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Biohazard symbol for all laboratories dealing with infectious material.

Laboratory-Acquired Illnesses, Accidents, and Incidents: Turning a Negative into a Positive

Life scientists and biosafety professionals have a critical role and responsibility in preventing occupational exposures that could lead to a laboratory-acquired illness (LAI), as well as preventing the release of pathogens outside the laboratory environment where they could present a hazard to public health or the environment. When accidents occur these individuals also have the responsibility to report to their respective management chains that an accident or illness has occurred. Institutional leadership must ensure that a mechanism is in place to investigate incidents and accidents, identify and implement corrective actions as well as having mechanism to communicate back to affected employees on actions taken. At the national level the governments should consider developing both mandatory and voluntary reporting and analysis mechanisms for LAIs and other laboratory incidents.

The founding principle of biological safety is the promotion of safe laboratory practices, procedures, and the proper use of containment equipment and facilities by workers in the life sciences to prevent occupationally acquired infections or release of organisms to the environment. Laboratory accidents that result in LAIs are clear indicators of the hazardous nature of working with biological agents, and could point to procedural errors or equipment failures (including design flaws) and hazards associated with ongoing work.

Any LAI is always an unfortunate and, in some cases, tragic event. However, when LAIs do occur, laboratory managers, biosafety professionals, and senior leaders of the institution have the solemn responsibility to determine, to the best of their abilites, the cause(s) of the events leading to the exposure and subsequent illness, and implement the necessary actions to prevent future occurrence to the extent possible.

Unfortunately, as many case studies and reviews of LAIs have shown, determining the exact exposure event can be a challenge, particularly if it does not occur in conjunction with a known accident or other incident (e.g., a lapse in good biosafety technique or equipment failure).

Institutions that have experienced LAIs should be encouraged to share such information and any lessons learned with the life science community, by describing these cases in scientific journals or reporting them in another accessible resource. Sharing the nature of the incident, its probable cause, and lessons learned with relevant stakeholders would allow the benefit to be derived from an unfortunate event. Historically, and to the present, it has been a challenge to document information on LAIs and other laboratory incidents because in many cases surveillance data are difficult to collect due to subclinical infections; and institutions may be reluctant to report LAIs or other incidents for fear of negative repercussions (regulatory action, loss of funding or negative press) upon the institution.

Historically speaking, the epidemiological review of laboratory infections had a slow start. The first case of typhoid in a laboratory worker occurred 30 years prior to the first survey of laboratory infections. In 1915, Kisskalt sent a questionnaire to "numerous colleagues" in Europe, and collected information on 50 cases of laboratory-acquired typhoid fever dating back to 1885; there were 6 deaths.[1] The mode of infection was known in 23 cases, and mouth pipetting was the cause in 16 of those. In 1929, Kisskalt reviewed 59 more typhoid cases and 24 additional LAIs due to other infectious agents.[2] Again, ingestion through a pipette was the most common means of infection. In 1918, Austrian physician F. Reinhardt described 21 different pipetting devices. He stated in his paper, "With the aid of the devices described, it is possible to work more quickly than with the oral pipette."[3]

It is interesting to note that, as a biosafety professional who started his career in the mid-1980s, on a few occasions I have had to discourage the unsafe practice of mouth pipetting, 70 or more years after it was identified as a known problem, and after numerous types of mechanical pipettes had become readily available.

Between 1949 and 1951, S. Edward Sulkin and Robert M. Pike conducted the first U.S. Public Health Service-funded investigation of LAIs.[4] They contacted 5,000 laboratories with the goal of disclosing unreported infections in the U.S. resulting from laboratory work; only half responded to the survey. One of the more interesting findings of this study was that only 35 percent of the 1,342 cases captured in the survey had previously been acknowledged in any publication, which potentially denotes a reluctance or indifference by institutions to report LAIs.

Three additional collective studies by Pike and Sulkin identified a total of 4,079 LAIs caused by 159 agents (10 of which accounted for greater than 50 percent of total LAIs); 168 of these cases resulted in death during the period beginning in 1930 to 1978.[5] Other valuable reviews between 1979 and 2011, such as those by Sewell (1995), Harding and Byers (2000), and Weinstein and Singh (2009) continue to add to this body of knowledge.[6-8]

The study by Harding and Byers is of particular interest; they identified 1,267 overt infections, including 22 resulting in death (5 of these were aborted fetuses due to maternal infection).[7] The authors were also able to identify additional 663 cases of subclinical infections. One of the commonalities illustrated by all of these studies, beginning with Kisskalt and continuing

through today, is that determining the risk of developing an LAI is difficult due to the lack of a systematic reporting mechanism at the local, regional, national, or international levels. If LAIs and other incidents were routinely reported, it would be more likely to better identify agent risks as well as common contributing factors such as procedural errors, insufficient training, equipment related issues, etc.

The value of LAI information in Risk Assessment

The information derived from reports of known LAIs and the exposure events that preceded them is most valuable when conducting a risk assessment for work using similar agent, similar equipment, or similar procedures.

For example, a recent, fatal LAI with a pigmentation-negative (pgm-) attenuated *Y. pestis* strain (KIM D27) involving a University of Chicago researcher can provide some useful information as it relates to risk of working with attenuated agents.[9]

Although a specific exposure event was not identified in this case, deficiencies in biosafety practices based upon co-worker interviews, including the inconsistent use of gloves, could have resulted in inadvertent transdermal exposure even though aerosol exposure could not be ruled out. The pathologic findings were consistent with septicemic plague, but did not reveal evidence of pneumonic plague. Although this case underscores the importance of following good biosafety practices even when working with attenuated agents, more importantly it emphasizes the need to consider the researcher's health status prior to conduct of a research protocol and during the course of the work.

In this case, the victim, a 60-year-old male with insulin-dependent diabetes mellitus,was diagnosed with hereditary hemochromatosis on autopsy. The authors of the MMWR report indicated that a "possible explanation for the unexpected fatal outcome" was that hemochromatosis-induced iron overload might have enhanced the virulence of the infecting KIM D27 strain by compensating for its iron-acquisition defects. This explanation is supported by studies indicating that the virulence of pgm-negative *Y. pestis* strains can be enhanced by the simultaneous injection of iron into experimental animals. The lessons learned in this case are that workers should self-report medical conditions that could put them at increased risk, and that an institution, as part of staff training, should identify medical conditions (such as diabetes mellitis, hemochromatosis) that could place workers at increased risk when working with specific agents.

Reporting and analyzing LAIs and other laboratory accidents at the institution level provides useful information about improving laboratory safety. By understanding why an exposure event occurred,it should be possible to prevent similar incidents in the future.

Another goal is to develop a resource for generating information about LAIs, accidents, and incidents and sharing lessons learned. In addition to known accidents, incidents, and LAIs, institutions should also be encouraged to record, analyze, and take appropriate corrective actions for incidents and reported unsafe conditions that, fortunately, have not yet caused an injury or illnesses. Definitions of what constitutes a laboratory incident differ. An incident can be defined as an unsafe occurrence arising out of or in the course of work where no injury or illness is caused, or an unforeseen upset condition. Yet another definition I have heard many times is that an incident is an accident that, by sheer dumb luck, did not result in an injury or illness.

All individuals who are involved directly in or who support research or diagnostic activities involving biohazards need to learn from past accidents and incidents in order to minimize or eliminate the possibility of future occurrence. Institutions need to ensure that laboratory safety is an institutional value, and that employees actively participate in developing and engaging in safe and responsible laboratory practices. One of the key aspects of employee involvement is that they can report accidents, illnesses, and unsafe conditions without fear of reprisal. Another aspect of maintaining employee participation is that the employer has in place processes to identify, document, and communicate corrective actions in a timely manner. Without this management and employee partnership, establishing a lasting organizational culture of safety would be difficult to achieve.

Beyond the Institutional Level: Laboratory Incident, Accident, and LAI Analysis and Reporting Systems

The expansion of laboratory capacity for life science research and diagnostic activities over the past decade, especially the expansion of high and maximum containment facilities worldwide, has raised awareness of the public and policy makers to the potential for worker exposure and accidental release of harmful biological agents into the environment.

In the United States, this awareness has resulted in numerous reports by various entities, both public and private, focusing on risks posed by research and diagnostic facilities, especially those that are working with high-risk biohazards (e.g., Transfederal Taskforce Report, GAO-10-850).[10,11] Some of these reports discuss the need for a centralized system for incident reporting, analysis, and information-sharing, which would be a valuable resource for scientists, biosafety professionals, and medical professionals in identifying laboratory-associated risks.

Similar to other nations, the United States has in place several mandatory accident- and illness-reporting requirements that have been codified by various Federal Agencies such as the Occupational Safety and Health Administration (OSHA), U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS), Centers for Disease Control and Prevention (CDC), and National Institutes of Health (NIH). These mandatory

systems either encompass a small subset of high-consequence biological agents (such as those identified by the CDC/APHIS Select Agent Program), or illnesses that can be associated with a known exposure event. Therefore, only a fraction of biological laboratory incidents are reported and many valuable lessons learned are never reported or shared with the life science community.

The solution to this problem, both in the United States and in other nations, would be the establishment of a nationwide, voluntary, incident-reporting system coupled with a centralized, integrated mechanism for analyzing incidents and sharing information and lessons learned from both mandatory reports by a nation's regulatory authorities and the voluntary reporting system.

Such a system would be able to utilize data from existing mandatory and voluntary reporting systems without duplication of efforts, and provide the ability to readily communicate lessons learned to the scientific and regulatory communities as well as to other relevant stakeholders.

Of course, there are also a number of hurdles one would have to consider in developing such a system, such as clearly defined terminology (what is an incident versus an accident, etc.), various reporter protection issues (ensuring anonymity, confidentiality, and de-identification of data), and ensuring that the information is accessible in a usable format to all relevant stakeholders.

Other industries (aviation, nuclear power, and patient care) have been successful in implementing such safety reporting systems to great advantage, such as the early detection of equipment failures and procedural errors, suggesting codes of best practices, and identifying potential oversight gaps. Therefore, it is high time that the life science community turns the "negative" of a laboratory incident or accident into a "positive" by embracing the concept of safety incident-reporting and analysis systems at the national level, with the eventual goal of national systems feeding into an international incident-reporting repository.

The views expressed in this article are solely those of the author and do not reflect the views or official position of the U.S. Government.

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Biosafety Curriculum for Post-Graduate Microbiology Students at a Pathology Institute

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Preface

Armed Forces Institute of Pathology (AFIP) is a referral diagnostic, academic and research institute. It has played a leading role in imparting post-graduate training in various disciplines of pathology including histopathology, microbiology, chemical pathology, hematology, virology and immunology. Fellowship, MPhil and PhD students not only from the Armed Forces but civilians as well undertake well defined and structured training in respective specialties.

Fellowship training is conducted under the prerequisites of College of Physicians & Surgeons Pakistan (CPSP), after clearing FCPS-I in Pathology. It comprises of fours years training under CPSP recognized Supervisor. During first year rotational training is conducted through various disciplines including: clinical pathology, virology, haematology, chemical pathology, histopathology and immunology.

The postgraduate trainees (PGs) in microbiology undergo a structured program comprehensively covering theoretical and practical aspects. Somewhat neglected aspect of the curriculum in the past had been inadequacy in biosafety training and whatever the PGs were learning was mainly through personal observation or experience.

Key Words

Biosafety, Biosecurity, Curriculum,

Rationale

The purpose of this document is to introduce and implement structured advanced training in biosafety for postgraduate students (FCPS – II) in microbiology in year 2 after the initial concept and introduction during rotational period of year 1. Subsequent to approval by the academic council of AFIP and Advisor in Pathology, it will be forwarded to CPSP, after necessary authorization the curriculum will be formally inducted.

Health Care Problem

Negligence in biosafety may lead to laboratory associated infections (LAIs) and in turn to laboratory staff and colleagues, and ultimately spread outside as well. The infectious agent could be of high pathogenic significance.

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Importance of the Problem

The qualitative criteria includes; deterioration in maintaining requisite microbiology laboratory biosafety standards along with professional obligations. Quantitatively the magnitude is directly proportional to inapt attitude and inversely proportional to awareness about biosafety and implementation of rules and regulations.

Literature Review

Needs assessment is done by details of available data required to evaluate qualitatively and quantitatively the issues pertaining to biosafety in laboratories. Thorough literature search has been done and discussion done with concerned.

Biosafety in simple words is the exercising of protection of self, staff and environments while working with infectious agents in the laboratory. Biosecurity entails the safety measures taken to ensure the proper storage of micro-organisms so that they may not fall in to precarious hands for any malicious use. The curriculum content about microbiology has been very effectively explained by Smith *et al*, describing the levels of skills as well.[1] This concept has been adopted while formulating the biosafety curriculum. Attitude and dedication are requisite part of behaviour while working in high containment laboratories, this mandates hands-on training.[2] Workforce Florida has very nicely incorporated concepts and techniques mandatory for laboratory safety and biohazards in to the curriculum.[3] Fascinated by their concept, short duration intense training has been adjusted in our curriculum. Zhao *et al* nicely explained the biosafety curriculum at the under-graduate level.[4] Concept of advanced educational biosafety programme along with training modules has been elaborated by Pobedimskaya *et al* (Russia).[5] The point to understand is that currently, there is a passionate international effort to introduce the subject as part of curricula both at graduate and post-graduate levels for the concerned specialties. In Pakistan, efforts have also been initiated at various levels for incorporation in to the syllabi.

Expert Role

Need to play an imperative role in this curriculum drafting as well as implementation, being a specialist in biosafety. Presently, there is dearth of specialists in our country in this challenging field. There is a definite need to train PGs in this evolving branch related to life sciences. Requisite qualifications and expertise in biosafety as well as academic experience, entrust

the concerned to play a leading role. Presently, there is no published curriculum in biosafety in Pakistan, so whatever is defined, will definitely have major contribution in future.

Educational goals

Overall - By the end of fellowship training in microbiology, PG students will be able to display biosafety practices while working with microorganisms.

Aims and objectives

The primary learning objectives of training in biosafety will address six competencies and two skills as defined in detail below:

Medical Knowledge

- Demonstrates basic knowledge of biosafety in Microbiology/Virology Laboratory.
- Meaningfully integrates and evaluates the problems for not abiding to biosafety measures.
- Presents and analyzes the fundamental knowledge of biosafety in relation to laboratory associated infections.
- Becomes familiar with basic techniques of biosafety.
- Participates in scholarly activities including presentation in clinicopathological conferences, multidisciplinary activities, participation in conferences and publications.

Practice-based learning and improvement

- Understands essential components of different biosafety levels.
- Practices safe methods.
- Evaluates the importance and adequacy of details available with infectious samples.
- Recognizes needs for personal protective equipment.
- Demonstrates knowledge of biosafety and biosecurity, and rules and regulations both national and intenational.
- Demonstrates knowledge of basic safety precautions and universal precautions against infectious agents.
- Demonstrates efficiency in packing and transport of infectious material.
- Effectively utilizes library resources, internet and relevant texts to update knowledge.

Community (Patient) Care

- Evaluates biosafety measures regularly.
- Thoroughly reviews biosecurity practices.
- Effectively assesses risk.
- Effectively documents any laboratory accident.
- Effectively restricts the entry of unauthorized personnel in lab.

Interpersonal and communication skills

- Effectively communicates with colleagues and clinicians.
- Effectively teaches junior students, lab staff and health care workers.
- Interacts effectively with administration, staff and laboratory technicians.
- Appropriately uses electronic information systems.

Professionalism

- Adheres to standing operative procedures (SOPs) and regulations.
- Accepts responsibilities for actions, admits omissions and mistakes.
- Maintains log of stored bacteria.
- Recognizes personal limits and need to consult others.
- Works effectively with other members of team, demonstrates courtesy, respects their views.
- Engages in continuing professional development.
- Implements improvements in response to feed backs
- Understands and complies with institutional biosafety policy
- Accepts responsibility and follow through tasks.

Systems – based practices

- Participates in institutional tutorials, in-house discussions and forum meetings for PG education, management and quality assurance.

Skill levels

The skill level will be divided in to two; level I for limited rotational period during first year of PG training and level II denotes competencies covered at advance level of training in year 2 of the fellowship.

Skill Level I

- Demonstrates proficiency in basic biosafety skills.
- Demonstrates knowledge of the essential components of biosafety.
- Demonstrates competency in working with biosafety cabinets.
- Knows the procedure for incidence reporting of any untoward event like accidental spills, pricks or injuries.

Skill Level II

- Demonstrates knowledge of the common situations requiring biosafety controls.
- Be able to independently work in biosafety cabinets type 2 & 3.
- Demonstrates knowledge about certification of cabinets.
- Demonstrates knowledge of requirements pertaining to retention of pathology specimens and records.
- Demonstrates proficiency in handling, processing and transport of infectious specimens and lab animals.
- Demonstrates knowledge of web-based or organization relate learning and CME tools in biosafety, whenever possible.
- Demonstrates full ability in disinfection and sterilization and capable of checking autoclaves.
- Demonstrates ability to train juniors and lab technicians in biosafety related issues.
- Demonstrates knowledge about biosecurity, management of stored microorganisms.
- Demonstrates the responsibility for oversight at personal level.

Targeted Need Assessment for Biosafety Curriculum

Learner

Postgraduate fellowship trainees in microbiology will be targeted. Previous knowledge about biosafety will be assessed as baseline. The teaching methods will be student centered and will cater to all learning styles.

Targeted Environment

- Teaching faculty.
- Infrastructure would include demonstration room, simulation room, laboratory with biosafety cabinet, and library with electronic access. Audiovisual equipment would be required.
- Concerned include Commandant AFIP, Head of Academic Division, Program Director, faculty, Accreditation Bodies (CPSP), Institutional Biosafety Committee, Biosafety Officer and mentors.
- Modes include directed learning, group discussion, forum, tutorials, video and practical demonstrations; feedback and assessment would be done.
- Stakeholders include:
  - PGs,
  - Laboratory Technicians,
  - Course Directors – appraisal, induction & faculty
  - Faculty – insight and development,
  - Administrators – resources, community
  - Accreditation bodies - community requirement

Information Methods

Previous data/baseline knowledge will be gathered through questionnaire for PGs for piloting. Feedback and assessment will be a regular feature for improvement.

Educational Strategies

Effective modes of teaching will include directed learning, focus/small group discussion, lectures, role models, video and practical demonstrations, seminars and workshops; matching with objectives has been depicted in the tables 1 to 3. Curriculum will include strategies that promote practice based learning and improvement. Reflections and self study will also be encouraged. These methods are feasible and within resources. The proposed organogram is depicted in figure 1 and curriculum map in figure 2. Table 4 shows the workshop to be conducted.

Learners Outcomes

By the end of year 2, each PG would be able to identify and demonstrate (KSA) issues related to biosafety as depicted in tables 1 to 3.

Process

By the end of year 2, PGs will have participated in:

- Lectures 12 (1 hr duration, once per month): Monday, first week.
- Tutorials 12 (1 hr duration, once per month): Thursday, last week.
- Small Group Discussions 20 (90 minutes duration, fortnightly): Wednesday.
- Simulated situations 4 (once per quarter): Friday.
- Demonstrations/Practical 30 (90 minutes, weekly): Tuesday.
- Self study 2 hours duration every Saturday.

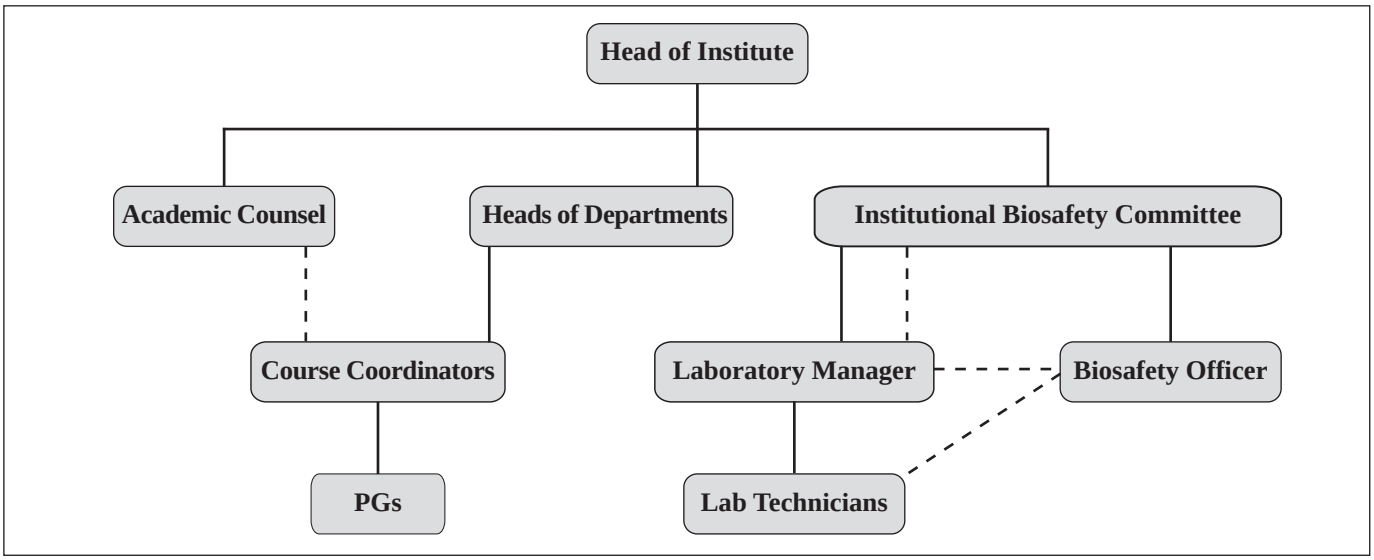
Program Process

By the end of year 2, 90% students will attend lectures x 10 (once per month), simulated situations x 4 (once per quarter), demonstrations x 26.

Implementation

An important aspect for successful launching is appropriate budgeting. Most of the facilities including infrastructure already exist, some of the additional expenses would include:

Figure 1: Proposed Organogram



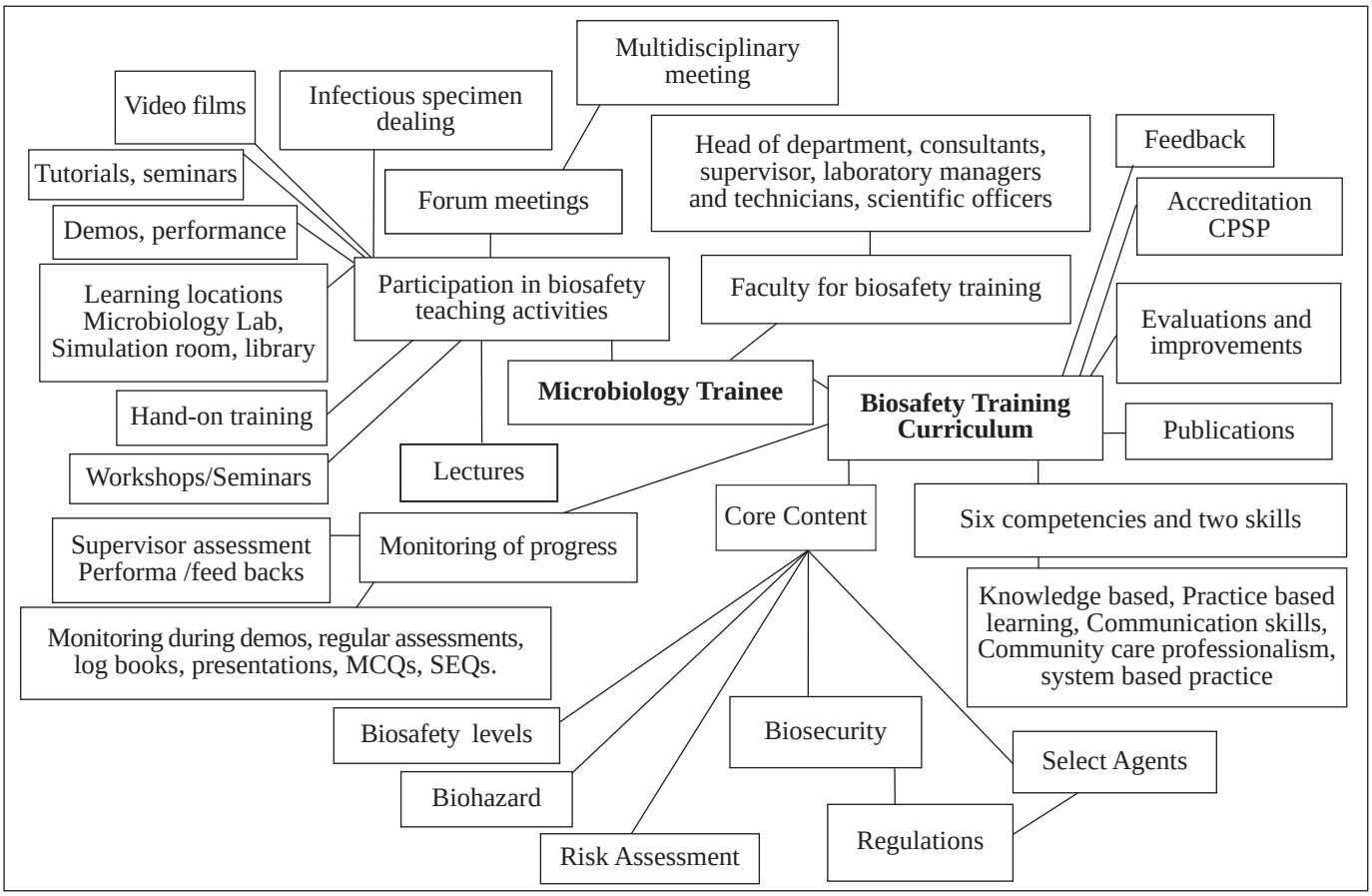


Figure 2: Curriculum map for biosafety training in microbiology

Table 1: Biosafety training (intended learning outcome)

By the end of year 2 PGs must have adequate knowledge & understanding of:
1. The pathological basis of infectious diseases and aerobiology.
2. Genetically modified organisms.
3. Potential hazards with laboratory practices.
4. Different biosafety levels and accordingly usage of biosafety cabinets.
5. Laboratory design and equipment.
6. Laboratory commissioning and certification.
7. Risk assessment & management.
8. Use of personal protective equipment.
9. Infectious waste management.
10. Transportation of infectious material.
11. Sterilization & Disinfection.
12. Animal Biosafety.
13. Rules and regulations regarding biosafety.
14. Code of conduct for life scientists.
15. Contingency plan.
16. Regulatory aspects of health and safety issues.
17. Health and medical surveillance including vaccination.

- Training of faculty through workshops (40,000 x 4) Rs 1,60,000
- External facilitation Rs 1,20,000
- AV Equipment Rs 1,50,000

Support

Support would be required from Commandant, Head of Department and Academic Division, Institutional Biosafety Committee, Curriculum Committee, Faculty and PGs internally, and CPSP externally for successful accomplishment of the targets. Administrative mechanisms will include communication, periodical meetings all levels, feedback and involvement of stakeholders through mechanisms like syllabus communication, website usage, regular memos, visits and evaluation of faculty and PGs.

Pilot Phase

Plan to introduce pilot phase would be carried out on existing year 1 PGs, phase in on new inductees and full implementation in a spiral manner.

Evaluation and Feedback

Curriculum evaluation users would be PGs in Microbiology,

Table 2: Knowledge and Professional Skills (Psychomotor)

By the end of year 2 PGs will be able to:	
Objectives	**Teaching strategies**
Advise proper usage of personal protective equipment and type of safety cabinet.	Small group discussions, Tutorials, Demonstrations
Interrogate any accidental spill of hazardous material.	Small group discussions, Forum meetings, Tutorials, Self Study of LAI cases, Guest Speakers
Identify issues to be addressed by the cross-infection.	Lectures, Small group discussions, Demonstrations during training, Tutorials
Describe sterilization and disinfection processes.	Lectures, Demonstrations, Practical work, Audio visual, Guest speakers, Workshop
Describe working of different safety cabinets.	Small group discussions, Demonstrations during work, Audio-visuals
Work in biosafety cabinet in required standard manner.	Hands-on training, Demonstrations , Audio-visual, Workshop

Table 3: Affective Domain

By the end of year 2 PGs will be able to:	
Objectives	**Teaching strategies**
Appreciate the importance of accuracy and requirement for attention to handle infectious material.	Small group discussions, Lectures, Forum meetings, Self Study of LAIs
Be aware of the different types of specimens and aerobiological affects.	Small group discussions, Audio visuals, Tutorials, Self Study
Be responsible to report any accident, spill or prick.	Lectures, Role models, Demonstrations, Tutorials, Reflection
Liaise with clinical colleagues in order to obtain clinical information prior to dealing specimens.	Small group discussions, Tutorials, Forum meetings
Explain colleagues regarding PPE and disposal of infectious waste.	Small group discussions, Tutorials, Self study, Forum meetings
Take an active interest in safe working practices for staff.	Lectures, Small group discussions, Tutorials
Caution in transporting the infectious specimens.	Demonstrations, Small group discussions, Tutorials, Workshops
Maintain proper record of stored microorganisms.	Small group discussions, Tutorials

Curriculum Developers, Program Director, Faculty, Dean, Administrators and Accreditation Body like CPSP. Formative and summative needs from individual and programme's point of view will be evaluated. Resources are available in the form of time, personnel, funds and equipment.

The evaluation design for the biosafety curriculum will be Single Group pretest & posttest for objectives O_1 --- X --- O_2. It will be feasible, cost effective, and can be applied to evaluate all the three domains (KSA), performance and perceptions.

Critical Evaluation Questions

Critical evaluation questions include: what percentage of PGs

Table 4: Biosafety Workshops

Workshops	Duration	Topics
First quarter	1 week	Theoretical basis Biosafety levels Biosafety cabinets Certification
Second quarter	2 weeks	"Hands-on training" Concentration on the issues of infectious material, dealing, aerobiology & PPE. Sterilization & disinfection Animal Biosafety
Third quarter	1 week	Biosecurity Risk assessment & management
Fourth quarter	1 week	Proper transportation Infectious waste disposal

is able to identify issues related to biosafety, and what percentage of the students is able to practically demonstrate biosafety procedures?

Method of Evaluation

Method of evaluation will include self assessment questionnaires, assessment (cognitive, affective and psychomotor) during practical and demonstrations, group discussions and feedback, perceptions and suggestions for improvement. These will be congruent with the objective questions, feasible to construct and implement in available environments, valid & reliable.

Data Collection Process

Curriculum Coordinator and nominated members of the faculty will collect the data to minimize the use of resources and maximize response rate. Data collection will be integrated in to meeting time. Data collection considerations will not influence the design of measurement instruments.

Data Analysis

Administrative assistant/statistician under the supervision of the Curriculum Coordinator will collate and perform required descriptive statistics.

Evaluation Report

Collated evaluation results with simple descriptive statistics will be compiled and distributed annually by the Curriculum Coordinator to the Program Coordinator shortly after completion of year 2 for using it in future planning and improvement in the following year's program. Copies will be sent to Commandant and further to CPSP.

Suggested Reading Material for Students

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Conclusion

To conclude this is a comprehensive curriculum for biosafety for the concerned post-graduate students. Concerted efforts will be launched for implementation and imparting proper training. The outcome will be thoroughly analyzed for any modification and better outcome.

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Pattern of Dengue Fever in Different Weather Conditions of Karachi

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Abstract

Objective
The purpose of this study was to find the relationship between weather conditions (temperature, humidity, and precipitation) and frequency of occurrence of dengue fever in Karachi from 2005 through 2009.

Methodology
This descriptive epidemiological study was conducted from April to June 2010. Weather data of Karachi (temperature, humidity, precipitation) from 2005 to 2009 was obtained from Pakistan Meteorological Department, Karachi, whereas dengue fever patients' data was collected from Provincial Dengue Fever Surveillance Cell, Ministry of Health, Government of Sindh. Correlation between occurrences of cases of dengue fever during understudy 5 years with variations in weather conditions was analyzed to find any relationship.

Results
A significant relationship existed between weather and frequency of dengue fever. There were 77% cases of dengue fever occurring in September-November period when humidity is around 80% and temperature 30-32ºC. Preceding months of July and August are usual recipient of monsoon rains.

Conclusion
The ambient temperature, humidity and breeding areas post monsoon results in increased mosquito activity with consequential higher incidence of dengue cases.

Key words
Aedes aegypti, Dengue Fever, Dengue Haemorrhagic Fever, Dengue Shock Syndrome.

Introduction

The importance of climate change and global warming has been realized by the scientific world.[1] It is a widely held view that these environmental changes adversely favor infectious diseases. Fluctuations in climate like temperature, precipitation, humidity have been linked with biological organisms and spread of infectious diseases.[2] Changes in seasons and climate may modify the distribution of important vector species and may increase the spread of disease to new areas. It is important to increase our knowledge of present climate – health relationship, particularly the seasonality of diseases since this will help in focusing our efforts towards limiting factors of diseases.

Over the last few years dengue infection has become a major international health problem. The World Health Organization estimated that more than 2.5 billion people were at risk of dengue infection.[3] Every year about 50 -100 million cases of dengue infection, 500,000 cases of Dengue Hemorrhagic Fever (DHF) and at least 12,000 deaths occur worldwide.[4] Presently dengue is endemic in 112 countries around the world.[5] Among all the viral diseases that spread through arthropods, dengue fever is the most important emerging disease of the tropical and subtropical regions, affecting urban and periurban areas.[6] During last few years Pakistan has emerged as a region of endemic dengue activity, the endemicity having advanced westward from India.[7,8] The effect of global warming, poor living conditions, intense and prolonged monsoon, rapid urbanization, frequent travel and lack of controlling measures contribute to the spread of mosquito vector driven infections.[9]

First major outbreak of dengue fever in Pakistan was reported in 1994-95.[10] An unprecedented increase in epidemic of dengue fever in the country occurred during the year 2005-2006, with a large number of cases reported from Karachi.[11]

The virus is transmitted to susceptible humans by bites of *Aedes aegypti* and *Aedes albopictus*, mosquito species found worldwide.[12-14] Significant increase in the mosquito larval population is seen during the rainy season. This may be a reason why the epidemics of dengue tend to coincide with the rainy season.[15]

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Methodology

This descriptive study was carried out from April to June 2010. Dengue fever patients' data from year 2005 to 2009 was collected from Provincial Dengue Fever Surveillance Cell, Ministry of Health, Government of Sindh. Only confirmed cases of dengue fever, dengue hemorrhagic fever and dengue shock syndrome that were admitted in hospitals of Karachi during the five year period were included in this study. All cases were classified according to the month of occurrence.

Weather data was obtained from Pakistan Meteorological Department, Karachi. It comprised of mean temperature and mean humidity of each month, along with monthly rainfall from 2005 to 2009. Association between occurrence of dengue fever during these 5 years with weather conditions (temperature, humidity, rainfall) was matched to find out any relationship.

Results

Weather parameters (temperature, humidity, precipitation) for each month from the year 2005 to 2009 as obtained from Pakistan Meteorological Department, Karachi are shown in figure 1 to figure 5. Month-wise occurrence of confirmed and combined cases of dengue fever, dengue hemorrhagic fever and dengue shock syndrome admitted in various hospitals of Karachi during the same period are represented in same graphs, whereas figure 6 shows association between the frequency of dengue fever and mean weather condition from 2005 to 2009.

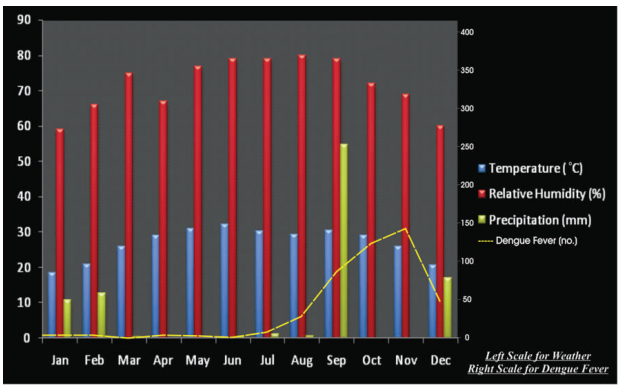

Fig 1. Correlation of dengue fever with weather parameters in 2005.

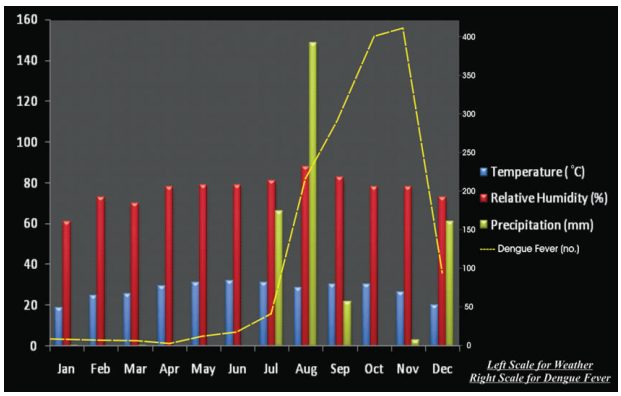

Fig 2. Correlation of dengue fever with weather parameters in 2006.

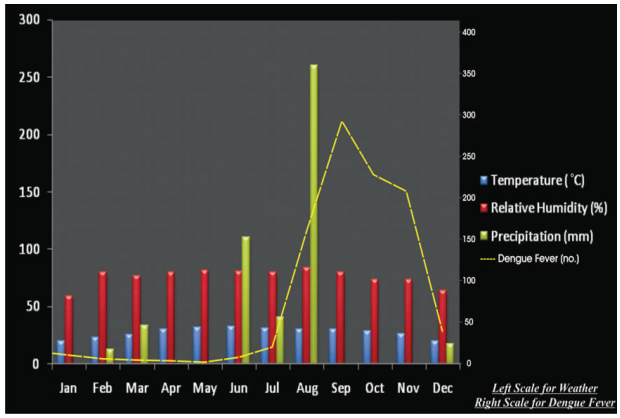

Fig 3. Correlation of dengue fever with weather parameters in 2007.

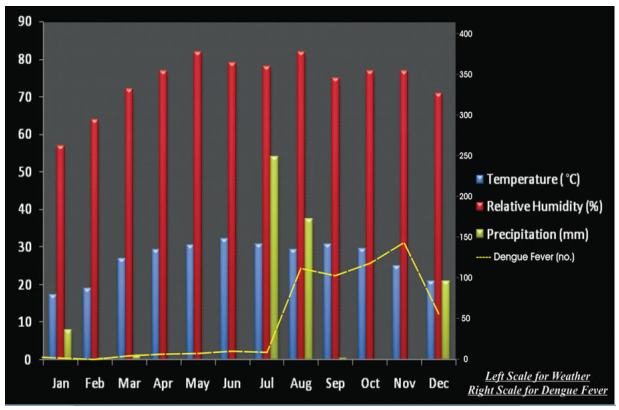

Fig 4. Correlation of dengue fever with weather parameters in 2008.

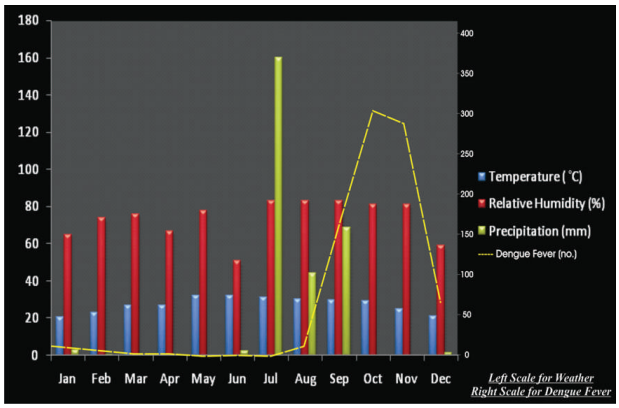

Fig 5. Correlation of dengue fever with weather parameters in 2009.

Discussion

Dengue fever is an acute infectious disease caused by an

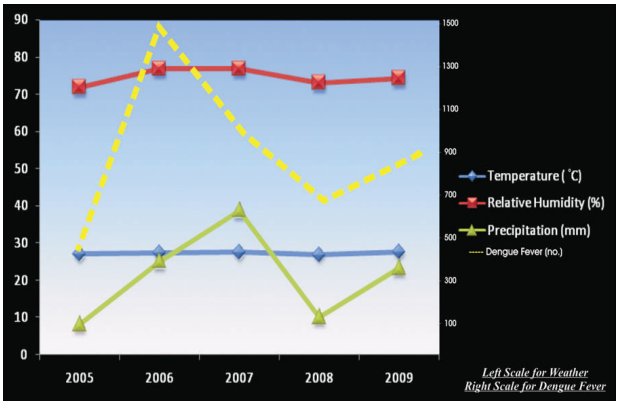

Fig 6. Correlation of mean weather conditions (2005-2009) with frequency of dengue fever.

arbovirus in the Flavivirus genus. There are four viral serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). Infection with one serotype of dengue confers life long immunity against further infection with that serotype, but no heterologous protection against other serotype.[2] Indeed, evidence suggests that second or subsequent infections with other dengue serotypes are significantly associated with more severe clinical manifestations.

Incubation period of dengue fever is 3-14 days and manifestations range from a sudden flu like illness with considerable myalagia or arthralgia, with or without rashes, intense headache lasting 3-7 days.[16] Dengue infection may become severe and at times fatal, characterized by hemorrhage and/or shock, known as dengue hemorrhage fever and dengue shock syndrome respectively. [17]

It is evident from the study data that most of the cases of dengue fever occurred in months of September through November. During understudy five years, a total of 4251 cases of dengue fever were diagnosed among admitted patients in different hospitals of Karachi. Among total patients, 77% cases occurred during three months from September to November. Only 112 cases (2.63%) were diagnosed during five months period that is from January to June in five years.

The emergence of one generation of *Aedes aegypti* usually takes about 15 days at 27ºC and extrinsic incubation period *in vitro* for dengue type 2 is 12 days at 30ºC.[2] Diagnostic dengue IgM antibodies becomes positive in 6 to 10 days after the onset of illness.[9] It takes minimum of about 35 – 40 days that a mosquito is able to cause a diagnosable disease of dengue fever in human beings. As we can see from data of precipitation that July and August are the months when Karachi receives most of its monsoon rains. This rain helps in breeding of vector which is then responsible for majority of the cases during September to November period. Early rains in June 2007 caused significant number of dengue cases (167) in August 2007. Temperature of around 30º-32ºC and humidity around 80% during July-September period served to propagate vector in spreading the disease. This finding is in line with findings of a national seroprevalence survey undertaken in Mexico in 1986 that pin pointed temperature as a key predictor of dengue transmission.[2] The authors found that median temperature during the rainy season strongly predicted dengue infection; a four fold increase in risk of infection was observed between 17ºC and 30ºC. Increase in temperature accelerates vector's metabolic activity and *Aedes aegypti* then needs to feed more frequently.[16] Their biting rate increases thus escalating the spread of infection.

The mean monthly temperature of Karachi city remains high during May to September period, favouring increased mosquito activity. Similarly, maximum humidity in Karachi is encountered during June-September period and monsoon rainfall is more evident from July to August. These factors prolong survival of most of the arthropods, help in mosquito growth and increased activity resulting in higher incidence of dengue cases during these months.

High incidence of dengue fever during August 2007 may be due to earlier rains that year. These findings are consistent to dengue epidemic in Chandigarh, India.[18] Similar findings were earlier reported in Karachi 2006.[19] Studies and observations elsewhere showed that higher rainfalls would wash out breeding pools, thereby decreasing mosquitoes population.[2] *Aedes aegypti* are adapted to urban environments, breed in water containers and relatively unaffected by precipitation.[3]

Present study involved data of only hospitalized patients in Karachi. Dengue cases consulting family physicians or seeking alternative treatment through homeopaths, hakeem (herbal healers), and traditional healers were not included in this study. Similarly, some of the patients included in this study were brought to Karachi from other parts of the country. As the diagnostic criteria for the confirmation of the cases of dengue fever was IgM, performed after minimum 7 days of onset of clinical symptoms, possibility of missing the late responders could not be excluded.[20] Nevertheless the data reflected trend in frequency of dengue cases which had quite significant relationship with weather.

Frequency of dengue fever outbreaks is likely to increase in Karachi because of favorable weather conditions, high indices of mosquito vectors and high prevalence of cases. Effective prevention and control of disease should include mass awareness programs, insecticidal sprays/fumigation especially after monsoons, filling of areas with pooled water, continued medical education for family physicians to recognize signs and symptoms of the disease, availability of facility of dengue virus isolation by cell culture and typing by reverse transcriptase polymerase chain reaction.

Acknowledgement
Would like to thank Pakistan Meteorological Department,

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ORIGINAL ARTICLE

Clinical Profile and Management of Dengue Fever at a Tertiary Care Hospital in Pakistan

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Abstract

Introduction
The first outbreak of dengue viral fever (DVF) in Pakistan was detected in 1994-95 and second in 2005-06. Since then, it has become endemic and has been recurring annually during summer months.

Objective
To study the clinical profile and management of all patients with suspected or confirmed DVF seen at the Liaquat National Hospital, Karachi in 2005-2006.

Method
All cases that met the WHO case definition of DVF during this period were documented on a pre-designed proforma. Patient demographics, clinical presentation, management, complications, mortality and relevant laboratory tests were included.

Results
Out of 336 cases of probable or confirmed cases of DVF, 71% were male. Mean age of the patients was 28.1 ± 13.4 years. Out of the 294 tests sent for Dengue IgM antibodies, 190 (64.6%) were positive while 104 (35.4%) were negative. Mean hemoglobin was 13.9 ± 2.4 g/dL, and mean platelet count was $49.3 \pm 39.7 \times 10^9/mm^3$. The mean duration of hospitalization was 4.1 ± 3.3 days. Fifteen patients (4.5%) died during the course of their hospital stay.

Discussion
Majority of patients were males. Fever was the most consistent symptom, other features being skin rash and bleeding. Thrombocytopenia was a hall mark of the infection but was not always associated with serious bleeding. Treatment of DVF remains symptomatic. Mortality in our series was 4.5%.

Key words: Dengue Viral Fever, Management

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Introduction
Dengue fever (DF) is an arthropod-borne viral infection caused by any of four distinct serotypes of dengue virus. Infection with one serotype affords no protection against the other 3 serotypes. Dengue virus is a single stranded RNA virus that belongs to the Flavivirus family with *Aedes aegypti* and *Aedes albopictus* being its primary vectors. The clinical spectrum of dengue viral infections includes asymptomatic infections, DF, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).

Dengue viral infections have been globally recognized as a growing health concern particularly from the public health perspective. According to statistics from the US Centers for Disease Control and Prevention (CDC), dengue is now endemic in at least 100 countries. The World Health Organization (WHO) estimates that two-fifths of the world's population is now at risk of acquiring dengue infections. About 50 to 100 million dengue viral infections, 500,000 cases of DHF and 22,000 deaths are incurred from the disease annually.

Pakistan has witnessed at least two major outbreaks of DHF (1994-1995 and 2005-2006). Sindh province appeared to be the main hub for dengue infections in the 2005-2006 epidemic. More than 4,500 patients with probable dengue fever were admitted to several hospitals across Sindh. Of the 40 deaths reported from dengue viral infections in Pakistan, more than 90% were from Sindh province. Additionally, Pakistan now faces the problem of outbreaks of dengue infections each year.

Despite the magnitude of the impact the disease has had in Pakistan, local data on clinical, laboratory and management aspects of DF are relatively limited. This current report aims to fill the gaps in local literature by analyzing the demographic, clinical and laboratory features in addition to various prognostic features of suspected cases of DF admitted to a tertiary care hospital in Karachi, Pakistan.

Methods
This study was carried out at Liaquat National Hospital (LNH),

Karachi and included all patients who were admitted with the working diagnosis of "probable dengue fever" from October 2005 to October 2006, the period of the second DF epidemic in Sindh. The cases were defined as "probable cases" on the basis of clinical suspicion in accordance with WHO guidelines. Information was collected by clinicians on a specially designed proforma which included details of socio-demographic characteristics, signs and symptoms at time of presentation, presence of concomitant infections such as malaria, as well as laboratory parameters and complications. The presence of dengue IgM antibodies was assessed using ELISA.

All the cases were closely followed up during their hospital stay. Parameters such as duration of stay, incidence of bleeding, development of complications and outcome were subsequently recorded for each patient.

All data was entered and managed using Microsoft Excel. Data was analyzed with SPSS version 16.0. For categorical variables, frequencies have been reported while for continuous variables, measures of central tendency and standard deviation were calculated. Associations were analyzed using Chi-square test, Fisher's exact test and Student's t-test where appropriate. Multiple logistic regression was used to determine factors associated with death in dengue fever patients.

Results

A total of 336 cases of probable DF were admitted to LNH, Karachi between October 2005 to October 2006. Mean age of the patients was 28.1 ± 13.4 years (range: 3 – 78 years) with 238(71%) males. The mean age for men was 27.6 ± 12.7 years and for women was 29.5 ± 15.2 years. Although we had planned to test sera from all suspected cases in the hospital laboratory for the presence of dengue IgM antibodies by ELISA, this antibody investigation results were available for only 294 patients (87.5% of the sample) owing to technical and logistic issues. Amongst these patients, 190 (64.6%) were positive while 104 (35.4%) were negative. Fifteen patients (4.5%) died during the course of their hospital stay. The majority of the cases (75.3%) occurred in the period of June 2006 to October 2006. This temporal distribution was consistent with the occurrence of summer/monsoon season in Pakistan.

To make a diagnosis of probable dengue infection, fever was taken as an essential criterion. The mean maximum temperature of the patients was 102.9 ± 1.1 °F (range: 99.2 – 105 °F). Other clinical symptoms reported in descending order of frequency were vomiting (75.3%), rash (53.3%), myalgias (52.7%), abdominal pain (49.4%), cough (17.9%), sore throat (2.1%), and diarrhea (1%).

Mean hemoglobin of the patients was 13.9 ± 2.4 g/dL (males 14.3 ± 1.9 g/dL; females 12.8 ± 2.1 g/dL). The mean platelet count was 49.3 ± 39.7 x 10^9/mm^3. The platelet count was ≤ 25,000 x 10^9/mm^3 in 185 patients (59.1%). The detailed laboratory parameters are presented in table 1.

Table 1: Laboratory parameters of patients with dengue fever

Laboratory parameter	n	Mean ± SD	Range
Hemoglobin (g/dL)	315	13.9 ± 2.4	2.6 – 16.9
Hematocrit (%)	84	46.6 ± 7.3	4 – 56
Total leukocyte count (x 10^9/mm^3)	322	4.8 ± 3.0	0.5 – 21.5
Platelet count (x 10^9/L)	312	49.3± 39.7	2 – 228
Alanine transaminase (U/L)	292	200.3 ± 153.3	125 - 545

The median duration of hospitalization was 3 days (range 1-29 days). Third spacing in the form of pleural effusion and ascites was seen in 9 patients (2.7%). Bleeding occurred in 82 patients (24.4%). Common bleeding sites were gastrointestinal (43.9%), gums (40.2%), epistaxis (29.3%) and menorrhagia (28.6%). There was a significantly lower proportion of bleeding in cases with platelets above 25,000 (17%) as opposed to cases with platelets below 25,000 (28%) ($p < 0.028$). However, there was no significant association of low platelet count with any specific bleeding site.

The majority of the patients (93.5%) had an uncomplicated clinical course. Amongst the 6.5% complicated cases, concomitant infections (n=15), unusual manifestations such as encephalopathy, renal failure or acalculous cholecystitis were recorded. Eight of the 15 cases with concomitant infections had malaria.

Association of complicated cases with mean alanine transaminase (ALT), minimum recorded platelet count, mean duration of stay and mean age was also evaluated. A statistically significant association was seen with mean ALT and mean duration of stay.

In the period of observation, fifteen deaths were observed (4.5%). Approximately 13% of those who died had co-existing conditions as opposed to 4% of those who survived. Similarly 6.7% of those who died had iatrogenic complications as opposed to 0.3% who survived ($p<0.01$). Nine (60%) deaths were from Dengue shock syndrome. The association of outcome in patients with parameters like age, maximum temperature, duration of hospital stay, mean hemoglobin value, total leukocyte count, minimum platelet count and ALT count was also ascertained. Significant associations were found only with low platelet counts ($p< 0.05$) and hemoglobin values ($p<0.01$) (Table 2).

Finally, using multiple logistic regression, the presence of a rash (adjusted OR=4.2, CI: 1.1, 15.6), as well as the presence of a gastrointestinal bleed (adjusted OR=21.3, CI: 6.1, 74.1)

Table 2: Association between outcome and various laboratory and clinical parameters

Parameter	Patients who survived (n=321)	Patients who died (n=15)	*p* value
Age (years)	28 ± 13	31 ± 19	Not significant
Maximum temperature (?F)	101 ± 1.1	103.1 ± 1.1	Not significant
Duration of hospitalization (days)	5 ± 3	7 ± 4	Not significant
Hemoglobin (g/dL)	13.9 ± 2.3	12.5 ± 3.6	< 0.01
Hematocrit (%)	46.5± 7	45.8 ± 9	Not significant
Total leukocyte count (x 10^9/mm^3)	5.1 ± 4.3	5.5 ± 3.2	Not significant
Minimum platelet count(x 10^9/mm^3)	65.2 ± 35.4	44.2 ± 30.2	< 0.05
ALT levels (U/L)	193 ± 98.1	437 ± 105	Not significant

were strong clinical parameters associated with death in dengue fever patients, after adjusting for their age.

Discussion

During the 2005-2006 outbreak, the metropolitan city of Karachi was worst hit. In our study population, 70% of the patients were male with a male-to-female ratio of 2.4:1. This pattern of male predominance parallels the results of an earlier study from Pakistan which detailed the features of the 1994-1995 dengue epidemic. A study from Saudi Arabia reported even higher male-to-female ratio (3.3:1) in patients with DF. This gender difference may be explained by possible innate susceptibility in males or environmental factors such as difference in clothing or outdoor activities due to cultural and religious reasons.

In our study, we observed two peaks for the number of suspected dengue cases. The highest number of suspected dengue cases occurred between the months of November-December and then again between June and October. Similar patterns have been reported in studies from other countries in the region, such as Bangladesh, which experiences similar climatic conditions. On the contrary, one series of 225 patients with confirmed dengue virus infection in Pakistan between 2000 and 2006 reported approximately 87% of the hospital admissions in the winter season from October to December. The mean age of the patients in this study was 28.1 ± 13.4 years. This is almost similar to another study from Karachi which reported mean age of patients with dengue infection as 31 ± 12.9 years. The most commonly occurring clinical features in our study were fever, vomiting, rash, and myalgias. The observed pattern in patients also correlates well with observations reported in earlier studies from Pakistan.

Amongst the laboratory parameters, severe thrombocytopenia (platelet count ≤ 25,000) accounted for more than 50% cases. Earlier studies have shown that the platelet count is one of the most frequently deranged hematological parameters in patients with dengue infections and more than 50% patients have severe thrombocytopenia (<50,000). However, thrombocytopenia should be interpreted in the complete clinical context of the patient and not in isolation. A significant association was seen between incidence of bleeding and low platelet counts (≤ 25,000).

In this series, 15 patients had a co-existent infection. About half of these cases were due to dual infection of malaria and dengue. Pakistan is a malaria endemic area and earlier reports from the country have found a high incidence of dual dengue and malaria infections. The breeding season and conditions for the vectors of both diseases are similar. Therefore, an integrated approach towards vector control and disease surveillance may be adopted.

In this study, parameters such as minimum platelet count and low hemoglobin level appeared to have an impact on patient outcome. Earlier studies have cited presence of shock, coma at presentation and seizures as important predictors of mortality. Although a significant association between raised ALT and complicated cases of dengue infection was seen in this study, the association of ALT with overall outcome was not significant. Deranged liver functions occur commonly in patients with dengue infections and transaminase levels should be measured in these patients. A recent study from Karachi found that severe hepatitis, as evidenced by ALT > 300 U/L, is a bad prognostic indicator in dengue infection in terms of duration of stay, mortality and complications. Our study also found a higher incidence of manifestations such as bleeding, renal failure and encephalopathy in dengue infected patients with severely deranged liver functions. Although we didn't find any significant association between total leukocyte count and outcome, a study from Karachi has shown that high total leukocyte counts maybe associated with mortality. Upper and lower gastrointestinal bleeding with concomitant generalized rash and thrombocytopenia were considered ominous signs.

In recent years, DF has emerged as an important cause of hospitalization and death in tropical and sub-tropical regions of the world especially in urban and peri-urban areas. The

disease holds particular importance in the health canvas of Pakistan. Considering the burden it adds to an already frail health system of this developing country, there is a dire need to conduct more research on the subject. Such research is vital to establish endemicity and epidemicity patterns of dengue infections in Pakistan and can help in devising better management strategies in the future. In addition, the knowledge of the public, especially in the lower socioeconomic areas, about dengue infections needs to be improved through well structured education programs and media campaigns. Provision of facilities for confirmation of cases and prompt management of complicated cases should be ensured as well.

Conclusion

Dengue is present year round in Karachi but its incidence begins to rise in the summer months, peaks in September and continues through November. Males appear to be affected by dengue infection more commonly as compared to females. Higher ALT levels and longer duration of stay was seen in patients with a complicated clinical course. Minimum platelet count and hemoglobin levels may have a value in prognostic outcomes in patients with dengue fever.

Conflict of interest

The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Frequency of Cutaneous Leishmaniasis in Patients with Suspected Skin Lesions

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Abstract

Objective
The objective of this study was to find the frequency of positive cases of cutaneous leishmaniasis in patients who were referred from the outpatient department of Khyber Teaching Hospital Peshawar during the year 2009.

Materials and Methods
It was a descriptive study performed in the Pathology Department of Khyber Medical College Peshawar. All those patients who had suspected skin lesion and attended the skin OPD of Khyber Teaching Hospital Peshawar were included in the study. Proper sampling was done and taken on slide, stained with Giemsa stain and examined under direct microscopy for leishmania.

Results
Out of total 120 cases, 60 (50%) were positive. In the positive cases male to female ratio was 2:1. Most of the positive cases were in age range of 15-40 years.

Conclusion
Chronic non-healing ulcer must be investigated for cutaneous leishmaniasis due to an increased incidence. In addition, the community should be educated about cutaneous leishmaniasis with emphasis on preventive measures.

Key words
Cutaneous Leishmaniasis, *Leishmania tropica*, Sand Fly.

Introduction

Leishmaniasis is a major health problem worldwide.[1,2] It is also a particular health problem in Pakistan, especially in the rural areas.[3,4] The infection is due to an intracellular protozoan parasite belonging to the genus Leishmania. There are four main clinical types of leishmaniasis: cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis.[5] The cases can be divided into urban and rural. The most common type in Pakistan is urban or anthroponotic leishmaniasis. The disease is transmitted by sand fly from human to human, but the rural or zoonotic leishmaniasis comes from interaction of man with animals. The cutaneous variety may present in unusual clinical variants that can be difficult to diagnose.[6,7] Geographic distribution of the sand fly vector determines the cutaneous leishmaniasis. Sand flies are more active in evening and at night and do not fly far or high and lives in dark, damp places. Only female sand fly is hematophagous (blood sucking). Once a sand fly is infected, it can transmit parasite to both humans and animals for the rest of its life.[8,9]

In Pakistan and India, simple cutaneous leishmaniasis is usually caused *by Leishmania tropica* and man is the most common reservoir.[10] The lesions are either solitary or multiple and occur on exposed areas of the body easily accessible to the sand fly. The lesion can be papules, nodules, ulcers or plaques.[5] The nodule comprises of parasite laden macrophages surrounded by lymphocytes which try to restrict spread of these organisms. This is a self limiting disease but it may take many months before complete healing with scar formation occurs. Usually life long cell mediated immunity is the ultimate response.[11]

Cutaneous leishmaniasis is endemic and highly prevalent in the provinces of Balochistan and Khyber Pakhtoon Khawa. In Sindh province, cases have been reported from Dadu, Larkana, Jacobabad and Karachi, while in Punjab region, the disease is mainly present in Multan and Dera Ghazi Khan.[12] The hall mark of cutaneous leishmaniasis is skin lesion which can heal in 6-12 months time. The duration of the lesion varies between different species.[13]

The main diagnostic test available at most of the places is microscopy of stained smear. Culture is only available at specialized centers. Skin tests (Leishmanin test or Montenegro test) are usually not useful in the clinical settings.[14] The present study was conducted to know how frequently *Leishmania* can be detected by preparing slides from the tissues of the patients

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with suspected skin lesions referred from skin out-patient department (OPD).

Materials and Methods

This descriptive study was conducted at Pathology Department of Khyber Medical College, Peshawar from 1st January 2009 through 31st December 2009. A suitable part of raised margin of the lesion of the patient was selected, cleaned with methylated alcohol and was squeezed between finger and thumb to make it blood less. A small nick was made with a lancet or surgical blade (no.25) at the raised or swollen edge, blood was wiped. The blood lancet or scalpel blade was then turned at 90° and used to scrape the wound so that some fragments of tissues and serum were collected over the blade. This was spread over a glass slide. Three slides were prepared for each patient. The slides were fixed in methanol, stained with Giemsa stain and examined by direct microscopy with oil immersion lens.

Diagnosis of cutaneous leishmaniasis was made on observing amastigotes of *Leishmania* (LD bodies) extracellularly or in monocytes (macrophages). Slides were examined thoroughly by laboratory technician followed by the microbiologist.

Results

This study included 120 cases with suspected skin lesions referred from skin OPD to the microbiology laboratory. Overall 60 (50%) cases were positive. LD bodies were present in different shapes like oblong, rounded and rod shaped.

Out of total positive cases, 42 were male and 18 were female with male to female ratio 2:1. Age-wise, distribution of positive cases is shown in table 1.

Table 1: Age wise distribution of positive cases (n=60)

Sex	< 15 years	15-40 years	> 40 years
Male	7	32	3
Female		18	0

Discussions

Both visceral and cutaneous forms prevail in Pakistan over several endemic foci with different climatic and geographic conditions. The frequency of leishmaniasis has increased recently in Pakistan especially in Khyber Pakhtoon Khawa province due to the influx of reservoir hosts. There are other additive factors, including the environmental, climatic, housing, sanitation, socioeconomic conditions and resistance to the available treatment in many cases.

Timely and proper diagnosis is mandatory for treatment. Parasitological diagnosis include rapid diagnostic tests; K39 antigen dipstick which is useful for visceral leishmaniasis. Nested PCR is available for diagnosis of cutaneous leishmaniasis. *In vitro* cultures can also be done from skin snips. Scarce diagnostic facilities are available at most of the places in Pakistan that too limited to stained smear examination. Non-availability of latest methodologies was a limiting factor in our study. It is well understood that not all active lesions would have positive smears, presumably because the number of parasites is small even though the infection may be active. The rate of positive smears decreases with the duration of the lesion; older lesion are less likely to be positive. The rate of positivity of the slides also depends on skills of the person preparing and examining the smear.

High percentage of positive cases in the present study reflects the fact that all cases were from clinically suspected cases. There are various studies available regarding CL; Rahman *et al* had earlier reported 95% of positive cases from subjects having ugly looking skin lesions.[15] This frequency was much higher than ours as it had subjects from Leishmania endemic belt of FATA. Another hospital based study reported a frequency of 7.18% of positive cases of CL.[11] Nawaz *et al* reported 6.9% positivity on population based study.[16]

Male to female ratio in our study was consistent with study by Zubair Khan, and so was number of maximum patients in the age group of 15-40 years.[11] The reason for this age group was more of chances of exposure at this active age.

Where ever the disease is prevalent suitable preventable measures must be employed and the community must be educated. Mostly the emphasis should be on preventive measures like use of insect repellents on the exposed skin, elimination of the reservoirs, cleanliness of buildings, use of mosquito nets and installation of mesh on doors and windows.

Conclusion

There is an increase in referred cases of CL in the Khyber Pakhtoon Khawa. Chronic non-healing ulcer must be referred for skin smear for CL and in expert hands skin smear preparation is a sound technique to diagnose these cases especially in the absence of latest modalities.

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ORIGINAL ARTICLE

Positive Urine Cultures after Renal Transplantation at a Transplant Center in Karachi

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Abstract

Urinary tract infection is the most common type of bacterial infection encountered among renal transplant recipients.

Methodology
This retrospective study was conducted at the Sindh Institute of Urology and Transplantation, Karachi from January 1st 2009 till December 31st 2009. All renal transplant patients found to have positive urine cultures over a one year period were included in the study. Patients' data were retrieved through microbiology computerized database.

Results
Among 163 renal transplant recipients, 248 episodes of positive urine cultures were documented during 2009. 114 patients had a single positive urine culture whereas recurrent urine culture positivity occurred in 49 recipients. Out of 248 episodes, one-third was asymptomatic. Post-transplant urological interventions were performed in two-thirds, including ureteral stent placement in 58% of patients. Potency of immunosuppression was not found to be a statistically significant risk factor. The majority (92%) of microorganisms recovered were gram negatives and over one-third were multidrug resistant predominantly extended spectrum beta lactamase producing. Post-transplantation surgical interventions and underlying structural abnormalities of the urinary tract were significantly associated with repeated positivity of urine cultures. Recurrent positive urine cultures were also associated with the development of interstitial fibrosis and tubular atrophy.

Conclusion
Risk factors for urinary tract infections among renal transplant patient include abnormal pre-transplant urological anatomy, and post-transplantation instrumentation of the urinary tract. A thorough pre-transplantation urological evaluation must be performed and repeated in post-transplantation stage if recurrent urine culture positivity occurs.

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Introduction

In renal transplant recipients, 25 to 63% of patients suffer a single episode of urinary tract infection (UTI) while a recurrence rate of up to 55% has been reported in post-transplant follow up studies.[1-3] Primary stone disease, repeated surgical interventions, abnormal urinary tract anatomy, catheters, ureteral stents and potent immunosuppression have been found to be contributory risk factors for bacteriuria. Due to drug induced immune modulation, these patients may not always show signs and symptoms of UTI even with significant bacteriuria.[4,5] However, asymptomatic bacteriuria requires close clinical monitoring for renal graft dysfunction. Persistent bacteriuria, especially with *E. coli*, is associated with interstitial fibrosis and tubular atrophy (IFTA).[6]

Recurrent UTIs in renal transplant recipients associated with multidrug resistant Extended Spectrum Beta-Lactamase (ESBL) producing organisms pose a therapeutic challenge since treatment usually requires prolonged parenteral antibiotic administration, some of which may be nephrotoxic such as the polymyxins. The purpose of this study was to assess the risk factors for urine culture positivity and its recurrence, associated clinical features and causative uropathogens in renal transplant recipients.

Material and Methods

This was a retrospective, cross sectional descriptive study conducted at Sindh Institute of Urology and Transplantation (SIUT) Karachi. Post renal transplant, all patients with positive urine cultures over a one year period from January 1st 2009 till December 31st 2009 were identified through the microbiology computerized database. Their medical charts were reviewed for clinical presentation, level of immunosuppression, urological interventions and/or abnormalities and graft function during each episode of urine culture positivity.

SIUT is a 500-bedded hospital dealing in adult and pediatric nephrology and urology and is the largest transplant center in the country. More than 3000 live related renal allograft surgeries have been performed over a span of 20 years. Renal transplant recipients are closely monitored at regular follow up visits in the outpatient clinic. In case of any rise (>20% above baseline)

in creatinine, patients are admitted for a complete clinical evaluation. Baseline laboratory investigations, cultures, imaging, immunosuppressant levels and graft biopsy are performed as required.

Urine samples received for cultures were inoculated on cysteine lactose electrolyte deficit (CLED) agar (Oxoid, UK) using 1µL loop. The plates were incubated at 37ºC and examined after 24 and 48 hours for colony counts. Any yield was identified using biochemical identification tests; API (20E \ 20NE \ 20 Strep) (BioMerieux, France). Susceptibility testing was performed following the guidelines by Clinical laboratory Standard Institute (CLSI).[7] Control strains were regularly applied.

Definitions

- Significant bacteriuria: at least $>10^5$ bacteria per mL of fresh unspun midstream urine.
- In a symptomatic transplant recipient, a pure culture of at least 10^3 bacteria per mL of urine with pyuria was considered significant.[4]
- Conventional immunosuppression: a regimen containing prednisone, cyclosporine and azathioprin.
- Potent immunosuppression: a regimen containing mycophenolate mofetil and/or Tacrolimus.

Results

Among 163 renal transplant recipients, 248 episodes of positive urine cultures were documented during the year 2009. Mean age was 30 years (±12 years) with 67 % males. All were live related renal transplantation cases except for two patients,

114 patients had a single positive urine culture whereas recurrent urine culture positivity occurred in 49 recipients within the study period. Out of 248 episodes, 87 (35%) positive urine cultures were not associated with any symptoms. Associated features included: fever 139(56%), raised peripheral white cell count 67(27%) and dysuria 60 (24%). Febrile illness along with bacteremia with isolation of same microorganism as recovered in urine was observed in twenty one (9%) episodes.

Associated urological and medical risk factors for positive urine cultures among 163 renal transplant recipients were evaluated (Table 1). Pre-transplant urological structural abnormalities were present in one-third, and post-transplant urological interventions performed in two-thirds which included ureteral stent placement in 58% of patients (Table 1). Patients on high immunosuppression had comparatively more repeated episodes, however, potency of immunosuppression was not found to be a statistically significant risk factor.

The majorities (92%) of microorganisms recovered were Gram negatives and over one-third was ESBL producers (Table 2). Surgical interventions post-transplantation and underlying structural abnormalities of the urinary tract were significantly

Table 1: Urological and medical risk factors for positive urine cultures (n=163)

Risk Factors	n(%)
Medical	
Conventional immunosuppression	93 (57)
High immunosuppression	70 (43)
Diabetic mellitus	5(3)
Pre-transplant structural abnormalities	**53(32.5)**
Native kidney stone disease	26(16)
Reflux of native kidney	7(4.3)
Neurogenic bladder	6(3.7)
Mitrofanoff	6(3.7)
Stricture urethra	7(4.3)
High neck bladder	1(0.6)
Post-transplant Urological Interventions	**102(62.5)**
Ureteral stents	94(58)
Multiple urinary tract surgeries	5(3)
Graft hydronephrosis with percutaneous nephrostomy	3(2)

Table 2: Microbial yield & its susceptibility pattern (n=248)

Microorganism	n (%)	Resistant organisms n(%)
Gram -ve organism	**230 (92)**	**81(35)**
E. coli	130 (56)	48(37) ESBL
Klebsiella spp	52(23)	29(56) ESBL
P. aeruginosa	27(12)	1(4) MDR
Typhoidal Salmonella	5(2)	-
Others	16(7)	3 (18) ESBL
Gram +ve organism	**13 (7)**	**1(8)**
Enterococcus spp	9(69)	1(11) VRE
Streptococcus spp	1(8)	-
S. aureus	3(23)	-
Candida spp	**5(2)**	

associated with repeated positivity urine cultures, as was infection with multidrug resistant (ESBL) producing organisms. Positive urine culture, if occurring in the early post-transplant period was found to predispose to recurrence. Recurrent positive urine cultures were also associated with the development of IFTA. Potency of immunosuppression did not appear to predispose to recurrence (Table 3).

Table 3: Comparison of risk factors in patients with single episode and repeated episodes of positive urine culture

Factors	Single n (%)	Repeated n (%)	p-Value
Immunosuppression			
Conventional	70 (61)	23 (47)	0.087
High	44 (39)	26 (53)	
Surgical & structural factors	33 (29)	26 (53)	0.003
DJ Stent	61(54)	33(67)	0.101
MDR Organisms	22(19)	59(44)	<0.001
Development of IFTA	11(10)	31(23)	0.005

Regarding treatment of positive urine culture, among 114 single episodes, 59 (52%) were symptomatic and treated on average for 10 days. Out of 134 recurrent episodes, 102 (76%) were symptomatic and all were treated on average for 15 days. Asymptomatic patients with positive urine cultures and elevated creatinine were considered to have UTI if no other cause could be identified for diminished graft function. These asymptomatic patients, on average, received antimicrobial therapy for two weeks.

Discussion

Among solid organ transplant recipients, the renal transplant population has a greater propensity for UTIs probably because of primary renal pathology, abnormal anatomy and instrumentation of the urinary tract.

Because of specific host-parasite interactions, uropathogenic *E. coli* has tropism for the urinary tract.[8] In our study, *E. coli* was the predominantly isolated organism which was consistent with other studies of post transplant UTIs.[9, 10] Over one-third of the isolates were ESBL producers comparable with other studies[11,12]. Increasing resistance of uropathogens is alarming in the transplant patient as ESBL producing bacteria are difficult to eradicate, incur high cost of antimicrobial therapy, prolong hospital stay and increase mortality. Moreover, multidrug resistant organisms, especially ESBL producing *E. coli* have been associated with recurrent UTI.[11-14] We also observed that infection with ESBL organisms predisposed to recurrent urine culture positivity as reported earlier by Pinheiro *et al.*[12] It is essential to implement good infection control practices to prevent the spread of ESBLs among this vulnerable population.

Although recurrent UTIs associated with nontyphoidal *Salmonella* have been reported in immunocompromised population, *Salmonella* Typhi bacteriuria is rare even in countries where it is endemic[13 -15]. The clinical significance is not clear and it is possible that patients, who are chronic carriers of *S.* Typhi, can develop acute UTI due to the same organism.

Urological and structural abnormalities are known risk factors for repeatedly positive urine cultures as demonstrated in our study[3, 16]. Over half of the patients in our study had placement of ureteric stents post-transplantation which was found to be a significant risk factor by Kamath *et al* as well[17]. If stenting is required, it must be performed with caution, possibly with antibiotic prophylaxis and placed for shorter duration. Vesicoureteral reflux (VUR) is considered an important risk factor for pyelonephritis in renal transplant recipient; in our study it was present in 4.3% patients only. Surgical correction of VUR before renal transplantation should be considered although in one study the frequency of febrile UTI after correction was still found to remain high as compared with those without VUR .[18]

It is advisable that evaluation for urological and functional abnormalities be performed pre-transplantation, and if recurrent UTIs are documented post-transplantation, re-evaluation should be performed.

Immunosuppression with more potent drugs predisposes to infections. However, our study did not show an increased incidence of urine culture positivity or recurrence rate with more potent immunosuppression.

A limitation of the retrospective design of our study was that we were unable to verify the method of urine sample collection. Therefore, it was difficult to assess whether a positive urine culture represented colonization, contamination or an actual UTI, especially in asymptomatic patients. At times there was patchy documentation and complete clinical history and name of the antibiotics could not be obtained.

Conclusion

Risk factors for urinary tract infections among renal transplant patient include abnormal pre-transplant urological anatomy, and post-transplantation instrumentation of the urinary tract. A thorough pre-transplantation urological evaluation must be performed and repeated in post-transplantation stage if recurrent urine culture positivity occurs.

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Dr Aamer Ikram

ORIGINAL ARTICLE

Medical Students' and Doctors' Perception of Fever in Children

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Abstract

Introduction
Fever is one of the commonest complaints for which a child is brought to the doctor. Misconceptions regarding causes, harmful effects and management of fever in children prevail in the health professionals. This study was carried out to assess and compare the knowledge and perception of fever amongst the medical students and the practicing doctors.

Methods
Final year MBBS students and practicing physicians were interviewed using a structured questionnaire regarding recognition, causes, harmful effects and management of fever in children. The information obtained from the two groups was compared and analyzed.

Results
There were 100 participants in the study; 52 medical students group and 48 doctors. Majority of the respondents in both the groups gave incorrect definitions of fever and high fever. Infection was identified as a cause of fever by 88.5% students and 91.7% doctors. Teething was identified as a cause of fever by 55.8% students and 29.2% doctors. Paracetamol was the favoured antipyretic by the majority of the respondents. Sponging was recommended as the adjunct treatment for management of fever by 96.2% students and 87.5% doctors.

Conclusion
Medical students and doctors lack adequate knowledge regarding fever in children. Curriculum for medical students should be revised so that they are in a position to effectively manage this common childhood problem.

Key words
Fever, Medical Students.

Introduction
Fever is defined as a rise in body temperature above the normal for that individual. Body temperature varies slightly from individual to individual, ranging from 97.9^0 F to 100.2^0F (rectal).[1] It also varies at different times of the day in the same individual, being lowest in the early morning, and highest in the late afternoon.[2]

Myths prevail regarding recognition, causes, harmful effects and management of fever in children in the lay people as well as amongst the health professionals.[3-6.] This study was carried out to assess and compare the knowledge and perception of fever in children amongst the final year medical students and the practicing doctors.

Materials and Methods
The study population was divided into two groups. The first group comprised of final year medical students who had completed their pediatric rotations (Students Group). This group had had exposure to pediatric patients during their 3rd and 4th and final year MBBS clinical rotations as part of their medical school curriculum. The second group consisted of practicing doctors with a minimum of six months of experience in treating pediatric patients (Doctors Group). Both groups were interviewed by one of the authors, using a structured questionnaire during the months of February and March 2009. The study was carried out at three separate campuses of a parent institution, which serve as teaching centers for medical students and post graduate trainees. The two groups were compared and information was analyzed using Statistical Package for the Social Sciences (SSPS) ver 11.0. A p-value of <0.05 was considered as statistically significant. For the purpose of this study, a temperature of $>100.4^oF$ was considered to be fever and a temperature of $> 103^0$ F to be high grade fever.

Results
A total of 100 questionnaires were filled, out of which 52 belonged to the medical students group and 48 to the practicing doctors group. The demographic data of the participants of the two groups is shown in table 1. The mean pediatric experience of the practicing doctors was 6.05 years ± 6.7 years with a range of 6 months to 28 years. The highest professional degree obtained by the practicing doctors included Bachelor of Medicine & Bachelor of Surgery (MBBS) 30, Diploma of College of

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Table 1: Demographic Data of Respondents

Total Number:		n=100
Groups:	Students	52
	Doctors	48
Gender:	Male	37
	Female	63
Age:	Range	22-56 years
	Mean±SD	23.3± 0.8 years (students)
		34.7 ± 7.6 years (doctors)

Physicians and Surgeons (DCPS) 10, Fellow of College of Physicians and Surgeons (FCPS) 5, Membership of the Royal College of General Practitioners (MRCGP) 2, and Diploma in Tuberculosis & Chest Diseases (DTCD) 1. The professional title of the practicing doctors included Resident Medical Officers 13, Resident trainees 13, Faculty members 7, Consultants 4, Registrars 4, General Practitioners 4, and Casualty Medical Officers 3.

The questionnaire and the responses given by the participants of the two groups are shown in table 2. Teething was identified as a cause of fever by 29 (55.8%) of the participants in the students group and 14 (29.2%) of the participants in the doctors group. Loss of consciousness and death were identified as harmful effects of fever by 27 (51.9%) and 20 (38.5%) of the participants in the students group respectively and by 15 (31.3%) and 10 (20.8%) of the participants in the doctors group respectively. Six participants in each group (11.5%) of the students and 12.5% of the doctors said that they would alternate Paracetamol and Ibuprofen as the drug of choice for treating fever in children. Nine students (17.3%) and two doctors (4.2%) would use an antibiotic as the drug of choice for treating fever.

Twice as many doctors than students thought that rectal route was more effective than oral route for giving antipyretic to the child. Forty nine (94.2%) students and 40 (83.3%) of the doctors thought that the main reason for giving antipyretic for fever was to prevent fits. Majority of respondents in both the groups would sponge the child as an additional measure to treat fever. Thirty three (63.5%) of the students and 18 (37.5%) of the doctors would only sponge the forehead of the febrile child and many, in both the groups, would use cold or ice water for sponging the febrile patient.

Discussion

Fever is a common problem for which a child is brought to a doctor. Most authorities do not consider a body temperature below 100^0 F to be fever. Our study showed that the majority of respondents in both the groups incorrectly identified a body temperature between 99^0 F and 100^0 F to be fever. This is in contrast to a study from Norway where majority of the general practitioners surveyed correctly identified fever as body temperature greater than 38^0 C (100.4^0F).[7] It is a well-established fact that tactile method is an unreliable method of detection of fever[8, 9]. Majority of the respondents in both the groups of our study were correct in this regard. This is different from our

Table 2: Questionnaire and the responses

	Student (n=52) No. (%)	Doctor (n=48) No. (%)	p-value
How do you define fever?			
98.6^0F	4 (7.7)	1 (2.1)	
99-100^0F	45 (86.5)	33 (68.8)	<0.01
>100.4^0F	3 (5.8)	14 (29.2)	
How do you define high grade fever?			
>101^0F	22 (42.3)	12 (25)	0.1
>102^0F	18 (34.6)	26 (54.2)	
>103^0F	12 (23.1)	10 (20.8)	
Is tactile method a reliable way of detecting fever?			
Yes	4(7.7)	5(10.4)	0.39
No	48 (92.3)	43(89.6)	
How do you recognize fever?			
By the general look/ touching the child	5(9.6)	9(18.8)	0.18
By measuring the temperature	47(90.4)	39(81.3)	
Which is the best thermometer (in terms of accuracy) for children?			
Mercury	49 (94.2)	41(85.4)	0.26
Digital	1(1.9)	1(2.1)	
Forehead strip	1(1.9)	0	
Ear	1(1.9)	5(10.4)	
Do not know	0	1(2.1)	
What is the preferred site for measuring			

(continue on next page)

	Student (n=52) No. (%)	Doctor (n=48) No. (%)	p-value
temperature in children <1year?*			
Mouth	2(3.8)	0(0)	0.13
Forehead	0(0)	0(0)	
Arm pit	12(23.1)	17(35.4)	
Anus	36(69.2)	26(54.2)	
Ear	1(1.9)	3(6.3)	
What is the preferred site for measuring temperature in children 1-5 years?			
Mouth	4(7.7)	4(8.3)	0.12
Forehead	0(0)	1(2.1)	
Arm pit	41(78.8)	37(77.1)	
Anus	6(11.5)	1(2.1)	
Ear	1(1.9)	5(10.4)	
What is the preferred site for measuring temperature in children >5 years?*			
Mouth	36(69.2)	30(62.5)	0.66
Forehead	2(3.8)	1(2.1)	
Arm pit	12(23.1)	14(29.2)	
Anus	2(3.8)	1(2.1)	
Ear	0(0)	1(2.1)	
What are the common causes of fever in children?**			
Exposure to cold	33(63.5)	18(37.5)	0.02
Exposure to sun	8(15.4)	15(31.3)	0.05
Teething	29(55.8)	14(29.2)	0.02
Infection	46(88.5)	44(91.7)	0.38
Other (drinking warm/ cold liquids)	13(25.0)	4(8.3)	0.05
What are the harmful effects of fever in children?**			
Seizure	51(98.1)	47(97.9)	0.95
Loss of consciousness	27(51.9)	15(31.3)	0.03
Confusion	23(44.2)	27(56.3)	0.23
Other CNS effects (brain damage/stroke /MR)	27(51.9)	26(54.2)	0.82
Dehydration	41(78.8)	30(62.5)	0.07
Death	20(38.5)	10(20.8)	0.05
Other (Blindness/ weight loss/ serious illness)	23(44.2)	9(18.8)	<0.01
What is your drug of choice to treat fever?**			
Paracetamol	47(90.4)	40(83.3)	0.29
Ibuprufen	18(34.6)	5(10.4)	<0.01
Alternating ibuprufen & paracetamol	6(11.5)	6(12.5)	0.88
Antibiotic	9(17.3)	2(4.2)	0.03
Aspirin	2(3.8)	0(0.0)	0.17
Which route of antipyretic administration is more effective?			
Oral	29(55.8)	14(29.2)	0.01
Rectal	10(19.2)	20(41.7)	
Both are equally effective	13(25)	14(29.2)	
The main reason you give antipyretic to treat fever is to:**			
Prevent fits	49(94.2)	40(83.3)	0.08
Prevent brain damage	18(34.6)	15(31.3)	0.72
Prevent death	11(21.2)	4(8.3)	0.07
Make the child comfortable	32(61.5)	26(54.2)	0.45
Make the parents comfortable	13(25)	11(22.9)	0.8
What additional steps would you recommend for fever management in children?**			
Fan cooling	6(11.5)	20(41.7)	<0.01
Decrease clothing	30(57.7)	34(70.8)	0.17
Shower/bathe the child	18(34.6)	26(54.2)	0.04
Sponge the child	50(96.2)	42((87.5)	0.11
forehead	33(63.5)	18(37.5)	0.07
axilla	2(3.8)	5(10.4)	
groin	0(0.0)	1(2.1)	
whole body	17(32.7)	23(47.9)	
do not know	0(0.0)	1(2.1)	
Type of water used for sponging*			
Tepid water	24(55.8)	33(68.8)	0.02
Cold water	5(9.6)	10(20.8)	
Ice water	14(26.9)	3(6.3)	
Water with spirit	4(7.7)	2(4.2)	

*Numbers less than the total, as some participants did not give any answer to the question.
**Numbers more than the total, as participants gave more than one answer to the question.

study of parental perception of fever where 43% of parents thought that they could determine if the child was febrile or not by touching the forehead.[4]

Mercury thermometer was adjudged to be the most accurate by majority of our respondents. This may be due to the reason that mercury thermometers are very cheap and widely used in most health care facilities in our area. Mercury thermometers are accurate, but are no longer recommended for use because of the potential toxicity of mercury in case of accidental leakage. Digital thermometers are now recommended by most authorities as they are relatively inexpensive, easy to read, and are very accurate.

Myths still prevail about the causes and harmful effects of fever even amongst the health care professionals. Exposure to sun, cold and teething were identified as causes of fever by both the doctors and the medical students. Likewise, loss of consciousness, brain damage and death were identified as harmful effects of fever by a good proportion of respondents in both the groups.

It was inspiring to know that Paracetamol was identified as the drug of choice to treat fever by majority of the respondents. This is consistent with other studies where Paracetamol was the most popular antipyretic recommended by physicians.[5,7] Paracetamol is a safe and effective antipyretic for children. Aspirin and Ibuprofen may cause side effects like gastric irritation, ulcers or bleeding. A small percentage of respondents chose alternating Paracetamol and Ibuprofen as the regimen of choice for treating fever in children. There could be potential problems in using this regimen resulting in over dosage of either drug and subsequent complications.[10] On the other hand, there is no advantage in alternating these medications over giving them alone.[10] Aspirin was chosen as the drug of choice by a small number of students. Aspirin should never be used as an antipyretic, as it increases the risk of Reye's syndrome in children.[11] Antibiotic usage was chosen by a small percentage of respondents, more so in the students group. Antibiotic should not be used for the treatment for fever in children, unless a bacterial cause of fever is strongly suspected or proven, most fevers in children are viral in origin.[12]

Regarding the route of administration of the antipyretic, the correct response of both oral and rectal routes being equally effective was correctly identified by only a quarter of respondents in each group. Significantly more doctors than students thought that the rectal route was more effective in lowering the temperature compared to the oral route.

It is a well known fact that use of antipyretic does not prevent febrile fits.[13] This is contrary to the response given by the majority of our respondents. Only 2/3rd of the students and approximately half of the doctors in our study thought that the main reason for giving an antipyretic was to make the child comfortable. Use of sponging for the treatment of fever is controversial. If sponging is at all recommended for fever management, it should be done after giving an antipyretic, using tepid water over the whole body. As shown in our study, sponging of the forehead alone or in combination with axilla or groin using cold/ice water or water with spirit/alcohol rubbing was a common practice recommended by the health professionals. This practice should be strongly condemned, as it makes the child very uncomfortable and may induce shivering.[14] Use of alcohol or spirit for sponging may also result in alcohol toxicity.[15]

Conclusion

Medical students and practicing doctors have misconceptions regarding fever management in children. Curriculum in the medical schools and post graduate training programs should be revised to focus more on the practical aspects of this important topic. Practicing doctors should be encouraged to attend continuing medical education courses on a regular basis to enable them to manage this common problem of children more effectively.

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CASE REPORT

Cerebral |Malaria Caused by *Plasmodium vivax*

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Introduction
Cerebral malaria is a dreadful complication of *Plasmodium falciparum* with high mortality and morbidity especially in children and pregnant women[1]. Although rare, but most of the *Plasmodium vivax* induced cerebral involvement has been reported in India and China[2,3]. More than 45 cases of *P. vivax* cerebral malaria have been published till today since 1920. We report a case of *P. vivax* infection complicated by cerebral involvement with intact sensorium in a young soldier. Patient was managed successfully with artemether based compound. To the best of our knowledge, this is the third reported case in Pakistan.

Key words
Artemether, Cerebral malaria, *Plasmodium vivax*.

Case report
A 27-year-old soldier presented to the emergency room of Combined Military Hospital, Dera Ismail Khan with 4 days history of fever and vomiting. Fever was high grade, sudden in onset, intermittent and was associated with rigors and chills. He had 4-5 episodes of yellowish coloured non-projectile vomiting for the last one day. At the time of arrival in hospital, he was disoriented in time, place but not in person with aggressive behavior and irrelevant talk (Glasgow coma score 14/15). His temperature was 39°C; pulse 114/minute (regular); blood pressure 100/70 mmHg; and respiratory rate was 20 breaths/minute. His neck was supple with no focal deficit and there was no rash over the body. His systemic examination was unremarkable with negative Kernig's and Brudzinski's signs. Laboratory findings demonstrated a hemoglobin level of 12.1 g/dL, plasma glucose 134 mg/dL, platelet count 44 x 10^9 /L and white cell count 4.6 x 10^9 /L (neutrophils 71%; lymphocytes 27%; monocytes 2%; eosinophils 1%; basophils 0.8%). His serum urea and creatinine were 4.2 mmol/L and 76 µmol/L respectively. Prothrombin time, activated partial tissue thromboplastin time, liver function tests and serum electrolytes were within normal limits. Cerebrospinal fluid chemistry was normal and Gram stained smear did not show any organisms. Leishman's stain of his peripheral blood film showed trophozoites and ring forms of *Plasmodium vivax* with index of 1%. A suspicion of cerebral malaria was made. Keeping in view the endemicity of falciparum malaria in our area, a possibility of mixed infection was made but repeat films showed *P. vivax* only. Serological testing with immunochromatographic (ICT) kit (SD Bioline Malaria Antigen, Korea) confirmed the presence of *P. vivax* only (Fig 1). Patient was advised injection artemether, 3.2 mg/kg I/M stat and then 1.6 mg/kg x once daily for two days followed by capsule Artem DS 40/240, 2 capsules x twice daily for three days. After 48 hours of therapy, his fever started settling and his neurological status improved remarkably. No *P. vivax* parasite was seen on thin blood film after 2 days of therapy. Patient was discharged on 7th day of hospitalization with full clinical, microbiological and hematological recovery.

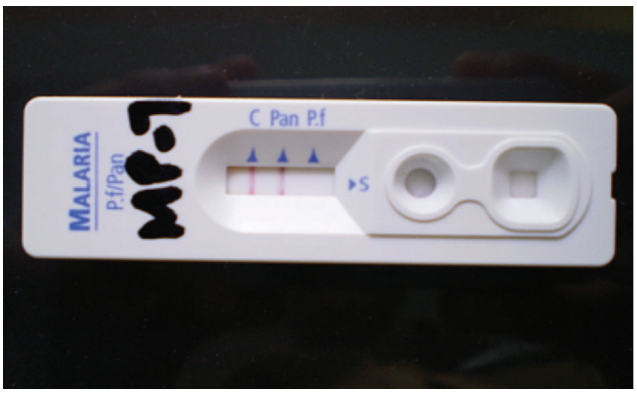

Figure 1: ICT kit showing positive band for *Plasmodium vivax* only.

Discussion
P. vivax is responsible for approximately 100-300 million cases each year[4]. Although *P. vivax* is mainly responsible for benign disease, cerebral involvement is the most consistent neurological manifestation of complicated vivax malaria. *P. vivax* can cause complications similar to those caused by *P. falciparum* and these include cerebral malaria, acute respiratory distress syndrome, severe anaemia, hepatic failure, renal failure, splenic rupture and severe thrombocytopenia[5-13].

In Pakistan, the first case report of cerebral malaria by *P. vivax* was documented by Islam *et al* in 1995[14]. In this case report along with cerebral malaria, other complications included disseminated intravascular coagulation and adult respiratory

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distress syndrome. The second case report published in 2002 was described by Beg *et al* in which *P. vivax* infection was complicated by seizures and multiple basal ganglia infarcts[1]. In our patient, the sensorium was intact and there was no focal neurological deficit. The first case report was described by Rossle in 1921 in which a young soldier had a lethal cerebellar hemorrhage[15]. While going through literature, it has been observed that maximum number of cases of *P. vivax* cerebral malaria have been reported in India.

Exact pathogenesis of *P. vivax* cerebral malaria is still poorly understood due to limited research in this field[4]. Cerebral involvement may be due to production of nitric oxide and adherence of parasitized red blood cells to the vascular endothelium[16]. Cytokines and leukotrines may be responsible for severe anaemia and hematological abnormalities[17]. A recent research showed that erythrocytes clumping, reduced deformability affecting microcirculation and increased aggregation is responsible for severe complications in *P. vivax*[18].

Mixed infection with *P. vivax* and *P. falciparum* is not so uncommon in this region of the country. In our case, a possibility of co-infection with *P. falciparum* was thought when the peripheral film showed *P. vivax* parasites. However, mono-infection with *P. vivax* was confirmed by repeat thin films and rapid serological method which is based on the detection of *Plasmodium vivax* lactate dehydrogenase (pLDH) and *Plasmodium falciparum* histidine rich protein II (*p.f* HRP-II). This rapid device method has been recently evaluated with high sensitivity and specificity of 96.4% and 98.9% respectively[19]. We could not perform PCR because of lack of this facility in our setup. Nevertheless, our case report suggests that *P. vivax* alone can cause complications like cerebral malaria in malaria endemic areas like ours.

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Articles should report original work in the fields of microbiology and infectious diseases.

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Abstract
Abstract should not exceed 200 words and must be structured in to separate sections. Avoid abbreviations or citing references in the abstract.

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Four to five standard key words must be provided.

Introduction
The section must clearly state the background and importance of the research along with objectives.

Materials and Methods
Mention the place and duration of study. design of study and details of interventions used must be clearly described. Provide details of subject selection (patients or experimental animals). Details must be sufficient to allow other workers to reproduce the results. Identify precisely all drugs and chemicals used, including generic name(s) and route(s) of administration.

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Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of five illustrations (in a combination of figures and tables) per article.

Discussion
Emphasize important aspects of the study and compare with other studies. Do not repeat the details from the results section. Discuss the implications of the findings and their limitations. Link the conclusions with the goals of the study but avoid unqualified statements and conclusion not completely supported by your data.

Acknowledgments
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Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. These should be comprehensive and fully referenced. Articles should contain Abstract, main mext divided into sections, Conclusions and References.

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Instructive cases with a message are published as case reports. Routine syndromes or rare entities without unusual or new features are invariably rejected. The text should contain no more than 1000 words and 2 illustrations or tables. The authorship should not exceed 4 persons.

Letter to the Editor
These may relate to material published in the IDJP, topic of

interest pertaining to infections diseases, and/or unusual clinical observations. A letter should not be more than 300 words, one figure and 3-5 references.

News and Views
Pertinent news and updates in infectious diseases from around the world (approximately 200 words).

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

References
Number references in the order in which they are first cited in the text. Label references in text, tables and legends by Arabic numerals (in superscript). References cited only in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification of the particular table or illustration.

Write all authors, complete title, journal name (standard abbreviation in italics), year, volume, issue, page numbers according to "**Uniform Requirements of Manuscripts submitted to Biomedical Journals**", as cited in *N Engl J Med* 1997; 336:309-15.

Tables and Figures
Data reported either in a table or in a figure should be illustrative of information reported in the text, but should not be redundant with the text. Table should be numbered consecutively in Arabic numerals. Tables and Figures legends should be self-explanatory with adequate headings and footnotes.

Illustrations
Illustrations should be numbered and given suitable legends. If possible, figures should be submitted in electronic format as either a TIFF (tagged image file format) or JPEG format. Minimum resolution for scanned artwork is:

Black & White Illustration
- Line, e.g. graphs: 600 dpi
  Halftone, e.g. photographs: 300 dpi

Coloured illustrations: 400dpi (images should be split CMYK)

Ethical Guidelines
The research project must be reviewed by Ethical Committee of the institute. All clinical research articles/studies involving human subjects submitted to the IDJP must adhere to ethical guidelines of their institutions and have informed consent from their patients. All scientific research that uses animals in their study protocols must include a statement on the ethical treatment of animals during the study.

Financial support: Authors must disclose any financial support received during the course of the study.

Conflict of Interest: If there is any conflict of interest then this must be disclosed by the author(s) at the end of the article.

Plagiarism
Make sure that your article is not copied or reproduced. Plagiarism in research is a crime and the matter will be taken seriously.

Instructions updated - December 2010.
Editor IDJ

MEMBERSHIP APPLICATION FORM

INFECTIOUS DISEASES SOCIETY OF PAKISTAN

No. _____

Name

Mailing Address

Institute/ Organization

Department & Division

Field of Interest

Designation | PMDC No.

Phone No. Resdence | Office

Cell | E-mail

Degree/ Diploma:

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

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

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For Office Use Only

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Membership No: _____ Reference No: _____

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Should be at least medical graduates registered with PMDC and having postgraduate qualification in any field.
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All the members shall have the right to:
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1. Participate in programs of the society.
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Please send your Application form by hand or by mail only.
Membership fee will only be received in cash/ cross cheque/ pay order or bank draft made out to Infectious Disease Society of Pakistan.

Mailing Address and Contact Nos:

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