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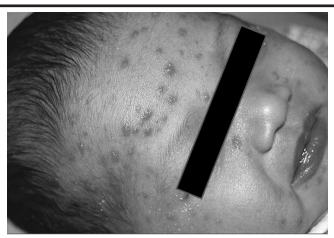

Neonate with multiple vesicular lesion of the face (Neonatal Varicella).

Courtesy: Department of Paediatrics & Child Health, AKUH, Karachi.

Under Graduate Medical Education- Where are we and Where to go?

Undergraduate medical education in our setup has failed to keep pace with the rest of the world. There is an obvious deterioration in this field which has progressively occurred over the years. The existing colleges in the public sector with two to three hundred students in each class reflect a poor picture of curriculum delivery and the state of student faculty contact can well be imagined.

The basic sciences faculty till near past consisted of people who became teachers mainly by virtue. Salaries in the public sector were meager. There is no criterion, any body who has a post graduate diploma is a teacher, no system of teacher training /grooming exists in our set up.

The mushrooming of colleges in the private sector has provided a hope in this bleak situation. Salaries and working environment has improved considerably both in basic sciences and the clinics. Now young enthusiastic doctors are seen running after post graduations inspiring for a career in basic sciences. This competitive environment has forced the public sector colleges to revisit their policies. Unfortunately the institutions are more focused on infrastructure, equipment and required numbers of faculty numbers as this is mandatory for PMDC approval. Not much attention is paid to the educational aspect in the colleges.

Existing short falls

Curriculum

Content

Content overload is the major factor in the confusion which exists in undergraduate teaching. Every topic which is there in the text book is part of the curriculum. The volume is so enormous that it produces what can be called as intellectual choking of the students.

Delivery

Text books are the main focus in content delivery which is done chapter-wise. Main tool of delivery is the lecture of one hour duration and practical performed on bodies, models and animals. Some of them have no relevancy to the practice of medicine. A considerable amount of knowledge crammed (not learned) is never applicable in their entire medical careers. Theory and practical are taught as separate modalities with hardly any link between them.

Each subject is taught in complete isolation. If the hip bone is taught in anatomy, physiologist may be teaching cardiac cycle. Each subject specialist is focused on the continuity and sequence in his/her own subject rather than relating teaching with the other disciplines. The student is left at own to create a linkage between structure, function and abnormal functions. There is no deliberate attempt to create relevancy in the classrooms. So at times the student wonders why he/she is studying all this content. The system is such that a number of students have an impression that classroom teaching has a negligible effect on the results of their examination. This leads to disinterest in attending the classes and loss of credibility of the faculty and the whole system in the eyes of the students.

The subject specialist has a desire to teach the student everything he/she knows and what all known may be many years old. There is little attempt to be updated by adding new concepts and removing the old ones. Most of the experienced faculty members have difficulty even in using computers.

Remedial measures proposed

Curriculum

Content

Extensive content revision is required. Guided by the regional epidemiology, unnecessary and redundant portions should be eliminated. Content has to be reduced to make it possible for the student to master it.

Delivery

Teaching text books cover to cover must give way to learning occurring in themes, developed from real life situations. Learning should take place in smaller groups. Didactic lectures should not be more than 10% of the learning program. Adequate time should be given to the students for self study to prepare for group discussions. Classroom session should be alive and integrated with assessment so that students who miss classes would find it difficult to pass in the examinations.

Teaching material should be prepared in such a way that it promotes analysis and application. The curriculum should be used to develop skills like critical evaluation and decision making. We need to produce lifelong learners and leaders in medical practice.

Evaluation

Major overhauling is required for the existing evaluation system. Weightage of the annual examination needs to be reduced and spread over the entire year. Students who are regular in learning throughout the session must receive credit. This will discourage the trend of cramming to pass the examination at the end of the session. This will also help in changing the focus of studies from just passing the exam to learning for the sake of acquiring knowledge and skill. The assessment needs to be prepared in such a manner that it measures the critical thinking and decision making of the students rather testing their short term memory recall. After all it is a well known fact that assessment derives learning. The type of examinations will determine what and how the students will study in their courses the year round.

We are talking about these shortcomings and reforms in under graduate medical education for quite a while but hardly a few institutions have taken it seriously. Apart from the fact that by ignoring the need of the hour we will be producing an inferior product, it is quite possible that in near future the medical schools who are neglecting the curricular reforms may lose the international recognition of their degrees, depriving their graduates for further studies abroad. To sleep over and wait for that to happen will be detrimental for these institutions. This is a highly competitive world and only those survive who can read the winds of change.

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ORIGINAL ARTICLE

Comparison of Thin Layer Agar 7H11 and MGIT 960 for the Detection of Rifampicin and Isoniazid Resistance in *Mycobacterium tuberculosis Complex*

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Abstract

Objective
To validate Thin Layer Agar containing Middlebrooks's 7H11 medium using fully automated MGIT 960 system for detection of rifampicin and isoniazid resistance in acid fast bacilli smear positive clinical specimens.

Methods
This was a cross sectional study conducted at department of Microbiology Armed Forces Institute of Pathology, Rawalpindi Pakistan from August 2011 to July 2012. All AFB positive specimens submitted in the department during the study period were inoculated onto Bactec Mycobacterial Growth Indicator Tube (MGIT) 960 system and Middlebrooks's 7H11 medium simultaneously. The results of both tests were recorded by independent readers who were blinded to each other. Subjects on anti-tuberculous treatment, blood and bone marrow specimens, samples of saliva and pus swabs and mycobacterim resistant to paranitrobenzoic acid (PNB) on Thin Layer Agar (TLA) and MGIT 960 system were excluded from the study. Results on MGIT 960 (culture-based technique) were taken as gold standard.

Results
A total of 211 specimens (including sputum, bronchoalveolar lavage, endobroncheal washings, fluids, CSF) were tested. Seven (3%) specimens gave contamination on TLA while four (2%) specimens were contaminated on MGIT 960 system; these were excluded from the final analysis. Results of TLA compared with gold standard MGIT 960 system showed an agreement of 66 % for isoniazid (INH) and 72% for rifampicin (RIF).

Conclusion
TLA containing 7H11 medium is a simple method, for the direct detection of INH and RIF resistance in *Mycobacterium tuberculosis* on smear positive clinical specimens.

Key Words
Mycobacterium tuberculosis complex, MGIT 960 system, Middlebrook's 7H11 medium.

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Introduction
Tuberculosis (TB) has been present in humans since antiquity.[1] It is estimated that ten million new TB cases and 2 million deaths occur each year, more than any time in history.[2] Furthermore, an estimated 2 billion people are thought to be latently infected, providing a large reservoir for active TB that will last for decades.[3] In Pakistan incidence of TB is 181 per 100000 population per year.[4] According to World Health Organization (WHO) global tuberculosis report 2011, number of new cases of MDR-TB in Pakistan is 3.4 % per year.[5] This has further been complicated by the emergence of multidrug-resistant tuberculosis (MDR-TB), defined as the resistance of the organism to INH and RIF, which has reached an alarming level in our population.[6,7] This demands appropriate treatment with second line anti-TB drugs but also accurate and reliable drug sensitivity testing.[8]

Timely availability of drug susceptibility testing (DST) is important in administering anti-TB treatment (ATT) to improve clinical results and survival rates.[6] The development of new diagnostic tools for the detection of resistance in tuberculosis is an important in WHO new global "Stop TB Strategy".[9] To attain the new goals recommended by the WHO for the treatment of MDR-TB, all laboratories should develop the capability to perform DST of first and second-line anti-tuberculosis drugs to detect MDR and extensively drug resistance tuberculosis (XDR-TB) using reliable methods.[10]

For diagnosis of MTB infections, slow and time consuming mycobacterial culture is still the gold standard.[11] Lowenstein-Jensen (LJ) medium has long being used for the culture of MTB with a sensitivity of around 60 % , but this medium cannot be used for the drug susceptibility testing of anti-TB drugs.[12] MGIT 960 is a fully automated commercially available system with reliability in the rapid detection of resistance to first and second line anti-tuberculous drugs. However, it requires costly equipment and consumables, which makes it unsuitable for resource poor countries like Pakistan.[6] To reduce the time of diagnosis of MTB, some new methods have been developed, which include microscopic observation drug susceptibility assay (MODS), Nitrate reductase assay (NRA), colorimetric redox-

indicator assay and thin layer agar (TLA).[13-16] In addition to the detection of growth within 10 days, it also allows the initial identification of *M. tuberculosis* based on its characteristic cording morphology, which can be observed microscopically.[17, 18] It does not require any sophisticated equipment and is better recommended for use in under-developed countries.[6] In this study we compared low cost method TLA with MGIT 960 system on clinical specimens, as an early guidance to physicians' help in prompt and appropriate therapy.

Methods

This laboratory based comparative study was carried out from August 2011 to July 2012 at the department of Microbiology Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan. AFIP caters to the needs of local clinics and hospitals of Rawalpindi, and also receives clinical samples for culture of *M. tuberculosis* from tertiary care hospitals like Combined Military Hospital Rawalpindi, Military Hospital Rawalpindi, Armed Forces Bone Marrow Transplant Centre Rawalpindi and from Northern areas of Pakistan. All AFB smear positive clinical specimens e.g. sputum, bronchoalveolar lavage, endobroncheal washings, pleural fluid, peritoneal fluid, tissues, CSF and pus specimens, submitted at AFIP for culture of *M. tuberculosis*, were included in the study while specimens from patients on ATT, blood and bone marrow specimens, samples of saliva and pus swab, mycobacteria resistant to PNB on TLA and MGIT 960 system were excluded. The study was approved by the Ethical Committee of the institute. Age and gender of the patients were recorded. The results of both tests were recorded by independent readers who were blinded to each other.

All sputum samples were collected in sterile wide mouth leak proof container aseptically. The specimens were subjected to ZN staining and AFB smear positive specimens were included in the study. *M. tuberculosis* H37Rv (ATCC 27294) was used as the reference susceptible control. Standard decontamination procedures were followed. [19]

Inoculation onto MGIT 960 tube was carried out as per the manufacturer's instructions. All the tubes were incubated at a temperature of 37°C for a period of six weeks**.** Once the instrument flashed a tube positive, it was removed after scanning of barcode and then subjected to ZN stain for AFB presence and sub-cultured on to Columbia agar (Oxoid, Basingstoke, UK) containing 5% horse blood (blood agar), incubated aerobically at 37°C overnight and examined for any contaminant growth. When AFB was identified in a smear prepared from a positive MGIT tube, the growth was further confirmed as *M. tuberculosis* complex (MTBC) by PNB test.

If the sample was positive for MTBC, DST for INH and RIF was performed using 7ml MGIT tubes. One tube was used a control whereas one tube was used each for INH and RIF. DST was performed as per recommended standard procedure. At the end of protocol incubation, all the negative tubes were subjected to ZN staining to look for any false negative tubes.

A quadrant petri plate was used and prepared with 5 ml of 7H11 agar per compartment, as per the protocol described by Satti *et al*.[20] One of the compartments served as the growth control (GC), second with 1 µg/ml RIF, the third compartment with 2.0 µg/ml INH and the last one with 500 µg/ml PNB. 10 µl of the inoculum was added to each compartment. Plates were sealed with parafilm and incubated at 37°C in a 5% CO_2 incubator. Plates were read twice weekly for 28 days using a conventional microscope (objective 10×). Once the GC compartment was positive, resistance was defined as any growth appearing in the compartments with drugs as compared to the GC compartment. A susceptible strain was defined as no growth appearing in the compartments with drugs compared to the GC. Any growth in the compartment containing PNB was considered as mycobacterium other than tuberculosis (MOTT) and was excluded from the study.

Sample size was calculated by using WHO sample size calculator by anticipated population proportion 90% (arbitrarily taken sensitive), absolute precision 4.5% and with 95% confidence interval; we needed a total of 200 samples.[21]

We calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) using SPSS (Statistical Package for the Social Sciences) version 18. Overall, percent agreement was calculated by adding positive cases by both methods and then dividing by total number of cases.

Results

During the period of study, 200 AFB positive specimens were evaluated on both MGIT 960 system and Middlebrooks's 7H11 medium, for the detection of INH and RIF resistance. Forty four percent (n= 88) specimens were from female patients with a female to male ratio of 1:1.3.

Sputum was the most frequent specimen 138 (69%), followed by endo-bronchial washing 28 (14%), tissue 5 (2.5%), pus 10 (5%), fluids 11 (5.5%), broncheoalveolar lavage 6 (3%) and CSF 2 (1%) of all the specimens tested.

Susceptibility results of our study the summarized in table 1. Overall agreement between the two methods was 72 % for detection of RIF resistance and 66 % for INH. Total MDR isolates on MGIT 960 were 49 and 37 on TLA.

Discussion

This is the first reported study in Pakistan on the use of TLA for direct detection of INH and RIF resistance from smear positive specimens. These two media have been compared before in this institute as well, but with respect to recovery rate and time of detection of *M. tuberculosis*.[20] We compared the same media for detection of INH and RIF resistance. A study

Table 1: Comparison of MGIT 960 system with TLA for INH and RIF sensitivity testing

	MGIT 960	TLA		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Agreement (%)
		R	S					
INH	R	39	40	71	72	49	87	72
	S	16	105					
RIF	R	67	37	69	64	64	69	66
	S	30	66					

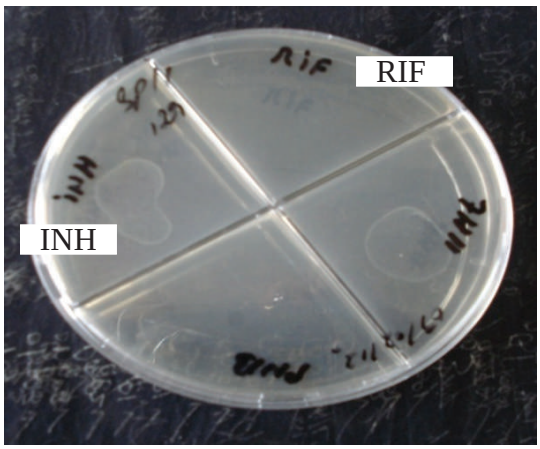

Fig 1: *M. tuberculosis* **strain showing resistance to INH only on TLA**

conducted at the Institute of Tropical Diseases Belgium, compared three commonly used media for the detection of *M. tuberculosis*, both in terms of time to detection and also for the rapid detection of INH and RIF resistance. These media included MGIT 960, TLA medium and LJ medium. The results of that study showed that median time of growth on TLA is comparable to that of MGIT 960 method. TLA sensitivity, specificity and predictive values for INH and RIF were 100 %, there by concluding that performance of TLA is comparable to other conventional DST methods used for MTB.[22] In our study, the sensitivity and specificity of TLA was less than that study.

TLA has been used for the detection of resistance of second line drugs. Although we restricted our project to the detection of resistance in INH and RIF because of logistic reasons, this media can be used for detection of resistance in other first and second line ATT. A study conducted in Belgium in 2009 evaluated this media for the detection of resistance in ofloxacin and kanamycin along with RIF. and reported high sensitivity and specificity, thereby indicating that TLA quadrant plate can be used for the rapid detection of resistance to these three TB drugs with a median of 10 days.[6]

A limitation of our study was that we did not record the growth rate of MTB on TLA. Nevertheless results of our study are encouraging and the technique can be used for screening of MDR-TB, larger studies are required to evaluate this method before it can be recommended for use.

Conclusion

TLA containing 7H11 agar is an easy, reliable and simple method for the detection of DST of MTB in different clinical specimens in the peripheral health care diagnostic facilities for screening of MDR-TB.

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ORIGINAL ARTICLE

Factors Contributing to Drug Defaulters in Tuberculosis Patients at Bahawal Victoria Hospital

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Abstract

Objective
To identify the factors associated with non-compliance to anti-tuberculosis (ATT) treatment under the direct observational therapy (DOTS) program at Bahawal Victoria Hospital Bahawalpur.

Methods
The demographic and clinical data of patients aged more than 14 years, presenting to the Bahawal Victoria Hospital Bahawalpur were retrospectively analyzed from January 2010 to December 2010. Phone calls were made to the patients who were enrolled in the DOTs programme at the BVH hospital and defaulted from treatment to know the reasons for default.

Results
Over a period of one year, 2350 patients were enrolled. We categorized the defaulted patients in three age groups < 30(22±2.27), 30-50 (38±2.8), >50(59±2.5) years. Majority of them were males (n=1219; 60.4%). Two hundred and sixty (11%) patients had acid fast bacilli positive. 434 (18.45%) patients defaulted from treatment. Most patients defaulted within the first three months (75.8%). Majority of patients who defaulted were of older age group >50years (41.4%), non-school enrolled 358(82.48%) females (mostly house wives), unemployed, students 227(22.99%), farmers 173(15.35%) and skilled workers 34(14.4%). It was also noted that patients residing far from treatment center (>10km) had a higher chance of default (26.47%) as compared to patients residing <10km (13.93%) P<0.001. Most patients 374 (86%) patients had pulmonary TB and only 60(13.8%) had extra pulmonary tuberculosis. Poverty, initial symptomatic treatment and use of alternative treatment were identified to be the most common reasons for default.

Conclusion
Old age, male gender, illiteracy, poverty, distance from treatment center, poverty, initial symptomatic treatment and use of alternative treatment are the main factors contributing towards drug defaulter in patients of tuberculosis at Bahawal Victoria Hospital Bahawalpur.

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Key Words
Tuberculosis, Directly Observed Treatment

Introduction

According to WHO estimates, over 2 million people die every year because of tuberculosis; and 95% of these deaths occur in developing countries.[1] Certain factors like emergence of HIV, malnutrition, poor vaccination coverage and efficacy, drug resistance and associated chronic conditions increase the burden and magnitude of this disease.[2]

Situation of tuberculosis in many countries of South Asia like Srilanka is serious where the default rate is 23%.[3] In Pakistan its incidence is estimated to be 231 per 100,000 and majority of patients are in the reproductive age group. Our country has an annual death rate of 38 per 100,000 due to tuberculosis.[4] According to a study default rate of tuberculosis in rural areas of Pakistan is 66%.[5] The reason behind this alarming situation is lack of effective TB control program in Pakistan and poor adherence to its treatment.[6]

National TB program was launched in 1960. It is working all over Pakistan.[7] The National TB Program (NTP) adopted DOTS strategy with the help of WHO in 1995.[8,9] The case detection rate for Pakistan rose from 13% in 2002 to 67% in 2007, close to WHO's target of 70%.[10] DOTS program is the only hope for controlling the problem of drug default in TB. DOTS has been successfully implemented in many countries but default from treatment is one of the reason of the failure of DOTS in Pakistan.[11] Keeping these facts in view, current study was conducted to determine the factors which contribute to drug default in a DOTS programme

Material and Method

Study Setting

The study was conducted at Pulmonology Department of Bahawal Victoria hospital (BVH). Bahawalpur City is situated in southern Punjab, with a population of 3.2 million. Bahawal Victoria Hospital is the only tertiary care center, working at district Bahawalpur. There are 20 diagnostic and 93 national TB program treatment centers functioning in district Bahawalpur. DOTS program was launched in the Pulmonology department of BVH, Bahawalpur in 2006. Every year almost more than 2 thousands new cases of TB report here. We do not have sputum culture facilities available in our center and diagnosis of

pulmonary TB is based on sputum examination according to the NTP guidelines.

Study Design
Cases of tuberculosis (pulmonary and extra-pulmonary), registered in the DOTS programme at the BVH hospital from January 2010 to December 2010 were retrospective reviewed. The subjects were contacted over the phone for information.

Inclusion Criteria
Patients aged > 14 years, residents of district Bahawalpur, diagnosed with any form of tuberculosis and in TB category (I or II) were enrolled in the study.

Exclusion Criteria
Patients with multidrug resistant tuberculosis, those with co-morbid conditions like chronic renal failure, chronic liver disease, chronic obstructive airway disease and patients with physical handicap.

Operational Definition

Defaulters
A patient who completed at least 1 month of treatment after at least 2 month of interruption of treatment at any time during treatment coarse.[12]

Category 1
New case of smear negative or smear pulmonary or extra pulmonary tuberculosis who had not taken anti tuberculosis treatment before.

Category 2
Previous treatment for more than four weeks in the past, and was found sputum smear positive pulmonary tuberculosis. This category includes relapse, failure, treatment after default and others (with smear positive). (Retreatment cases).[13]

Results
Total 2350 patients were enrolled over a one year period, of which 260 patients were AFB positive. Total 434/2350 (18.45%) patients defaulted from treatment, 282(64%) were males and three fourth of them 329/434 (75.80%) defaulted during first three months of their treatment. Majority of patients who defaulted were >50years (41.4%). Similarly 358(82.48%) patients who defaulted were non-school enrolled. Most of the patients were females (mostly house wives), unemployed and students 227(22.9%). Almost one third were poor farmers (15.35%) and one third were skilled workers 34(14.4%). Patients residing>10km from the treatment center had a significantly higher chance of default as compared to patients residing <10km (p<0.001) (Table 1). Majority (86%) had pulmonary tuberculosis and remaining had extra pulmonary tuberculosis (pleural effusion, lymphadenopathy, meningitis, TB abdomen and TB spine). The reasons for default from treatment and the timing of default after start of therapy are shown in table 2 and 3.

Discussion
Poor compliance to anti tuberculous treatment is one of the major obstacles in the control of TB. Social and demographic features play an important role in the adherence to tuberculosis treatment. Tuberculosis is a disease of the poor and if adequate social

Table 1: Distribution of defaulters according to their occupation, distance from the treatment center and educational status

Occupation	Defaulters n= (434)	Completed treatment n =(1916)	Total patients n=(2350)	p-value
Dependent (house wives, students and unemployed)	227	760	987	<0.1
Farmers	173	954	1127	
Skilled workers/ civil servant	34	202	236	
Distance from the treatment center				
1-10km	1500	205	1291	<0.001
>10km	850	225	625	

Significance level =P<0.05

Table 2: Reasons for default from treatment identified on telephone contact with the patients

Reasons for default	n (%)
False telephone number	69(15.8)
Lack of interest in treatment due to poverty	150(34)
Initial symptomatic improvement	112(25)
False believes	23(5.4)
Unsatisfied from behavior at treatment center	30(6.9)
Using alternative treatment	50(11.5)

Table 3: Time of default in months after start of antituberculous therapy

Time of default (months)	n (%)
2	231(53.2)
3	93(21.4)
4	67(15.4)
5	25(5.7)
6	13(2.9)

support is not provided the risk of default increases, as is evident in our study and also documented from other countries.[14]

Default in the older age group could be their multiple co morbidities, ill health, unavailability of transport and dependency to others; as documented earlier by Demissie.[15] Chances of default are maximum at the end of the intensive phase, possibly due to symptomatic improvement in condition.[16] Although majority of patients know the sign and symptoms of the disease but they are naive about the poor outcome of the disease if default after initial symptomatic improvement.[17] We observed that females defaulted more than males; a similar trend has been observed in other countries.[14] There is a need to understand the reasons for default specifically in this population so that the issues can be adequately addressed and adherence to treatment improved. Earlier reports from developing countries highlight stigmatization as a major cause of default in females.[18]

Bahawalpur is an agricultural city. Most of the patients coming for the treatment of tuberculosis belong to the nearby villages. Although the facility of DOTS is available to the patients at centers near their homes but most patients are registered and treated in BVH probably because of lack of confidence in their treatment center, unavailability of doctors and supply and quality of medicines. Results of other studies with respect to distance from the treatment center are similar to our findings; the economic cost of travel to the treatment center hinders compliance.[18] For similar reasons unemployment and poverty increase the chances of default.[19] Major limitation of our study was that it was a retrospective review and we could not determine the cause of default in almost one fifth of the subjects because of a fake telephone number, and further details of default in females and reasons for dissatisfaction from treatment at the DOTs center could not be identified.

Conclusion

Sociodemographic features lead to default in tuberculosis treatment, adequate infrastructure of DOTs ensuring a social support network is likely to improve adherence to treatment.

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ORIGINAL ARTICLE

Infection Associated Hemophagocytic Histiocytosis - Clinicopathological Correlation

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Abstract

Objectives
To describe the etiology, grading and clinic-pathological features of hemophagocytosis associated with infections.

Methods
Sixty-two patients suffering from infection of varied infectious etiology (viral, tuberculosis, enteric fever, malaria, leishmaniasis) were included in the study over a two-year period (2004-06). Bone marrow aspiration was done in all cases along with complete blood counts. Bone marrow smears were examined for HP and its grade and effect on hematological parameters was noted.

Results
The cause of hemophagocytosis (HP) was found to be viral in 17 patients followed by tuberculosis (9), and enteric fever (13). The remaining patients had malaria, visceral leishmaniasis, or brucellosis. Most of the patients showed either moderate, (grade II, n=31) or severe degree, (grade III, n=21) of HP. Hemophagocytic syndrome (HPS) was present in 21 patients. Patients with higher grades of HP had profound effect on hematological parameters; particularly hemoglobin and platelet count, resulting in the depression of these cell lines.

Conclusion
Viral, bacterial and parasitic infections play an important role in the causation of histiocytic hyperplasia with HP. It may present as HPS with multisystem disorder, which could be fatal if not properly diagnosed and treated.

Key Words
Hemophagocytosis, Hemophagocytic syndrome, Infection

Introduction
Phagocytosis of all blood cells, mature and developing, by activated histiocytes of the mononuclear phagocyte system is known as HP. It has been reported in the literature under various diagnostic terms, such as histiocytic medullary reticulosis, malignant histiocytosis and infection associated HP.[1] This phenomenon may result from immunologic activation of the mononuclear phagocyte system (reactive) or may be due to a neoplastic proliferation of histiocytes (malignant). Sometimes this may occur as a result of genetic or chromosomal derangement.[2] HP with histiocytic hyperplasia may present as HPS.[3] It was first identified in 1979 in association with viral infection in immunocompromised patients.[4] Subsequently it was recognized as a reactive process in a variety of other infections including bacterial fungal and parasitic.[5]

There is scanty literature about the causes, grading and clinical features of HP from Pakistan. We aim to describe the etiology, grading and clinic-pathological features of HP associated with infection from Pakistan.

Materials and Methods
The study was carried out at Pakistan Institute of Medical Sciences and Riphah University allied teaching hospitals from March 2004 through March 2006. Sixty two patients with underlying infections were included in the study. Detailed history was taken and a complete physical examination was done in each case. Complete blood counts, reticulocyte count, absolute values, RBC morphology were performed in each subject. For cell counts and absolute value hematology analyzer Sysmex K1000 was used.

Bone marrow aspiration was done in all the patients and trephine biopsy was carried out in relevant subjects. All the bone marrow aspiration smears (Leishman's stained) were examined for the presence and grading of HP. The parameters looked into were cellularity of bone marrow in general, cellularity and maturation of erythropoiesis, myelopoiesis and megakaryocytes. The smears were also examined for lymphocytes, abnormal cells, histiocytes and parasites. The phenomenon of HP was graded in the following manner: numbers of phagocytic cells were counted in 10 random fields and average of such cells per high power field was calculated. The intensity of HP was graded depending upon the number of hemophagocytic cells per high power field (Table 1).

Other relevant tests in individual cases were done to establish the type of infection like Widal test, viral screening, bacteriological culture and serological tests. Liver function tests, coagulation studies, lipid profile, serum ferritin levels were done in order to establish the presence or absence of HPS.

The grading criteria are shown in table 1. Grading of intensity of HP was done on bone marrow aspiration smears in order to determine the severity of this phenomenon. The mounted slides

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Table 1: Grading criteria

Grading	No. of Hemophagocytic Cells/HPF
Grade I (Mild)	1-2
Grade II (Moderate)	3-5
Grade III (Severe)	6-8
Grade IV (Very severe)	7-8

were first scanned under low power (x10) in 10 random fields and presence or absence of HP was noted. Slides were then examined under high power (x 40) to grade the intensity of HP.

At the time of bone marrow aspiration, 2 ml of aspirated material was mixed with brain heart infusion (BHI) medium for routine culture in relevant cases. Lowenstein Jensen medium was inoculated with 0.5 ml of bone marrow aspirate in cases suspected of having TB. The culture bottles were sent to microbiology department for further processing.

Results

Sixty-two patients with different underlying infections showing HP in the bone marrow were included in the study. The age of the patients ranged from 1.8 to 63 years with male to female ratio of 1.8:1. Fever was the most common symptom at the time of presentation, occurring in all cases. This was followed by cough (63%), generalized weakness (58%), diarrhoea, bleeding and night sweats. Majority of the patients had mild to moderate anemia (65%) at the time of bone marrow examination. Most of the patients had splenomegaly (62%). Hepatomegaly, lymphadenopathy, jaundice, bruises and purpuric spots were other important physical findings at the time of presentation. Hemoglobin levels ranged from 17 to 308 x 10^9/L at the time of bone marrow examination; platelet count range and from 17 to 308 x 10^9/L.

The underlying etiology of HP in the subjects is shown in table 2 and figure 1. Out of the 62 cases, most of the patients had either moderate Grade II or severe Grade III HP (Table 2). HPS was present in 21% patients. Mostly viral infections were associated with HPS. These patients had depressed cell counts presenting with pancytopenia, liver dysfunction, organomegaly and coagulopathy (Table 3).

The hematological parameters were depressed in majority of the patients in Grade II and Grade III groups. Mean hemoglobin, TLC and platelet counts were significantly reduced in Grade II and Grade III ($P < 0.05$) as compared to Grade I (Table 3).

Three adult patients were diagnosed with brucellosis, one having mild HP and two patients had moderates HP. Serology was positive for Brucella infection. None of the patient presented as HPS. Peripheral pancytopenia was found in one patient.

Out of the nine patients diagnosed with tuberculosis; 6 had

Table 2: Underlying etiology and grade of infection-associated hemophagocytosis (n= 62)

Etiology	n (%)	Grade I	Grade II	Grade III
Viral	17 (27.4)	3	10	4
Enteric	13 (21)	2	5	6
Tuberculosis	9 (14.5)	2	4	3
Malaria	8 (12.9)	-	3	5
Visceral leishmaniasis	9 (14.5)	1	5	3
Brucellosis	3 (4.8)	1	2	-
Unknown etiology	3 (4.8)	1	2	-
Total	62	10	31	21

Table 3: Hematological parameters with varying grades of intensity of HP

MEAN	GRADE I	GRADE II	GRADE III	P-value
Hb gm/dl	10.2	9.1	6.3	<0.05
TLC x 10^9/L	11.4	8.3	4.5	<0.05
Platelet count x 10^9/L	189	152	128	<0.05

pulmonary disease and three had miliary tuberculosis. Two patients with disseminated TB had granuloma in the BM trephine biopsy specimens. AFB culture in the BM was positive in 2 patients. Three patients presented as HPS. Blood culture was positive in 5 patients for *Salmonella typhi* and bone marrow culture in four of these subjects.

Eight (3.2%) patients were diagnosed with malaria in the present study. Four patients had *Plasmodium vivax*, two had *Plasmodium falciparum* infection and two patients had mixed infections. Two patients with falciparum infection with grade III intensity presented as HPS with peripheral cytopenia, hepatosplenomegaly, liver dysfunction and hyperferritinemia.

Discussion

Clinical features and biochemical events of HPS include fever of unknown origin, cytopenias, hepatosplenomegaly, coagulopathy, hyperferritinemia, liver dysfunction and derangement in lipid metabolism. It may be primary as observed in familial hemophagocytic lymphohistiocytosis, X-linked lymphoproliferative syndrome, Chediak-Higashi syndrome and Griscelli syndrome, or secondary to infection, malignancy, autoimmune disease, drugs, non-malignant hematological conditions and variety of other diverse disorders.[6]

Hemophagocytic histiocytosis, although an uncommon

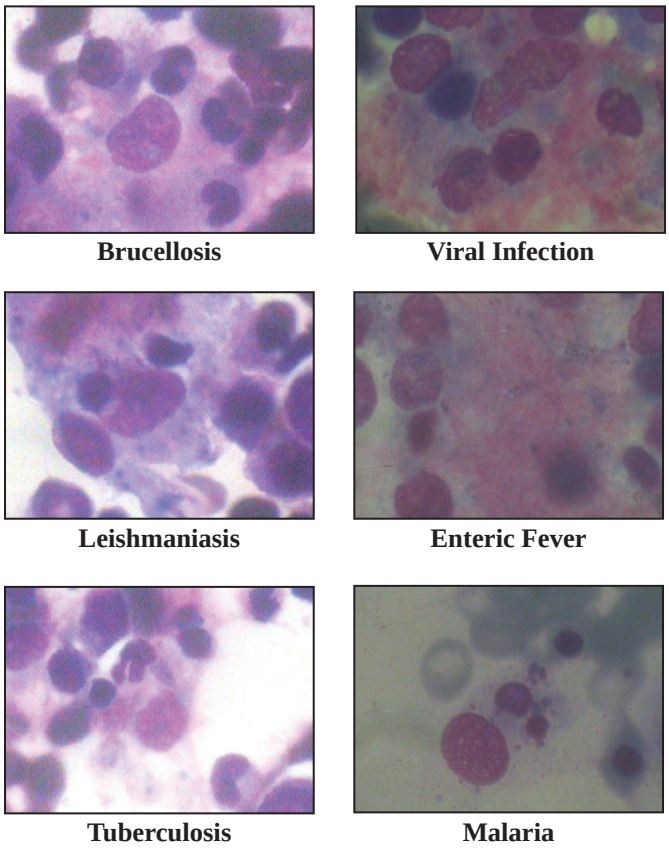

Fig 1. Showing phenomena of HP in various infections

condition, has been reported in several clinical situations mainly as a reactive phenomenon, sometimes with fatal outcome. The term initially used for this process was histiocytic medullary reticulosis and it was invariably believed to be neoplastic. Infection associated HP is a multisystem disease process. There is usually an illness 2-6 weeks before the manifestation of HP becomes apparent. It may present as a full-blown syndrome with all the constitutional signs and symptoms or as histiocytic hyperplasia with HP.[6]

The viral agents commonly implicated include Epstein-Barr virus, cytomegalovirus, herpes simplex, Varicella-Zoster, adenovirus II, HIV, parvovirus B19, dengue virus, rubella, influenza A, coxsackie A9 and hepatitis A virus.[7] HP may also develop in association with bacterial infection such as tuberculosis, enteric fever, brucellosis and variety of other Gram negative or positive bacteria.[1-12] Parasitic infection in which HP has been observed includes leshmaniasis and malaria.[13, 14] In the present study, 17 patients with viral infection showed variable intensity of HP. Risdall *et al*. studied 19 patients with active viral infection, bone marrow smears in those cases showed histiocytic hyperplasia with prominent HP.[4] Most of those patients presented as HPS. Other workers have also isolated different viral agents including Herpes Simplex, Epstein Barr virus, CMV, HIV, Parvovirus B19 as a cause of virus associated HPS.[15-18] In our study, majority of the cases with viral infection had severe HP. All HPS patients in our study, presented with bicytopenia or pancytopenia. Hepatosplenomegaly, liver dysfunction, hyperferritinemia, lipid derangement, and coagulation disturbance were other findings of HPS. The bone marrow was mainly hypercellular or normocellular in these cases of HP. The macrophages in the bone marrows of all these patients were benign looking with low nucleus: cytoplasmic ratio, inconspicuous nucleoli and abundant cytoplasm. Erythrophagocytosis was mainly present along with phagocytosis of leukocytes and platelets in some of the patients.

There are several reports of HPS due to tuberculosis.[19, 20] In the present study, patients with tuberculosis with increased intensity, grade III had depression of cell counts. Thirteen patients with enteric fever were diagnosed in the present study, with variable degrees of HP and presented as HPS. Erythropoiesis and granulopoiesis were depressed in these patients with histiocytic proliferation and prominent HP in the marrow. Association of erythrophagocytosis with enteric fever is well known. Risdall *et al*, also reported three patients with enteric having HPS.[4]

Although an uncommon occurrence, association of visceral leishmaniasis with HPS is also well known. Peripheral cytopenia in visceral leishnaniasis could be multifactorial. Hypersplenism, parasitic infiltration, bone marrow fibrosis and above all exaggerated HP are the important factors resulting in pancytopenia.[21]

Presence of HP in malaria has been sparingly reported in the literature, perhaps because of its uncommon occurrence in the west. Zvulunov *et al* reported a case of falciparum malaria with HP resulting in pancytopenia and proposed that systemic parasitic infections, particularly falciparum malaria, should be included in the differential diagnosis of pancytopenia and infection associated HPS.[14] In the present study also, patients of falciparum malaria had HPS and pancytopenia.

HPS in children with brucellosis, presenting as pancytopenia has also been reported.[12] It is thought that HP associated with non-viral infection could be related to over production of cytokines such as TNF-alpha and INF gamma. Bone marrow failure, hypersplenism, granulomas and hypercytokinemia, may result in peripheral cytopenia in such patients.

It is presumed that pathogenesis of peripheral cytopenia in infection associated HPS is multifactorial and largely depends upon the type of underlying infection. In viral illness, for example, the antigens may directly target the cells. Other possible mechanisms include hypersplenism, immune destruction of cells, bone marrow infiltration and suppression by microbes, phagocytosis of hemopoietic cells and above all hypercytokinemia . Additional studies are however necessary to clarify the possible role of bone marrow microenvironment, the pathogenesis of HPS leading to pancytopenia and the role of T lymphocytes and their associated cytokines profile.

Conclusion

Viral, bacterial and parasites infections play an important role in the causation of histiocytic hyperplasia with HP. It may present as HPS with multisystem disorder with profound effects on hematological parameters particularly on hemoglobin level and platelet count.

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ORIGINAL ARTICLE

Seasonality of Malaria and the Effect of Climatic Change on Malaria Incidence in Karachi

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Abstract

Background
Malaria is endemic in Karachi, with cases presenting to health care facilities through out the year. However, there are particular months of the year in which there is a surge, and medical institutes are over burdened with cases of malaria. Seasonal differences are responsible for breeding and multiplication periods of malaria vector.

Objective
To study the seasonality of malaria and the effect of climatic changes on its incidence.

Materials and Methods
This observational study carried out at The Indus Hospital in Karachi. Patients with confirmed malaria aged 14 yrs and above were enrolled between June 2010 and May 2011. Data was obtained on a data extraction form; and temperature, humidity and rainfall in Karachi for the same period was obtained from the metereologic service.

Results
A total of 970 patients of malaria were documented. The peak season for malaria was from July to October, with maximum number of cases noted in September. Mean temperature during that month was 29^{0}C and mean humidity 66%. Also, moisture status was found to be the significant predictor of malaria cases.

Key words
Malaria, Malarial Incidence.

Introduction

Malaria is one of the most common arthropod borne tropical diseases worldwide. It is responsible for high morbidity and mortality in developing countries especially among children below five years and pregnant women. It was estimated in 2010 that around 3.3 billion people worldwide are at risk of malaria with total reported cases of malaria being 216 million.[1] With the intensification of malaria prevention programmes success was noted with reduction of 50% cases in 44 countries out of the 109 endemic countries.[1] The trend of malaria, however, fluctuates and the reason for failure of complete eradication of this disease is in part due to the increase in resistance to anti-malarial drugs and insecticides. Other factors responsible for the high prevalence of malaria especially in tropical developing countries include lack of surveillance and infrastructure, occurrence of natural disasters, deforestation and climatic changes.[2]

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The female anopheles mosquito is the vector responsible for transmitting malaria; however not all species play host to the plasmodium parasite. In Pakistan, 38 species of anopheles have been identified.[3] The three most common species responsible for transmitting malaria are *Anopheles subpictus* followed by *A. culicifaciens* and *A. stephensi*.[4] The most common characteristics of breeding sites associated with these species are turbid water, fauna and inorganic matter that is found in waterlogged areas and animal ponds.[4] The life cycle of the parasite within the mosquito also depends on the climatic factors. It takes around 8-30 days for the parasite to complete the cycle from gametocyte stage to sporogony. Climatic factors conducive for its life cycle include rainfall, a minimum temperature of 16-18^{0}C, and humidity between 55% minimum to 80% maximum.[5-8]

Karachi is the largest city of Pakistan and one of the most populous cities in the world, with a population of around 21 million.[9] It is situated along the coast of the Arabian Sea and therefore does not experience extremes of temperatures. The humidity also remains high from March to November. The combination of high temperature, increased rain and humidity seem ideal for the breeding of anopheles mosquitoes and may account for the exceptionally high number of cases seen here especially during these months.

This study was aimed to assess the relationship between malariometric indices and environmental factors so that an early alert system for malaria outbreaks can be devised, thus helping to utilize preventive methods in a timely manner and streamline hospital services to better deal with malaria epidemics.

Methodology

This was a retrospective medical chart review conducted at The Indus Hospital, a non-governmental organization charity hospital in Karachi, serving a catchment population of nearly 250,000.

Malaria case data was collected from the medical records of

all patients aged 14 years and above, who visited the outpatient or emergency department of the hospital with acute onset of high grade fever (>100^{0}F), and tested positive for malaria between July 2010 and June 2011. Cases were aggregated on a monthly basis.

The association between climatic variability and the number of malaria cases presented at The Indus Hospital, Karachi was investigated using publically available meteorological data for the same period for Karachi.[10] Information accessed included daily maximum, minimum, and mean temperatures, humidity, dew point, wind speed and precipitation. Monthly mean daily maximum temperature (T max) was calculated as the mean of daily maximums for each month. Monthly mean daily minimum temperature was calculated as the mean of daily minimums for each month. Similar calculations were done for humidity and dew point.

Gold standard malarial parasite thick and thin smears and rapid diagnostics tests for confirmation of malaria status were used on all patients in the same laboratory. The incidence of malaria was recorded with respect to monthly variability and was then compared to the meteorological indices such as mean temperature and humidity for the year 2010-2011.

Secondary variables like age, gender, and thrombocytopenia were also noted. Ethical approval for the study was obtained form the Interactive Research & Development's Institutional Review Board.

Statistical analyses were done using SPSS version 16 (version 16.0; Chicago, IL, USA). Baseline and clinical characteristics of all out patients as well as those hospitalized were presented with mean and standard deviation for continuous variables such as age, and frequencies and percentages for discrete variables such as gender, type of malaria, area of residence, clinical features etc.

Correlation analysis was conducted to describe the degree of linear relationship between monthly total malaria cases to various meteorological measures including temperature, humidity and dew point. A spearman's correlation coefficient (rho) of at least 0.8 was taken to indicate a very strong strength of linear relationship, whereas a correlation coefficient value between 0.6 up to 0.8 was taken as a moderately strong relationship between the variable of interest. The coefficient of determination (r^2) was used to explain the proportion of the variance that the two variables had in common.

Multiple linear regression analysis was done to determine the best model. Variables that were not significant in the combined model were removed to come up with a final model. Principal component analysis was applied to represent malaria cases. Cronbach's Alpha was applied to check inter-reliability of principal components.

Results

In this one year study, we received a total of 970 patients who were diagnosed with malaria. Of these cases, 890 had smears positive for only vivax, 25 for only falciparum and 17 had vivax and falciparum co-infection. 72 patients were diagnosed using MPICT and hence were not specified (Table 1). A total of 43 patients required hospitalization. 61% of the vivax malaria patients had thrombocytopenia (platelet count was less than 150,000/mm^3) and 21% of these had severe thrombocytopenia (platelet count less than 50,000/mm^3). The number of malaria cases were significantly correlated with temperature, humidity, dew point and sea level pressure as shown in table 3. Maximum humidity strongly correlated with

Table1: Baseline demographics and clinical presentation of malaria patients

Baseline Characteristics	All Patients n= 970	Hospitalized Patients n=43
Gender		
Male	671 (69.2%)	23 (53.5%)
Female	299 (30.8%)	20 (46.5%)
Age		
Median Age (IQR)	30 (22-42)	40 (25-55)
Type of malaria		
P. Vivax	890 (91.8%)	30 (73.2%)
P. falciparum	25 (2.6%)	7 (17.1%)
Both	17 (1.8%)	6 (14%)
Unknown type	72 (7.4%)	12 (100%)
Quarterly presentation of malaria		
July to Sept	428 (44.1%)	21 (48.8%)
Oct to Dec	429 (44.2%)	11 (25.6%)
Jan to Mar	29 (3.0%)	4 (9.3%)
Apr to Jun	84 (8.7%)	7 (16.3%)
Residential address		
Indus Hospital catchment area	832 (85.8%)	32 (74.4%)
Non catchment area	92 (9.5%)	10 (23.3%)
Unknown area	46 (4.7%)	1 (2.3%)
Thrombocytopenia		
≤ 20000	34 (3.5%)	8 (18.6%)
21-50000	163 (16.9%)	8 (18.6%)
51-100000	374 (38.8%)	11 (25.6%)
100-150000	221 (22.9%)	2 (4.7%)
>150000	173 (17.9%)	14 (32.6%)
Leucopenia		
<3.9	309 (32%)	11 (25.6%)
4.0-10.9	632 (65.5%)	23 (53.5%)
>11000	24 (2.5%)	9 (20.9%)

malaria cases among all the environmental factors showing that number of cases increased significantly with increasing humidity with a lag of 1 month and this result was also supported by multiple linear regressions (Table 3 and Fig 2). However, using PCA it was found that the moisture factors all together were significant predictors of malaria cases (Table 4). There was a profound seasonal variation in the number of cases with a peak during June to October (mean 160.8 cases per month), the highest number of cases occurring in the month of September (n=207). This might be due to humidity which was maximum (86.3%) and temperature was minimum (32.7^{0}C) in August causing malaria vectors to live longer (Table 2)

Using multiple linear regression, the variable maximum humidity explained 69.8% of the variation (R^2 =0.698). As the max humidity increase by one unit, number of malaria cases increases by 11.7 units. It means that malaria cases depend only on maximum humidity.

Malaria cases = -836.937+ 11.661* Max.Humidity R^2= 0.698 -------- Eq (1) (P-value=0.001)

Due to presence of multicollineartiy in the dataset, principal component analysis was applied to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables.

Table 4 lists the eigenvalues associated with linear components (factors) after extraction and after rotation. The eigenvalues associated with each factor represent the variance explained by the particular linear component and also displays their eigenvalue in term of the percentage of variance explained. PCA extracts all factors with eigenvalues greater than 1.

Table 2: Seasonal variation in malaria cases – tabular

Months	Malaria Cases n	Avg Max Temp ^{0}C	Avg Max Dew point	Avg Max Humidity %
Jun 10	94	34.8	25.83	81.83
Jul 10	148	34.35	26.65	85.19
Aug 10	186	32.74	25.55	86.23
Sep 10	211	34.03	23.97	83.9
Oct 10	165	35.33	22.23	82.97
Nov 10	53	31.67	15.93	71.63
Dec 10	12	27.26	10.16	69.42
Jan 11	6	25.94	10.26	74.32
Feb 11	11	27.64	14.79	77.14
Mar 11	15	32.61	17.81	74.58
Apr 11	34	35.17	20.73	77.63
May 11	35	34.87	24.55	79.65

Table 3: Correlation of malaria cases with meteorological measures

	Malaria cases	
	Correlation Coefficient	P-value
Max Temperature (C)	**.580***	**.048**
Mean Temperature (C)	**.727****	**.007**
Min Temperature (C)	**.720****	**008**
Max Dew point	**.755****	**.005**
Mean Dew Point	**.720****	**.008**
Min Dew point	**.790****	**.002**
Max Humidity	**.811****	**.001**
Mean Humidity	**.720****	**.008**
Min Humidity	**.720****	**.008**
Max Sea Level Pressure (Pa)	**-.776****	**.003**
Mean Sea Level Pressure (Pa)	**-.776****	**.003**
Min Sea Level Pressure (Pa)	**-.776****	**.003**
Max visibility (km)	**-.567**	**.054**
Mean visibility (km)	.407	.189
Min visibility (km)	.455	.138
Maximum wind speed (m/h)	.378	.226
Mean wind speed (m/h)	.392	.208

Table 4: Eigenvalues and total variance explained after extraction and rotation of factor loadings using principal component analysis.

Total Variance Explained			
Component	Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %
1	12.994	81.214	81.214
2	1.154	7.210	88.424
	Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %
1	7.475	46.721	46.721
2	6.673	41.703	88.424

Before rotation: Factor 1 accounted for considerably more variance than the second (81.2% Vs 7.2%) however after extraction it accounted for only 48% of variance compared to 42% (rotation has the effect of optimizing the factor structure and one consequence for these data is that the relative importance of the three factors is equalized.)

Rotated matrix rotation using Varimax rotation with Kaiser Normalization is shown in Table 5. This matrix contains the loading (weights) of each variable onto each factor where values

less than 0.4 were suppressed from the output. Multiple linear regression analysis was repeated using forward method by using principal components (FA's) as inputs. FA's are the weighted factor scores calculated by sum of rotated factor loading multiplied by variable.

Malaria cases= -249.940 + 1.426*
FA2 (Moisture Status) **R^2= 0.623** **---- Eq (2)**
(P-value= 0.002)

Here coefficient of determinant is 0.623 i.e. moisture status shows 62.3% of the variation in the malaria cases. Above equation shows that as the moisture in the air increases by one unit, number of malaria cases increases by 1.4 units. It means that malaria cases depend on moisture status.

Table 5: Matrix rotation using vaimax rotation with Kaiser Normalization of environmental factors and moisture status

Rotated Component Matrix[a]	Component	
	FA1 Environmental factors	FA2 Moisture status
Mean wind speed (m/h)	.897	
Maximum wind speed (m/h)	.891	
Min visibility (km)	.816	.427
Min Sea Level PressurehPa	-.808	-.520
Mean Sea Level PressurehPa	-.807	-.526
Max Sea Level PressurehPa	-.801	-.537
Min Temperature (C)	.710	.687
Mean Temperature (C)	.686	.665
Max Temperature (C)	.586	.563
Max Humidity		.961
Mean Humidity	.462	.838
Mean Dew Point	.620	.778
Min Dew point	.604	.777
Max Dew point	.637	.756
Min Humidity	.522	.752
Mean visibility (km)	.548	.579

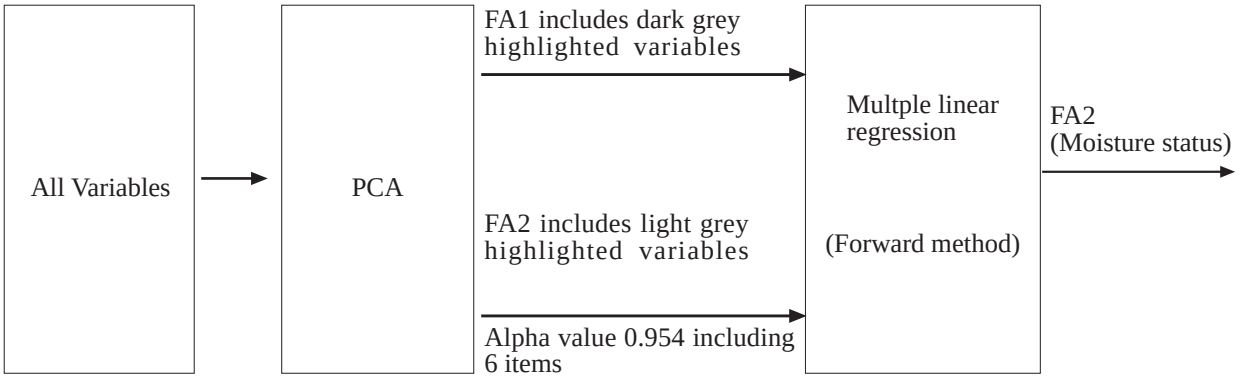
Discussion

Karachi is considered as a region with marked seasonality for malaria that is defined as 75% or more episodes of malaria occurring in less than six consecutive months in a year.[11] This study concurs with that showing 82% of patients presented in six consecutive months.

Our study showed that there was a significant correlation between humidity, temperature and the number of malaria cases with a 0-3 month lag period. Studies conducted previously in Bangladesh and India have also shown similar results defining the lag period as 0-3 months.[12-13] The Bangladeshi study, however, found no correlation between humidity, temperature and cases, after adjusting for confounding variables. The lag period could be explained by the fact that the life cycle of the malarial parasite in the mosquito takes around 8-30 days and the mosquito needs to survive more than at least 7 days for the completion of parasite development from gametocyte stage to sporogony stage.[6] Consequently the incubation period for the clinical presentation of malaria in the human further takes between 7-30 days depending on the species.[14] Temperature is a key factor in malaria transmission and directly affects growth, reproduction and spread. This study showed that malaria transmission rose after temperatures reached 30^0C. Ye et al suggested a possible reason for increased frequency of malaria in temperate season was that as the temperature rises above 27^0C the vector increases its frequency of feeding to every two days due to increase in blood digestive rates.[15]

The rise in minimum temperature was associated with a rise in the number of cases in the following month (lag of 1 month). Studies conducted in China and Burundi yielded similar results.[16,17] Relation with minimum temperature was also seen in other studies done in Madagascar, South West Ethopia and China.[5,18,19]

The correlation between minimum temperature and malaria cases may be justified by past research showing that the normal life cycle stops when the temperature falls below 16^0C, which in our cohort was noted from November to March explaining the concomitant fall in the clinically presenting numbers during these months.[6,15]



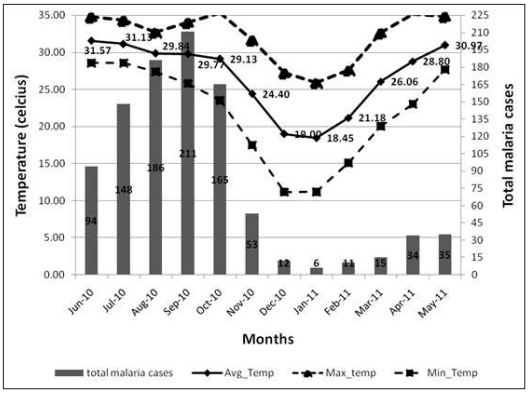


Figure 1 : Relation of monthly malaria cases with temperature

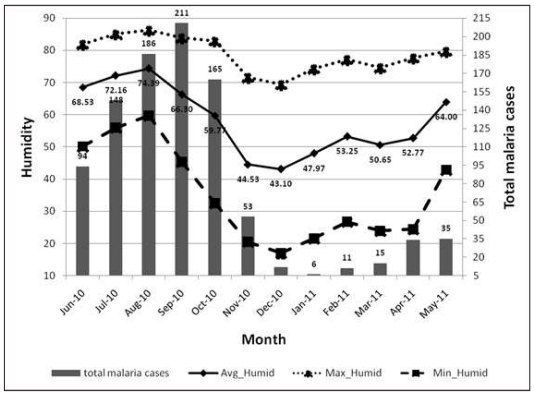


Figure 2 : Relationship of monthly malaria cases with humidity

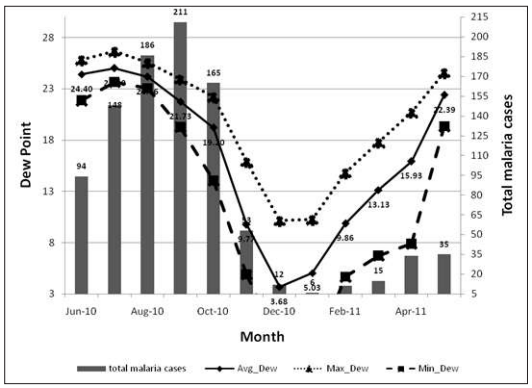


Figure 3: Relationship of monthly malaria cases with dew point

It was found from our study after adjusting for confounders (Table 5), that moisture (humidity and dew point) still played a role in increasing malaria transmission. This differed from results in Bangladesh where they found no relation with humidity after adjusting for confounders.[12] The strongest positive correlation of the number of malaria cases was with maximum humidity (R^2=0.881, p=0.001) (Table 3). A study done in the African highlands showed a correlation between humidity and malaria frequency that concurred with our results.[20] This phenomenon can be explained by the fact that the anopheles does not survive below 60% humidity, while its survival is 90% at temperatures 16^0C to 36^0C.[21,22]

The results of principal component regression that showed the effect of moisture were of particular interest as they might, in the future, be used to gauge the expected rise in malaria cases (rise by 1, increasing the rise of malaria cases by 1.4) so that hospitals in the malaria catchment areas may be better prepared to accommodate the increase in malaria incidence in those months.

Limitations of the study: This study was done only over a 12-month period. In order to understand better the implications of climatic factors on transmission of malaria another study should be carried out over several years, which would give us a complete overview of malaria seasonality in a given environment. Our patient population was from a single hospital, although the hospital caters to a large portion of Karachi, bias could arise. Future studies should be done on a larger scale using data from multiple hospitals throughout the city.

Conclusion

Karachi has a marked seasonality of malaria. The frequency of malaria is correlated to temperature and moisture, especially maximum humidity. These correlations may be used in the future as early alert systems so that hospitals may be better prepared when dealing with a high incidence of malaria.

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Efficacy of Methicillin Resistant *Staphylococcus aureus* Screen Latex Agglutination test

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Abstract

Objective
This study was conducted to evaluate the efficacy of methicillin resistant *Staphylococcus aureus* screen latex agglutination test as a rapid diagnostic modality for identification of methicillin resistant *Staphylococcus aureus*.

Place and Duration
The cross-sectional descriptive study was conducted at Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi on clinical specimens collected from November 2008 to November 2009.

Materials and Methods
Clinical isolates of *Staphylococcus aureus* were presumptively identified using colony morphology, microscopy of Gram's stain, catalase, coagulase and DNase tests. Methicillin resistant *Staphylococcus aureus* (MRSA) was identified by oxacillin disk diffusion test carried out according to the recommendations of the Clinical Laboratories Standards Institute (CLSI). The isolates were confirmed as MRSA through detection of *mec*A gene by PCR according to the agreed guidelines. The clinical isolates were subsequently tested with MRSA-Screen Latex Agglutination Test (Denka Seiken Company Tokyo, Japan) with a monoclonal antibody against the epitope PBP 2a.

Results
A total of 100 MRSA strains were evaluated. Mean age of the patients was 32.4 ±18.1 years (Mean ± SD) with male to female ratio was 3.3: 1. Majority of the MRSA were isolated from pus/ pus swab, received mostly from patients of post traumatic wound infections. *mecA* gene PCR assay identified 94 of the isolates as *mecA* positive *Staph aureus* and 6 as *mecA* gene negative. MRSA-Screen Latex Agglutination Test had an efficacy of 92% and a sensitivity of 93.62% and a specificity of 66.67%. The positive predictive value (PPV) and negative predictive value (NPV) were found to be 97.78% and 40%, respectively.

Conclusion
MRSA-latex agglutination test is highly sensitive, rapid, and reliable. This test is easy to perform at clinical microbiology labs where high tech PCR facility for detection of *mec*A gene is not available.

Key Words
Anti PBP2a, *mecA* gene, MRSA.

Introdution
S*taphylococcus aureus* has emerged worldwide as a major community and opportunistic pathogen.[1-5] More or less 20% strains are methicillin resistant *S. aureus* (MRSA).[2] MRSA increases morbidity and mortality and causes nosocomial infections worldwide.[3,4] European hospitals started reporting outbreaks of MRSA infections during early 1960s, and later MRSA clones were reported around the world.[5,6] The multidrug-resistant phenotype of MRSA strains and their intrinsic β-lactam resistance make them difficult as well as expensive to treat. MRSA infections account for approximately 35.6% and 42% in Pakistan and United States respectively[6,7].

Methicillin resistance in *S. aureus* is primarily associated with acquisition of *mecA* gene responsible for production of penicillin binding protein 2a (PBP2a). PBP2a has an unusually low binding affinity for all β-lactam antibiotics, substituting the native PBPs and allowing continuous cell wall assembly.[8] Amplification of *mecA* gene by Polymerase Chain Reaction (PCR) is the gold standard for recognition of methicillin resistance in MRSA.[9,10] PCR is an expensive test, and is restricted to reference labs in our country.[10]

We planned our study to evaluate the efficacy of a commercially available assay, the MRSA Screen Latex Agglutination Test (Denka Seiken Co., Ltd., Tokyo, Japan) as a reliable method for detection of MRSA, isolated at Armed Forces Institute of Pathology (AFIP) Rawalpindi. The MRSA-Screen Latex Agglutination Test uses latex particles sensitized with a monoclonal antibody against the epitope PBP2a.[11] It was evaluated against the gold standard, *mecA* gene amplification by PCR according to the agreed guidelines.[12,13]

Objective
This study was conducted to evaluate the efficacy of MRSA-Screen Latex Agglutination test as a rapid diagnostic modality for identification of MRSA for resource poor settings.

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Materials and Methods

This cross-sectional descriptive study was conducted at Department of Microbiology, AFIP, Rawalpindi on clinical specimens collected from 4th November 2008 through 3rd November 2009. One hundred clinical isolates of MRSA from various clinical specimens sent for culture and sensitivity were included in the study. Laboratory samples irrespective of gender and age that yielded growth of MRSA were included while duplicate isolates from the same patient were excluded.

Clinical specimens of the patients like pus, blood, body fluids, urine, sputum, tissue, pus swabs, catheter tips and tubes received for culture and sensitivity from various wards and outpatient departments of military and civil hospitals of Rawalpindi were processed for Gram's staining and cultured on appropriate media and incubated for 24- 48 h at 37^0C. Isolates were identified by colony morphology, microscopy of Gram's stain, catalase, coagulase and DNase tests and were subjected to routine antimicrobial susceptibility testing.[14]

S. aureus isolates were tested for methicillin resistance by modified Kirby-Bauer disk diffusion technique using 1 µg oxacillin disk (Oxoid, Basingstoke, UK) and Mueller-Hinton agar (Oxoid, Basingstoke, UK) containing 2% NaCl. The plates were incubated at 35^0C for 24 h. Susceptibility to oxacillin was interpreted as per CLSI criteria. A zone diameter of > 14 mm was taken as susceptible, 11-13 mm as intermediate, and < 10 mm as resistant.[15]

We considered the presence of *mecA* gene as the 'gold standard' method for identification of MRSA. The PCR was performed for detection of *mec*A gene according to the agreed guidelines.[13] Growth of *S. aureus* in brain heart infusion, after overnight incubation, was used for extraction of DNA by kit method (Symbiosis Asti Italy). Primer pair used for the amplification product of DNA were (5-CTCAGGTACTGCTATCCACC-3´) and (5-CACTTGGTATATCTTCACC-3´); amplification was carried out in Master Cycler, Eppendorf, Hamburg, Germany. MRSA-Screen Latex Agglutination test (Denka Seiken Company Tokyo, Japan), was performed according to the manufacturer's instructions.[11] From the fresh subculture of each specimen, 5µL was taken in 200µL of first extraction reagent in a microcentrifuge tube. It was emulsified and boiled for 3 min. The suspension was then allowed to be cooled to room temperature. Then 50µL of a second extraction reagent was added and mixed. The suspension was then centrifuged at 1500 × *g* for 5 min. 50µL aliquot of the supernatant was added to each of the two circles on a test card and mixed with 25µL of the anti PBP2a sensitized latex and 25µl of the negative control latex, mixed for 3 min on a shaker and observed for visible agglutination.

The data was analyzed with the help of statistical package SPSS version 14.0. Mean and standard deviation were calculated for age, and frequencies for gender, *mecA* gene and MRSA-Screen Latex Agglutination test. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and efficacy were calculated.

Results

A total of 100 MRSA strains were evaluated. Mean age of the patients was 32.4 years ± 18.1; range was 5 to 75 years. Male to female ratio was 3.3:1. *mecA* gene PCR assay identified 94 *S. aureus* isolates as *mecA* gene positive and 6 as *mecA* negative. Break point MICs of MRSA were within the CLSI defined range i.e. (R =4µg /mL and S =2µg/mL)

Majority of the MRSA identified were isolated from pure pus/ pus swabs received from patients suffering from trauma or injuries like wound infections in orthopaedic patients (n=35) followed by surgical site infections (SSIs) (n=29) (Table 1). About 85% MRSA isolates were recovered from patients admitted in Combined Military Hospital Rawalpindi while remaining isolates were from out-patient-departments of different hospitals in Rawalpindi.

Table 1 : Source of MRSA isolation (n=100)

Clinical sites/ diseases/ conditions	Numbers
Wound infection (Orthopaedic)	35
Surgical site infection (SSI)	29
Chronic suppurative otitis media	8
Abscess	6
Diabetic foot	5
Cancer	4
Bed sore	2
Burn	2
Skin infection	2
Nasal carriage	1
Miscellaneous (*mecA* gene negative)	6

Table 2: MRSA-Latex Agglutination results (n=100)

MRSA-Screen Latex Agglutination	*mecA* gene positive	*mecA* gene negative	Total
Positive	88	2	90
Negative	6	4	10
Total	94	6	100

MRSA-Screen Latex revealed 90 isolates positive from all the specimens; among them 2 isolates were *mecA* gene negative (Table 2).

The test had an efficacy of 92%; specificity of 66.67% and sensitivity of 93.62%. The PPV and NPV were found to be 97.8% and 40%, respectively.

Discussion

Emergence of MRSA has mainly resulted due to the increased use of β-lactamase resistant penicillins.[16] The widespread use of methicillin and other related penicillins was curtailed in the late 1960s.[17] MRSA, however, subsequently became a global problem; 1980 onwards, it has been recognized all over the world as the major agent for nosocomial infections.[18,19] Rapid and accurate detection of MRSA is necessary for proper management and prevention of transmission of this pathogen in clinical settings.[20]

Our study showed that detection of PBP2a by the MRSA-Screen Latex Agglutination test is sensitive as well as specific for the detection of methicillin resistance in *S. aureus*. The accurate diagnosis of MRSA in the laboratory is of extreme importance for the effective management of patients, as well as the consequential interpretation of surveillance data. Currently, surveillance data are difficult to interpret because there is no uniform system for the detection of MRSA, and laboratories in different settings differ in their standard operating procedures and interpretations of results.[21] In a study conducted in Korea, with PCR as the gold standard method, MRSA-Screen Latex Agglutination test demonstrated 100 % sensitivity and specificity.[22]

PCR being expensive, labor intensive and time consuming test, presently is restricted to reference laboratories.[9] Phenotypic identification methods have to be resorted for routine laboratories. Although many methods for detection of MRSA have been adopted, the heterogeneous nature of resistance to methicillin/ oxacillin by many strains makes their recognition difficult.

The MRSA Screen Agglutination Test is a rapid, and reliable method for specific detection of MRSA which can be used for routine diagnostic setups. It is easy to perform test requiring no sophisticated instrument.

In our study, when compared with PCR technique, the MRSA-Screen Latex Agglutination test showed efficacy of 92%, and an accuracy of 97.78%. This finding is supported by the study conducted in South Korea which showed 100% sensitivity; under appropriate conditions >97% of resistant strains could be detected by MRSA-Screen Latex Agglutination test method.[22]

Conclusion

MRSA-Screen Latex Agglutination is easy to perform test at clinical labs where facility for high tech PCR amplification technique is not available. It is sensitive, rapid, and reliable test.

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CASE REPORT

Fever of Unknown Origin Ten Years Later Due to Gossypiboma

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Abstract

Gossypiboma is an accidentally retained surgical sponge left during surgery. It is a rare intra-abdominal surgery complication. We present a case of a 65 year old lady who presented with 2-3 weeks history of low grade fever, weight loss and ill-defined abdominal mass in the right upper abdominal quadrant. Her laboratory investigations for infection were negative. Abdominal CT scan showed a mass with fluid and gaseous contents adjacent to the transverse colon. A surgical exploration revealed a gossypiboma which was deemed secondary to a cholecystectomy done 10 years prior to presentation. Gossypiboma should be considered in the differential diagnosis of low grade fever with intra-abdominal mass and weight loss especially if there is past history of abdominal surgery.

Key word
Gossypiboma, Intra-abdominal, Pyrexia of Unknown Origin

Introduction

Intra-abdominal surgery is associated with various types of early and late complications including cardiopulmonary events, deep venous thrombosis, acute abdominal wall dehiscence and surgical site infections.[1] One rare intra-abdominal surgical complication is "gossypiboma". This refers to a foreign body which in most situations is a surgical sponge left unintentionally inside the body during surgery and mostly within the abdominal cavity.[2] The exact incidence of gossypiboma is unknown as most of the cases are asymptomatic or under reported owing to its medical and legal consequences.[3] Gossypiboma can present within few days to months or rarely years after surgery. It can mimic an abscess in case of acute presentation while behaves like intestinal obstruction or as an intra-abdominal mass if presented late.[3, 4] Mostly the diagnosis is difficult because of low clinical suspicion.[4] A positron emission tomography /computerized tomography scan can show a hypo metabolic area surrounded by increased 2-[fluorine-18] fluoro-2-deoxy-D-glucose uptake, mimicking tumor necrosis.[5] Surgical removal is the standard way of management.[3, 4]

We report a case of a woman who presented with a 3 week history of fever and weight loss. She had an abdominal surgery a decade ago. Imaging of the abdomen revealed a likely gossypiboma which was confirmed on exploration and its removal led to cure.

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Case History

A 65-year-old lady who was a known diabetic and hypertensive with bilateral knee osteoarthritis was referred for the evaluation of fever for 19 days. She had undocumented weight loss and daily fever spike without rigors and chills. The maximum recorded temperature was of 101.5^0 F. Her primary physician treated her initially with moxifloxacin (5 days) followed by clarithromycin (6 days) without any relief. Later she developed a dry cough along with vomiting (3-4 episodes/day) for a couple of days. At presentation she had become almost bed bound due to weakness and lethargy. Her initial laboratory investigations showed hemoglobin of 8.8 g/dl, WBC of 5,600/mm (neutrophils 68%, lymphocytes 28%), and platelets of 175000/mm; ESR 35 mm, normal urinalysis, negative blood and urine cultures and a normal chest x-ray. She was referred to adult infectious diseases clinic for management. Her past medical history revealed that she had a cholecystectomy done ten years prior to presentation. She also gave a history of a right sided ischemic stroke two years prior and had residual dysarthria at presentation. She appeared non-toxic but lethargic. She was hemodynamically stable. Right upper abdominal palpation revealed an ill-defined tender minimally mobile intra-abdominal mass of about 7x10 cm in the right upper abdominal quadrant. Rest of her systemic examination was unremarkable.

Based on above clinical findings a contrast enhanced abdominal computerized tomography was done which showed evidence of prior cholecystectomy with a few clips in the gallbladder fossa. There was a 9 cm mass with fluid and gaseous contents in the right upper abdomen occupying the right aspect of transverse mesocolon, reaching up to the gallbladder fossa with surrounding inflammatory change but no evidence of rupture. The duodenum, the distal stomach as well as the right aspect of the colon were pushed secondary to the mass effect (Figure 1). The differential diagnosis included an abscess, duodenal diverticulum or possibility of infected gossypiboma although the cholecystectomy was in the distant past.

She underwent upper gastrointestinal endoscopy to rule out possible duodenal diverticulum, which revealed a mass bulging into supero-posterior aspect of first part of duodenum with surface ulceration (Figure 2). An exploratory laparotomy was

done with findings of adhesions due to previous surgery and a 10 cm circumscribed thick walled cavity involving the transverse mesocolon, duodenum, gallbladder fossa and head of pancreas which contained a foul smelling swab. After adhesionolysis to restore anatomy, the abscess wall was dissected out from mesocolon, duodenum and liver along with its contents. The swab had eroded into the first part of the duodenum so partial gastrectomy and gastrojujenostomy (Bilroth II) was performed. A 10 cm segment of small bowel with its mesentery was sent to laboratory. Serial sectioning revealed thickened wall with purulent exudate in the lumen. The mucosa was ulcerated. The mesentery and serosa showed extensive exudations and adhesions with few strips of adherent cotton fibrosis. A piece of surgical gauze was present and measured 10.0 cm in its greatest dimension. It was soaked with purulent material.

Histologic examination of the gastrectomy sample showed extensive ulceration with underlying necrotizing inflammatory granulation tissue and foreign body type giant cell reaction to gauze material in the jejunum and gastric antrum. No evidence of granulomatous or neoplastic process was seen (Figure 3). Pus culture was negative and she received antibiotics for 7 days. She was discharged successfully after 10 days of hospital stay and was doing well on follow up after 1 year.

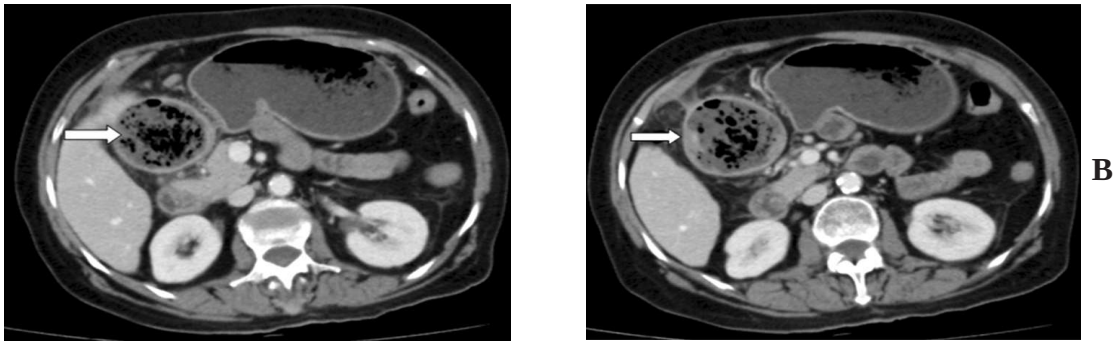

Fig 1. CT scan of abdomen showing (A) a large thick walled cavity approximately 90 mm with fluid and gas contents (B) pushing the duodenum and the distal stomach.

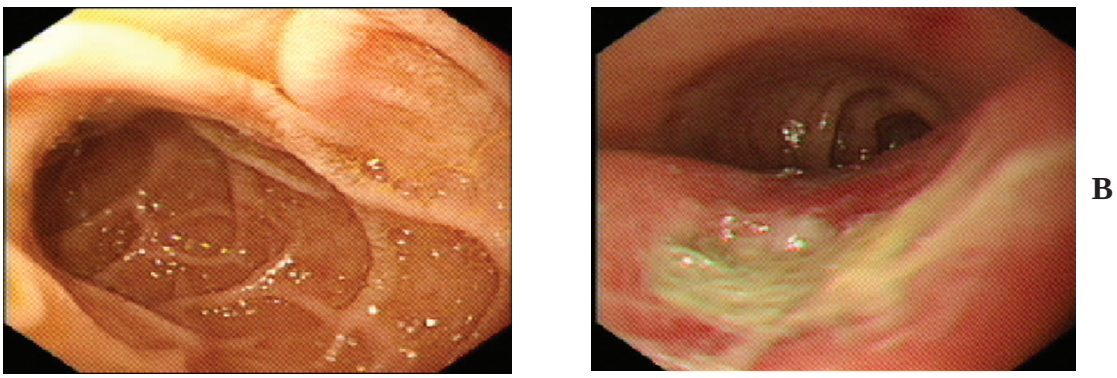

Fig 2. Endoscopy of first part of duodenum (A) with surface ulceration and a mass (B) bulging into supero-posterior aspect of it.

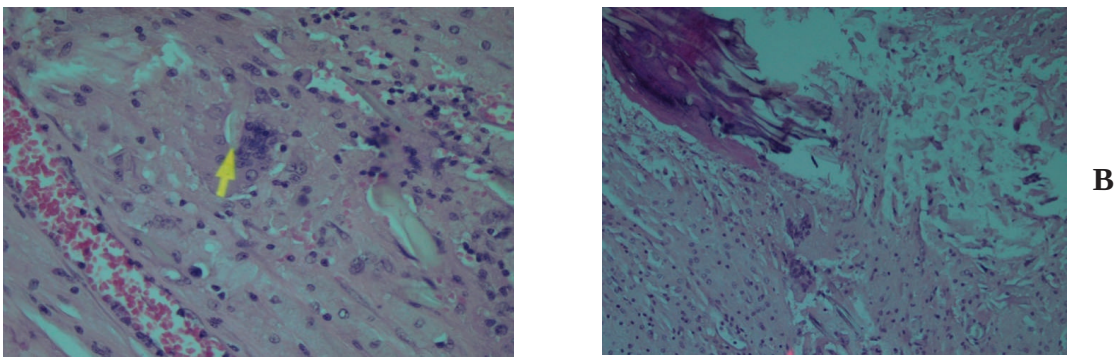

Fig 3. Foreign body type giant cell reaction to gauze material (arrow in A) in gastrectomy sample with underlying necrotizing inflammatory granulation tissue (B)

Discussion

General abdominal, urologic, gynecological, spinal and thoracic surgeries can rarely result in gossypiboma formation.[4,6] Gossypiboma is a rare surgical complication but can have serious medical and medico-legal consequences.[3,7] Most publications are either case series or case reports. A thirteen year (1990-2003) medical review from a Jordanian hospital reported an incidence of 1:5027 for all inpatient operations.[4] Intra-abdominal gossypiboma has variable modes of presentations depending on the location and nature of reaction. It can be acutely symptomatic, producing an acute exudative inflammatory reaction leading to an abscess or fistula formation, however most cases remain asymptomatic or have vague symptoms.[6] A foreign body type giant cell reaction with fibrosis and encapsulation may present late or remain silent.[8] In our case the latter phenomenon was documented on histopathology of intestinal specimens. Presentation as long as 40 years after surgery has been reported.[6,8] In acase series from a Turkish hospital, 13/14 presented with nonspecific abdominal pain and intestinal obstruction.[3] Another case series of 12 cases from Pakistan reported various presentations including intestinal obstruction (58%), discharging sinus (42%), intra-abdominal abscess (17%), peritonitis (17%) and abdominal mass (8%). [9] There are reports of gauzeoma / foreign body granuloma formation while transmural visceral migration is also seen when left for a long time.[7,9] To our knowledge there are no case reports of gossypiboma presenting with fever as the only significant clinical finding. Our patient presented with fever almost 10 years after cholecystectomy. Similar cases with erosion or even fistula formation have been documented in the literature.[10]

Cross sectional imaging is helpful for the diagnosis and management of retained foreign bodies.[6] CT scan can show thin or thick walled cavities with air bubbles having a spongiform appearance.[5,6] The same was observed in the CT scan of our patient. Management largely depends on the mode of presentation and includes urgent surgery if there are features of peritonitis or intestinal obstruction. Mostly an elective exploratory or diagnostic laparotomy is carried out as happened in our case.[3] Gossypiboma formation can be avoided by careful handling and counting of surgical gauze at the end of surgery, and by using radio opaque surgical textile materials.[10]

Conclusion

Gossypiboma is rare but it can present as diagnostic dilemma as a vaguely defined abdominal mass or fever of unknown origin with past history of abdominal surgery. Prevention rather than cure is the best remedy for gossypiboma.

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CASE REPORT

Neonatal Varicella: When to Treat and with What?

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Abstract

Neonatal varicella is rare and can pose serious and life threatening complications and sequel. Prompt diagnosis and treatment are required to decrease the complications and risk of death. We are reporting a case of 35-week neonate with generalized vesicular rash due to maternal varicella. He was treated with intravenous acyclovir because of possible encephalitis. His lumbar puncture was unremarkable. Blood and CSF cultures showed no growth. He responded well to treatment, discharged and followed in the clinic without any neurological sequelae.

Key words
Varicella Zoster; Neonatal Varicella; Acyclovir

Introduction

Primary varicella (chickenpox) is an acute, highly infectious disease caused by Varicella-Zoster virus (VZV) belonging to human herpes virus family.[1] Clinically it presents as a febrile illness with a generalized, pruritic, and vesicular rash over the skin but can disseminate and cause pneumonia, hepatitis, encephalitis and severe coagulopathy resulting from hepatic failure and thrombocytopenia.[2, 3] VZV is a highly transmissible virus, leading to approximately 90% of susceptible household contacts becoming infected after exposure.[1] The incubation period usually is 14–16 days (range 10-21days).[2,3] Chickenpox is communicable for one to two days (up to five days) before the onset of the rash, continuing until all the lesions are crusted; however the communicability may be prolonged in patients with altered immunity.[4,5] For neonates and immunosuppressed individuals, the risk of disseminated or hemorrhagic varicella is increased.[4,5] Varicella in neonates presents as congenital or neonatal varicella. Congenital varicella occurs if mother is infected in the first or early second trimester resulting in multiple anomalies causing congenital varicella syndrome (limb hypoplasia, cutaneous scarring, eye abnormalities and central nervous system damage) and is associated with a high mortality.[1,3,6] Varicella infection can be fatal for an infant if the mother gets varicella within 5 days before to 2 days after delivery because of absence of transfer of varicella associated antibody to the new born threatening severe infection. These infants should be given varicella-zoster immunoglobulin (VZIG) or immunoglobulin (IVIG) at birth or as soon as the maternal symptoms appear.[6, 7] However despite the administration of VZIG, infants exposed to maternal varicella in this high-risk period should be monitored very closely and intravenous acyclovir should be given if there are any signs of illness. If the mother develops chickenpox after delivery she can possibly transmit the disease to the neonate via droplet infection (postnatal VZV infection) that has a relatively low morbidity in full term babies;[1] however premature and very low birth weight neonates are at increased risk for severe disease during the first 6 weeks after birth.[5,8] Postnatal neonatal varicella in term neonates does not require VZIG however neonate should be closely followed for disease progression and complications. Acyclovir should be given whenever there is a suspicion of disease progression. We are reporting a case of postnatal varicella with complication in a preterm neonate.

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Case History

A 27-day-old male preterm (35 weeks) neonate presented with generalized vesicular rash that started from face and then progressed gradually to involve the trunk and limbs. He also had low-grade fever documented up to 101^{0}F with reluctance to feed for one day. The mother also had similar eruptions when he was 10 days old, which was diagnosed as varicella and was managed conservatively. The mother was a primigravida with no antenatal, natal and postnatal issues. The neonate had a birth weight of 3 kilograms and was discharged home on second day of life. Physical examination on admission revealed an active vigorous baby with flat anterior fontanel, a vesicular rash consistent with VZV, few were pustular indicative of super infection and an unremarkable systemic examination (Figure 1). He was started on intravenous acyclovir along with intravenous cloxacillin for seven days because of possible secondary skin and soft tissue infection. On the second day of admission he became lethargic with generalized increase in tone. Laboratory investigations revealed normal leukocyte count with lymphocytosis; normal electrolytes and calcium, blood culture showed no growth; cerebrospinal fluid was also unremarkable (glucose 51 mg/dL; WBC 6 µL; RBC 300 µL), CSF culture showed no growth. Viral cultures were not possible while fluid PCR was not sent because of crusted lesions at the time of admission. Intravenous acyclovir was continued for 21 days. He responded well to treatment with drying of the lesions and was discharged with no neurological sequelae. He had no side effects of therapy. At one month of his follow-up he was doing well.

Discussion

Vesicular rashes in the neonates are challenging in terms of

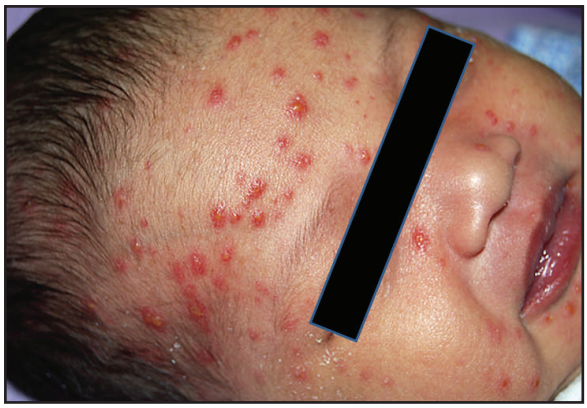

Fig 1. Neonate with multiple vesicular lesion of the face. (Parents had consented for the photograph).

diagnosis and management. Herpes virus infections are important diagnostic considerations.[2] It is a rare condition with an incidence of 2–6 per 100, 000 live births per year.[7-9] Diagnosis of neonatal varicella is essentially based upon the clinical features and maternal history of chicken pox. The newborn in our study, whose mother contracted varicella 14 days post-delivery was a very clear clue for diagnosis in our case. The neonate should be observed closely if the child remains asymptomatic; however prompt and early treatment should be started if the neonate develops a rash. VZIG or IVIG is not indicated in post-natal varicella if the neonate presents after 72 hours, hence no immunoglobulin was given in our case. According to American academy of Pediatrics guidelines immunoglobulin (VZIG) is recommended for those newborns that are born from mothers with a varicella infection 5 days before delivery and 2 days after delivery.[1,4,10] Varicella-zoster immunoglobulin prophylaxis is indicated for the neonate within 10 days of initial exposure if the mother has a varicella rash in the perinatal period, and the VZV antibody status of the mother or infant is negative.[1] It is established that VZIG reduces the severity of disease, but does not always prevent neonatal infection.[1,5] Neonates born 2 days before or 4 days after the onset of maternal varicella can develop serious illness. Without treatment, the death rate is approximately 30%.[11] Therefore it should be recognized and promptly treated.

Effective antiviral treatment is available for herpes infections. Acyclovir is the drug of choice for the herpes infection.[2,4] It is not recommended for the treatment of uncomplicated chickenpox in healthy and immunocompetent infants and children. Physicians caring for neonates with a vesicular rash must be vigilant to ensure prompt isolation and ensure that appropriate treatment is administered without delay.

Conclusion

Postnatal varicella in premature neonates can be complicated. Prompt recognition and treatment is paramount for successful neonatal outcome.

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Plagiarism in Scientific Research: Harm Done

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Dear Editor,

The importance of novelty in literary work and humanities (to an extent) is aptly described by Paul Gauguin who states that *"Art is either plagiarism or revolution"*. The Merriam -Webster online dictionary defines plagiarism as "*the act of using another person's words or ideas without giving credit to that person:Êthe act of plagiarizing something*". A scientific article may be no less than a work of art. However there is a clear difference between science and art; the difference between the two ways of "knowing" and looking at nature.[1] While originality of words is essential for the spirit of art, the primary goal of biomedical research is to report scientific findings in an " *accurate and honest manner"*.[2] Theft of ideas and perspectives of another author clearly amounts to plagiarism.[3] But is the case the same when publications are duplicated and original data or previous work is restated with appropriate references?

Plagiarism is one of the forms of scientific misconduct, the others being falsification, fabrication and selective publication.[4] Plagiarized work can broadly be categorized into two forms; one that is referenced and one that is not.

There is not an iota of doubt that a failure to reference previous work is plagiarism.[5] This includes directly copying others work word for word without referencing (text based plagiarism),[6] reconstituting a paragraph using words copied from different sources and then presenting as one's own (patchwork plagiarism), and redundant (multiple) publication without referencing previous publication. In all forms, there is a lack of originality of ideas and the motive behind them may be a desire for increasing "original" publications. It is abominable simply because of the intent of the plagiarist.

There is a hazy line that differentiates appropriately referenced work from plagiarism. One commonly discussed form is self plagiarism, where reports of previous studies are published or previously used words are intentionally re-used by the author multiple times. This may be driven by the thought that the author had *"said it so well the first time that it makes no sense to say it differently a second time"* as said by Samuelson[2] or the *"intentional reuse to persuadereaders and editors to take serious problems seriously"*.[7] There is a lack of consensus among editors whether this is true plagiarism or not and policies vary across journals. It is clear, however, that duplicate publications lacking transparency in mentioning the need to replicate previous work, clearly amounts to deception[8] and authors need to take into consideration the copyright policies of the journals publishing the articles.[9] There are other more serious forms of plagiarism where work may have been inappropriately cited. For instance source citation may be obscure or incomplete; such that it cannot be located or investigated further. This may be accidental or purely intentional. Whatever the cause it has important implications for understanding of the idea under discussion. Another instance is when an exact restatement of someone else's work, published or unpublished; in one's own words using blanket references has been done despite referencing (minimalist plagiarism/improper paraphrasing). It may directly result, either from a lack of originality in the writer's own idea. Often it is attributable to difficulty by the writer in stating things in literary language, a common problem of writers of a foreign language.[10] This may not be considered plagiarism by some but it actually provides no further information to the topic of discussion.

Plagiarism in any form, across any discipline of science, whether of words or ideas, is a practice harmful to progression of science. The reinforcement of old processes is as important as the development of new ones but needs to be done carefully, by a meticulous and conscientious effort to referencing previously indexed work and maximizing publication transparency across journals .

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Infectious Diseases Course for General Practitioners Held by IDSP

The Infectious Diseases Society of Pakistan held its annual short course for general practitioners on 3-8 September, 2012 at Sind Institute of Urology and Transplantation for the fourth consecutive year. The theme of the week long activities was "Countering Infection At All Ages", each day devoted to age-specific infectious diseases and their management in clinic settings. Dr Fatima Mir and Dr Faisal Mehmood were course convener and co-convener respectively. The audience was a mix of physicians and general practitioners working in clinic settings in medium to low income communities.

Day one included sessions on fever, its interpretation appropriate approach in Pakistani settings; common misconceptions about infectious diseases and optimizing clinical diagnosis by appropriate use of the laboratory. Day two comprised of sessions addressing common neonatal and pediatric infections encountered in clinics. These ranged from neonatal infections ranging from serious infections like meningitis, pneumonia and sepsis to pustulosis; infectious diarrhea in children; respiratory infections in childhood and an update on progress of Polio Eradication Initiative in Sindh, Pakistan and how general practitioners could contribute by reiterating necessity of vaccinating each child each campaign. Day three was devoted to adolescents and young adults. Participants made their way to the venue despite pouring rain in Karachi. Common adolescent infections, sexually transmitted infections, tuberculosis and community acquired pneumonia were discussed in interactive sessions. Day four dealt with infections in pregnancy. A general discussion on keeping pregnancy infection free, was followed by talks on malaria and perinatal infections (TORCHES) screening. Day five included geriatric concerns like skin and soft tissue infections, urinary tract infections and adult vaccination. The last day was included pediatric vaccination in addition to EPI vaccines, dengue and Naegleria (topical public health concerns) and infection control in outpatient settings. The week long activities had an avid audience from start to finish who kept the proceedings lively with challenging questions and shared experiences. The IDSP thanks all participants, presenters (experts) and especially the audience who made the week long course a resounding success.

2nd International Forum on Infectious Diseases (IFID) 2012

The 2nd IFID was conducted by GETZ Pharma in cooperation with IDSP under an MOU at Manila, Philippines on 20th September 2012 with the theme of "Respiratory Tract Infections: Emerging Scenarios in Developing Countries". More than 100 delegates from 8 countries (Myanmar, Sri Lanka, Cambodia, Vietnam, Philippines, Kenya, Kazakhstan and Pakistan) participated. The 5 scientific sessions focused on local respiratory diseases and all sessions' and were lead by eminent speakers. A panel of experts moderated each session with active participation from the enthusiastic audience. President IDSP, Dr Ejaz A. Khan and Consultant Microbiologist Dr Nazar Ullah Raja represented IDSP and chaired a session.

Overall the conference was regarded as a success with high quality relevant papers. There were many positive things about this conference. It was a good forum to create awareness of ID related issues in Pakistan. Important topics with practical solutions were discussed. Many suggestions were given to build on this platform to promote various aspects ID in Pakistan in future as well.

Mycology Workshop

Microbiology Department of Shifa International Hospital held a mycology workshop in collaboration with the Department of Pathology Microbiology, AKUH at Shifa College of Medicine, Islamabad under the umbrella of Infectious Diseases Society of Pakistan (IDSP) on Saturday 15th September 2012. This workshop was supported by a grant from HEC and USAID.

It was widely attended by the participants coming from Shaukat Khanum Memorial Hospital (SKMH) Lahore, Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan Institute of Medical Sciences (PIMS) Islamabad, Shifa International Hospital (SIH) Islamabad, Islamic International Medical College Rawalpindi, and KRL Hospital Islamabad.

The objective of the workshop was to raise awareness about fungal diagnostics among the young microbiologists and technical staff to cater to the needs of oncology, bone marrow, liver and kidney transplant units of various hospitals.

More than 27 participants attended the workshop and had hands on experience in identification of fungal isolates. Organizers of the workshop, Dr NazarUllah Raja, Dr Ejaz A Khan and Dr Afia Zafar handed over the certificates to the participants.

Dr Ejaz A Khan, Dr NazarUllah Raja and Dr M Usman applauded and appreciated the efforts of Dr Afia Zafar and her colleagues Dr Kausar Jabeen, Dr Joveria Farooqi and Miss Sidra who travelled from Karachi to Islamabad for the workshop.

Instructions to Authors

Scope

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

Criteria for publication

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

Submission of the Manuscript

Manuscripts must be formatted according to submission guidelines given below, which are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (originally published in N Engl J Med 1997;336:309-15). The complete document appears at www.icmje.org. Please submit one complete copy of the manuscript and all enclosures to **The Managing Editor, Infectious Diseases Journal of Pakistan, Department of Pathology and Microbiology, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan.** An electronic copy of the manuscript must also be sent to maahin1@yahoo.com and pak_idj@yahoo.com**.** All manuscripts submitted to IDJP must be accompanied by an Authorship Declaration stating that *'The authors confirm that the manuscript, the title of which is given, is original and has not been submitted elsewhere.Each author acknowledges that he/she has contributed in a substantial way to the work described in the manuscript and its preparation'*. Upon submission a manuscript number will be assigned which should be used for all correspondence.

Manuscript Categories

I. Original Articles

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

Title page

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

Abstract

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

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

Background

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

Materials and Methods

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

Results

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

Discussion

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

Acknowledgments

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

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

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

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

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

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

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

References
Number references consecutively in the order in which they are first mentioned in the text. Identify references in text, tables and legends by Arabic numerals (in superscript). References cited only in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification of the particular table or illustration. Bibliography should be given in order.Authors, complete title, journal name (Abbr), year, vol, issue, page numbers. According to "Uniform Requirements of Manuscripts submitted to Biomedical Journals", as cited in N Engl J Med 1997; 336:309-15.

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

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

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

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

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

Instructions updated - April 2012.
Editor IDJ

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