Utility of Carba NP test (Inhouse/RAPIDEC commercial kit) in the identification of carbapenemase producing clinical isolates

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Introduction: Carbapenems are the drug of choice for the treatment of many multidrug resistant hospital acquired infections. Resistance to carbapenems is also not uncommon and is increasingly being reported nowadays. Though various tests like modified Hodge test are available for the detection of carbapenemases, they lack sensitivity and specificity. Recently the Carba NP test has been introduced for carbapenemase detection which has been approved and included in CLSI guidelines. It is based on *in vitro* hydrolysis of imipenem by a bacterial lysate, which is detected by changes in pH values using the indicator phenol red.

Aims and Objectives: The present study aims to identify the prevalence of carbapenemase producing isolates and the utility of Carba NP in-house as well as commercial RAPIDEC Carba NP test in the identification of these carbapenemases.

Materials and Methods: A total of 91 isolates were isolated during the study period (May-Oct2018) which were further tested for carbapenemase production by both In-House Carba NP test as well as commercially available RAPIDEC Carba NP(Biomerieux). In addition, 25 carbapenem sensitive isolates were also tested. *Klebsiellapneumoniae* BAA ATCC 1705 was used as positive control and ATCC 1706 as negative control.

Results: Out of the 91 carbapenem resistant strains tested, 72 were identified as carbapenemase producers. Out of them *Klebsiellapneumoniae* accounted for 59.7% of the total carbapenemase producing organisms followed by *Pseudomonas aeruginosa* (25%) and *Escherichiacoli* (6.9%) followed by *Acinetobacter*and *Enterobacter* which constituted less than 5%. Among enterobacteriaceae, 43 out of 59 carbapenem resistant *Klebsiella* were carbapenemase producers whereas all the 5 carbapenem resistant *E.coli* were positive for Carba NP test.

All the positive isolates identified by In-House Carba NP test were also positive by commercial RAPIDEC test and vice versa and none of the 25 carbapenem sensitive strains tested were positive for Carba NP test by either method indicating 100% correlation between the two methods studied.

Conclusion: To conclude both the Carba NP in-house as well as commercial RAPIDEC Carba NP test were equally effective in the identification of the carbapenemase producers among the Gram negative bacilli.

Identification of Potential Anti-microbial Agents Against MRSA: An In-silico Investigation

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Introduction: *Staphylococcus aureus*, is among the most common causes of nosocomial infections. Amidst all the medical advancements, MRSA (methicillin-resistant strain of *S. aureus*) continues to wreak havoc worldwide. Glycolytic enzymes, like glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) is required for the pathogen's survival. GAPDH is well studied as a housekeeping enzyme, but recently its new properties like localization on the cell surface, binding to cellular molecules and role in apoptosis have been revealed.

Objective: (a) To explore catalytic and substrate binding domain of GADPH enzyme of MSRA252 strain (PDB ID 3LVF). (b) Development of pharmacophore models for high throughput virtual screening of FDA approved drugs. (c) Identification of FDA (Food and Drug Administration) approved drug as potential GADPH inhibitor employing molecular docking and molecular dynamics simulations studies.

Methods: All the *in-silico* studies were performed using Maestro suite of Schrodinger. Crystal structure of holo GADPH 1 was taken up for this study with resolution of 1.7Å. Substrate binding pocket was analysed and a 5-feature e-pharmacophore model was developed based on receptor-ligand complex. A 7-feature pharmacophore model was also developed by selecting the important interactions of NAD (nicotinamide-adenine-dinucleotide) with GADPH 1. Compounds were ranked based on phase screen (PS) score. Around 97 compounds with PS score above 1.8 were selected as hits. Selected hits were put for molecular docking studies in extra precision mode. Compounds were further ranked upon dock score (DS) and protein-ligand interactions were also analysed. Molecular dynamics simulations study was performed for the best identified hit for 20ns.

Result: ZINC000004216238 (Flurabidine), ZINC000001995484 (Valganciclovir) and ZINC000049783788 (Valrubicin) with dock score of -8.125, -7.967 and -7.891 respectively, were identified as the best hits.

Conclusion: These hits can be tested for their *in-vitro* activity and further modified to increase the potency and selectivity towards GADPH 1 enzyme.

Antibiotic Susceptibility Pattern and Biofilm Production by Clinical Isolates of *Salmonella enterica* Serovars

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Abstract:

Introduction: *Salmonella enterica* has more than 2500 serovars, and causative agent of enteric fever is *S*. Typhi and *S*. Paratyphi. *S*. Typhi persists as a biofilm on gall stones.

Objective The study aims to find out the biofilm forming abilities and antibiogram of *Salmonella enterica* serovars (n=55) isolated from human blood and stool samples.

Methods: Antibiogram and biofilm formation by Salmonella isolates from clinical samples were studied by disc diffusion and microtiter plate methods respectively. Polymerase chain reaction was done to detect *invA* and *spvC* genes in the isolates.

Results: Of the 55 isolates studied, 36 (65.45%) were *S.* Typhi, 13 (23.63%) were *S*. Paratyphi A, two (3.64%) were *S.* Typhimurium, and four (7.28%) were *Salmonella* spp*.* which could not be serotyped. Resistance to ciprofloxacin and nalidixic acid were found to be 81.8% and 92.7% respectively. Moreover, 98.18% of the strains were susceptible to chloramphenicol and co-trimoxazole. One each of *S*. Typhi, *S*. Paratyphi A and *S*. *enterica* isolates formed biofilm at 28^{o}C. Two *Salmonella* spp., one *S*. Paratyphi A and eight *S.* Typhi produced weak biofilm respectively in the absence and presence of bile at 37°C. All the isolates were positive for the *invA* gene. *S.*Typhimurium serovars had *invA* and *spvC* genes.

Conclusion: Bile may contribute for biofilm formation and persistence of the organism on gall stones which may lead to carrier state of Salmonella. Changing antibiotic susceptibility pattern of Salmonella serovars is also seen in our geographic area

Role of Bleach Concentration, Method for Detection of Acid-Fast Bacilli (AFB) in Sputum Using LED-Fluorescence Microscopy.

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Introduction: With millions of people being affected annually, tuberculosis (TB) is deemed as a global health crisis for both developing and developed countries. Identifying active pulmonary tuberculosis (PTB) is important for diagnosis and reducing disease transmission. Sputum microscopy is a first line test used for PTB laboratory diagnosis. Ziehl-Neelsen (ZN) staining and Auramine O staining have been used for detection of AFB. Household Sodium hypochlorite (NaOCl) or bleach can be used in concentration of sputum specimens which increases the yield of microscopic detection.

Methods: This prospective study was carried out in the Department of Microbiology, Kasturba Medical College, Manipal. 239 sputum samples were collected from suspected PTB patients. Smears were prepared from the direct sputum samples and stained with ZN and Auramine O methods. Bleach was added to the remaining sputum samples before centrifugation. ZN and Auramine O staining was performed from the sediment and were observed under light and LED-fluorescence microscope respectively.

Results: Out of the 239 sputum specimens, 20 (8.36%) samples were smear positive by both direct ZN staining and LED- fluorescence microscopy. After bleach centrifugation technique, ZN staining showed 28 (11.71%) smear positives and Auramine O staining showed 24 (10.04%) smear positives. This method also helped in the clearance of the smear background enhancing better visibility thus leading to an increased detection rate.

Conclusion: Bleach concentration has been found to be a better method in detecting *M. tuberculosis* especially from sputum samples reported negative by direct microscopic methods. It can digest the sputum products and inactivate the mycobacteria without altering their structures. Being an effective bactericidal disinfectant, it provides better safety to laboratory personnel during processing of samples.

A Large-Volume Sputum Collection and Dry-Storage Device for Tuberculosis Molecular Diagnostic Testing

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Introduction:

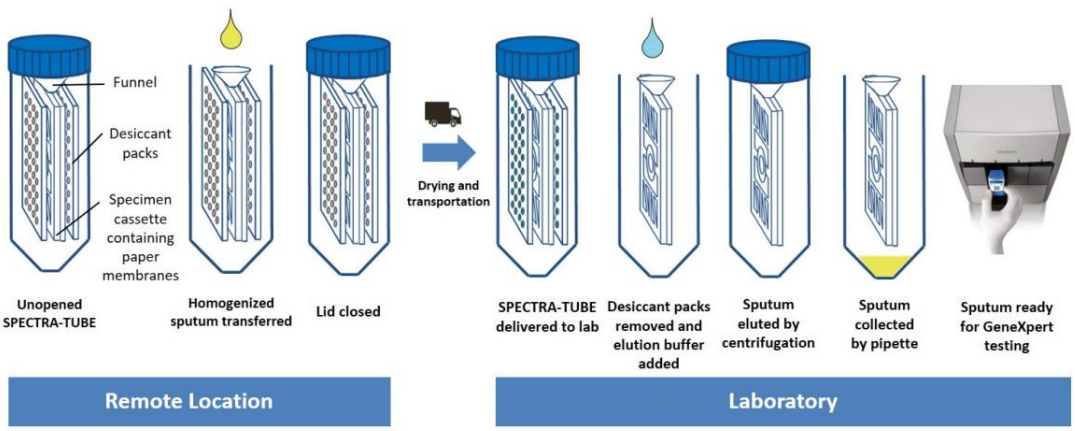
In developing countries, biological specimens need to be transported to central diagnostic laboratories for detection of infectious diseases. It takes days to weeks and in the absence of cold chains, specimens are often putrefied and unfit for analysis. Dry-storage of biological specimens has relied upon Dried Blood Spot technology, which can collect small volumes of specimen, increases the risk of contamination due to open-air drying and requires punching to retrieve the sample for analysis. This is limited to blood and stabilization for sputum has not been widely explored.

Objective:

To determine an ideal substrate and develop SPECTRA (Specimen Transportation) device for large-volume sputum collection and dry-storage.

Methods:

Artificial sputum was synthesized, consisting of 1.8% methylcellulose and 10% egg yolk emulsion. Dried membranes were rehydrated and Qiagen extraction was performed, followed by qPCR. The SPECTRA device was designed - a funnel for sputum addition, a paper-and-plastic cassette that holds sputum, and desiccant packs for drying, all packaged inside 50-ml Corning tube. Clinical specimen testing was performed in collaboration with Bigtec labs, Bangalore.



Results:

1cm^2 paper membranes were tested for recovery of dried orange dye based on elution. Stability of dried sputum spiked *Mycobacterium smegmatis* mc^{2}155 on Standard 17 glass fiber was determined for five days. Standard 17 was found to be most efficient at releasing dried reagents and qPCR analysis of five days storage revealed 8% Msm DNA loss compared to positive control. The compatibilty of SPECTRA device was assessed using tuberculosis infected clinical specimens. *Mycobacterium tuberculosis* H37Rv DNA was successfully detected after five day storage of sputum in the device.

Conclusion:

Glass fiber, unlike cellulose membranes are an ideal substrate for sputum storage. The membranes apart from molecular testing, can also culture Mycobacterium. Since there is no established solution that can dry and stabilize sputum, SPECTRA device is the first of its kind.

Epidemiological characteristics of presumptive tuberculosis patients in a tertiary care center in Karnataka, India

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Abstract

Background and Aims

India is the country with the world's highest absolute TB incidence and Karnataka state has a high HIV co-infection rate. Moreover, in India prevalence of diabetes mellitus (DM) exceeds 20% in some areas. We aimed at investigating the impact of HIV and DM as risk factors for TB manifestation (i.e. solitary pulmonary (PTB), solitary extra-pulmonary (EPTB) or concurrent PTB plus EPTB).

Methods

We prospectively evaluated presumptive TB patients at Kasturba Medical College, Manipal, India including point-of-care ultrasound for EPTB. Relevant case data including laboratory, microbiological and sonographic results were analyzed, and patients were categorized as solitary PTB or solitary EPTB or concurrent PTB plus EPTB.

Results

425 patients aged ≥ 16 years were enrolled in 2016. Age ranged from 16-86 (median 43) years, 77% were male and 20% were HIV-positive. PTB was diagnosed in 29%, 18% were diagnosed with EPBT and 17% with concurrent PTB and EPTB; 30% were categorized as unlikely TB. 22% of patients were categorized as diabetic. DM diagnosis was most frequent in patients with PTB. EPTB correlated with HIV-infection, which was

also a prevalent condition in patients with unlikely TB (32%). Differential diagnoses of TB in unlikely cases included various infectious and malignant conditions.

Discussion

In Karnataka, TB is a major public health concern with a high co-prevalence of HIV and DM. This is the first prospective study with regards to these entities in this setting and to use a precise classification of TB manifestation type. Our data suggests, that DM is a risk factor for pulmonary TB (PTB), but not for EPTB.

Genetic Variability of Attachment (G) Genes of Human Metapneumovirus Strains Circulating During 2016-2018 in India

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Introduction: Human metapneumovirus (HMPV) belongs to the family *Pneumoviridae,* of the order Mononegavirales. The clinical features of HMPV infection vary from mild upper respiratory tract infection to life threatening severe bronchitis and pneumonia. It mainly infects children under the age of 5 years but elderly people with underlying illness and immunocompromised individuals are also at a risk of infection. HMPV is divided into four subtypes: A1, A2, B1 and B2. HMPV-A2 is further divided into HMPV-A2a, A2b and A2c. Genotypes A2a, A2b, B1 and B2 were reported in India, among which A2b is the predominant genotype. However, limited amount of data is available on the current circulating genotypes of HMPV in India.

Objective: To find out the circulating genotypes of Human metapneumovirus in India.

Methods: Throat swab samples positive for HMPV, archived at Manipal Institute of Virology as a part of hospital based acute febrile illness surveillance study, from April 2016 to August 2018 were used. We performed conventional polymerase chain reaction for twenty samples targeting the complete G gene and they were subjected to Modified Sanger sequencing. Phylogenetic analysis was done using MEGA version 7.0.26 by Maximum Likelihood method.

Results: All the twenty sequences were belonging to A2c subgroup. Phylogenetic analysis showed that strains from the study have genetic relation with circulating strains in Japan, China and Croatia.

Conclusion: The study suggest that A2c is the circulating strain in India from the period April 2016 to August 2018. This is the first study identifying the A2c sub-group in Indian subcontinent. Though this strain was reported earlier in India, they were assigned under genotype A2b.

Prevalence of Leucocidin genes in Staphylococcus aureus Osteomyelitis

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INTRODUCTION:

Osteomyelitis (OM) is a serious bone and joint infection caused by *Staphylococcus aureus.* It is the most common pathogenic bacteria, due to its ability of elaborating diverse virulence factors like exotoxins and several adhesion factors. Among the exotoxins, leukotoxins specifically Panton-Valentine leucocidin (PVL) studied widely. The prevalence and role of other leukotoxins: γ-hemolysin (Hlg) and LukED are not commonly explored in the past which can cause severe destructive OM. Present study was done to explore the prevalence of leukotoxins (PVL, Luk ED and HLg) and their contribution in osteomyelitis.

METHODOLOGY:

A prospective study was conducted for six months duration. Patients having *S. aureus* osteomyelitis were included in our study. *S. aureus* was identified by MALDI-TOF and leukotoxin genes were detected by conventional PCR method. Patients' clinical manifestations and laboratory data, combining with the corresponding microbiological data were analyzed to find out the contributory role of leucocidins in *S. aureus* OM.

RESULTS:

Total 34 *S. aureus* isolates were analysed for the presence of leukotoxins collected from OM cases. Out of 34, 22 (64.7%) were MRSA. 18 of 34 isolates (52.9%) possessed leukotoxins. 16 isolates (88.9%) had luk D/E gene, 4 had PVL gene (22.2%). 66.7% of the leucocidin positive isolates presented with chronic OM (12/18) and were more tended to infect male patients (15/18, 83.3%) of age group of 25-44 yrs (9/18, 50%). Presence of LukE/D significantly contributed to implant associated OM (p value=.02) and finally led to removal of implant.

Discussion & Conclusion:

Athough *S. aureus*, is the primary cause of OM, but role of their leukotoxins in OM is largely unknown. Among the leukotoxins, PVL is found to be present in small percentage in osteomyelitis but explored more in the past. Research on Luk ED has remained minimal. In this study also prevalence of PVL is less compare to Luk DE. It was found more with in the S.aureus isolates causing chronic infection among young patients having implants.

Repurposing of Approved Target Specific Antivirals Against Dengue Virus

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Introduction

Dengue is a mosquito-borne viral fever caused by all the four serotypes of dengue virus. This emerging disease is of an international public health concern as it is endemic to more than 100 countries in different parts of the world. Thus, approved antivirals targeted directly and specifically to viral structural and non-structural proteins can help in reducing the global disease burden.

Objective

To identify inhibitors against RNA dependent RNA polymerase (RdRp-NS5) of Dengue virus by in-silico molecular docking.

Methodology

Nine RdRp inhibitors of Hepatitis C virus (HCV) were screened against the co-crystallized structure of Dengue virus RdRp using Schrödinger. Glide module was used to perform Extra Precision (XP) and Induced fit docking (IFD).

Results

Seven compounds were identified based on XP docking scores and ligand interactions. Two compounds, dasabuvir and nesbuvir, were selected for IFD based on ligand interactions. Dasabuvir had 12 poses and the docking scores were in the range of -10 to -15. Nesbuvir had 14 poses and the docking scores were in the range of -9 to -11.

Conclusion

This docking study shows that anti-HCV antivirals, nesbuvir and dasabuvir can be strong inhibitors against dengue virus. Further in-vitro work will be done to check the inhibitory activity of these drugs.

Title: Cervical Lymphadenopathy and Absence of Posterior Pituitary Bright Spot in CNS Tuberculosis: A Case - Control Study.

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Introduction:

Current diagnostic methods used in CNS tuberculosis are limited by the paucibacillary nature of this form of tuberculosis. Recently discovered Glymphatics has been shown to play an important role in the movement of CSF along with the substance present in it to cervical lymph nodes. Hence we planned a study to look for the association of cervical lymphadenopathy with CNS tuberculosis. In addition to other MRI features of TB meningitis, it has been seen in children with TB meningitis that the posterior pituitary bright spot is absent in nearly half of the patients. This finding has not been described in adults.

Objective:

To study prevalence and characteristics of cervical lymphadenopathy and the absence of posterior pituitary bright spot in patients with CNS tuberculosis when compared to a control group.

Methods:

A case-control study was performed with 100 patients with CNS tuberculosis admitted over the past 5 years (January 2014-April 2019) and compared them with 200 control arm (matched in 1:2 ratio) of patients with normal MRI brain. The MRI images in a randomised sequence were presented to a blinded radiologist to report presence and characteristics (size, shape, heterogeneousity in T2, number and level) of cervical lymphadenopathy along with absence of posterior pituitary bright spot. The data was subsequently analysed to look for association with CNS tuberculosis.

Results:

The prevalence of cervical lymphadenopathy in cases was 9% and in controls was 16% with no statistical significant difference among the two groups (OR 0.646 CI 0.292-1.431). Among the various characteristics of cervical lymphadenopathy, presence of heterogeneous signal in T2 imaging was found to be more common in cases than in controls (OR-2.377 CI 0.9234-6.117). Absence of posterior pituitary blind spot was significantly associated with CNS tuberculosis (OR-9.546 CI 5.067-17.985).

Conclusion:

The presence of heterogeneous signal in T2 imaging in cervical lymph nodes and the absence of posterior pituitary bright spot were found to be significantly associated with CNS tuberculosis and these could be used as additional aids in the radiological diagnosis of CNS tuberculosis.

To evaluate the utility of serum C-Reactive Protein levels in the early detection of severity of dengue

Abstract

Objective*:* Dengue is the most prevalent human arboviral disease. It has broad clinical presentation with unpredictable clinical evolution and outcome. The cornerstone management of dengue patients involves detection of the disease at initial phase followed by timely management. The aim of the study was to determine the relationship between C-reactive protein (CRP) level and the severity of dengue and the potential use of CRP in predicting severe dengue infection in adults.

Methods: A cross-sectional observational study was performed on dengue patients admitted in Department of medicine, Kasturba hospital, manipal. All patients of age above 18 years, with serologically proven dengue infection were included in the study. The detailed laboratory parameters pertaining to dengue were recorded. WHO 2009 classification of dengue was used to classify the patients into non-severe dengue and severe dengue. CRP levels were estimated and compared between groups i.e. severe and non-severe dengue. CRP cut-off value was detected using the receiver-operator curve along with sensitivity and specificity.

Results: Total 221 patients with mean age of 38 years were included in the study. The ratio of male to female dengue patients was 3:1, 11.3% patients suffered from severe dengue, 56% cases had non-severe dengue without warning signs, and 34.3% had non-severe dengue with warning signs. The most common symptoms observed were headache (57.4%), abdominal pain (31.2%), vomiting (35.7%), skin rash (27.6%), hepatomegaly (28.1%), and splenomegaly

(22.2%). Median CRP was significantly high in patients with severe dengue compared to non-severe dengue (96 mg/dL vs. 4.9 mg/dL). CRP cutoff value of 16.2mg/L had good sensitivity (80%) and specificity (79%) in predicting severe dengue infection. Several complications such as acute kidney injury, pancreatitis, acute respiratory distress syndrome and myocarditis were found to be associated with high CRP levels.

Conclusion: CRP level could be used as a potential biomarker to predict severity of dengue in adults.

Exploring Effectiveness of Mass Drug Administration Programme against Lymphatic Filariasis in Raichur District, Karnataka

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Abstract

Introduction: Lymphatic filariasis is one of the oldest and most debilitating neglected tropical diseases known to mankind. In India, around 630 million people are at risk of Lymphatic Filariasis spread across 256 endemic districts in 16 States and 5 Union Territories. Mass Drug Administration (MDA) programme against lymphatic filariasis is a strategy adapted by Government of India to eliminate this scourge by breaking the chain of transmission of disease.

Objective: To assess effectiveness of MDA programme.

Methods: This cross-sectional study was conducted across four clusters (three rural and one urban) spread over three endemic talukas of Raichur district Karnataka in October 2018. Multi stage random sampling was used to select the clusters. 60 houses in each of the selected clusters were surveyed to gather information on coverage, compliance, directly observed treatment, reasons for non-consumption, source of information on MDA, adverse drug reactions were collected in a pre-tested structured proforma by interview technique.

Results: Among the 240 households visited, a total of 1222 persons were identified as beneficiaries for MDA programme. Among the beneficiaries, 617(50.5%) were males and 605 (49.5%) were females. 25.12% of the beneficiaries were under 15 years of age. Of the total 1222 eligible beneficiaries identified, only 1147 (93.9%) had received DEC and Albendazole tablets as part of the MDA Programme. 1065 persons had consumed the tablets distributed to them; thus, the Compliance rate was 87.2%. The coverage and compliance rates were significantly higher in rural areas compared to urban area.

Conclusion: Raichur district has attained higher level of coverage and compliance for mass drug administration, but the difference in these indicators between rural and urban areas is a disturbing phenomenon, which has to be addressed through intensive behaviour change communication strategy.

Key words: Lymphatic Filariasis, Mass Drug administration, Raichur, Coverage, Compliance

Title: Nested PCR for Diagnostic Confirmation and Clinical Characterisation of Scrub Typhus in South India.

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Word count : 299

Introduction: Scrub typhus(ST) causes seasonal outbreaks of acute febrile illness with frequent systemic involvement, across India. The reference standard for its diagnosis (IgM IFA) is imperfect, point of care tests are lacking , and there is limited access to and variable sensitivity of PCR .

Objectives: To establish a nested PCR targeting a gene encoding a 56-kDa protein for confirmation of scrub typhus among patients with suspected scrub typhus.

Methods: We enrolled adult patients with acute febrile illnesses with suspected scrub typhus based on WHO suggested criteria, and exclusion of common acute febrile illnesses. IgM antibodies against *O.tsutsugamushi* were tested using Scrub detect IgM ELISA. Nested PCR(nPCR) was performed on DNA extracted from buffy coat.

Results: 48 suspected cases of scrub typhus were evaluated with both IgM ELISA and nPCR. 46 of these were positive either on IgM or nPCR. 34 cases(70.8%) were IgM positive whereas 27 cases (56.3%) were positive on nPCR. A positive nPCR added 12 cases to our yield who were IgM negative. Patients presented with acute febrile illness alone or associated with pulmonary, hepatic, renal or CNS involvement. Eschars were present in 19 cases(39.6%)., MODS was seen in 13 cases(27%) and there were 4 deaths(8.3%). nPCR was positive even in illnesses lasting 15 days or more and it helped confirm the diagnosis of scrub typhus in IgM negative patients mimicking leptospirosis, dengue, cholestatic jaundice, and PUO in a pregnant woman.

Conclusion: nPCR helped to confirm diagnosis of scrub typhus, adding 25% to our case yield in IgM negative cases. nPCR positivity was not limited to those with early illness as nPCR was often positive even in those with fever≥15 days. nPCR helped diagnosis of ST in those with presentations similar to leptospirosis, dengue, typhoid fever and in fever in pregnancy.

Association of Total Vitamin D Status, Vitamin D Receptor (*VDR*) Gene Methylation, VDR Expression, and Interleukin 1-beta (*IL-1β*) Expression in Children with Tuberculosis Disease

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Abstract:

Aim: Deficiency in circulatory vitamin D level is associated with a weakened immune response and increased susceptibility to tuberculosis (TB) disease especially in children. Epigenetic modification, particularly DNA methylation on immune defense genes negatively influences disease susceptibility. In this study, we hypothesised that vitamin D receptor (*VDR*) gene promoter methylation influences the availability of active vitamin D, and *VDR* and *IL-1β* genes expression levels in children with TB disease thus resulted in decreased immunity to TB disease.

Objective Methods: A cross-sectional comparative study was conducted in which 43 children with TB and 33 age and gender-matched healthy controls were recruited. The concentration of circulating total vitamin D level was measured in plasma, while the levels of *VDR* gene promoter methylation, VDR and IL-1β mRNA expression were measured in peripheral blood. Appropriate statistical tests were performed to analyse the test variables.

Results: Active TB included 25 (58%) pulmonary TB cases and 18 (42%) extra-pulmonary TB cases. Median vitamin D plasma level in TB cases (18.39 ng/mL) was significantly lower (41.34 ng/mL) than in healthy controls ($P< 0.0001$). Median distribution of *VDR* methylation in TB cases (75%) was significantly lower to healthy controls (10%) ($P<0.0001$). Due to hypermethylation in *VDR*, significantly decreased expression in levels of VDR and IL-1β mRNA (1.7 and 1.4 fold) were observed in TB cases respectively and ROC analysis showed statistically significant in discriminating ability of TB from healthy controls (AUC = 0.977, Sensitivity: 88%, Specificity: 100%, and cut off value: 37.5). A positive correlation was observed between *VDR* and *IL-1β* gene expression levels (r value – 0.435) ($P<0.002$) in TB cases.

Conclusions: In this study, lower circulatory vitamin D level, hypermethylation at *VDR* promoter, decreased expression of *VDR* and *IL-1β* genes were significantly observed in TB cases than controls thus weekend the immune response to TB disease.