge impact.[7] As pediatricians, children and parents wait, it is anticipated that approximately 40 new or improved vaccines will be available by 2015 including vaccines against major killers such as malaria, TB, HIV, pandemic flu and other viral and parasitic diseases. Many of these vaccines are in phase 2 or 3 stage and we expect to hear encouraging news in the coming years.

Our case above illustrates very well that as an infectious disease consultant, I find something exciting everyday as I see, hear, read, explore or enquire from patients, residents, colleagues, books, journals, lectures, conferences or the ever expanding internet, Pubmed and other relevant sites. This part is easy but the challenge is to share with others who may have not heard or read. I try to tell each of the intriguing stories to someone around me and hope to give others something to use and build on it. Our lust for better and newer knowledge knows no boundaries and that's how we thrive to live for another similar but brighter day.

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ORIGINAL ARTICLE

Antimicrobial Susceptibility of Gram Negative Organisms in Urine Cultures at Armed Forces Hospital in Saudi Arabia

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Abstract

Background
The main aim of this study was to analyze the antimicrobial susceptibility of bacterial isolates from urine specimens examined at the Armed Forces Hospital, Al-Kharj, Saudi Arabia.

Methodology
Consecutive mid-stream urine specimens submitted to our laboratory by 10332 patients at the Armed Forces Hospital from November 2006 to October 2009 were included. The specimens were cultured and the isolates were identified using standard microbiological techniques. The antibiotic susceptibilities of the isolates were determined.

Results
The number of patients with suspected urinary tract infection who yielded positive cultures from their mid stream urine specimens was 959 (9.3%) out of 10332. The commonest isolates were *Escherichia coli* (55.1%), *Klebsiella pneumoniae* (17.9%), *Enterobacter spp.* (7.8%) and *Proteus mirabilis* (5%). Other bacterial pathogens were *Pseudomonas aeruginosa* (3.8%), *Acinetobacter spp.* (1.2%), *Citrobacter spp*. (1.1%), *Morganella morganii* (0.6%), Group B β-haemolytic *Streptococcus* (4.4%), *Staphylococcus aureus* (1.6%) and group D *Streptococcus* (1.5%) respectively.

Conclusion
In vitro drug sensitivity tests showed Norfloxacin and Nalidixic acid to be very effective for most of the strains of the bacterial pathogens and may be used in the empirical treatment of UTIs in Al-Kharj city. Imipenem may be used in cases un-responsive to commonly used antibiotics.

Key words
Bacterial Resistance, *Escherichia coli*, Urinary Tract Infection,

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Introduction

Urinary tract infection (UTI) is among the most common bacterial infections for which patients seek medical advice. The exact prevalence of UTI is age and sex dependent.[1] The range of clinical effect varies from asymptomatic bacteriuria to acute pyelonephritis.[2] There are estimated 150 million UTI cases per annum worldwide.[3] UTIs result in approximately 8 million physician visits and more than 100,000 hospital admissions per year in the United States.[4] These are the most common bacterial infections in women and account for significant morbidity and health care costs.[5] The vast majority of UTIs are seen in female outpatients and many are treated empirically by physicians if their symptoms suggest acute uncomplicated bacterial cystitis.[6] UTIs, including cystitis, pyelonephritis, asymptomatic bacteriuria, and acute urethral syndrome, constitute one of the most frequent causes of illness in humans.[7-9] Many Gram positive and negative organisms are implicated in causing UTI.[10] The predominant organisms associated with UTI in Saudi Arabia are Gram negative bacteria mostly resistant to commonly used oral agents.[11-12]

Although UTI is a major problem for the community at large, there is lack of reports about the causative agents of UTIs and their treatment in Al-Kharj, Saudi Arabia. The main aim of this study was to identify the causative agents and their antimicrobial susceptibilities among Saudi military personnel and their families reporting at Armed Forces Hospital, Saudi Arabia with suspected UTI.

Material and Methods

Ten thousand three hundred and thirty two consecutive freshly voided mid-stream urine (MSU) specimens were submitted by the Military personnel and their families attending Armed Forces Hospital (AFH), Al-Kharj, Saudi Arabia during the period November 2004 to October 2007. All these patients were suspected of suffering from symptoms of UTI (cystitis or pyelonephritis or both). All MSU specimens were processed for culture and sensitivity upon medical request from the caring clinicians. Patients with suspected UTI were instructed for MSU collection (posters in the collection areas re-inforce the method). Specimens were collected in wide mouth screw-on-top containers and stored at 4^0C until transported to our laboratory

in Eight-quart Coleman coolers.

Standard microbiological techniques were used for culture and identification of the isolates.[13] A standard calibrated platinum wire loop delivering 0.001 mL of uncentrifuged urine was used to inoculate sheep blood agar (SBA) plates containing 6% blood and cysteine lactose electrolyte deficient (CLED) (Oxoid, UK) media. These plates were incubated aerobically at 35-37^{0}C for 18-24 hours[14]. The plates were read at the end of the incubation period. The criteria for considering a culture as having a "significant growth" was yield of more than 100 colony forming units of pure growth/mL MSU inoculum on the plates corresponding to a colony count of more than 10^5 colony-forming units per 1 milliliter.[14]

All isolates were identified using standard biochemical tests. In addition commercially available biochemical kits, API 20E (Analytic Profile Index system, La Balme les Grottes, France) were also used for the identification of enteric pathogens.

Antibiotic sensitivity testing was performed using the disc diffusion method on 85 mm Mueller-Hinton agar (Oxoid, UK) plates with agar depth of 4 mm. The bacterial suspension that was prepared for antibiotic sensitivity testing on Mueller-Hinton agar was adjusted to the recommended turbidities for all species.[15] The antibiotic disc applied included: Ampicillin 25 µg, Trimethoprim-Sulphamethoxazole (1.25/ 23.75 µg), Cephalothin 30 µg, Amoxicillin-Clavulanic Acid (20/10 µg), Cefuroxime 30 µg, Cefotaxime 30 µg, Piperacillin 100 µg , Nalidixic Acid 30 µg, Nitrofurantoin 300 µg, Norfloxacin 30 µg, Gentamicin 10 µg, Amikacin 30 µg and Imipenem 30 µg. The Clinical Laboratory Standards Institute (CLSI) break points were used for interpretation of susceptibility patterns as sensitive or resistant[16]. Statistical Package for Social Sciences (SPSS, version 10.1) was used for data analysis. Chi-square test for independence was used to test the relationship between sex (males versus females) and culture result (significant/ insignificant growth). Z-test of proportions was used to test the sample proportion against population proportion. $P<0.05$ was considered statistically significant.

Results

The microbiology laboratory of Armed Forces Hospital at Al-Kharj, in the central province of Saudi Arabia received and examined 10,332 urine specimens, between November 2004 and October 2007. Of these, 7326 (70.9%) specimens were obtained from females and 3006 (29.1%) from males. Significant growth of pure culture of organisms was obtained from 959 out of 10332 patients (9.3%). Within these 959 patients, UTI was significantly higher among the females, 693 (72.3 %), than in the males, 266 (27.7 %). Urinary tract infection cultures with significant growth were diagnosed among 693 out of 7326 female subjects (9.5%) compared to 266 out of 3006 male subjects (8.8%). The result of the chi-square for independence was not significant ($p>0.05$) indicating that culture results were independent of sex. There were no significant differences between two sample proportions.

Most of the isolates were Gram negative organisms; 888 (92.6%), and Gram positive organisms constituted 71 (7.4%) of all the isolates. The species distribution is shown in table 1. None of the specimens yielded polymicrobial growth.

Among Gram negatives all isolates showed high sensitivity to Norfloxacin and Nalidixic acid, except for *Proteus* and *Pseudomonas spp*. Cefotaxime sensitivity was low in *Pseudomonas spp.*, *Klebsiella spp.*, *E. coli* and *Citrobacter spp*. Most *E. coli* and *Klebsiella pneumoniae* isolates were sensitive to Imipenem and Amikacin, but almost all were resistant to Ampicillin, Cotrimoxazole, and variably sensitive to Amoxiclav, Gentamicin and Piperacillin. *Pseudomonas aeruginosa* were found to be 100% sensitive to Imipenem and 77.7% to Amikacin (Table 2).

Discussion

This study showed the pattern of organisms causing UTI among Military personnel and their families at Al-Kharj city. The rate of isolation of the urinary tract pathogens was in general agreement with others[17]. The number of female patients with UTI was more than the males, but the difference was not statistically significant. Higher prevalence of UTI among females was due to the factors that predispose women to UTI more than men. [18] The most predominant urinary tract pathogens in both sexes in this study were *E. coli* and *Klebsiella* spp., in concordance with other studies reported by Shamweel and Farooque, and Ahmed and Ragaa.[19,20]

In vitro drug sensitivity demonstrated a high prevalence (~80%)

Table 1: Uropathogens isolated from MSU at Alkharj Hospital

Isolate	n(%)
Escherichia coli	528 (55.1)
Klebsiella pneumoniae	172 (17.9)
Enterobacter spp.	75 (7.8)
Proteus mirabilis	48 (5.0)
Pseudomonas aeruginosa	36 (3.8)
Acinetobacter spp.	12 (1.2)
Citrobacter spp.	11 (1.1)
Morganella morganii	6 (0.6)
Group B β-haemolytic *Streptococcus*	42 (4.4)
Staphylococcus aureus	15 (1.6)
Group D *Streptococcus*	14 (1.5)

Table 2: Antimicrobial susceptibility pattern of Gram negative pathogens isolated

Organism	n	% sensitive against												
		CTN	**AMP**	**AMC**	**GEN**	**AK**	**CXM**	**CTX**	**SXT**	**PIP**	**NIT**	**NA**	**NOR**	**IMP**
Escherichia coli	528	0.6	11.3	39.7	31.2	51.1	76.1	43.3	20.8	NT	58.7	86.1	87.5	70.3
Klebsiella pneumoniae	172	45.9	0	47.0	53.5	59.9	70.3	63.3	19.7	NT	44.8	89.5	91.3	90.7
Enterobacter spp.	75	8.0	20.0	22.6	73.3	NT	70.6	78.6	46.6	30.6	24.0	89.3	100	77.3
Proteus mirabilis	48	41.6	33.3	62.5	79.2	NT	68.7	87.5	37.5	NT	0	66.6	95.8	79.1
Pseudomonas aeruginosa	36	NT	NT	0	13.8	77.7	NT	NT	NT	52.7	44.4	36.1	80.5	100
Acinetobacter spp.	12	25.0	16.6	66.6	91.6	NT	75.0	83.3	33.3	25.0	16.6	83.3	91.6	91.6
Citrobacter spp.	11	81.8	45.4	54.5	100	NT	72.7	54.5	63.6	45.4	100	90.9	100	100
Morganella morganii	6	0	33.3	33.3	100	NT	66.6	100	33.3	NT	0	100	100	100

NT=Not tested, CTN=Cephalothin, AMP=Ampicillin, AMC= Amoxicillin-Clavulanic Acid, GEN=Gentamicin, AK=Amikacin, CXM=Cefuroxime, CTX=Cefotaxime, SXT= Trimethoprim-Sulphamethoxazole, PIP=Piperacillin, NIT= Nitrofurantoin, NA= Nalidixic Acid, NOR= Norfloxacin, IMP= Imipenem

of resistance among the *E.coli* strains to Trimethoprim-Sulphamethoxazole, a drug considered to be of choice in urinary tract infection. The high prevalence of resistance to the commonly used antibiotics such as Trimethoprim-Sulphamethoxazole and Ampicillin has caused considerable alarm. The factors favouring development of antibiotic resistance include previous use of an antibiotic by the individual or widespread use of antibiotics in the community. The latter may lead to a shift in the species resistance pattern of organisms prevalent in a community[20]. Antibiotic misuse is very common in developing countries, mainly because of failure to restrict the use of antibiotics in hospitals and to control their sale and use in the community[21-23]. Continued blind empiric therapy with failure to send a urine culture may be the key reason for the emergence of resistance to Ampicillin, Trimethoprim-Sulphamethoxazole, Amoxicillin-Clavulanic Acid, and Cephalothin.

There was a differential decrease in *in vitro* sensitivity of organisms to Norfloxacin, Nalidixic Acid and Cefuroxime indicating that fluoroquinolones are a good choice for empiric therapy of UTI. *Pseudomonas aeruginosa*, a common cause of hospital-acquired UTI, was found to be less sensitive to the common antibiotics but sensitive to Imipenem (100%) and Amikacin (~78%), corroborating with the results of others.[24-26] *Pseudomonas aeruginosa* was relatively more susceptible to the second line of anti-pseudomonas drugs with more resistance to the first-line antibiotics, such as Nalidixic acid and Gentamicin. This may be due to widespread use of common antibiotics in the hospital and cross-resistance among different bacteria. The other antibiotics showed high level of resistance against all the isolates in this study. We could not demonstrate the efficacy of antibiotic treatment in patients with UTI in this work as it was not in the scope.

Conclusion

Norfloxacin or Nalidixic acid may be used for empirical treatment of UTIs in Al-Kharj city. Imipenem may be used in cases un-responsive to commonly used antibiotics. Antibiotic resistance patterns need to be continuously monitored in the community and as such guide the empirical treatment of patients.

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ORIGINAL ARTICLE

Yield of Endobronchial Biopsy in Diagnosis of Tuberculosis in Clinically Suspected Patients

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Abstract

Objective
To assess the yield of endobronchial biopsy by fiberoptic bronchoscope for diagnosis of tuberculosis in clinically suspected patients.

Methods
The study was carried out at the Department of Pulmonology and Critical Care, Military Hospital Rawalpindi Pakistan from January 2010 through August 2010. All patients with clinically suspected tuberculosis and three consecutive negative sputum smears for acid fast bacilli were included in the study. The patients underwent bronchoscopy and biopsy of the suspected lesion was submitted to laboratory for histopathological examination. The histological diagnosis of chronic granulomatous inflammation and chronic caseating granulomatous inflammation was taken to be consistent with the diagnosis of tuberculosis.

Results
Thirty patients fulfilling the inclusion criteria reported during the study period. 12 (40%) were males and 18 (60%) females. Mean age of the patients was 50 (±18) years with range 18 to 80 years. On histopathological evaluation of the biopsy samples, 25 (83%) out of 30 patients were diagnosed as having tuberculosis.

Conclusion
Our observations suggest that fiberoptic bronchoscopy is a reliable and effective procedure with adequate yield in the diagnosis of tuberculosis in clinically suspected patients.

Key words
Caseating Granulomatous Inflammation, Endobronchial Biopsy, Fiberoptic Bronchoscopy, Tuberculosis.

Introduction
Tuberculosis (TB) has been declared as the emergency of the millennium by WHO and is the leading cause of death in adults due to a single infectious agent.[1] Its incidence is increasing worldwide especially in the developing countries including Pakistan. This situation has further been complicated by the others factors such as introduction of multidrug resistant (MDR) TB, AIDS, growing poverty and epidemics such as floods etc.[2-5]

TB is a chronic illness and increasing incidence of this disease puts a lot of burden on the hospitals as well as human resources. Main reason for the chronic nature of this disease is delayed diagnosis resulting in debilitated state of the patient. It is therefore important that an early diagnosis of this disease should be made so that proper treatment is administered at an early stage.[6,7]

Zeihl Nelson (ZN) staining of sputum smear to detect acid fast bacilli (AFB) has been employed as a key test for confirmation of clinically suspected patients of TB. It has been observed that AFB is not detected in sputum smears of all of the patients suffering from pulmonary TB.[8-11] Other methods such as sputum for AFB culture, use of fiberoptic bronchoscope followed by bronchoalveolar lavage or bronchial brushings for AFB and bronchoscopic biopsy of the suspected lesion for histopathological examination have been used for confirmation of TB.[12-14] Many studies have been conducted to assess the diagnostic yield of these methods and bronchoscopic biopsy has proved to be an effective method for diagnosing TB in sputum smear negative patients having clinical suspicion of TB.[15-17] The purpose of our study was to evaluate the diagnostic effectiveness of bronchoscopic biopsies in sputum AFB negative but clinically suspected patients of TB in our set up.

Material and Methods
The study was carried out at Department of Pulmonology and Critical Care, Military Hospital, Rawalpindi, from January 2010 to August 2010. All those patients with suspected TB on the basis of history, clinical, radiological and laboratory findings but having three consecutive negative sputum smears for AFB were included in the study. The clinical findings and presenting complaints in the history included productive cough, hemoptysis, low grade fever, weight loss and night sweats etc. Positive findings on X-ray chest and positive Mantoux test further added to the clinical suspicion. Patients having extra pulmonary TB, patients already on antituberculosis treatment or with severe dyspnoea, bleeding diathesis, history of cardiac diseases were

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not included in the study.

Patients fulfilling the inclusion criteria were subjected to fiberoptic bronchoscopy using flexible Olympus P-60 fiberoptic bronchoscope. The bronchoscopy was performed by the same clinicians in all the patients. Patients were premedicated with 2% xylocaine spray and thorough examination of tracheobronchial tree was performed with bronchoscope. Biopsies of suspected lesion were performed and adequate tissue was obtained. The tissue was transported in 10% formalin to laboratory.

Histological diagnosis of each biopsy was noted and the diagnosis of chronic granulomatous/ chronic caseating granulomatous inflammation was taken to be consistent with diagnosis of TB. Mean and standard deviation of quantitative variables such as age was calculated. Percentages of qualitative variables such as patient gender and histological diagnosis were also calculated.

Results

Thirty patients reporting in the Pulmonology Department of MH Rawalpindi during the study period fulfilled the criteria and were included in the study. 12 (40%) were males and 18 (60%) females. Mean age of the patients was 50 (±18) with range of 18 to 80 years.

Eight (28%) of 30 patients presented with history of productive cough, 7 (23%) with persistent low grade fever, 4 (13%) with hemoptysis, 4 (13%) with weight loss and 7 (23%) with combination of above findings including night sweats.

The histological diagnoses included chronic caseating granulomatous inflammation, chronic granulomatous inflammation and others including non specific inflammation, congestion etc. The first two diagnoses were taken to be consistent with tuberculous etiology. 14 patients were diagnosed as chronic caseating granulomatous inflammation on histological examination of bronchoscopic biopsies, 11 as chronic granulomatous inflammation and 5 as others/ non specific inflammation (Table 1). Out of 30 patients, 25 (83%) were diagnosed to be suffering from TB as confirmed through bronchoscopic biopsy. Gender wise distribution of histological diagnoses is summarized in figure 1.

Table 1: Histological diagnosis (n = 30)

Histological Diagnosis	n	Percentage
Chronic caseating granulomatous inflammation	14	47%
Chronic granulomatous inflammation	11	36%
Others	5	16%

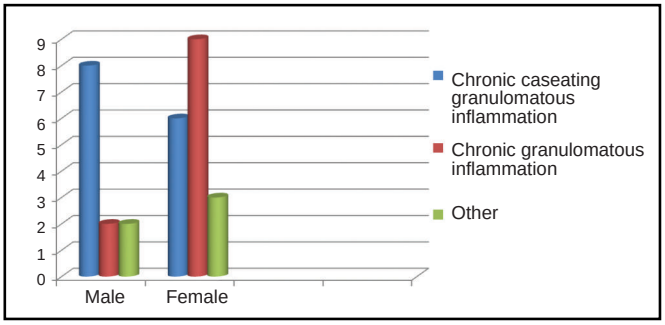

Fig 1. Gender wise distribution of histological diagnoses (n=30)

Discussion

The incidence of tuberculosis has been increasing worldwide and Pakistan being no exception. The emergence of MDR TB, AIDS, inadequate diagnostic facilities, noncompliance to treatment and poor socioeconomic conditions further aggravate the incidence.[2-4] According to WHO TB report of 2010, there were estimated 9.4 million incident cases of TB globally in year 2009 and most of these cases were in Asia.[1] The number of incident cases of TB in Pakistan during 2009 was 420,000, mortality was 60,000 and HIV prevalence in incident cases was 1.5 %.[1] The absolute number of TB cases continues to increase every year.

Keeping in view such high number of TB cases and their mortality, the need to diagnose TB correctly and timely has become more significant. Early diagnosis of TB not only results in early start of treatment but it also prevents complication such as disease spread, increasing morbidity, permanent tissue damage such as lung fibrosis and mortality and therefore lessens the burden on hospital resources.[18]

Sputum for AFB has been used as an investigation of choice for the confirmation of TB in clinically suspected patients. It is a cheap, simple and rapid method for detection of AFB. However studies have shown that not all patients suffering from pulmonary TB demonstrate AFB on sputum smear despite rigorous search for the microorganism.[19,20] According to studies, 30 to 50 % of patients infected with TB are not diagnosed through direct sputum examination.[7-9,21] According to Khan *et al*, the percentage of sputum positive cases was 54% in 2001 and less than 10% in 2004.[5] Bronchoalveolar lavage or bronchial washings smear for AFB can help in diagnosis of TB. The percentage of patients confirmed by bronchial washings smear is also variable and not all patients suffering from TB are confirmed by this method. The percentage of AFB positive patients on ZN staining of bronchial washings is 48.3%, 42% and 50% according to Bachh *et al*, Ansari *et al* and Caymmi *et al* respectively.[17,18,21] Sputum and bronchial washings for AFB culture are confirmatory but time consuming, require skilled laboratory staff, expensive equipment and need

meticulous monitoring.

Histopathological evaluation of the bronchoscopic biopsy of the suspected lesions has proved to be another reliable and rapid method for the diagnosis of TB. According to Bachh *et al*, the percentage of patients diagnosed to be suffering from TB through bronchoscopic biopsy was 48 %. This percentage was 24% by biopsy alone and 34% if combined with bronchial brushings according to Iqbal.[22] According to Wallace *et al*, immediate diagnosis of TB was made in 48% of patients by endobronchial and transbronchial biopsies.[23] In the same study, endobronchial and transbronchial biopsy provided the diagnosis of TB in 20% of patients who were having negative sputum cultures for myocbacteria. According to Caymmi *et al*, 51.4% of patients were diagnosed by bronchoscopic biopsy and this yield further increased to 80% when combined with sputum culture and ZN staining of bronchial washing.[21] In our study, in 25 (83%) out of 30 clinically suspected patients, diagnosis of TB was made through bronchoscopic biopsy. This percentage is quite high as compared to above mentioned studies. Fiberoptic bronchoscopy is an operator dependant procedure and if it is carried out properly by experts, yield through bronchoscopic biopsy can be high. Another aspect in this regard is that the diagnostic yield further increases if combined with bronchial washings for AFB and therefore these two methods should be used in combination for the diagnosis of TB.

Conclusion

In conclusion, our observations suggest that fiberoptic bronchoscopy is a reliable and effective procedure with adequate yield in expert hands for the diagnosis of TB in clinically suspected patients.

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ORIGINAL ARTICLE

Assessment of Knowledge of Different Disciplines of First Year University Students regarding Dengue Fever

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Abstract

Objectives
To assess the knowledge of first year university students about dengue fever and preventive strategies adopted by the students against dengue fever virus.

Materials and Methods
This was a cross-sectional survey conducted on first year university students enrolled in different disciplines (science and arts disciplines) through simple random sampling during the year 2011. For analysis purposes the study population was divided into two groups based on the discipline of study; group 1 (science) and group 2 (arts).

Results
One hundred students participated in the study. 99% knew about dengue fever. Sufficient knowledge about dengue fever and its preventive practices was found in 14% of the students. Out of these, 71.4 % belonged to group 1 while 28% were from group 2.

Majority (76%) of the individuals correctly identified mosquito bite as the mode of spread. Most common clinical symptom identified was fever, (51% in group 1 and 49% in group 2). Regarding availability of dengue vaccine, 66% in group 1 reported that dengue vaccine was not available as compared to 35% in group 2. Interestingly 58% of the group 2 individuals correctly identified standing clean water as the breeding site for dengue vector. Most common preventive measure was use of insect sprays/coils (82%). Covering water tanks and containers was reported by almost two third (73%) of the individuals as the most effective measure for eradication of vector breeding site. Real life preventive practices were also consistent with the knowledge of preventive measures.

Conclusion
Health education and awareness play an important role in motivating the masses to participate in measures for prevention and control of dengue. Since dengue can be prevented by simple day to day measures therefore, preventive aspects should also be emphasized upon. Involvement of students from schools, colleges and universities by educating them about prevention will augment efforts of creating awareness regarding dengue fever.

Key words
Dengue Fever, Knowledge

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Introduction

Dengue fever is a vector borne viral infection caused by a Flavivirus transmitted by a mosquito *Aedes aegypti*. First Dengue epidemic was reported in 1954 from Philippines.[1] Various factors are responsible for increasing incidence of this disease including absence of screens on windows or doors, existence of mosquito breeding sites at the premises and empty containers in houses.[2] Currently it is recognized as one of the emerging infectious diseases of public health importance worldwide. Dengue fever is endemic in over 100 countries in the world.[3, 4] According to CDC more than 2.5 billion people are at risk of acquiring this infection worldwide.[5] Annually, the number of reported cases of Dengue fever ranges from 50 to 100 million worldwide, with an average case fatality rate of five percent.[6, 7]

In South Asia, the first reported epidemic was in 1989 in Srilanka.[8] In Pakistan the first dengue outbreak was reported in 1994-95 and since then Pakistan has emerged as an endemic region for Dengue fever.[9] A recent survey in province of Punjab showed 1382 confirmed cases of dengue fever.[10] Despite the increasing number of dengue outbreaks in Pakistan, data on knowledge and preventive practices among adult population is insufficient. A survey conducted at Karachi showed that only 38.5% of our adult population had sufficient knowledge about the disease.[11] The overall knowledge regarding dengue fever was reported to be 52% in university students from Philippines.[12]

As there is no treatment or vaccine against the disease, core prevention programs must be implemented to control the spread of infection. Health professionals and students related or

unrelated to health and allied fields can play a crucial role in disease prevention, early recognition of symptoms, health education and promotion. To the best of authors' knowledge, limited data exists regarding knowledge of dengue fever among university students in Pakistan. Therefore this study was conducted aiming to assess the knowledge of first year university students about Dengue fever and preventive strategies adopted.

Materials and Methods

This cross-sectional survey was conducted during 2011 on first year university students studying at University of Karachi, enrolled in different disciplines (Science and Arts). Students were selected through simple random sampling. The sampling frame was available in the form of lists of all first year students from Science and Arts disciplines of Karachi University. For comparison and analysis purposes, the study population was divided into two groups based on the discipline of study; group 1 (science) and group 2 (arts).

The sample size was calculated using WHO sample size estimation software, assuming the knowledge of dengue fever reported from Phillipines as 52 percent.[12] Students having past history or family history of dengue fever were excluded from the study. A pre-tested self administered questionnaire was used to assess the knowledge of the students. The questionnaire consisted of 21 questions divided into four broad categories: demographic profile; knowledge regarding clinical manifestations, transmission and vector control; preventive practices; and sources of information. The purpose of study was explained to the students prior to the administration of questionnaire and they were informed that the data would not be used for any other purpose. Approval was taken from ethics review committee of the university. The questionnaire was administered simultaneously to all the students of a batch. Level of knowledge was analyzed by giving score to each question; each correct answer was given a score of one. A student achieving score of 60% (equivalent to 13 correct answers) or more was considered as knowledgeable.

Data entry and analysis was done in SPSS version 12.0. Frequencies were calculated for all the variables (gender, level of knowledge regarding clinical manifestations, vector control and preventive strategies, source of information). Stratification of sample was done into two major disciplines as science and arts for the purpose of analysis. Chi-square was applied to determine if there was any significant difference in knowledge of the two groups, and p-value of <0.05 was considered significant.

Results

One hundred students participated in the study with response rate of 83%. Almost all had previously heard about dengue fever. While assessing the awareness on dengue spread, clinical manifestations and treatment, 99% students knew that dengue was transmissible. 76% students correctly identified mosquito bite as the source of spread. Most commonly identified clinical symptom was fever (72%) followed by headache (44%) and nausea/vomiting (42%). Bleeding from gums/nose was identified by only 17% as the common symptom of dengue fever; 58.8% were from group 1. Regarding treatment options, 43% reported use of antibiotics as first line treatment of the disease. A significant number (65.8%) from the group 1 correctly identified that dengue vaccine was not available. Comparison between the groups is depicted in table 1. Table 2 represents the data showing knowledge regarding characteristics of vector including

Table 1 : Awareness on Dengue spread, symptoms and treatment

Variable	Science Students n (%)	Arts Students n (%)	*p*-value
Mode of spread			
Mosquito bite	38 (49.4)	39 (51.6)	0.39
Contaminated food and water	5 (33.3)	10 (66.7)	0.25
By direct contact with dengue patient	2 (40)	3 (60)	0.75
Common symptoms			
Fever	38 (52.8)	34 (47.2)	0.03
Nausea/ vomiting	26 (61.9)	16 (38.1)	0.007
Headache	27 (61.4)	17 (38.6)	0.006
Bleeding from gum and nose	10 (58.8)	7 (41.2)	0.24
Joint/ muscle pain	9 (33.3)	18 (66.7)	0.12
Rash	6 (46.2)	7 (53.8)	0.99
Medicines against Dengue			
Antibiotics	21 (48.8)	22 (51.2)	0.62
Antimalarials	14 (48.3)	15 (51.7)	0.77
Antipyretics	16 (61.5)	10 (38.5)	0.07
Dengue Vaccine availability			
Yes	7 (25.0)	21 (75.0)	0.005
No	25 (65.8)	13 (34.2)	

Table 2: Knowledge regarding vector characteristics and preventive measures

	Science students n (%)	Arts students n (%)	*p*-value
Common breeding sites			
Plants	13(37.1)	22(62.9)	0.19
Water containers	37(48.1)	40(51.9)	0.45
Tyres	2(50.0)	2(50.0)	0.90
Type of water for vector breeding			
Standing clean water	16(42.1)	22 (57.9)	0.54
Standing dirty water	24(44.4)	30 (55.6)	0.73
Running dirty water	5(83.3)	1 (16.7)	0.06
Most frequent time for mosquito bite			
Sunrise	9 (45.0)	11 (55.0)	0.92
Sunset	27 (50.9)	26 (49.1)	0.29
Both dawn and dusk	7 (77.8)	2 (22.2)	0.05
Afternoon	8 (66.7)	4 (33.3)	0.13
Night	10 (55.6)	8 (44.4)	0.37
Season of highest transmission			
Winter	7 (33.3)	14 (66.7)	
Summer	8 (50.0)	8 (50.0)	0.62
Rainy season	24 (50.0)	24 (50.0)	
Knowledge of Preventive Measures			
Prevention against mosquito bite			
Insect sprays/coils	39 (47.6)	43 (52.4)	0.50
Insect nets	29 (50.9)	28 (49.1)	0.26
Insect repellants	27 (62.8)	16 (37.2)	0.003
Use of fans	17 (70.8)	7 (29.2)	0.005
Windows/door screening with net	34 (46.6)	39 (53.4)	0.85
Covering body with clothes	26 (49.1)	27 (50.9)	0.52
Eradication of breeding site			
Cover water containers	35(47.9)	38 (52.1)	0.52
Prevent stagnation of water	33(61.1)	21 (38.9)	0.001
Remove garbage/trash	23(47.9)	25 (52.1)	0.71
Changing water in flower pots	21(53.8)	18 (46.2)	0.21

breeding sites of vector, mosquito bite time and season of highest transmission for the disease. 38% of the individuals correctly identified standing clean water as the breeding site of vector. Forty three percent students recognized mosquito bites at sunset/dusk while 11% identified sunrise/dawn as the mosquito bite time. Rainy season was reported for highest transmission for the disease by almost half (47%) of the students with almost equal proportion from each group.

Knowledge about preventive practices was also assessed. Most common preventive measures reported were use of insect sprays/coils (82%), and screening of windows and doors with nets (71%) as shown in table 2. The two groups differed significantly on knowledge regarding use of fans and insect repellants as preventive measures (p-value <0.05). Covering water tanks and containers was reported by almost two third (73%) of the individuals as the most effective measure for

eradication of vector breeding site. Television and radio turned out to be the most common source of information about dengue fever followed by newspapers.

Real life preventive practices were consistent with the knowledge about these practices with a large majority depending on mosquito sprays/coils (72%). Figure 1 demonstrates distribution of preventive practices in university students according to the discipline of study.

Sufficient knowledge about dengue fever and its preventive practices was found to be in 14% of the students. Out of these, 71.4 % belonged to the discipline of science while 28% were from non-science faculties. A significant association was found between the discipline of study and knowledge score (Table 3).

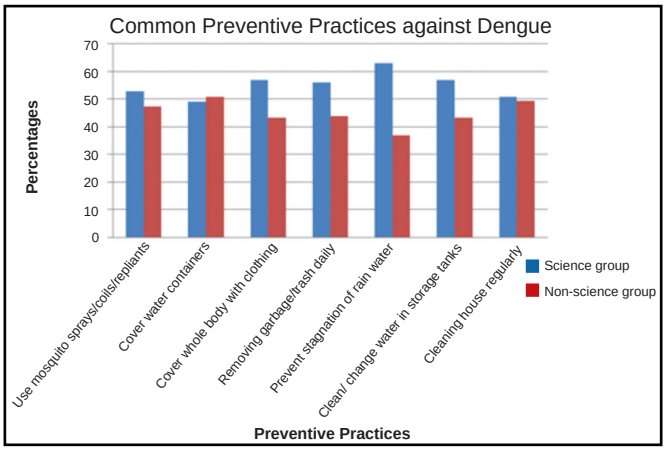

Fig 1: Distribution of preventive practices in university students according to the discipline of study

Table 3: Knowledge scores versus discipline of study

Knowledge Status	Discipline of study				*p*-value
	Science		Arts		
	n	%	n	%	
Sufficient knowledge (knowledge score >13)	10	71.4	4	28.6	0.040
Insufficient knowledge (knowledge score <13)	36	41.9	50	58.1	

Students coming from the Cambridge system of education (A-Levels) were found to be more knowledgeable (38.5%) as compared to in HSC/ intermediate (10.3%). No significant associations were found between knowledge scores and gender of the respondents, parental education and source of information.

Discussion

Almost all the students had heard about dengue which is probably due to the fact that dengue has re-emerged in the recent years. It could also be contributed to the mass awareness campaigns launched by media during the recent outbreaks. Despite being aware of the fact that transmission of dengue is via mosquito, many misconceptions existed among the students regarding its transmission patterns, clinical manifestations and treatment.

WHO states that the typical time of dengue transmission by *Aedes aegypti* is at dawn or dusk.[13] A great majority in our study identified night as the peak time of transmission. This is because malaria is endemic in our setting and most people think that the vector for dengue is same as that of malaria.[11] A substantial number of students in our study sample correctly reported mosquito as the vector for dengue which is comparable to results from Madiha *et al*.[14] Better knowledge percentages were obtained from earlier studies at Hong Kong and India.[15, 16]

Awareness regarding clinical manifestations of dengue fever is important for disease recognition and seeking health care in timely fashion.[17] Clinically dengue can manifest in the form of 3 syndromes, dengue fever, dengue hemorrhagic fever and dengue shock syndrome. No matter what type of clinical syndrome it is, fever remains the most common manifestation of dengue. In our study, fever was correctly identified as the most common symptom followed by headache which is consistent with reports by Acharya *et al*.[18] Knowledge of bleeding from different sites of the body and rash as a clinical parameter was under reported. As fever can be a presenting feature of many other infectious diseases prevalent in our community, it is important to impart knowledge about associated clinical features of dengue.

Majority of the students were unaware of the fact that tyres and plants were important breeding sites for mosquitoes. Most common choices for prevention from mosquito bite in our study were mosquito sprays and coils followed by mosquito nets. These results are consistent with another Pakistani study conducted on adult population.[11] Covering or eliminating water containers as a measure for eradication of breeding site was the most efficient means of control, similar to study from Brazil.[19] When it came to preventive practices, the results were consistent with the knowledge in the respective categories.

Science students were better in terms of knowledge scores as compared to the arts group as reported previously.[20] Though maternal education was not significantly associated with knowledge score yet more than half of the students with sufficient knowledge had mothers with graduation or above. Studies have shown that higher levels of parental education results in better understanding and comprehending abilities and thus transferring information to their offspring.[20]

Approximately two third of the participants cited television as a source of their information regarding dengue, correlating with data gathered in Kuala Lumpur and Thailand.[21-22] This makes mass media like television as an important tool for dissemination of knowledge.

Conclusion

Health education and awareness play an important role in motivating the masses to participate in measures for prevention and control of dengue. Involvement of students of educational institutes (schools, colleges and universities) will enlighten the people around them and thus augment community outreach awareness programs.

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Neonatal Sepsis: Bacterial Isolates and Resistance Pattern

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Abstract

Background
Neonatal sepsis is one of the most common reasons for admission to neonatal units in developing countries. It is also a major cause of mortality in both developed and developing countries.

Objective
To evaluate the magnitude of neonatal infections in neonatal nursery of a tertiary care medical unit and the pattern of antibiotics use in these cases.

Methodology
This was a cross-sectional retrospective study. All the cases of neonatal infections admitted from January 2007 through December 2009 were included in the study. Relevant information like age, sex, birth weight, diagnosis, antibiotic use and outcome were noted in the predesigned performa. Infection within first week of birth was regarded as the early onset infections and infections thereafter were considered as late onset infections.

Results
During 36 months period, out of 796 neonatal blood cultures sent, 140 came out to be positive. 89 were early onset infections while 51 were late onset infections. Majority of the babies were referred. The most common organism isolated was *Pseudomonas aeruginosa* (51.4%) followed by *Enterobacter* spp. (22%), other Gram negative rods and *Staphylococcus aureus* (3.5%). The most frequently used antimicrobial agents were combination of third generation cephalosporins, aminoglycosides and imipenem. In Gram negative group, best susceptibility was for cephalosporins followed by ciprofloxacin and imipenem. Susceptibility was remarkably low to ampicillin. The Gram positive group showed better susceptibility to aminoglycosides, vancomycin and tiecoplanin. Regarding outcome, 75% cases recovered well, 10% left the hospital against medical advice and 15% died in the hospital.

Conclusion
Pseudomonas aeruginosa and *Enterobacter* spp. were the most common organisms causing neonatal sepsis during the study period and there was high degree of resistance to commonly used first line antibiotics.

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Introduction

The reported incidence of neonatal sepsis varies from 7 to 38 per 1000 live births in Asia, from 6.5 to 23 per 1000 live births in Africa, and from 3.59 to 8.9 per 1000 live births in South America and the Caribbean. By comparison, rates reported in the United States and Australasia range from 6-9 per 1000.[1] The commonest cause of death in the neonatal period is septicemia including meningitis, respiratory infections, diarrhoea, and neonatal tetanus, accounting for 30-50% of all neonatal deaths followed by birth asphyxia and pre-maturity.[2] The pathogens implicated in neonatal sepsis in the developing countries differ from those seen in the developed countries. Overall Gram negative organisms are more common in the developing countries and Group B streptococcus is generally rare.[1] Among Gram negative organisms, the rate of *E.coli* infection varies from 15.7-77.1%; *Klebsiella* spp. infection from 8.9-64%; and *Pseudomonas aeruginosa* from 8.9-38.3%.[2] As the bacteriological profile of neonatal septicemia varies, a rational protocol for sepsis management must be based on adequate knowledge of local causative organisms and their antibiotic sensitivity pattern. This study was conducted to determine the bacterial pathogens in cases of neonatal sepsis in our unit and to find their antimicrobial sensitivity pattern.

Methodology

This retrospective cross-sectional study was carried out in the department of Neonatal Intensive Care Unit of Ziauddin Hospital, Kemari campus, from January 2007 through December 2009. All neonates suspected on clinical grounds and first blood culture positive were included in the study. Babies who had been given antibiotics prior to admission were excluded. Complete history and physical examination including obstetric history, maternal and neonatal risk factors were recorded. Infection in the first week was considered as early onset infection and beyond that period was considered as late onset infection. Babies were classified as indoor if born in the hospital or outdoor if born outside but admitted to the nursery.

Diagnosis was mostly clinical. Complete blood counts and blood cultures were sent in all cases. Other investigations such as cultures of cerebrospinal fluid (CSF) and urine were sent in selected cases. Chest radiograph, arterial blood gases, serum electrolytes and renal function tests were performed as indicated. Antibiotics were started empirically and later modified according to culture sensitivity reports.

Cultures were processed by standard techniques; inoculated onto blood agar, chocolate agar, and MacConkey's agar (Oxoid, UK) and incubated at 37°C. Culture positive isolates were further tested for identification and antimicrobial susceptibility was done using Kirby-Bauer disc-diffusion method.

Results

During this 36 months period, a total of 796 neonates were admitted with suspicion of sepsis. These babies were having clinical symptoms of sepsis such as lethargy, poor activity, inability to suck, abdominal distension, etc. 96 (68.5%) babies weighed 2.5 kg or more and 44 (31.4%) less than 2.5 kg. 140 blood cultures yielded isolates. Among these cultures positive babies, 53 (37.8%) babies were delivered in our hospital while 87 (62.2%) were born at some other place and were brought to nursery for treatment. 80 were females and 60 were male babies. Among culture positive cases, 89 (63.6%) suffered from early onset infection while 51 (36.4%) came with late onset infection. Infections included: septicemia, pneumonia, diarrhoea, meningitis, umbilical cord sepsis, pyoderma, and oral candidiasis. *Pseudomonas* spp. was isolated in 71 (50.7%) neonates, *Enterobacter* spp. 31 (22%) cases while *Staphylococcus aureus* and *Acinetobacter* spp. each in 5 (4.28%) cases. Other organisms isolated were *Klebsiella* spp., *E coli, Staphylococcus epidermis* and *Staph lugdunenesis*. Table 1 shows antibiotics resistance to different antibiotics.

Discussion

Neonatal sepsis is one of the most common reasons for admission to neonatal units in developing countries, and it is also a major cause of mortality in both developed and developing countries. The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram negative organisms are more common mainly *Klebsiella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* spp.[4] Of the Gram positive organisms, *Staphylococcus aureus, coagulase negative staphylococci, Streptococcus pneumoniae,* and *Streptococcus pyogenes* are most commonly isolated.[4] In developed countries Gram positive organisms are predominant in both early as well as late onset neonatal septicemia.[4]

In our study, Gram negative organisms were more common as compared with other studies.[1,2] Main pathogens were *Pseudomonas aeruginosa* and *Enterobacter* spp. Presence of *Pseudomonas* spp. has been reported by earlier local studies in contradiction to many other studies reported from Pakistan and other developed countries.[5-7] Rizwan reported *E. coli, Klebsiella* spp. and *Pseudomonas aeruginosa* as main pathogens constituting 77% of the total isolates. An Indian study reported *Pseudomonas* spp. as the most common isolated organism.[3] Afroza reported *Klebsiella pneumoniae* as the leading pathogen causing neonatal sepsis in Bangladesh and neighboring countries.[8] Other studies also reported *Klebsiella* spp. to be the main causative organism.[9,10] Studies done in developing countries have reported *E.coli* as the predominant agent.[11]

Enterobacter spp. was the second commonest organism in our study. In an Indian study it was detected as the leading pathogen.[12] *Enterobacter* spp. was detected in varying percentages ranging from 1.5 to 14.6 by various authors.[13,14] *Enterobacter* septicemia should be emphasized and taken into account for its rising incidence. In the present study, Gram positive isolates accounted for 18.57%. *Acinetobacter* spp. and *coagulase negative staphylococci* each accounted for only 5% of all pathogenic isolates in comparison to Mondal *et al* who reported 15.2% and 21.2% respectively.[11]

The rate of *Staph aureus* infection in the present study was 4.8%. Similar reports varying from 3.7- 7% have been found previously.[15] However, Karthikeyan and Kumar reported *Staph aureus* as a predominant pathogen causing early neonatal sepsis in 50% of cases.[16] Group B *streptococci* were not isolated by us similar to most of the studies from Pakistan and other

Table 1: Resistance pattern of isolates

Isolate	Amino-glycosides	Cephalo-sporins	Quinolones	Oxacillin	Tazo/ pipera	Vanco-mycin	Genta-mycin	Tiecoplanin	Ampicillin
Pseudomonas spp	RR						RRRR		
Enterobacter spp	RRRR	RRR	RRRR		RR				
Staph aureus	RR		RRR	RR					RR
Klebsiella spp	RR						RR		RRR
Gram positive cocci		RR	RR						
Staph epidermidis									RR
Staph lugdunensis	RR		R						

Key: RRRR (all isolates resistant), RRR (75-50% isolates resistant), RR (25-50% isolates resistant), R (< 25 isolates resistant)

developing countries.[17,18]

Most of the babies were referred to our hospital. Our hospital is surrounded by areas where scanty antenatal and natal care facilities are available. Most of the deliveries took place in homes under unhygienic conditions.

A previous study conducted in gynecology department of our hospital during 2008-09 to assess the microbiological flora in women during labour showed higher proportion of women had *Candida albicans* followed by *Klebsiella* spp. and *Staph aureus* as the main microorganisms, demonstrating that the source was probably not maternal.[19]

In the present study, amikacin and cephalosporins showed reasonably good sensitivity for Gram negative isolates. The Gram positive organisms were sensitive to aminoglycosides, vancomycin and tiecoplanin. Various studies from Pakistan presents similar antibiotics sensitivity patterns.[5,20,21] A study from Jordan also supports these findings and recommends amikacin combined with a third generation cephalosporin as best treatment for neonatal sepsis.[22]

Antibiotic resistance is a worldwide problem.[1] Several reports have shown increase in multiresistant bacteria causing neonatal sepsis in developing countries.[23] The resistance is reported more for *Klebsiella* spp. and *Enterobacter* spp. The reason for this is probably easy access to antibiotics and inappropriate, improper and irrational use of broad spectrum antibiotics. Our study showed high degree of resistance of Gram negative organisms to first line antibiotics. About 50% of *Staph aureus* were resistant to ampicillin, quinolones and gentamicin. The Gram negatives showed low resistance to quinolones.

To overcome the neonatal septicemia, there is need to implement certain preventive measures and strategies. Hand washing has been shown to be effective, and several guidelines are available. Health personnel require education, continuous reminding, and feedback if compliance is to be maintained. Early discharge policies for the low risk newborns as a means of reducing staff workload and exposure to nosocomial infection need to be undertaken. Minimizing invasive procedures has also shown an impact in reducing nosocomial infections. There is also need to implement strict antibiotic policy. Consideration can also be given for intra partum antibiotic prophylaxis and the use of antiseptic solution to disinfect the birth canal. There is also need for promotion of clean deliveries and restriction of antibiotic use.

Conclusion

Pseudomonas aeruginosa and *Enterobacter* spp. were the most common organisms causing neonatal sepsis during the study period and there was high degree of resistance to commonly used first line antibiotics. Local antibiotic policies should be prepared and regularly updated.

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ORIGINAL ARTICLE

The Pattern of Malaria and Role of Rapid Diagnostic Test in Diagnosis

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Abstract

Introduction
In our country nearly half a million cases of malaria occur annually causing fifty thousand deaths. Therefore, malaria is a major public health concern of Pakistan. Clinical diagnosis of malaria may be unreliable because of diverse presentations.

Objective
To know the pattern of malarial infection and to determine sensitivity and specificity of rapid diagnostic test for detection of *Plasmodium falciparum and Plasmodium vivax* malaria.

Materials and Methods
The study was carried out at Combined Military Hospital Nowshera, Khyber Pakhtunkhwa, Pakistan, from July through October 2011; the malaria season. Three hundred fifty patients with clinical suspicion of malaria were included in this study. For each patient, thick and thin blood films were prepared directly from finger prick blood samples. These slides were examined by two experienced technologists followed by microbiologist. Rapid diagnostic test(RDT) by immunochromatographic method was performed directly with 15µL of the finger prick blood samples. Sensitivity and specificity were calculated.

Results
Out of 350 patients, 266 (76%) were found positive on direct microscopic examination. The RDT was performed in all cases and it detected malarial antigen in 273 (78%) patients; 64 (18.3%) patients with *P. falciparum* infection, 158 (45.2%) with *P. vivax* infection and 51 (14.5%) with mixed infection while 77 (22%) were negative. Compared with microscopy, sensitivity of the RDT for *P. falciparum* was 98.1% and *P.vivax* 94.5%; specificity for *P. falciparum* was 98.2% and *P. vivax* 99.4%. For mixed infections, sensitivity was 95% and specificity was 95.8%. Overall sensitivity was 95.5% and specificity 77.3%; positive predictive value 93%, and negative predictive value 84.4%.

Conclusion
P. vivax infection was more common in our setup. RDT was found to be sensitive in detection of malarial infection; therefore, it can be used as a support tool to diagnose malaria where quality staining and microscopy is not certain.

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Key words
Malaria, *Plasmodium falciparum*, *Plasmodium vivax*, Rapid Diagnostic Test.

Introduction
Despite massive efforts by private and public sector, malaria remains the leading global health problem. Approximately, 250 million cases of malaria are recorded each year, with an annual toll of 781,000 deaths[1]. The major burden of the malarial infection is borne by the population living in highly endemic areas[2].

World Health Organization (WHO) has classified Pakistan as a country with moderate malaria prevalence. In our country, malaria causes at least 50,000 deaths out of an estimated 500,000 reported cases every year. Malaria caused by *Plasmodium vivax*, generally found in rural areas, can easily be treated if diagnosed early.[1] Weak health infrastructure, poverty and lack of surveillance are the reasons for the high mortality.[1,3] Therefore, malaria remains a major public health concern for Pakistan. Prompt and accurate diagnosis is necessary for rational therapy. It will not only reduce the morbidity and mortality but also reduce the impending drug resistance. Clinical diagnosis may be unreliable because the presentation is diverse, and it may be difficult to distinguish it from viral fevers, enteric fever, or even leptospirosis.[4]

Microscopic examination of stained thick films by a skilled microscopist has remained the standard laboratory method for the diagnosis of malaria.[5] It is cost effective and simple but its reliability is doubtful, mainly at low levels of parasitemia and in the interpretation of mixed malarial infections.[6] In many endemic regions of Pakistan, there are problems like lack of skilled staff, non-availability of microscopes and inadequate quality control. Due to these shortcomings, most of the suspected malarial patients are treated merely on the basis of a clinical suspicion.[7] Simple and cost effective diagnostic tests for malaria are required to overcome these deficiencies of direct microscopy and clinical diagnosis.[8]

Rapid Diagnostic Test (RDT) detects malaria antigen in a small

amount of blood by immunochromatographic assay. RDTs are cheap, simple to perform and are easy to interpret.[9] The number of febrile patients visiting Combined Military Hospital (CMH), Nowshera increases dramatically during and soon after the monsoon season. This study was conducted to know the pattern of malarial infection and to determine sensitivity and specificity of RDT for detection of *P. falciparum* and *P.vivax* malaria.

Materials and Methods

The study was performed from July through October 2011 at Combined Military Hospital, Nowshera, Pakistan, after approval from ethical committee. Three hundred and fifty patients with clinical suspicion of malaria, attending medical outpatient department of the hospital were included in this study. Diagnosis of clinical malaria was based on presence of fever (≥37.5°C) at the time of presentation, or within the previous 48 hours with rigors and chills in the absence of overt viral or bacterial infection. All patients who had been treated for malaria in the previous four weeks were excluded from the study.

For each patient, thick and thin blood films were prepared directly from finger prick blood samples. Thick and thin films were stained with Giemsa (10%) and Leishman stains respectively, for 10 minutes and examined by two experienced technologists and confirmed by microbiologist. The thick film was considered negative if no parasite seen in at least 100 high-power fields.

RTD by immunochromatographic method (*Plasmodium falciparum*/Pan rapid test device; MK BIO, San Diego, USA) was performed directly with 15µL of the finger prick blood samples. Tests were performed by expert technicians according to the manufacturer's instructions and the results were read by the pathologist. Briefly, whole blood was added to a sample pad. A buffer reagent was added to induce cell lysis and allow *P. falciparum* HRP-2 antigen (histadine rich protein) and pan-malarial antigens to bind to colloidal gold-labeled antibody. Additional buffer caused the blood and immune complexes to migrate up the test strip and cross monoclonal antibody lines. Buffer was further added to clear hemoglobin from the strip and facilitate reading. The test was considered valid if the control line was visible and positive if the HRP-2 and /or pan malarial antigen lines were visible. An immunochromatographic test diagnosis of *P. vivax* malaria was made if only the pan malaria antigen line was visible. A diagnosis of *P. falciparum* malaria was made if the HRP 2 line was visible, with or without the pan-malaria antigen line. Co-infection with both *P. falciparum* and *P. vivax* was interpreted when three lines including control were visible. Appearance of a coloured band at the control region served as procedural control indicating proper volume of specimen. The technician and pathologist were blinded to patient's diagnosis and to previous microscopy and RDT test results.

Data were analyzed using the SPSS 14.0. For sensitivity and specificity, test kits were compared with microscopy (standard) results. The sensitivity was calculated as the proportion of positive test results obtained among samples containing malaria parasites by microscopy; the specificity was calculated as the proportion of negative test results obtained among samples whose thick blood films were negative. Positive and negative predictive values were calculated as the proportion of true positive results among all positively reacting samples and as the proportion of true negative results among all negatively reacting samples, respectively.

Results

Three hundred and fifty patients with presumptive clinical diagnosis of malaria were included in this study. The mean age was 19.5 years (range 2 to 67) with male to female ratio of 2.2:1. Sixty two percent of the patients were between 11-30 years of age. Figure 1 shows a breakdown of malaria cases in different age groups.

Out of 350 patients, 266 (76%) were found positive on direct microscopic examination (Fig 2 & 3). Among them, 60 (17.1%) were infected with *P. falciparum*, 166 (47.4%) with *P. vivax* and 40 (11.4%) were mixed infections while 84 (24%) were negative. The RDT detected malarial antigen in 273 (78%) patients; 64 (18.3%) patients with *P. falciparum* infection, 158 (45.2%) with *P.vivax* infection and 51 (14.5%) with mixed (*P. falciparum and P. vivax*) infection (fig 4) while 77 (22%) were negative. Comparison of microscopy and RDT for detection of malaria parasites is shown in figure 5.

On direct microscopy, 84 were negative for malarial parasites

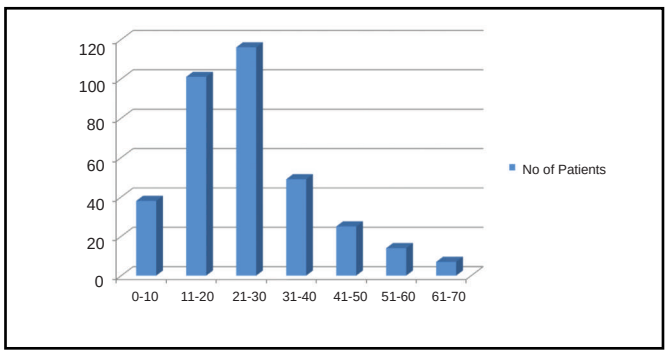

Fig 1: Frequency of malarial cases according to age groups (*n*=350)

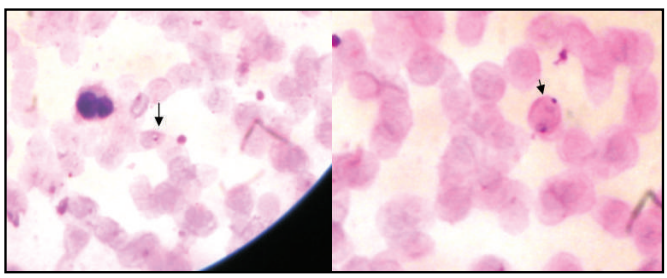

Fig 2: Giemsa stain slide showing ring trophozoites of *P. vivax* (left) and *P. falciparum* (right) x100

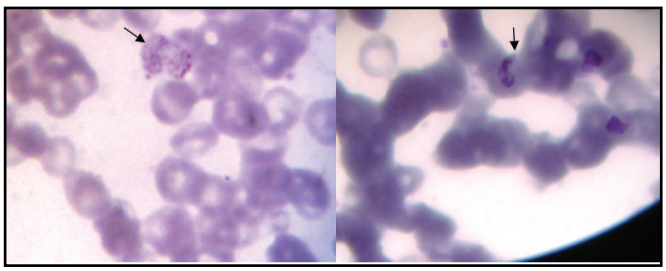

Fig 3: Leishman stain showing trophozoites of *P. vivax* (left) and gematocytes of *P. falciparum* x100

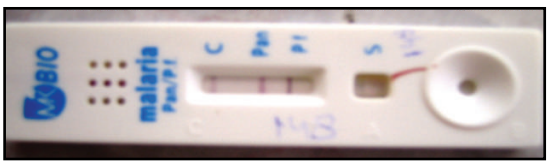

Fig 4: RTD showing mixed infection

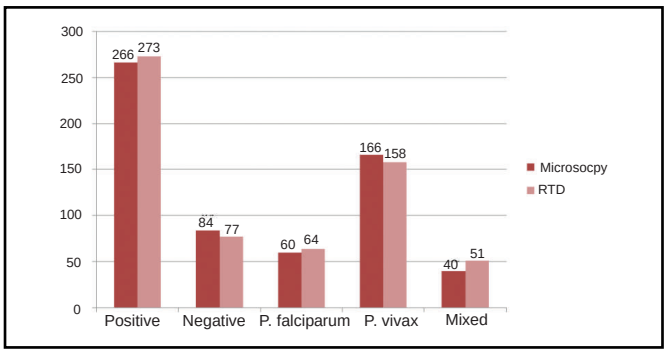

Fig 5: Comparison of results between microscopy and RTD

whereas 77 cases were found negative by RTD. Overall true positive cases were 254 while true negative were 65.

Compared with microscopy, sensitivity of the RDT for *P. falciparum* was 98.1% and *P. vivax* 94.5%; specificity for *P. falciparum* was 98.2% and *P. vivax* 99.4%. For mixed infections sensitivity was 95% and specificity 95.8%. Overall sensitivity was 95.5% and specificity 77.3%; positive predictive value 93% negative predictive value 84.4% (Fig 6).

Using microscopy as the standard test, RDT failed to detect malarial infection among twelve patients; two with mixed infection, nine having *P. vivax* and one patient with *P. falciparum.* On the other hand RDT detected nineteen cases of malaria among samples which were initially negative by microscopic examination; five with falciparum infection, nine with vivax infection and thirteen patients with mixed infection.

Discussion

Giemsa stained smear is still the most suitable diagnostic method to detect malaria till today. It is inexpensive, easy to perform, able to detect malaria species and can also quantify parasites. The detection threshold for *Plasmodium* spp varies in Giemsa

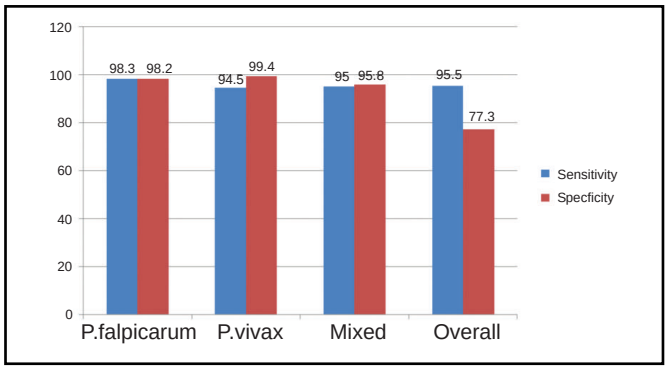

Fig 6: Comparison of sensitivities and specificities

stained thick blood film. In research laboratories, it is 4-20 parasites/µL but under field conditions it is about 50-100 parasites/µL blood.[10-12]

In RDT, histadine rich protein 2 (HRP-2) is the most common malaria antigen targeted and is specific for *P. falciparum*. Monoclonal antibodies against p-LDH are also commercially available for the detection of *Plasmodium* spp (pan-malaria), *P. falciparum* and *P. vivax*. The parasites can be detected as low as 5–15 µL of blood. Test line configuration and interpretation of RDT results vary with products.

Our study showed malaria was more common between 11 to 30 years of age; this might be due to more outdoor activities and as such exposure to mosquitoes. Similarly, male predominance may also be attributed to same reason. Rapid rise of malarial infection was seen during and soon after monsoon season which corresponded to the previous study conducted by Bouma *et al* in the province of Khyber Pakhtunkhwa (KPK)[13]. Our study showed that *P. vivax* infection was more common than *P. falciparum* at Nowshera, KPK. Same results were seen in previous studies conducted in Pakistan.[14-16]

Compared with direct microscopy (Giemsa stain), RDT performed better with *P. falciparum*. The results of our study showed that RDT detects *P. falciparum* malaria with high sensitivity and is highly specific as well. Similar results have been recorded for other RDT kits detecting the HRP-2 antigen of *P. falciparum.*[17-19] RDT failed to detect *P.vivax* infection in nine patients. The sensitivity of the RDT Malaria for *P.vivax* malaria conducted at village level in Myanmar was 66.7 to 79%, and that within Europe was 76%.[18,20] Auxiliary studies with a large number of patients with *P. vivax* infection from various endemic areas are required before agreeable recommendations can be made for the use of RDT in diagnosing vivax malaria.

In our study, RDT detected mixed malarial infections among thirteen samples which were negative by microscopic examination. False positive tests have been reported in prior studies.[17,20] False positive reactions may occur among patients

who have been recently treated for malaria or in the presence of chronic infections and circulating rheumatoid factors.[21] The overall sensitivity as compared to specificity was superior by RDT. Previous studies conducted among symptomatically diagnosed malaria patients to investigate the performance of the RDT, relative to microscopy have shown variable results.[22-23]

A study was conducted in Ghana to see the relationship of line intensity of RDT with parasites index.[24] We observed that line intensities for the HRP-2 antigen and pan malarial antigen could be related to parasite density. Semi-quantitative estimation of these antigens in plasma may prove to be useful for the rapid prediction of parasite count.

Thrombocytopenia was also related to malarial infection as shown in previous studies.[25-26]

Our study results supported the use of RDT for the detection of malarial antigens as an adjunct diagnostic tool in addition to clinical assessment of patients and Giemsa staining especially in uncertainty. These tests are swift, simple to perform and easy to interpret. Direct microscopy, however, must be taken as the gold standard as it detects all *Plasmodium* spp and offers clear distinctions between parasite growth stages which are essential for the therapeutic decisions.

Conclusion

P. vivax infection is more common in our setup. RDT was found to be sensitive in detection of malarial infection; therefore, it can be used as a support tool to diagnose malaria where quality staining and microscopy is not certain.

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ORIGINAL ARTICLE

Early Diagnosis of Acute Dengue Infection through Peripheral Film: An Affordable & Reliable Alert for Third World Population for the Deadly Virus

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Abstract

Objective
To evaluate the importance of plasmacytoid cells in peripheral film for early diagnosis of acute dengue infection.

Methodology
This prospective observational study was carried out at Shifa International Hospital, Islamabad and Shifa College of Medicine, Islamabad from August 2010 through December 2010. A total of 228 patients of both genders were enrolled. Consecutive patients with acute febrile illness of less than two weeks duration at Shifa International Hospital Islamabad were selected. Complete blood count with peripheral film examination and dengue serology were performed for the enrolled patients.

Results
Out of 228 patients, 86 patients were positive for dengue IgM signifying acute dengue infection. 44 (51.2%) proven dengue infection patients showed very prominent finding of 5-15% of plasmacytoid cells on their peripheral films with variable thrombocytopenia and/or leucopenia. Only 5 out of 142 (3.5%) febrile patients with negative dengue serology showed a few plasmacytoid cells (making up <5% of their differential count).

Conclusion
Presence of plasmacytoid cells in the peripheral film of the patients presenting with acute febrile illness has excellent correlation with Dengue positivity and should always be sought for as important diagnostic tool.

Key words
Dengue Fever, Complete Blood Count, Peripheral Blood Film, Plasmacytoid Cells.

Introduction

Dengue fever has emerged as a major public health concern across the globe in terms of mortality, morbidity and public health cost. According to World Health Organization (WHO) estimates, approximately 50 million dengue cases are diagnosed worldwide every year and around 500,000 cases of dengue hemorrhagic fever (DHF) require hospitalization per annum with mortality rate of 2.5 %.[1]

In Pakistan, the situation is haunting as late diagnosis of this fatal infection is the cause of even graver consequences in form of mortality and morbidity. Considering the lower socioeconomic status of most of the patients in Pakistan, who have continuous exposure to mosquito bite, approach to good health care facility is remote.[2] Demographic features of dengue fever have changed tremendously in Pakistan over the past two decades. Small scale studies from all over the country have reported different aspects of individual outbreaks during this time. However there is dearth of data looking at the overall trend of dengue virus infection in the country.[3]

Furthermore, confirmatory tests for dengue infection are scarcely available even in primary and secondary Health Care Centers in the country. As the infection shows a seasonal pattern and providing screening test round the year is not cost effective for most of the low & medium budget laboratories, physicians mostly rely on clinical suspicion due to non-availability of authentic diagnostic tests.[4] Peripheral film examination is available at almost all levels of medical facilities. Peripheral film findings relating the clinical scenario to the definitive diagnosis thus gain importance for the clinicians. During the present outbreak of DHF in Pakistan, our centre received many patients with acute febrile illness, some presenting with typical symptoms of acute headaches, myalgias, nasal bleed and retro-bulbar pain, while rest of them were atypical, presenting with fever only. Most of the patients showed documented and proven peripheral count abnormalities, including leucopenia and thrombocytopenia.[5] A unique finding in all these patients peripheral film was presence of many plasmacytoid cells, ranging in number from 5-40% of their differential count. As this finding showed excellent correlation with serological positivity for dengue IgM, a case review and data collection was planned in order to get a nearly clue to the diagnosis of this fatal infection. During our search of peripheral film findings a positive correlation was established for presence of plasmacytoid cells in diagnosed dengue cases in 56% of the subjects. Due to high prevalence, fatal outcome and lack of diagnostic facilities for this viral epidemic, we planned this observational study for all suspected dengue febrile cases. The

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objective of this study was early identification of fatal infection, which is vital in terms of preventing disease progression and mortality.

Material and Methods

This descriptive observational conducted study was carried out at Shifa International Hospital (SIH) Islamabad, from August 2010 through December 2010. Formal approval of the study was taken from Hospital Ethical Committee as the study intended to improve diagnostic work up for early detection of this life threatening infection. Consent of all the patients included in the study was taken at time of hospital admission. We investigated 228 cases of acute febrile illness presenting in our outpatient departments. All patients of both genders and age ranging from one year to 90 years with history of fever for less than two weeks were included in the study. Patients with confirmed diagnosis of bleeding diathesis, multiple myeloma, plasma cell leukemia, were excluded.

Patients were scrutinized for complete blood count (CBC), peripheral blood film, dengue IgM & malarial microscopy/ICT. Samples were withdrawn at SIH and tested in Hematology and Chemical sections of SIH laboratory. CBC samples were performed on a 5 mL venous blood sample collected in EDTA and were analyzed on Hematology Analyzer Cell Dyne (RUBY) Peripheral films were stained with Geimsa stain, manual and automated differentials were performed on all blood samples. Dengue viral serology was performed by ELISA technique using INC Immunodiagnostics, Microwell Dengue IgM USA kit. In our study, thrombocytopenia was defined as platelet count below $150X10^9$/L and leucopenia as white cell count below 4000/µL. Data was analyzed by using SPSS version 14; relevant descriptive statistics like frequency, mean, and percentages were calculated.

Results

Out of 228 patients, 86 were positive for dengue IgM, however among these patients CBC reports of only 79 patients were available and plasmacytoid cells were present in 44 out of 79 patients (55.6%). Malarial parasite was detected in 5 patients; plasmacytoid cells were present only in one patient. Though plasmacytoid cells were present in 98 patients in peripheral smears out of 228 (42.9%), CBC record of 19 patients was not available. Number of patients with positive Dengue IgM in comparison with total number of patients and number of patients with plasmacytoid cells as compared with total number of patients with CBC reports is illustrated in figure 1. Table 1 illustrates demographic details of the patients.

Besides usual haematological findings of leucopenia & thrombocytopenia, our Dengue positive patients showed presence of plasmacytoid cells; large lymphoid cells with markedly basophilic abundant cytoplasm, with coarse chromatin clumps in eccentric nuclei with perinuclear halo as shown in figure 2A and 2B.

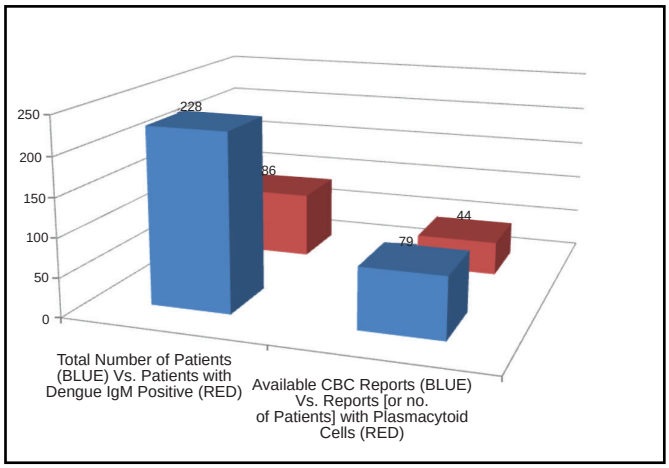
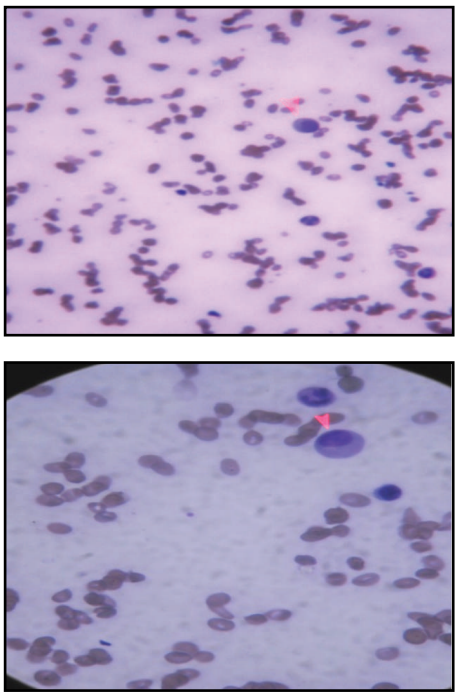


Fig 1 : Patients with Dengue IgM Positive and Plasmacytoid Cells in comparison with total patients

Table 1: Demographics details of patients

Male Patients (out of 86)	Female Patients (out of 86)	Male:Female
52 (60.5%)	34 (39.5%)	3:2
Mean Age	Min Age	Max Age
37.79±16.39	3 years	90 years



2A

2B

Fig 2A: Plasmacytoid cell at 10X in peripheral film of dengue patient;
2B: Plasmacytoid Cell at 40X in peripheral film of dengue patient

Discussion

According to World Health Organization annual report 2009, dengue infection has spread dramatically around the world in last decade; 2.5 billion people, almost two fifth of the world's population, are at risk from dengue infection with fatality rate of 20%, adopting almost endemic situation in South-East Asia and Western Pacific.[5,6]

In Karachi, largest city of Pakistan, first confirmed epidemic of dengue infection was reported in 1994.[7] The largest outbreak with maximum mortality occurred in 2006.[8] In 2010 epidemic, 3893 cases were found positive for dengue viral infection out of 5760 acute febrile cases in Karachi. According to statistics provided by the Health Department, during the recent outbreak of the disease as many as 1,400 cases of dengue were reported in Lahore, while Faisalabad was the second worst-hit city with 52 cases, followed by Multan with 26. Fourteen cases surfaced in Sheikhupura, 11 in Nankana Sahib and 5 in Chakwal.[9] Dengue virus is now endemic in Pakistan, circulating throughout the year with a peak incidence in the post monsoon period.[3] Four antigenically different serotypes of dengue virus (DEN1 to DEN 4) have been identified.[1] Dengue fever is an acute febrile illness with manifestations ranging from asymptomatic to mild self-limiting illness of short duration to bleeding tendencies.[10] The most important clinical characteristics include: fever and asthenia (100%), headache (98%), myalgias (84%), arthralgias (72%), morbilliform rash (61%) and retro-ocular pain (65%). Increased hepatic enzymes, ALT, AST and LDH have been reported.[11]

This study provided the significance of a simple peripheral film finding i.e., reactive plasmacytosis in early dengue detection using CBC as a screening test. This would provide readily available diagnostic clue for this viral infection. In comparison to a peripheral film examination worth PKR 100/-, other confirmatory tests like ELISA for dengue viral antibody detection (PKR 3000/-) and RT-PCR (PKR 5000/-) are expensive and loose importance due to non-availability of technical support and failure to maintain year round inventory of test kits.[12]

Many factors contribute to enhanced fatality of dengue infection including non-availability of vaccine and lack of specific antiviral medication with probable vaccine preparation time of five years.[7] In this scenario, only early diagnosis of this haemorrhagic viral infection affords chance of reducing mortality and morbidity. Presence of plasmacytoid cells in blood film of these patients with acute febrile illness would alert the clinicians, to diagnose early and prevent complications like DHF and dengue shock syndrome.[13]

Plasmacytosis with bicytopenia in peripheral blood film is a rare finding which is most often associated with plasma cell leukemia but is rarely associated with other malignancies, infectious diseases or drug reactions.[14] These cells (fig 3) are instrumental in antiviral innate immunity and shaping Th1

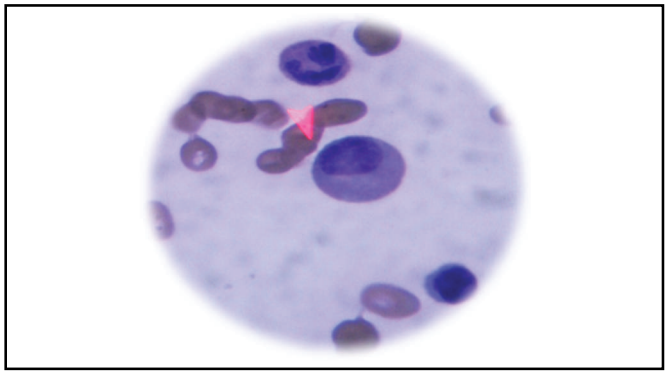


Fig 3: Plasmacytoid cell (arrow) under 100 X

adaptive immune responses. In Pakistan, reporting peripheral film plasmcytosis is followed by extensive workup to exclude plasma cell dyscrasias. Most of our reported patients were scanned for protein electrophoresis and bone marrow examination. The immune-histochemical profile of these plasma cells showed both kappa & lambda light chain presence in these cells ruling out cancerous plasma cell proliferations and augmenting the link between peripheral plasmacytosis and viral infection.

Factors including hot, humid climate after monsoon, crowded areas and poverty contribute to rapid spread of the disease, therefore in developing countries like Pakistan, active case surveillance is important for early detection and implementation of dengue control programme.[15] Rapid test devices based on immunochromatographic method supplied in the public sector hospitals are not reliable diagnostic tools for screening. More reliable tools are demanded during this viral epidemic in order to avoid false negative results.[16] Hematological parameters, leucopenia and thrombocytopenia are important supportive tools for dengue detection as serology being expensive and scarcely available, remains out of reach for a large percentage of population.[17] These peripheral blood parameters when combined with reactive plasmacytosis would establish the probable diagnosis of dengue infection in most cases of acute febrile illness. More studies at much larger scale exploring the same finding of reactive plasmacytosis with acute febrile illness are recommended to further strengthen this diagnostic relationship.

Conclusion

Early detection would prevent many patients progressing to fatal complication of dengue. We recommend that peripheral film should be given due consideration for early diagnosis of dengue infection. In cases where a choice has to be made between blood tests and serology, depending upon cost and technical availability, findings of plasmacytosis should always be taken in consideration as screening parameter for DHF and prevention of the disease.

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CASE REPORT

Dengue Encephalopathy in Infants: Case Series

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Abstract

Dengue fever has emerged as a huge burden on healthcare system in Pakistan. Besides its typical presentation as per WHO classification, central nervous system involvement is not an uncommon manifestation, but encephalopathy associated with dengue viral infection especially in infants is a rare entity. We report two cases of dengue encephalopathy both less than 1 year of age. Both infants had distributive shock and high grade fever on presentation and were positive for dengue IgM antibody. The infants received supportive treatment; 8 months old infant recovered and was discharged, while 3 month old infant continued deteriorating and died.

Key words

Dengue Fever, Encephalopathy.

Introduction

As spread of dengue fever and dengue hemorrhagic fever (DHF) is increasing, atypical manifestations are also on the rise though under reported because of the lack of awareness. The relationship between DHF and neurological disturbances was first described in 1976.[1,2] Encephalopathy in DHF is an atypical manifestation and may appear in various forms, including altered sensorium, convulsions, neck rigidity, pyramidal signs, headache, papilledema, myoclonus and behavioral disorders.[3] Pathophysiology of neurological involvement includes various factors: direct tissue lesion caused by the virus; capillary hemorrhages; disseminated intravascular coagulation; and metabolic disorders.[4] The exact mechanism by which dengue enters brain should be further elucidated. The outcome of dengue encephalitis may be an uneventful recovery in most of the cases; neurological sequelae or death has been reported in some cases.[2,4,6-9] We report this case series as we have recently faced an outbreak of dengue fever, although neurological manifestation is rare but when encountered should be diagnosed promptly.

Case reports

Case I

An 8-months-old male infant, was admitted in Aga Khan University Hospital in August 2010 with five days history of fever and four days history of fits (two episodes, generalized tonic clonic) and bulging anterior fontanelle. On admission, child was irritable and sick looking with bulging anterior fontanelle and brisk deep tendon reflexes, while rest of neurological examination was unremarkable. He was febrile (38ºC), tachycardiac (heart rate: 155/min), tachypnoeac (respiratory rate: 44/min) with 104/64 mm Hg blood pressure. There was no rash or palpable lymph nodes. Liver was palpable 7 cm below right coastal margin while rest of the examination was normal. Complete blood count revealed normal total leucocyte count with lymphocytosis with atypical lymphocytes (3.2%) and thrombocytopenia (platelets 13,000/mm^3). Coagulation profile was deranged with prothrombin time of 14.2/11.0 (INR 1.37) and activated partial thromboplastin time 75.1/30. Electrolytes were deranged; hyponatremia (sodium 127meq/L) and hyperkalemia (potassium 5.7meq/L) while renal function were normal. A diagnosis of meningitis/encephalitis was made. Initial management involved supportive and symptomatic management; intravenous fluids, anticonvulsants and antibiotics (3rd generation cephalosporin). On third day of admission, we noticed facial edema, with bilateral pedal swelling and flushing of the lower extremities. Meanwhile, cerebrospinal fluid examination was found normal whereas serum dengue IgM was positive. Antibiotic was discontinued while rest of the management continued. He became afebrile, tolerated diet orally and anterior fontanelle became normal and was discharged after 5 days.

Case II

A 3-months-old male infant was admitted in Aga Khan University Hospital in August 2010 with six days history of flu like illness, cough, fever and two days history of episodic seizures; generalized tonic clonic. On admission child was sick, irritable, seizing, febrile (39.6ºC), tachycardiac (heart rate 200/minute) with 90/40 mmHg blood pressure. There were no rashes on body or palpable lymph nodes, while liver was palpable 3 cm below the costal margin, and tone was high in all four limbs with clonus. Initial blood picture showed hemoglobin 9.4 gm/dL; hematocrit 28.0%; WBC 6,900/cm; neutrophils 64%; lymphocytes 26.5%; platelets 5,99,000/cm; prothrombin time 20.0/11.0; activated partial thromboplastin time 48.6/30.0; INR 1.97; sodium 136meq/L; potassium 5.7meq/L; chloride 114meq/L; bicarbonate 13.6mg/dL; BUN 6.0mg/dL; and creatinine 0.2mg/dL. A diagnosis of meningitis/encephalitis was made. Initial management included IV fluids, antibiotics (3rd generation cephalosporin), anticonvulsant, and supportive treatment. On second day of admission, the infant developed drowsiness and apnoea, child

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was shifted to Pediatric ICU, intubated and ventilated. During PICU admission, he had issues of low blood pressures for which he was kept on ionotropic support, low GCS, and consistently low platelets with hematuria which further complicated the course. Pediatric neurology team was consulted. EEG showed delta and theta wave slowing; CT scan brain was normal. Lumbar puncture was advised at admission, which was refused initially but later done after consent. CSF showed glucose 92 mg/dL, protein 131mg/dL, TLC 4/ HPF, and RBCs 300/ HPF. CSF culture did not yield any growth and CSF Herpes PCR was negative. However, dengue IgM turned out to be positive. Unfortunately baby continued to deteriorate with consistently low GCS. As there was no improvement in clinical condition, withdrawal of support was done on parents' request and the baby expired after 6 days of hospitalization.

Discussion

Encephalopathy in DHF is an atypical manifestation and may appear in various forms, including altered sensorium, convulsions, neck rigidity, pyramidal signs, headache, papilledema, myoclonus and behavioral disorders. The initial diagnosis in our cases was meningitis/encephalitis, but development of edema with flushing of extremities over the lower limbs with low platelets in case I and issues of low blood pressures, low GCS, increased protein in CSF, and low platelets with hematuria in case II raised the possibility of dengue viral infection, which was confirmed by positive dengue IgM antibodies. Both the infants had issues of extreme irritability, which is not typical of dengue fever. The severity of neurological disease caused by different dengue serotypes has been examined in a number of studies. Dengue serotypes 2 and 3 have primarily been reported to cause neurological symptoms.[4,7] Direct CNS invasion may occur by disruption of the blood-brain barrier at hemorrhagic foci or by mutation at a specific point in the envelope glycoprotein of the dengue virus allowing it to pass through the blood-brain barrier.[10,11] The exact mechanism by which dengue enters the brain needs further elucidations. The outcome of dengue encephalitis may be an uneventful recovery, neurological sequelae or death.[2,4-9]

Fatality in the second case could be attributed to very young age and DHF in association with encephalitis. Dengue encephalitis should be suspected in infants presenting with extreme irritability, clinical examination favouring CNS involvement in presence of normal CSF during dengue fever season. Early detection and hemodynamic support in such cases can be a lifesaving approach. CSF dengue IgM/PCR has higher diagnostic value as reported by other case reports.[1, 5] In our cases, positive serum dengue IgM along with other diagnostic criteria like platelets, hematocrit and clinical presentation, helped in diagnosis.

Conclusion

Dengue encephalitis should be suspected in infants presenting with extreme irritability, clinical examination favouring CNS involvement in presence of normal CSF especially those occurring during dengue fever season.

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CASE REPORT

Successful Treatment of Carbapenem-Resistant *Acinetobacter* spp. Meningitis with Prolonged Course of Polymyxin-B and Doxycycline

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Abstract

Post- neurosurgical multi-resistant *Acinetobacter* meningitis, generally treated with intrathecal +/- intravenous polymyxin, is a therapeutic challenge, hampered by spiraling treatment costs, difficulties in intrathecal /intraventricular drug delivery and possible biofilm formation on indispensable foreign devices (intraventricular drains or ventriculo-peritoneal shunts). We describe a child with carbapenem-resistant *Acinetobacter* meningitis and ventriculitis after partial removal of a midbrain pilocytic astrocytoma. Eradication of infection was achieved with prolonged combination therapy (intraventricular polymyxin and oral doxycycline).

Key words

Acinetobacter, Meningitis, Polymyxin B, Ventriculoperitoneal Shunt.

Introduction

Pediatric nosocomial meningitis is associated with insertion of foreign body devices, neurosurgical intervention, craniocerebral trauma, intrathecal chemotherapy and nosocomial bacteremia. Of these, 30% are attributed to *Acinetobacter* spp.[1] *Acinetobacter* meningitis is a therapeutic dilemma for physicians due to the emergence of strains resistant to multiple antibiotics including carbapenems. Polymyxins are increasingly being used to treat carbapenem-resistant strains but poor cerebrospinal fluid (CSF) penetration after intravenous drug delivery makes intrathecal drug administration necessary and adds to logistic difficulties. Though duration, dosage and efficacy of polymyxins in *Acinetobacter* meningitis are not established, it remains the primary drug of choice. Feasibility may dictate use of the intravenous route for drug delivery, even though the route is associated with poorer efficacy and CSF penetration compared to intrathecal administration. We report on our first experience in successful treatment of persistent nosocomial *Acinetobacter* meningitis in a patient having an indispensable ventricular device, with prolonged combination therapy (intrathecal polymyxin-B and oral doxyxycline).

Case Report

A 9-year-old girl was admitted for surgical removal of a posterior fossa tumor. Histopathology revealed a pilocytic astocytoma. Post-surgery, an external ventricular drain (EVD) was placed.

On 5th postoperative day, she developed fever and drowsiness. Intravenous ceftriaxone (100mg/kg/day) was initiated. Leakage around EVD site pointed towards tube displacement. Urgent reinsertion yielded straw colored CSF at high pressure (>30cm H_2O). CSF Gram stained smear showed Gram negative bacilli. Meropenem replaced ceftriaxone at 120 mg/kg/day in divided doses. Culture grew aminoglycoside- and carbapenem-resistant *Acinetobacter* spp sensitive only to polymyxin-B, and carbapenem-sensitive *Enterobacter cloacae*. Intravenous and intrathecal polymyxin-B were added to meropenem. The child had no fever or focal deficit at the end of first week of intravenous antibiotics. She was eventually discharged after completion of 3 weeks of intravenous meropenem and polymyxin, including one week of intrathecal polymyxin, and ventriculo-peritoneal shunt (VPS) insertion (Fig 1).

One week later, the patient presented to Emergency with abdominal pain and fever. CSF and VPS cultures grew *Acinetobacter* spp. An EVD was reinserted and intrathecal (50,000U qd) polymyxin-B was restarted.

Even though no subsequent CSF culture yielded *Acinetobacter* spp., repeated Gram stains intermittently showed Gram negative bacilli (Fig 1). Immediate signs of hydrocephalus on intermittent clamping of the EVD did not allow removal of foreign body throughout therapy. Major concerns over inadequacy of polymyxin-B monotherapy led to trial and error over various

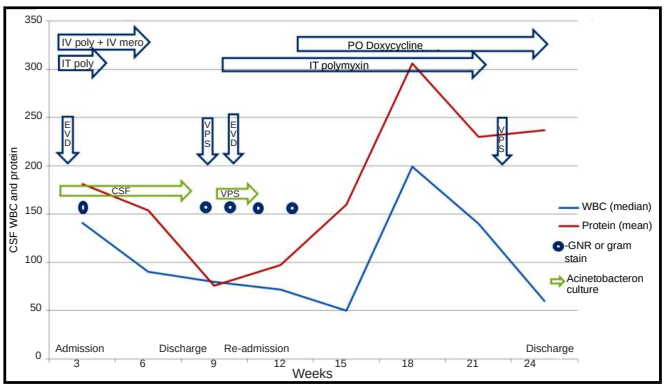

Fig 1: Clinical course of patient with multiresistant acinetobacter meningitis

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combinations (intrathecal polymyxin with rifampicin, high dose cefoperazone-sulbactam and co-trimoxazole). Eventually, CSF Gram stains normalized and cultures yielded no growth after adding doxycycline 100mg once daily (total of 12 weeks) to intrathecal polymyxin. The isolate was found to be tetracycline-susceptible after testing the drug due to reports of *in vitro* activity against *Acinetobacter* spp. The child remained well and relapse-free till 12 months post-discharge.

Discussion

Duration of therapy for *Acinetobacter* meningitis is based on Infectious Disease Society of America (IDSA) recommendation of three weeks for any gram negative meningitis. The optimal number of negative cultures to indicate successful eradication is not known even though three consecutive negative cultures drawn at separate occasions are often taken as indicator.[2]

Our case illustrates that three weeks of therapy may not be sufficient for eradication of *Acinetobacter* spp from the CSF especially when carbapenem-resistant strains and presence of foreign devices complicate matters. Intravenous route of administration has also been associated with treatment failures. Response rates may need to be further studied in animal models.

Another reason for a variable response rate to polymyxins may be a difference in activity of formulations available. Colistin (polymyxin E) and polymyxin B have different potencies, and previous responses to 3 weeks of therapy have been reported with colistin and not polymyxin B.[3]

Literature shows synergistic combinations of polymyxin B with rifampicin, azithromycin, sulfamethoxazole-trimethoprim and meropenem.[4] In our case, combination therapy with rifampicin, cefoperazone-sulbactam and sulfamethoxazole-trimethoprim in addition to intrathecal polymyxin failed to sterilize CSF. Recent reports have shown tetracyclines (doxycycline and minocycline) to be highly active *in vitro* against local MDR *Acinetobacter* strains.[5] However, their use is limited by toxicity, especially in the pediatric population. The addition of doxycycline to intrathecal polymyxin for a further 12 weeks was effective in our case. This *in vivo* effect advocates the use of polymyxin and doxycycline as synergistic agents despite lack of reports on *in vitro* synergism. This potential clinical synergism should be explored further in situations where non-CNS *Acinetobacter* infections are complicated by presence of foreign devices with biofilm formation.

Our patient despite prolonged polymyxin therapy did not suffer renal toxicity. Previous reports of colistin-related nephrotoxicity may have been due to understudied pharmacokinetics and higher doses. This case shows that although clinicians should be alert to nephrotoxic potential of polymyxin-B, its actual incidence in the pediatric population may be low.

Conclusions

Shunt-associated multidrug-resistant *Acinetobacter* meningitis may require more than standard 21 days of therapy recommended by the IDSA. Therapy may require addition of a synergistic agent to polymyxin B. Doxycycline appears to be a good therapeutic option but the combination needs more in-depth *in vitro* and *in vivo* analysis.

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