

MEETING ABSTRACTS

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EPOSTER PRESENTATIONS

E1

HAART restores the frequency of regulatory T cells (Tregs) among HIV/AIDS patients

M Suthandhira¹, H Durgadevi¹, OR Krishnarajasekar², K Raja², B Rayvathy², Sowmya Swaminathan³, Luke Elizabeth Hanna³, S Anbalagan³, Elanchezhian Manickan^{1*}

¹Department of Microbiology, Dr. ALM PG IBMS, University of Madras, Taramani, Chennai, India; ²Govt. Hospital of Thoracic Medicine, Tambaram Sanatorium, Chennai, India; ³National Institute for Research in Tuberculosis (ICMR), Chetpet, Chennai, India

E-mail: emanickan@yahoo.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E1

Background: Immunosuppression is the hallmark HIV infection associated with gradual loss of CD4+Tcells. The effector immune response is regulated by CD4+CD25+Foxp3+Tcells (Tregs). Enumeration of Tregs frequency showed equivocal results before and here we showed clearly their frequency by flowcytometry.

Methods: One hundred and eight individuals comprising of 71 HIV positive patients (44 patients undergoing HAART and 27 without HAART) and 37 HIV negative healthy controls. PBMCs were isolated and stained with CD4/FITC, CD25/APC, FoxP3/PE antibodies (BD Pharmingen) and analyzed for Tregs frequency by flowcytometer (BD FACS Calibur).

Results: Our study showed that healthy controls had a Tregs frequency of 4.1%. When Tregs frequency analyzed among HIV/AIDS patients who are yet to receive HAART we found a significant reduction in Tregs population i.e. 1.2%. Encouragingly this deep plunge in Tregs population was found to be restored and surged upon HAART up to 12.6%. However, these changes in percentages did not alter based on their gender or age or absolute CD4 count of the study population.

Conclusion: HAART is known to decrease viral load and improve the patient's CD4 cells count. Here we report that HAART has improved the

Tregs frequency. It is yet to be known that the consequence of resurging Tregs during HIV/AIDS.

E2

Comparative codon usage analysis of HIV-1 and HIV-2 genomes

KK Vidyavijayan, S Hassan, LK Precilla, S Swaminathan, LE Hanna
Department of Clinical Research, National Institute for Research in Tuberculosis, Chennai, India

E-mail: hannatrc@yahoo.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E2

Background: Compared to HIV-1, HIV-2 infection is associated with slower disease progression and transmission, longer latency period and low or undetectable plasmatic viral levels. In an attempt to understand underlying reasons for the varying pathogenicity between HIV-1 and HIV-2 at the molecular level, we analyzed the differences in codon usage pattern of all nine coding genes.

Materials and methods: Thirty five full length HIV-2 genome sequences deposited during the period 1986-2008 were downloaded from LANL. The effective number of codons (NC), GC, GC3s and Relative Synonymous Codon Usage data corresponding to each gene were calculated using Codon W and Correspondence analysis (CA).

Results: The NC for all the nine genes ranged from 43 – 55.2, indicating that the genes of HIV-2 are also poorly biased, as has been reported earlier for HIV-1, resulting in low levels of expression of these proteins in general. The NC value of the *tat* gene of HIV-2 was higher than that HIV-1. The CA of all nine genes revealed that the factors influencing the expression of different genes were not the same.

Conclusion: The codon usage pattern of HIV-2 is distinctly different from that of HIV-1, and appears to be majorly shaped by the genomic composition rather than translational selection. The high Nc value of *tat* is suggestive of a lower level of expression of the protein in HIV-2 as compared to HIV-1, implying that this could be one of the viral factors responsible for lower levels of HIV-2 replication and delayed disease progression.

E3

Computational studies on non-nucleoside analogs of pyrimidine as NNRTIs against HIV-1

Ritika Srivastava, Nidhi Singh, Anuradha Singh, Madhu Yadav, Ramendra K Singh^{*}
Nucleic Acids and Antiviral Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad (UP) - 211002, India
E-mail: rksinghsrk@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):E3

Background: HIV is a retrovirus having ss RNA as the genetic material, which gets converted into ds DNA in the presence of reverse transcriptase (RT) enzyme. RT has been found the most attractive target for development of anti HIV-1 agents. Its inhibition is considered as one of the most practical approaches to prevent HIV infection.

Methods: NNRTIs are non-competitive inhibitors and bind at allosteric site. Designing of non nucleoside analogs of pyrimidine as NNRTIs has been done on the basis of Lipinski's Rule of Five & using the software DS 3.0. The molecules have been docked with HIV-RT using DS 3.0 in order to generate computational data and assess their suitability.

Results: Docking experiments have shown good interaction of non nucleoside analogs of pyrimidine as NNRTIs. Analysis of the docking results revealed that these molecules adopted butterfly conformations while interacting at NNIBP of HIV-1 RT & formed hydrogen bonds with amino acids, Lys101, Lys103, Tyr181, Tyr318 and π -stacking interactions with Tyr181, Tyr188, Phe227 and Trp229.

Conclusion: The molecular modeling revealed the interaction level of these molecules comparable to that of nevirapine. Hence, we predict these molecules as potential NNRTIs against HIV-1 RT.

E4

Computational modelling, green synthesis and biological activity of arylsulfonilamides as NNRTIs against HIV-1

Anuradha Singh¹, Madhu Yadav¹, Nidhi Singh¹, Ritika Srivastava¹, Rajinder Kaur², Satish K Gupta², Ramendra K Singh^{1*}
¹Nucleic Acids and Antiviral Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad- 211002, India; ²National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, India
E-mail: rksinghsrk@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):E4

Background: The development of new and potent anti-HIV compounds has become almost obligatory for the scientific community due to rapid emergence of drug resistant mutations. In pursuance of developing new anti-HIV molecules, we herein report the design, synthesis and anti-HIV properties of a series of potent arylsulfonilamide derivatives as NNRTIs.

Methods: Development of arylsulfonilamides as NNRTIs involved both the computational and synthetic methods. On the basis of extensive docking experiments, ten promising compounds out of **55** initially taken were synthesized using green protocols and their *in vitro* anti-HIV activity assessed in TZM bl cells by luciferase assay and reverse transcriptase (RT) inhibition assay against wild type HIV-1 RT.

Results: The compounds showed very promising *in silico* results as reflected by their lower ΔG values-high binding affinity, significant scoring functions high RT- ligand stabilization energy and close interatomic contacts through strong H-bonds with Lys103, His235, Tyr318, Lys101 and Val179; π - π interaction with Tyr181 and π -cation interaction through Lys101, which all together predicted high EC_{50} values. However, the molecules showed unimpressive inhibitory action against HIV-1 under *in vitro* conditions. The encouraging part of this study was that these compounds behaved as NNRTIs as per our expectations, on the basis of results obtained during HIV-RT assay.

Conclusions: In the present study, it has been observed that promising *in silico* results are not always corroborated by the desired *in vitro* results. Nevertheless, it is a part and parcel of drug discovery process, where successful drug development is nearly a hard nut to crack.

E5

Tenofovir induced renal damage is associated with activation of NF- κ B inflammatory signaling pathway and PARP overactivation

Hemalatha Ramamoorthy¹, Bina Isaac², Premila Abraham^{1*}
¹Department of Biochemistry, Christian Medical College, Bagayam, Vellore, Tamilnadu, India; ²Department of Anatomy, Christian Medical College, Bagayam, Vellore, Tamilnadu, India
E-mail: premilaabraham@yahoo.com
BMC Infectious Diseases 2014, **14**(Suppl 3):E5

Background: Tenofovir is recommended as a first-line therapy in HIV treatment. However, its long term use is associated with proximal tubular injury and renal dysfunction. Tenofovir has been shown to target the proximal tubular mitochondria, resulting in severe mitochondrial injury and overproduction of ROS and RNS. ROS are potent stimuli for the activation of NF- κ B, a key transcription factor, which is known to mediate inflammation. The NF- κ B response proinflammatory genes include iNOS, COX, TNF α , and others such as PARP-1. In the present study, we investigated whether NF- κ B inflammatory signaling pathway plays a role in tenofovir nephrotoxicity.

Methods: Rats were administered 2 daily doses of tenofovir (300mg/kg body weight) by gavage for 35 consecutive days, while control rats received water alone. On the 36th day, the rats were killed and the kidneys were used for the following assays. (a) NF κ B protein expression by WB and immunostaining methods, mRNA expression by RT-PCR and activity by ELISA, and I κ B- α protein expression and mRNA expression, (b) iNOS protein expression by WB, immunostaining methods, and mRNA expression by PCR, (c) COX-2 protein and mRNA expression, and (d) PARP-1 protein.

Results: The results of the present study reveal that NF- κ B signaling pathway is upregulated in the kidneys of rats chronically treated with tenofovir, as evidenced by a statistically significant increase in the mRNA and protein expressions of NF κ B, and its target proinflammatory genes, iNOS, COX-2, TNF α and PARP-1 as compared with control.

Conclusion: NF- κ B inflammatory signaling pathway may play a role in the pathophysiology of tenofovir nephrotoxicity.

E6

Docking studies of flavonoid derivatives as potent HIV-1 integrase inhibitors

N Premjanu^{*}, C Jaynthy, C Soniyagandhi
Sathyabama University, Chennai, Tamilnadu, India
E-mail: janu6kishore@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):E6

Background: HIV-1 integrase is responsible for the transfer of virally encoded DNA into the host chromosome. The process of integration occurs through 3 essential steps: formation of the preintegration viral DNA complex, 3'processing and 5' strand transfers. Provirus ancestral pol protein is a component of preintegration viral DNA complex, which has reverse transcriptase domain, Ribonuclease H like domain, integrase, N terminal and Zinc binding domain. To complete the integration process, strand transfer should take place. This work involves *in silico* prediction of the flavonoids as inhibitors of integrase activity.

Methods: In this experiment, docking studies were performed by modeling Pol protein: P10266 (HERV-K_5q33.3 provirus ancestral Pol protein), Q9UQG0 (HERV-K_3q27.3 provirus ancestral Pol protein), Q9BXR3 (HERV-K_7p22.1 provirus ancestral Pol protein) and docking with the flavonoid compound AC1NSUMK, Quercetin 3 arabinoside and compared with Raltegravir.

Results: Docking studies illustrate the binding between the Pol protein and flavonoid compound. In docking, the interactions were found in P10266 with AC1NSUMK at GLN378, GLN338, GLY283 and TYR375 with -7.71 kcal/mol. P10266 with Raltegravir at VAL369 and THR371 with -7.68 kcal/mol binding energy. The binding site resembles a saddle shape cleft which has positively charged residues.

Conclusion: The protein along with the AC1NSUMK with four hydrogen bonds proves to be a most stable complex inhibiting the activity of integrase. The interaction shows that it binds to the protein and specifically interferes with its strand transfer activity. Hence, this may be one of the potential candidates for inhibition of HIV activity.

E7

Longer sequence insertions in p6 gag play a role in immune escape in HIV-1 subtype C

Shilpee Sharma¹, GA Shambhu Prasad¹, Ravi Vijaya Satya², Viswanath Ragupathy³, Shanmugam Saravanan⁴, Kailapuri G Murugavel⁴, Pachamuthu Balakrishnan⁴, Suniti Solomon⁴, Indira Hewlett³, Udaykumar Ranga^{1*}

¹Jawaharlal Nehru Centre for Advanced Scientific Research, HIV- AIDS Laboratory, Bengaluru, India; ²Bioinformatics – Databases and Algorithm Development, Qiagen, MD, USA; ³Food and Drug Administration, Laboratory of Molecular Virology, Bethesda, MD 20892, USA; ⁴YRG Centre for AIDS Research and Education, Chennai, India
E-mail: udaykumar@jncasr.ac.in

BMC Infectious Diseases 2014, **14**(Suppl 3):E7

Background: HIV-1 is capable of evading the CTL immune response by introducing mutations in residues both within the epitopes and in sequences flanking the epitopes. The present study aims at identifying the mechanisms of CTL immune escape primarily in the asymptomatic phase of HIV-1 subtype C in drug naive patients from an Indian clinical cohort.

Methods: In a prospective study, a cohort of select seropositive drug naive subjects is being monitored at YRG CARE, Chennai for a period of two years with repeated sampling at 6-month intervals. The viral RNA was extracted from plasma and Gag was amplified, followed by Sanger sequencing. The samples of interest were further subjected to next-generation sequencing using Illumina MiSeq and analyzed using the CLC Genomics Workbench software.

Results: Twenty plasmid clones of gag were sequenced from one of the subjects at four different time-points. We observed multiple viral strains that did or did not contain an insertion of 14 amino acid residues in the PTAP domain of p6 gag. The PTAP duplication was further confirmed in 6 other subjects using the next-generation sequencing.

Conclusion: The preliminary data suggest that subtype C is capable of causing sequence insertions of longer length in the PTAP domain of the p6 gag, unlike other viral subtypes that insert sequences of shorter length at this location. The bioinformatics analysis is suggestive of the role of the amino acid insertions in immune escape in the chronic phase of the HIV-1 infection.

E8

Genotypic characterization of HIV-1 C variants in PBMCs and cervicovaginal cells

Varsha M Prabhu, Varsha S Padwal, Shilpa M Velha, Atmaram H Bandivdekar*
Department of Biochemistry, National Institute for Research in Reproductive Health, Mumbai, India
E-mail: batmaram@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E8

Background: HIV-1 is primarily a mucosal pathogen since more than 80% of infections occur through genital exposure. Vaginal intercourse, though an inefficient mode of transmission, contributes more new infections worldwide than any other route. Also, the female reproductive tract has been identified as a compartment that harbors variants distinct from blood. Present study aims to highlight viral compartmentalization between these compartments reflecting inadequate ART penetration.

Methods: In this study blood and cervicovaginal swabs were collected from 8 female subjects. CD4 counts and viral loads were determined. Translated amino acid sequences of C2 v3 region of env gene of proviral HIV-1 C in PBMCs and genital cells were analyzed using N-Glycosite and Geno2pheno [Co receptor] 1.2 programs for presence of N linked glycosylation sites and co receptor preference, respectively.

Results: Characterization of translated amino acid sequences of C2 v3 region of env gene of HIV-1 C shows variation in the number of N linked glycosylation (NLG) sites and uniform co receptor preference. Viral load varies in blood and genital secretions.

Conclusion: Genotypic characterization of viral variants in blood and female reproductive tract can provide information regarding their association with sexual transmission of HIV. Difference in the number of NLG sites observed may influence the affinity for host cell co receptor.

Discrepancies in viral load of blood and genital secretions suggest that ART may not be uniformly effective in suppression of viral load in different compartments of the same individual.

E9

Genotypic characterization of HIV variants from PBMCs and spermatozoa

Jyoti P Sutar, Varsha S Padwal, Shilpa M Velhal, Atmaram H Bandivdekar*
Department of Biochemistry, National Institute for Research in Reproductive Health, Mumbai, India
E-mail: batmaram@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E9

Background: Several studies have shown that detectable levels of HIV are observed in semen in spite of undetectable viral load in blood following antiretroviral therapy (ART). Also, different HIV variants have been detected in blood and semen of the same individual, suggesting that drugs may not be uniformly effective to control the viral load and infectivity in different tissues and secretions, which in turn may affect sexual transmission of HIV. Hence, genotypic characterization of HIV variants in PBMCs and sperm of the same individual may provide information about the association of these variants with sexual transmission of HIV.

Methods: Present study describes characterization of translated amino acid sequence of C2 v3 region of env of HIV-1 C in PBMCs and Spermatozoa of 14 and 5 individuals, respectively. The sequences were analysed for presence of N linked glycosylation sites using the program N-Glycosite. Also, Co receptor usage predictions were done using an online tool, Geno2pheno [Co receptor] 1.2.

Results: Significant differences in the number of N linked glycosylation (NLG) sites were observed in PBMCs and spermatozoa of the same individuals as well as samples collected from different individuals.

Conclusion: This study describes presence of compartmentalization between blood and semen which may influence the effect of ART in control of HIV/AIDS and influence sexual transmission of HIV.

E10

Expression of cytokine-mRNA in PBMC of HIV-1 subtype C infected individuals with opportunistic viral infections from South India

Jaiprasath Sachithanandham^{1*}, Veena Vadhini Ramalingam¹, Jaganathan Raja¹, Ooriyapadickal Cherian Abraham², Susanne A Pulimood³, Rajesh Kannangai¹
¹Departments of Clinical Virology, Christian Medical College, Vellore, India; ²Internal Medicine, Christian Medical College, Vellore, India; ³Dermatology, Christian Medical College, Vellore, India
E-mail: jaiprasath@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E10

Background: HIV disease progression is associated with marked change in the level of plasma cytokines. The study reported here investigated the level of mRNA expression of different cytokines: TNF alpha, INF-gamma, IL-10 and IL-21 in the peripheral blood mononuclear cells (PBMC) among the HIV infected individuals with viral opportunistic infections.

Methods: We quantified the mRNA levels of 4 cytokines in PBMC directly from the blood of individuals infected with HIV and normal healthy controls using TaqMan based RealTime PCR.

Results: The mRNA expressions of all the 4 cytokines in HIV-1 infected individuals were significantly higher compared to healthy controls (*p* value range 0.0004-0.01). The mean level of IL-10, INF-gamma and TNF alpha were higher in HIV infected individuals with low CD4 counts (<300 cells/ μ L). The IL-10 expression showed a significant negative correlation with CD4 counts (*r* = -0.25, *p* = 0.04) while IL-21 showed a positive correlation with CD4 counts (*r* = 0.26, *p* = 0.03). There was significant negative correlation between the CMV viral load and IL-21 expression.

Conclusion: The study showed a significant negative correlation between CD4+ T cell counts and IL-10 mRNA expression showing a shift towards TH2 profile during the late stages of the HIV disease in subtype C infected individuals. This study on HIV-1 subtype C infected individuals with opportunistic viral infections like EBV and CMV adding new information in such specific opportunistic infections.

E11

Polymorphisms in reverse transcriptase and protease genes of HIV-1 subtype C from Mumbai

Ritwik Dahake^{*}, Shraddha Mehta, Sneha Yadav, Abhay Chowdhary, Ranjana A Deshmukh
Department of Virology, Haffkine Institute, Mumbai-400012, Maharashtra, India
E-mail: ritwikdahake@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):E11

Background: Primary or transmitted HIV-1 drug resistance has caused an alarm in developed countries. While certain HIV-1 subtype C polymorphisms in relation to consensus subtype B sequences are known, there still exists a debate on the validity of these mutations being associated with/mistaken as, primary resistance. In this preliminary study, we have determined polymorphisms in reverse transcriptase (RT) and protease (PR) genes of HIV-1 subtype C from Mumbai, India.

Methods: The study was performed using plasma samples from 24 antiretroviral therapy-experienced and drug-naïve patients employing a 'home-brew' semi-nested reverse-transcriptase-PCR followed by sequencing and sequence analysis. Analysis and interpretation of polymorphisms and other drug-resistance mutations was carried out using the Stanford HIV drug-resistance database. We also analysed Surveillance Drug Resistance Mutations (SDRMs) for PR gene.

Results: We determined polymorphisms at a mutational frequency of 0.0670 ± 0.014 and 0.1337 ± 0.042 , while 12.5% and 16.6% samples harboured drug-resistance mutations in RT and PR genes respectively. Substitutions greater than 50% were at positions D121, K122, T165, K166, K173, D177, T200, Q207, R211 for RT and L19, V82, M36, R41, L63, H69, L89, I93 for PR gene. Additionally, PR gene SDRMs were observed in 15.0% samples.

Conclusion: Our study re-iterates that polymorphisms in HIV-1 subtype C from India may also include a number of major and minor/accessory mutations associated with resistance. We recommend that HIV subtype-specific drug-resistance databases be created to empower routine and unambiguous surveillance of drug resistance prior to initiating antiretroviral therapy, especially when including Protease Inhibitors.

E12

Use of multi-primer / multi-gene PCR method for the detection of *Mycobacterium tuberculosis* among female genital tuberculosis patients in India

Venkanna Bhanothu^{1*}, Jane Theophilus¹, Roya Rozati², AVikram Aiman³
¹Department of Zoology, UCS, Osmania University, Hyderabad-7, AP, India; ²MHRT Hospital & Research Centre, Hyderabad, AP, India; ³Gandhi Medical College, Hyderabad, AP, India
E-mail: banothu.venkanna@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):E12

Background: Female genital tuberculosis (FGTB) is a silent disease that evidences itself only when it is investigated. Demonstration of the etiologic agent by H & E staining or Z-N staining for acid fast bacilli, smear microscopy, and culture of sputum and other body fluid specimens were often less specific.

Methods: A prospective case-control study was undertaken. A total of 302 endo-ovarian tissue biopsies collected from 202 infertile women highly suspected of having genital tuberculosis on laparoscopic examination and from 100 control women of reproductive age. All specimens tested by conventional/phenotypic methods were later compared with multi-gene/ multi-primer PCR method using four sets of primers for detection of *Mycobacterium tuberculosis* (MTB) in a single tube-single step reaction.

Results: The conventional methods showed 99% to 100% specificity with a low sensitivity, ranging from 21.78% to 42.08% while H & E staining showed a sensitivity of 51.48%. Multi-gene PCR method was found to have a much higher sensitivity of 70.29% with MTB64 gene, 86.63% with 19kDa antigen gene at species and TRC4 element at regional MTB complex level and 88.12% with MPT59 α -antigen/32kDa protein gene at genus level. The specificity of multi gene/multi primer PCR was 100%.

Conclusion: Multi-gene PCR was found to be a powerful technique for diagnosis and differentiation of mycobacterial infection among endo-ovarian tissue biopsies taken from infertile patients with FGTB. We suggest site specific sampling and amplification of the 19kDa antigen gene in combination with TRC4 element as a successful multi-gene/ multi-primer PCR method for the diagnosis of FGTB.

E13

Early infant diagnosis: gateway to survival for HIV positive infants

Jayanthi Shastri^{*}, Sachee Agrawal, Sandhya Sawant, Manish Pathak
Department of Microbiology, T.N.Medical College and B.Y.L.Nair Charitable Hospital, Mumbai, India
E-mail: jsshastri@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):E13

Background: Under PPTCT, babies born to sero-positive mothers were followed up and tested at 18 months of age. Under Early Infant Diagnosis (EID) program, HIV exposed babies are subjected to HIV-1 DNA PCR on DBS and whole blood as early as 6 weeks of age followed by initiation of ART.

Methods: Infants born to HIV positive mothers were tested according to NACO guidelines. Infants <6 months of age (n=136) were tested by DBS HIV-1 DNA PCR; DBS positive were confirmed by whole blood PCR. Infants 6 to 18 months (n=68) were tested by antibody test and if positive were confirmed by DBS HIV-1 DNA PCR. Detailed history including type of delivery, Single Dose Nevirapine (SDN) and breast feeding was taken.

Results: The HIV transmission rate was 11.28% (23/204); 13 infants <6 months of age and 10 infants $\geq$ 6 months of age. In infants <6 months of age, who did not receive SDN the positivity was 38.46% (5/13) whereas in those who received SDN it was 6.5% (8/123), [p=0.0012]. In infants $\geq$ 6 months the positivity rate was significantly higher in breast fed 60% (6/10) as compared to non breast fed 6.9% (4/58), [p=0.0001]. 60.9% (14/23) infants were delivered at home and did not receive SDN.

Conclusion: In resource limited settings, SDN given to mother in labor is a good option.. Mothers intending to continue breast feeding should be provided extended ART. EID and prompt ART institution ensures favourable outcomes in HIV exposed infants.

E14

Hybrid BFPSO algorithm based estimation of optimal drug dosage for antiretroviral therapy in HIV-1 infected patients

K Kamalanand, P Mannar Jawahar^{*}
Madras Institute of Technology Campus, Anna University, Chennai, India
E-mail: pmjmit@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):E14

Background: Although the use of anti retroviral drugs has prolonged the life of infected patients, the side effects can be severe. The objective of this work is to efficiently estimate the optimal drug dosage for ART using mathematical model based method.

Methods: In this work, an artificial intelligence technique known as the hybrid Bacterial Foraging Particle Swarm Optimization (BFPSO) algorithm is used in conjunction with the three dimensional patient specific HIV model, for estimation of optimal drug concentration. In the mathematical model, it is assumed that the drug acts directly on the viral burden. The optimization problem is formulated in such a way that the HIV-1 viral load as well as the total drug concentration is minimized.

Results: Based on the computer simulation studies performed for a particular case at the seventh year of infection, it was found that in the scenario without therapy, the CD4 cell count dropped to a value of 366 cells/mm³. However, when ART is administered based on the hybrid BFPSO algorithm, the CD4 cell count increased to a value of 782 cells/mm³, with minimal usage of the drug concentration.

Conclusion: Results demonstrate that, the optimal drug dosage estimated using the proposed methodology is efficient in improving the immune system and minimizing the viral burden. This work seems to be of high clinical importance since, at present; ART is the widely used method for treatment of HIV-1 infection.

E15

Design, synthesis and biological evaluation on *N*-heteroaryl compounds as probable NNRTIs against laboratory adapted strains and the primary isolates of HIV-1

Anuradha Singh¹, Madhu Yadav¹, Ritika Srivastava¹, Nidhi Singh¹, Ashwini Godse², Rahul Suryawanshi², Ashwini Dhamanage², Smita Kulkarni², Ramendra K Singh^{1*}
¹Nucleic Acids & Antiviral Research Laboratory, Department of Chemistry, University of Allahabad, India; ²National AIDS Research Institute, Pune, India
E-mail: rksinghsrk@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E15

Background: The emergence of drug resistant HIV strains and suitability of NNRTIs as potent anti-HIV molecules used in HAART did attract our attention towards developing NNRTIs. We, therefore, focused on developing some new NNRTIs utilizing benzimidazole and pyrimidine moieties to match the hydrophobic nature of the allosteric pocket in HIV-RT.

Methods: Compounds designed on the basis of extensive computational studies, were finally synthesized through facile synthetic route and characterized using various chromatographic and spectral techniques. All synthesized molecules have been screened against HIV-1 using TZM bl assay and laboratory adapted strains HIV-1 IIB (X4, subtype B), HIV-1 Ada5 (R5, Subtype B) and the primary isolates HIV-1UG070 (X4, Subtype D).

Results: Cell based assay showed that majority of the compounds were active at micro molar concentrations (1.39-17.39 μ M) and the SI value ranged between 10.77-17.39 against lab adapted strains and 5.8-13.91 against primary isolates. The studies on structure-activity relationship were also consistent with the experimental data.

Conclusions: In view of the results obtained, these compounds may be developed as potent inhibitors of HIV-1 replication with suitable structural/pharmacophore modifications.

E16

Host factors influencing the activation of tuberculosis in HIV positive individuals with latent TB infection

Kamakshi Prudhula Devalraju, Sharada Ramseri Sunder, Vijaya Lakshmi Valluri*, Arunabala Chowdary
LEPRA India – Blue Peter Public Health & Research Centre, Hyderabad, AP, India
E-mail: vijayavalluri@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E16

Background: TB kills 1.3 million persons annually, including 275,000 people in India. An estimated 40% of the population in India has LTBI (Latent Tuberculosis Infection), a significant percentage of them being infected with HIV. HIV infection increases the likelihood of LTBI progressing to active TB. Vaccination and immunotherapeutic strategies are alternative approaches that contribute greatly to TB control in HIV+ persons with LTBI.

Methods: Peripheral blood was drawn from, HIV+ and HIV- individuals with and without LTBI. PBMC and CD14+ monocytes were isolated and 2 million cells were cultured with γ irradiated *M. tuberculosis* H37Rv, CFP-10. Cultures were terminated after 96 hours, and cells were stained for CD4, CD25, FOXP3 and D4GDI. IFN- γ , IL-17, IL-22 were estimated in the supernatants by ELISA. RNA was isolated from CD14 cells and Realtime PCR was performed to quantitate c-maf expression. $P < 0.05$ was considered statistically significant.

Results: In response to *M. tuberculosis*, FoxP3+ cells expand in HIV+ and healthy LTBI+ donors. In contrast, D4GDI+FoxP3+ cells expand only in healthy LTBI+ individuals. IL-17 and IL-22 are significantly high in HIV-LTBI+ individuals, increased c-maf expression in monocytes was observed in HIV+ LTBI+ individuals

Conclusions: IL-17 and IL-22 can be helpful to control reactivation of tuberculosis in HIV+ individuals.

E17

Reiter's syndrome as a manifestation of immune reconstitution inflammatory syndrome in an HIV infected individual

J Shankari*, V Sudha, K Manoharan
Department of Dermatology, Institute of Venereology, Madras Medical College, Chennai, India
E-mail: drshankari@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E17

Introduction: Reiter's syndrome is a triad of arthritis, urethritis, and conjunctivitis. The prevalence of Reiter's syndrome in HIV infected patients varies between 1.7% and 11.2%. This syndrome can also occur as a part of immune reconstitution inflammatory syndrome (IRIS).

Case: A 50 year old male, known HIV patient on ART for past 4 months, presented with complaints of scaly raised lesions over palms, soles and genitalia with history of burning micturition and swelling of right knee for over one month. On examination patient was thin built, anaemic. Right knee joint effusion with tenderness was present. Palms and soles revealed keratoderma blennorrhagicum. Nails were yellowish and dystrophic. Oral cavity showed candidiasis and oral hairy leukoplakia. Genital examination showed circinate balanitis. Clinical diagnosis of Reiter's syndrome was made. Haemoglobin was low, ESR and CRP were elevated. CD4 counts were raised when compared to previous values. RNA load showed 2 log decreases. Urine culture showed no growth. Chest x ray had features of tuberculosis. Symptomatic improvement was seen after treatment with ofloxacin, isotretinoin and indomethacin.

Discussion: In HIV infected patients, Reiter's syndrome may follow a severe clinical course after immune reconstitution associated with ART. However, because of possible higher antigen dependence, clinical outcome may be more favourable if a specific infectious agent can be identified and treated with antibiotics.

Conclusion: We are presenting this case to highlight the occurrence of Reiter's syndrome as a rare manifestation of IRIS in an HIV infected individual.

E18

N-Heterocyclic analogues as peptide deformylase inhibitors: Molecular modeling, synthesis and antibacterial evaluation

Anuradha Singh, Madhu Yadav, Preeti Singh, Ritika Srivastava, Nidhi Singh, Rajesh Verma, Ramendra K Singh*
Nucleic Acids and Antiviral Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad-211002, India
E-mail: rksinghsrk@gmail.com

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Background: Global emergence of worsening antibiotic resistance has become a serious problem because of emergence of multi drug resistance (MDR) strains. The emergence of MDR strains has forced the scientific community to search for new antibiotics.

Methods: A new series of *N*- heterocyclic compounds has been derived from benzimidazole and pyrimidine nuclei optimized with the Discovery studio 3.0 software to investigate the interactions between the target compounds and the amino acid residues of *Escherichia coli* PDF Ni (PDB: ID 1G2A), and then synthesized. Further, all compounds were examined for their antibacterial activities against Gram-positive, *S. viridians*, and Gram-negative bacterial strains, *E. coli*, *P. mirabilis* and *K. pneumoniae* using the microdilution broth susceptibility test method and subjected to polynomial regression.

Results: The compounds showed promising *in silico* results as reflected by their significant scoring functions and close interatomic contacts through strong H-bonds with Ile 44, Gly 45, Gly 89, Cys 90, Glu95, Cys129 and Arg 97; pi-pi interaction with Arg 97 and His 132. Antibacterial results indicated that these molecules possessed significant activity against all the tested species with MIC values ranging between 0.22 and 2.62 μ M. The regression coefficients ($R^2 = 0.78$ to 0.89) derived for each strain were correlated significantly.

Conclusion: The molecules proved as effective inhibitors of bacterial PDF, which has been corroborated by docking studies.

E19

Alphalinolenic acid, a potent inhibitor of fatty acid synthase antimycobacterial agent

Mallika Jainu, A Hemaprabha*, S Vinutha, Sarah Rajitha, TS Ranjani
Department of Biomedical Engineering, Sri Siva Subramaniya Nadar College of Engineering, Kalavakkam, Chennai -603110, India
E-mail: ahems.8900@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E19

Background: The mycobacteria become resistant since their slow growth phase increases its ability to adapt and acquire new resistance mechanisms.

Presently the first line therapy against TB includes the use of isoniazid and rifampin, which are probably the most effective mycobacterial drugs available today and then there is a very small chance to treat the disease since other newer drugs are seriously limited by gastrointestinal, renal and/or neurological toxicities. Therefore, the search for new ways to treat TB is of primary importance and urgency. Alpha linolenic acid (ALA) is a potent inhibitor of fatty acid synthase (FAS) in a variety of prokaryotic and eukaryotic cells.

Methods: Using a standardized mycobacterial susceptibility test, we have observed that ALA inhibits the growth of several species of mycobacteria, including tuberculous species such as *Mycobacterium tuberculosis* (H37Rv and clinical isolates) and *Mycobacterium bovis* BCG.

Results: Alpha linolenic acids are toxic to mycobacteria, such as *Mycobacterium tuberculosis* H37Rv, with minimum inhibitory concentrations (MICs) of 25-50 µg/mL and *Mycobacterium bovis* with minimum inhibitory concentration of 6.25 – 25 µg/mL.

Conclusion: Our data demonstrate that ALA as a prototypical inhibitor, significant antimycobactericidal effects was seen with *Mycobacterium tuberculosis* and *Mycobacterium bovis*. A possible antibacterial mechanism was postulated to proceed via disruption of the bacterial cell membrane resulting in a change in membrane permeability.

E20

Mycobacterium bovis Bacille Calmette-Guerin infection modulates GRK2/3 dependent cytokine secretion

Manisha Yadav^{1*}, D Nagarajuna¹, Rakesh Singh Dhanda²

¹Dr. B.R. Ambedkar Centre for Biomedical Research, University of Delhi, Delhi, India; ²Department of Translational and Regenerative Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India
E-mail: myadav@acbr.du.ac.in

BMC Infectious Diseases 2014, **14**(Suppl 3):E20

Background: *Mycobacterium tuberculosis* has evolved highly specialized mechanisms to proliferate in the host during infection. In this process, the infection of alveolar epithelial cells is a necessary step for mycobacteria dissemination; however the mechanisms of mycobacterial epithelial interactions are incompletely understood. Previously, we characterized the role of epithelial G protein coupled receptors (GPCR) CXCR1 and CXCR2 during mycobacterial infection. However the role of GPCR kinases (GRK) 2/3 and GRK4-6 in response to mycobacterial infection has not been investigated.

Methods: The GPCR kinases expression (GRK2/3 and GRK4-6) after *Mycobacterium* infection was quantified by RT-PCR and Western blot analysis. Further, the secretion of cytokines IL-8 and TNF-α was quantified in supernatants by ELISA.

Results: Mycobacterial infection in lung epithelial cells increased secretion of IL-8 and decreased TNF-α upto 72 hours. Further, the infection in the epithelial cells was modulated by a combined up regulation of GPCR kinases (GRK) 2/3 genes and suppression of the GRK 4-6 gene expression. These results were confirmed at protein levels. In addition, the blocking of chemokine receptors decreased the inhibition of GRK 2/3 expression suggesting that mycobacteria manipulate epithelial responses by desensitizing the receptors and the cytokine secretion.

Conclusions: In conclusion, we have identified a role for GRK 2/3 dependent cytokine secretion in the initial phase of mycobacterial infections in the lung epithelial cells.

E21

In silico analysis of carboxamide derivatives of piperidine as potential antagonists of CCR5

A Harishchander^{*}, J Michael Joshua, U Benjamin Jason, D Alex Anand
Department of Bioinformatics, Sathyabama University, Chennai, India
E-mail: harishchander.a@gmail.com

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Background: Analysis and identification of competitive antagonist in the co receptor binding of HIV is a challenging task towards overcoming the tropism of HIV. Hence analyzing the structure activity relationship between CCR5 and the derivatives of carboxamide is an initiation towards discovering the future inhibitors of CCR5.

Methods: We have identified the interaction between CCR5 and existing sulphonyl derivatives of carboxyl amine and it was followed by construction of various derivatives of carboxamide with trans confirmation of carboxyl phenol. Further, the constructed molecules were screened on the basis of various molecular descriptors. The potential derivatives were identified using molecular dynamics simulations in 1 nano second using Discovery Studio software suite (version 2.0).

Results: Regression Analysis of various molecular descriptors showed a threefold increase in the competitive binding of carboxamide derivatives with CCR5 and the increased activity of existing sulphonyl derivatives is single fold. Binding Energies of carboxamide derivatives were less than 2 KCal of sulphonyl derivatives. Conformational analysis using Molecular Dynamics Simulation of sulfo carboxamide derivatives confirms a twofold increased stability when compared with sulphonyl derivatives of piperidine.

Conclusion: Construction of analogs based on sulfo carboxamide using chemsketch generated fourteen compounds. *In silico* analysis of these piperidine based Sulfo carboxamide derivatives show a better competitive inhibition than existing sulphonyl derivatives of carboxyl amine. Further *in vivo* tests are required to analyze the effective concentration for constructing effective derivatives of carboxamide based piperidine as potential antagonists of CCR5 in future.

E22

The *PknI* and *DacB2* double deletion mutant of *Mycobacterium tuberculosis* leads to alteration of cell morphology and susceptibility to antibiotics

Srinivasan Kandasamy^{*}, Sujatha Narayanan

Department of Immunology, National Institute for Research in Tuberculosis (NIIRT) (formerly TRC), ICMR, Chetpet, Chennai-600031, India
E-mail: biosrinik@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E22

Background: *Mycobacterium tuberculosis* is a slow growing infectious pathogen. It takes twenty hours for a single cell to divide into two. Its cell division is complex involving a number of proteins. Although, the physiological roles of several serine/threonine phosphorylation connected to cell division and peptidoglycan synthesis have been studied the exact mechanism is not clear. *PknI* and *DacB2* located in a same cluster have been shown to play a role in cell division and cell wall synthesis. The aim of this present study was to construct the double deletion mutant (DKO) of *PknI* and *DacB2* and study the effect on cell morphology and antibiotic susceptibility.

Methods: Specialized phage transduction method was used to construct DKO strain of *PknI* and *DacB2*. The cell morphology was observed in solid agar plate, light microscopy and electron microscopy. The MIC was determined by resazurin based microplate assay.

Results: The DKO was confirmed by PCR and southern blotting methods. The light and electron microscopy study revealed that DKO showed irregular shape and smoother colonies in comparison to *M. tuberculosis* H37Rv. The DKO was more susceptible to isoniazid compared to *M. tuberculosis* H37Rv and DKO showed the same sensitivity pattern as H37Rv strain to other drugs.

Conclusion: In the present study, we have successfully constructed a novel DKO strain of *Mycobacterium tuberculosis*. *PknI* and *DacB2* were found to have a role in maintaining cell morphology and isoniazid resistance.

E23

In silico analysis of drug resistance in wild type and mutant HIV-1 subtype d protease

Punnagai Munusami^{*}, CS Vasavi

Bioinformatics Division, School of Bio Sciences and Technology, Vellore Institute of Technology University, Vellore-632014, India
E-mail: punnagai.munusami@vit.ac.in

BMC Infectious Diseases 2014, **14**(Suppl 3):E23

Background: A general cause for therapeutic failure in people is due to the high resistance of HIV protease to antiretroviral drugs. The aim of the present work is focused on two objectives: to study the mutations responsible for the drug resistance using Stanford database and how the

existing ART drugs are resistant to subtype d (wild type, major and minor mutations) using docking studies.

Methods: The mutation frequency of subtype d in untreated persons was obtained from Stanford DR database, <http://hivdb.stanford.edu/>. Based on the database the wild type PTD sequence was generated. Crystal structure 3LZS showed more similarity based on BLASTp program. The protein structure 3LZS was used as a template to build wild type, major (L10V, N37D, K69Y), minor (K20I, L33I, P39T, Q61N), major+minor mutants of PTD using Modeller9v7. The docking studies of protease inhibitors (atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir) with the wild type and mutant models were carried using AutoDock 4.2.5.

Results: The docking studies revealed that contribution of high torsional energy in atazanavir affects the binding energy and leads to high level resistance to the wild type and the mutants. Indinavir showed susceptibility for the wild type PTD whereas nelfinavir showed susceptibility to all mutants and the susceptibility was due to less number of torsions and increased van der Waals, hydrogen bonding, desolvation and electrostatic energy contributions.

Conclusion: The developed wild type and mutant model of PTD provides an insight to understand the effect of mutations and resistance towards the protease inhibitors.

E24

Development of dual inhibitors targeting DprE1 and AHAS for treatment of *Mycobacterium tuberculosis* infection

Rupesh V Chikhale^{*}, Amit M Pant, Sunil S Menghani, Pramod B Khedekar
Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj
Nagpur University, Nagpur, India
E-mail: rupeshchikhale7@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E24

Background: The emerging multidrug resistant (MDR) and extensively drug resistant (XDR) *Mycobacterium tuberculosis* (MTB) infection is increasing with greater complexity, estimated 220000-400000 tuberculosis cases emerged in 2011 globally. A number of lead compounds have been developed for treatment of MDR and XDR TB, but no new chemical entity has emerged for clinical use. Recently DprE1 and AHAS have been identified as promising drug targets.

Methods: The methodology involved *in silico* studies, synthesis and *in vitro* evaluation for inhibition of *M. tuberculosis*. *In silico* studies involved protein preparation for DprE1, AHAS, docking and analysis of docking results. Sixty two substituted (thiazolidine-2-yl amino) benzthiazolylphenylhydrazine carbothamide derivatives were studied. *In vitro* evaluation was carried out by modified agar diffusion method.

Results: About 62 compounds were synthesized based on molecular docking studies. In case of DprE1 maximum interactions were found with His132, Asn385, Gly133, Leu134, Leu363, Val365, whereas in case of AHAS maximum interactions were shown between Arg318, Gly138, Lys197, Trp516 and Phe147. All compounds were synthesized in satisfactory yield and structurally elucidated. The range of MIC was found between 40-80 mg/L with percentage inhibition in range of 80-95%.

Conclusion: Experimental results reveals that newly developed compounds exhibited promising antitubercular activity which can be further explored for development of potent drugs.

E25

Production of cross neutralizing single chain fragment variables (scFv) from HIV-1 infected Indian children

Sanjeev Kumar¹, Rajesh Kumar¹, Muzamil Makhdoomi¹, Lubina Khan¹,
SS Prakash¹, Ramachandran Thiruvengadam¹, Mohit Singla², Rakesh Lodha²,
SK Kabra², Subrata Sinha³, Kalpana Luthra^{1*}

¹Department of Biochemistry, All India Institute of Medical Sciences (AIIMS),
New Delhi, India; ²Department of Pediatrics, All India Institute of Medical
Sciences (AIIMS), New Delhi, India; ³National Brain Research Centre (NBRC),
Gurgaon, Haryana, India

E-mail: kalpanaluthra@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E25

Background: Monoclonal antibody based vaccines are effective and highly specific in disease targeting. Presently, most of the existing

broadly neutralizing antibodies are generated against non subtype c viruses. HIV-1 subtype c accounts for more than 90% of infection in India. The disease progression in children is faster than adults.

Methods: Nine ART drug naïve HIV-1 subtype c infected children were recruited. PBMCs were isolated from all the subjects and pooled. RNA was isolated and cDNA was synthesized followed by amplification of VH and VL chain genes and scFv construction. A human recombinant scFv phage display library of 108 clones was constructed. Diversity of the phage library was checked by DNA sequencing and biopanned with RSC3 core antigen. 60 random clones were screened by phage ELISA. Expression of the scFvs was assessed by SDS-PAGE and Western blotting.

Results: The diversity of the phage library was more than 90%. Eight scFv monoclonals showed positive binding in phage ELISA and two best binders were further characterized. Both scFvs didn't show any reactivity with unrelated antigens. DNA fingerprinting analysis showed both scFvs were distinct. A 32kDa band was obtained in SDS-PAGE and Western blot. Both scFvs showed cross neutralizing activity against a standard panel of pseudoviruses.

Conclusion: Here, we have for the first time generated a human recombinant scFv phage display library containing neutralizing clones from HIV-1 infected children. Further characterization of these scFvs generated against CD4 binding site for their epitope mapping would be helpful in the development of an effective HIV-1 vaccine.

E26

Neuro-psychiatric co morbidity in HIV/AIDS; and effect of alcohol in CD4 count in HIV patients

Biswadeep Borthakur^{1*}, Dhruva Jyoti Borah², Supria Kumar Mandal¹,
Kamala Deka¹, Saumitra Ghosh¹, Dhruva Jyoti Bhuyan¹, BB Rewari², D Singh¹
¹Assam Medical College, Dibrugarh, Assam, India; ²National AIDS Control
Organisation, New Delhi, India

E-mail: biswadeebp@yahoo.co.uk

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Background: The exponential increment of various co-morbid disorders among HIV positive individuals includes various medical subspecialties and mental health is one of them. HIV/AIDS affects mental health by its complex interactions which may be the result of the psychological response to the diagnosis or complex neurobiological interactions. Therefore the current study aims at systematic evaluation of psychiatric morbidity in HIV/AIDS patients.

Methods: Patients attending ART centre during the period of 1 year were selected for the study by random sampling method. After obtaining written consent MINI-PLUS intentional scale was applied to evaluate the psychiatric co-morbidity.

Results: Of 75 patients MINI-Plus interview revealed co-morbidities of major depressive disorder (10.66%), generalized anxiety disorder (25.33%), panic disorder (1.33%), alcohol dependence (29.33%) and abuse (5.33%), nicotine dependence (32%), opioid & marijuana (1.33%) and schizophrenia (2.66%) and cognitive impairment (5.33%). Both the sex were almost equally affected by the psychiatric morbidities except substance abuse were more prevalent in male participants but patients with primary and secondary education suffered more from anxiety disorder. Interestingly, patients with co-morbid alcohol abuse and dependence had lower CD4 count than non alcoholic counterpart ($P = 0.0426$).

Conclusion: HIV/AIDS with psychiatric morbidity, out of which generalized anxiety disorder and major- depressive- disorder are common but alcohol and nicotine dependence and abuse, is the highest of co-morbidity. Co-morbid alcohol use problem is associated with low CD4 probably mediated through immune-modulator effect of alcohol. Treatments of co morbidity will definitely ensure a better quality of life and treatment outcome.

E27

M. tuberculosis Peptide based Point-of care test for TB

Jesus M Gonzalez^{1,2}, Bryan Francis¹, Sherri T Burda³, R Sam Niedbala^{1,2},
Suman Laal^{1,3,4*}

¹TB Biosciences, 205 Webster Street, Bethlehem, PA, USA; ²Lehigh University,
Department of Chemistry, Bethlehem, PA, USA; ³Veterans Affairs Medical
Center, New York Harbor Health Care System, New York, NY, USA;

⁴New York University Langone Medical Center, Departments of Pathology and Microbiology, New York, NY, USA
E-mail: Suman.Laal@nyumc.org
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Background: A rapid, simple and affordable point-of care (POC) test that can be implemented in peripheral health care settings to replace/improve upon microscopy for TB diagnosis or to rule out TB (triage) remains unmet.

Methods: Immunodominant regions of 3 *Mycobacterium tuberculosis* specific cell-wall proteins that are highly immunogenic in HIV- TB+ and HIV+TB+ patients have been mapped. Antibodies to these regions are absent in subjects with latent TB, BCG vaccination, other pulmonary diseases. To examine the feasibility of devising a peptide based rapid POC test, selected peptides were conjugated to a carrier protein using standard EDC/NHS coupling chemistry, purified by size exclusion; the peptide conjugation confirmed using MALDI MSMS before adsorption onto colloidal gold and striping onto nitrocellulose. The assay parameters were adjusted to determine positive/negative status within 15 minutes via visual or instrumented assessment.

Results: The prototype POC test was evaluated with sera from ~400 TB patients, non TB subjects from the groups defined in the methods section. The current prototype POC test provides >90% sensitivity and specificity in the above populations.

Conclusion: WHO recommends against use of all current commercial serological TB tests since they fail to achieve adequate sensitivity and specificity. These results demonstrate that, as for HIV rapid tests, carefully selected immunodominant epitopes of highly antigenic *M. tuberculosis* specific proteins can be the basis of antibody detection based rapid POC TB test. Test optimization to improve accuracy to meet WHO specifications for a POC test to replace microscopy or a triage test to rule-out TB are ongoing.

E28

Assessment of oxidative stress parameters in HIV infection

Shreewardhan Rajopadhye^{1,2*}, A Rosalind Marita¹, MA Ansari³, Abhay Chowdhary¹, Sucheta Dandekar²

¹Department of Biochemistry, Haffkine Institute, Mumbai-400012, Maharashtra, India; ²Department of Biochemistry, Seth G.S.Medical College & KEM Hospital, Parel, Mumbai-400012, Maharashtra, India; ³Department of Pathology, Seth G.S.Medical College & KEM Hospital, Parel, Mumbai-400012, Maharashtra, India

E-mail: shrajopadhye@gmail.com

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Background: Both viral and host factors are responsible for oxidative stress in HIV disease, which in turn activates the replication of HIV provirus by various pathways. Oxidizing stress is a pathologic phenomenon resulting from imbalance between the system producing active oxygen species and those defending the organism. The present study was aimed to assess oxidative stress markers in HIV patients.

Methods: The study included 30 HIV sero-positive patients, 30 healthy volunteers served as controls. Patients were categorized on the basis of their absolute CD4 counts into 3 groups - Group-1 (>500 CD4 cells/mm³), Group-2 (200-499 CD4 cells/mm³), and Group-3 (<200 CD4 cells/mm³). Lipid peroxidation was estimated using serum malondialdehyde as a marker, serum nitric oxide levels were assessed by Griess reagent method, serum reduced GSH by Beutler *et al*, serum C reactive protein, serum AOPP by Witko Savark method and serum proteins by Bradford method. Statistical analysis was done using the Student's t test and one-way ANOVA.

Results: Significant decrease ($p < 0.004$) in GSH levels and significant increase ($p < 0.0008$) in NO levels was observed in HIV infected group when compared to controls. However, no significant changes were found in levels of AOPP, MDA, and CRP in the study groups. Significant increase ($p < 0.0001$) in MDA levels in group 3 and in GSH levels ($p < 0.0395$) in all 3 groups was seen as compared to controls.

Conclusion: The findings indicate that considerable amount of oxidative stress are induced and changes in NO and GSH levels may contribute to the immunopathophysiology during HIV infection.

E29

Assessment of the anti-HBV like property of BEHP – a phthalate derivative and its *in silico* characterization

AS Smiline Girija^{1*}, V Duraipandian², K Pandi Suba³, G Hariprasad⁴, R Raghuraman³

¹Department of Microbiology, Meenakshi Ammal Dental College, Meenakshi University, Chennai, India; ²Entomology Research Institute, Loyola College, Chennai, India; ³Central Research Laboratory, Meenakshi Ammal Dental College, Meenakshi University, Chennai, India; ⁴Department of Microbiology, Government Thoothukudi Medical College, Thoothukudi, India

E-mail: smilinejames25@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E29

Background: Recent advances in the insilico virtual screening paves way towards efficient discovery of new drugs on the basis of drug target evaluation and analysis. The present study is thus an attempt to assess the anti-HBV like activity of Bis 2-(ethyl hexyl) phthalate (BEHP) from the black pigmented ink of South Indian squid, *Loligo duvauceli*.

Methods: Fresh ink glands from *Loligo duvauceli* were dissected and the ink was subjected to crude solvent extraction. Chromatographic profiles were assessed by TLC and HPTLC. Fractionation of the bio active compounds was done by Silica gel column chromatography and the active fraction was subjected to GC-MS analysis. *In vitro* analysis of the anti-HBV like activity was assessed by pre incubating the HBV positive serum samples with the active fraction by performing ELISA. *In silico* virtual screening was analyzed with the latest bio informatics tools and databases.

Results: TLC and HPTLC profiles yielded a compound with the Rf value of 0.76. The antimicrobial fraction upon GC-MS analysis revealed a chromatogram with nine peaks with BEHP possessing a highest percentage of area normalization (91%) with other few minor constituents. The ELISA results were negative for all the pre incubated HBV positive serum samples. Docking scores and the *in silico* analysis supported the preliminary screening results of ELISA and indicated the anti-HBV like activity of BEHP.

Conclusion: This present study suggests that BEHP could be a novel anti-HBV agent and a promising candidate to combat viral hepatitis in near future with further clinical and toxicity evaluation.

E30

HbAHP-25, a peptide designed *in silico*, exhibits potent anti-HIV activity *in vitro*

Tahir Bashir¹, CS Kumar², KVR Reddy^{1*}

¹Molecular Immunology & Microbiology (MIM), National Institute for Research in Reproductive Health, Mumbai, India; ²Department of Bioinformatics, Dr. D.Y. Patil University CBD Belapur, Navi Mumbai, India

E-mail: reddyk@nirrh.res.in

BMC Infectious Diseases 2014, **14**(Suppl 3):E30

Background: Identifying and / or designing molecules that can inhibit HIV infection and be safe to the host cells is highly desired.

Methods: HbAHP-25 was designed *in silico* against CD4 binding domain of gp120 of HIV-1 by molecular docking using Z dock and PROSA softwares. ELISA and SPR were used to determine the binding ability of HbAHP-25 to gp120. Anti-HIV activity of this peptide was checked by two different assays, viz: a) On TZM bl cells, using luciferase assay; b) On CEM-GFP cells and PBMCs using p24 antigen assay. MTT assay, TER/microsphere assay and Immunofluorescence were used to determine the effect of HbAHP-25 on cell viability, epithelial monolayer integrity and permeability.

Results: Five peptides were designed, and one of the peptides, HbAHP-25, exhibits significant anti-HIV activity against various strains of HIV-1, such as HIV-1 Ada, HIV-1 NL4-3, and HIV-1 IIIB. ELISA and SPR revealed a direct interaction between HbAHP-25 and gp120, thereby inhibiting its interaction with CD4 receptor. The peptide didn't affect cell viability even at higher concentrations; nor did it affect epithelial monolayer integrity or permeability. HbAHP-25 also did not interfere with any tight junction proteins such as ZO-1 and Claudin-1, thus maintaining cell integrity as well.

Conclusion: The peptide has potent anti-HIV activity, and can be explored as a potential therapeutic /prophylactic/preventive agent.

E31

HPV associated oral lesions among HIV/AIDS patients - a cross sectional study

T Smitha^{1*}, CV Mohan²

¹Department of Oral Pathology, V.S.D.C.H, Bangalore, India; ²Dental Care and Research Centre, Basavanagudi, Bangalore, India

E-mail: dr.smitha@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E31

Background: Oral HPV infection results from increase in oral based immune suppression in the HIV co infected individual that cannot be restored and is possibly compromised further by HAART. Unfortunately, little is known about the prevalence and immune response against oral HPV infection. The aim of the present study was to study the prevalence of HPV associated lesions in a group of patients with HIV who were on HAART.

Methods: Cross sectional, descriptive and observational study was carried out among HIV/AIDS patients attending the ART centre, KIMS, Bangalore from January 2010 to June 2012. Previous informed consent was taken and after which a detailed oral exam, was carried out in patients considering WHO diagnostic criteria. Demographic and clinical profile of HPV associated oral lesions was recorded according to the WHO diagnostic criteria.

Results: Among 318 patients with 540 lesions, majority of the lesions belonged to one of the five categories of ulcers (13.3%), warts (3%), candidiasis (36.45%), pigmented and white lesions (47.2%). Among the warty lesions screened the most common lesion was verruca vulgaris (8 cases), followed by papilloma (4 cases) and focal epithelial hyperplasia (1 case). Oral SCC was detected in two patients and they were confirmed by histopathology. The warts biopsied showed characteristic papilloma features with two cases showing dysplastic changes.

Conclusion: There was a large reduction in the presentation of oral ulcers, and there appeared to be a relative increase in papillomatous lesions and a decrease of other mucosal diseases over the period of study.

E32

Seroprevalence of viral hepatitis in jaundiced pregnant women in a tertiary care hospital

Rudrapathy Parthiban^{*}, Raveendran Vinod, Saraswathi, Javangula Umarani, Sri Venkateshwarra Medical College Hospital & Research centre, Pudukcherry, India

E-mail: parthi71975@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E32

Background: Viral hepatitis represents an important health problem in the developing countries. The objective of this study is to determine the seroprevalence of viral hepatitis pattern in jaundiced pregnant women.

Methods: A total of 128 jaundiced pregnant women admitted as in-patients were included in the study. The test samples were screened for viral hepatitis markers; HBsAg, Anti-HAV (IgM), Anti-HCV, Anti-HDV, Anti-HEV (IgM) by commercial ELISA kits. For HBsAg positive women, Anti-HBc IgM, HBeAg and Anti-HBe were done. HBV DNA analysis was done by polymerase chain reaction.

Results: Of the 128 test serum samples screened, 53.1% were positive for HBsAg, 15.62% were positive for HEV IgM and 3.25% were positive for HAV IgM. None of them were positive for Anti-HCV & Anti-HDV. Among the 68 HBsAg positive pregnant women, the prevalence of HBeAg, anti-HBe, and anti-HBc were 54%, 45.6% and 89%, respectively. Of the 31, HBeAg negative women, 11 had only anti-HBe, 9 were positive for anti-HBe and HBV DNA and 11 were negative for anti-HBe and HBV DNA.

Conclusion: The present study conducted has evolved a comprehensive picture on the virological pattern of viral hepatitis in jaundiced pregnant women. Hepatitis B virus (53.1%) seems to be the major causative agent followed by Hepatitis E virus (15.62%) in the 128 study subjects. Authenticated documentation on pregnancy hepatitis, maternal and fetal outcome in symptomatic viral infected mothers are essential features to plan and implement health measures package in a country.

E33

Prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* infection in pregnant adolescent women and its association with pregnancy outcomes

Priti Ghope^{1*}, Subash C Sonkar², Kirti Wasnik², Pratima Mittal¹, Daman Saluja²

¹Department of Obstetrics & Gynecology Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi-110029, India; ²Dr. B. R. Ambedkar

Center for Biomedical Research, University of Delhi, Delhi-110007, India

E-mail: priti.ghope@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):E33

Background: Sexually transmitted infections (STIs) are major cause for reproductive morbidity, infertility, long term disability and death amongst men, women and infants globally. *T. vaginalis*, *N. gonorrhoeae* and *C. trachomatis* are well established agents of STIs. In present study, we determined the prevalence of these infections in asymptomatic pregnant adolescent women using PCR and studied the association of these infections with maternal and fetal outcome.

Methods: Dry vaginal swabs from 232 asymptomatic pregnant adolescent women in the age group of 17 to 19 years, attending the obstetrics outpatient clinic of VMMC and Safdarjung Hospital, New Delhi were collected. Genomic DNA was extracted and used as template for PCR amplification using primers targeting *pfb*, *gyr A* and *orf1* gene for diagnosis of *T. vaginalis*, *C. trachomatis* and *N. gonorrhoeae*, respectively.

Results: Out of 232 samples, 14 (6.03 %) women had infection: 1 woman had mixed infection, 3 women (1.29 %) tested positive for *T. vaginalis*, one woman (0.43%) tested positive for *C. trachomatis* and 10 women (4.31 %) were infected with *N. gonorrhoeae* whereas 218 women (93.96%) were uninfected. Amongst 160 patients who have delivered, following clinical outcomes were observed: 44% women gave birth to fetus with low birth weight (2-2.5kg = 67(41.35%), 1.5-1.9kg = 3(1.85%), <1.5 kg = 2 (1.23%), Preterm labour pain = 24(15%), Preterm delivery = 24 (15%), Leaking pervaginum = 12 (7.5%) Premature rupture of membrane = 1 (6.2%), Pneumonia = 1(6.2%), NICU stay = 25(15.62%).

Conclusion: Guidelines should be formed to screen and treat patients during antenatal care for these infections to avoid adverse maternal and fetal outcomes.

E34

Prevalence of HPV in the oral cavity of HIV seropositive Indian women undergoing HAART

Jaya Mukherjee^{1*}, Ritwik Dahake², Deepa Das¹, Gopal Sharma¹, Bhagyashri Purandare¹, Abhay Chowdhary²

¹Department of Oral Medicine Diagnosis and Radiology, YMT Dental College and Hospital, Navi Mumbai-410210, Maharashtra, India; ²Department of

Virology, Haffkine Institute, Mumbai-400012, Maharashtra, India

E-mail: drjaya_mukherjee@yahoo.co.in

BMC Infectious Diseases 2014, **14**(Suppl 3):E34

Background: Oral manifestations (such as warts) of HIV infection are evident of disease progression, occurring in 30–80% of affected population. Recent evidence indicates that Human Papillomavirus (HPV) related diseases are increased in the oral cavity of HIV seropositive individuals irrespective of HAART. This study was undertaken to evaluate prevalence of oral HPV infection in Indian HIV seropositive women with/without oral warts.

Methods: Detection of HPV was carried out from saliva of 60 women divided into equal groups of HIV seropositive with/without warts and age/sex matched healthy controls with/without warts by nested PCR using consensus primers MY09/11 and GP5+/6+ for L1 gene followed by sequencing and sequence analysis. The sequences were typed using the HPV L1 typing tool available on <http://pave.niaid.nih.gov/#pav>.

Results: Only one sample was detected to be positive for HPV (prevalence of 6.67%) and was from the HIV seropositive women with warts group. The HPV serotype was determined to be the low risk non-oncogenic HPV Type 6 by the L1 typing tool and multiple sequence alignment.

Conclusion: While this was a preliminary study to determine prevalence of HPV in HIV seropositive women, our study has determined a positive correlation with multiple warts and oral sex as factors associated with

presence of HPV. Our study also questions whether HAART has a beneficial impact on HPV induced cancer. We are of the opinion that this study is the first of its kind in Indian women and emphasize that extensive studies are necessary to establish the incidence of HPV in HIV seropositive population from India.

E35

Lower genital tract infections during pregnancy and adverse pregnancy outcomes: a hospital based observational cohort study

Chaitanya Tellapragada^{1*}, KE Vandana¹, Parvati V Bhat², Chytrha Rao¹, Asha Kamath¹, Satheesha Nayak², V Shashidhar¹, Shashidhar Acharya³, Chiranjay Mukhopadhyay¹

¹Department of Microbiology and Community Medicine, Kasturba Medical College, Manipal University, Manipal, India; ²Department of Obstetrics and Gynecology, Melaka Manipal Medical College, Manipal University, Manipal, India; ³Department of Community Dentistry, Manipal College of Dental Sciences, Manipal University, Manipal, India
E-mail: achyuth21@gmail.com

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Background: Maternal lower genital tract during pregnancy is a complex niche of microbes that normally inhabit or cause infections in few instances. Association of various microbial flora and adverse pregnancy outcomes is being increasingly explored. The study was aimed to determine the prevalence of lower genital tract infections (LGTI) among pregnant women and to determine the common etiologies of LGTI and their association with adverse pregnancy outcomes, Pre term birth (PTB) and Low birth weight (LBW).

Methods: A hospital based observational cohort study comprising 486 asymptomatic, antenatal women in the age group of 18-35 and the gestational period of 8-24 weeks was carried out. High vaginal and endocervical swabs were tested for Nugent's score, culture and PCR for Mycoplasmas and Chlamydia. All the women were followed until delivery.

Results: Prevalence of LGTI s among study population was 134(28%) comprising BV (2%), candidiasis (13%), trichomoniasis (8%) and anaerobic vaginitis (9%). Nugent's scoring delineated study subjects as Grade I (84%), Grade II (14%) and Grade III (2%). Prevalence of various microbes were; *G.vaginalis* (2%), *Candida sp* (16%), anaerobic GNB (12%), anaerobic GPB (9%) and Mycoplasmas (8%). Prevalence of PTB and LBW were; 30 (6%) and 59(13%) respectively. Presence of anaerobic GNB and GPB (Nugent's Grade II organisms) was statistically significant with both PTB ($p = >0.01$, 0.005) and LBW ($p = 0.001$, 0.026).

Conclusion: Incorporation of a simple diagnostic modality like Nugent's scoring system in antenatal care can help in early diagnosis and management of asymptomatic LGTIs; thereby reduce associated adverse pregnancy outcomes.

E36

Surface functionalized nanoparticles of NVP an improved strategy to tackle deadly HIV infection

Bhagyashree R Dalvi^{1*}, Shilpa M Velhal², Atmaram B Bandivadekar², Absar Ahmad³, Padma V Devarajan¹

¹Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai, India; ²National Institute for Research in Reproductive Health, Mumbai, India; ³Biochemical Sciences, National Chemical Laboratory (CSIR), Pune, India

E-mail: bhagyashreedalvi@gmail.com

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Background: The therapeutic efficacy of nevirapine (NVP) is hampered by its poor solubility; poor ability to target infected cells and inherent toxicity. The objective of the present study was surface functionalization with a new targeting ligand to enable enhanced efficacy.

Methods: Surface functionalization of GMS nVP auNPs was studied by fluorescence spectrophotometry. Nanoformulations and NVP were evaluated for cytotoxicity at 1, 2 and 24 hours and uptake study at 0.5, 1 and 2 hours. *In vitro* anti-HIV activity was evaluated in TZM bl cell line against HeLa/LaV virus.

Results: GMS nVP auNPs with > 75% entrapment efficiency & < 300nm particle size were prepared and surface modified nanoparticles showed

>85% binding to the ligand. In cytotoxicity study nanoformulations showed higher cell viability at all time points as compared to NVP. Cell uptake study revealed higher uptake of nanoparticles as compared to NVP only. *In vitro* anti HIV assay of surface modified revealed 10 fold increased activity as compared to NVP.

Conclusion: Nanosize and surface functionalization both play an important role in enhancing anti HIV potential of GMS nVP auNPs.

E37

Biliary tract malignancies in HIV infected persons: a case series

Vinay Kulkarni^{1,2*}, Ritu Parchure¹, Prasad Bhoite¹, Harshal Gadhihar²

¹Prayas Health Group, Pune-411004, India; ²Deenanath Mangeshkar Hospital, Pune-411004, India

E-mail: vinay@prayaspune.com

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Background: Malignancies of biliary tract have been rarely reported in HIV disease.

Case series: We report 3 cases of biliary tract malignancy associated with HIV infection from Prayas Amrita clinic, an NGO based HIV clinic in Pune, India. Two of the cases were diagnosed with gall bladder malignancies (GBM) and the third one had cholangio carcinoma (CC). All presented with advanced stage of malignancy with metastases and had poor prognosis. All were males with median age at presentation being 47 years (range - 46-48 years) They were diagnosed to have HIV for a median duration of 102 months (range: 69-118 months) . Median nadir CD4 count was 77cells/cmm (range: 71-163 cell/cmm). They were on first line ART for median 63 months (range: 41-72 months). A patient with GBM was Hepatitis B co infected. Prior AIDS related events of anemia, immune thrombocytopenic purpura and tuberculosis were present in the patient with CC and progressive multifocal leukoencephalopathy was in one of the patients with GBM. Being managed in resource limited setting; the absence of other risk factors is corroborative and lacks evidence.

Conclusion: These 3 cases of biliary tract malignancies were seen recently in a relatively short span of time. All were men in late forties, infected with HIV for a long time and had no other known risk factors. This could be a pointer towards changing scenario of malignancies among HIV infected patients associated with prolonged survival.

E38

Squamous cell carcinoma of tongue in HIV patients- a report of 3 cases

SP Selvajothi Ranjitham^{*}, K Gnanambigai, P Kamaleswari, Elizabeth Joshua, Umadevi K Rao, K Ranganathan

Department of Oral Pathology and Microbiology, Ragas Dental College, Chennai, India

E-mail: spselvajothi@gmail.com

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Case series: Oral manifestations of HIV disease are broadly classified as "strongly associated with HIV", "less commonly associated with HIV" and "can be seen in HIV infection". Malignancies reported in HIV infection include Kaposi's sarcoma, Non-Hodgkin's lymphoma and squamous cell carcinoma of the oral cavity. At our Ragas- YRG CARE center, in a cohort of 5760 cases which were screened, 6 patients of Non-Hodgkin's lymphoma and 4 patients with oral squamous cell carcinoma were reported. We present 3 cases of oral squamous cell carcinoma on the lateral border of the tongue with varied clinical presentations. A comparison of the demographic data, habits, HIV history, clinical presentation, investigations, histopathological diagnosis and treatment of the three cases were made; 2 patients had no history of tobacco use, suggesting role of factors such as HPV co infection.

E39

Lamivudine sensitivity - a case report

Leelavathy Budamakuntla^{1*}, Prabhakar Basappa², Vidya Shankar², Nirmala², Ravi Kumar¹, Shwetha Suryanarayana¹

¹Department of Dermatology, Bowring & Lady Curzon Hospital, Bangalore Medical College& Research Institute, Bangalore, Karnataka, India; ²Department

of Medicine, Centre of Excellence for ART, Bowring & Lady Curzon Hospital, Bangalore Medical College & Research Institute, Bangalore, Karnataka, India
E-mail: drleelaskincare@rediffmail.com
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Background: Lamivudine is a dideoxy nucleoside analogue – NRTI, widely used in the treatment of HIV infection. It is a best tolerated drug with long term safety profile and most preferred in all regimens. Skin hypersensitivity reactions to lamivudine are rare and reported to be around 9%.

Case report: A 29 year old HIV positive female was initiated on ART on 5/11/2012 with first line regimen, (TDF+3TC+NVP) as patient was anaemic. Patient came back with Nevirapine rash and ART was stopped on 20/12/12. Nevirapine was substituted with Efavirenz after rashes healed on 2/1/13. Patient developed severe itching with rashes just after three doses and hence ART was stopped. After one month, she was counseled, hospitalized and was rechallenged with only TDF+3TC; patient again developed severe itching with only one dose. Hence, patient was challenged with only lamivudine. With just one dose of lamivudine, patient developed hypersensitivity reaction within few hours with facial oedema, redness, tightness of skin with maculopapular rash and generalized blanchable erythema, giddiness, and hypotension and collapsed. She was resuscitated with dopamine, IV fluids and antihistamines.

Conclusion: Nucleoside reverse transcriptase inhibitors (NRTIs) such as lamivudine can cause skin rash in 1-10% of patients. Lamivudine related cutaneous side effects are probably under estimated. Lamivudine hitherto considered a safe drug can cause serious side effects in some patients as reported here. Hence, careful observation and timely intervention is mandatory to avoid fatalities in those patients receiving NRTI based antiretroviral therapy.

E40

Improving dual protection among women infected with HIV

Beena Joshi^{1*}, Gajanan Velhal², Sanjay Chauhan¹, Ragini Kulkarni¹, Shahina Begum¹, YS Nandanwar³, Michelle Fonseca³, Sujata Baweja⁴, Dilip Turbadkar⁴, Anita Ramachandran⁴, Asha Dalal⁵, Jayanti Shastri⁶, Sachee Agrawal⁶, Manisha Panhale¹, Vasundhara More¹, Pravin Sanap¹, Renuka Panchal¹, Suman Kanoujiya¹

¹Department of Operational Research, National Institute for Research in Reproductive Health, ICMR, Parel, Mumbai, India; ²Department of PSM, TN Medical College and BYL Nair Hospital, Mumbai, India; ³Department of OBGYN, LTM Medical College and Sion Hospital, Mumbai, India; ⁴Department of Microbiology, LTM Medical College and Sion Hospital, Mumbai, India;

⁵Department of OBGYN, TN Medical College and BYL Nair Hospital, Mumbai, India; ⁶Department of Microbiology, TN Medical College and BYL Nair Hospital, Mumbai, India

E-mail: nirrdor@yahoo.co.in

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Background: The PPTCT program in India focuses on prong 3 (Provision of Nevirapine to pregnant infected mothers) and reports quote that it reaches only 32% of pregnant mothers who need it. Preventing unintended pregnancies among HIV positive women (Prong 2) could help reduce the burden on Prong 3. To improve use of dual protection and prevent unintended pregnancies among women infected with HIV, an operational research study was implemented in two randomly selected tertiary hospitals in Mumbai (supported by ICMR).

Methods: The main intervention in this experimental control study was linking ICTC/PPTCT with family planning services by capacity building of providers (counseling and testing medical eligibility to use methods), provision of IEC material, referral slip, maintaining MIS and testing the acceptability of dual methods among 300 women infected with HIV attending ICTC/PPTCT.

Results: Sixty percent of the referred participants reached Family planning centres. Statistically significant improvement in knowledge about contraception and dual protection, three times increased acceptance of dual methods, significant increase in consistent use of condoms and lesser numbers of unwanted pregnancies and births were observed in the experimental than control group. The cumulative failure rate of contraception in the experimental group reduced resulting in

preventing 12 women from risk of getting pregnant in first year of use compared to control.

Conclusion: The study demonstrated the feasibility to establish this linkage and improved dual method use among participants. The study recommends expanding the current mandate of PPTCT to focus on prong 2 and improve use of dual methods.

E41

Synthesis of biogenic gold nano conjugate for increased efficacy and sustained drug release profile of saquinavir

Rohan Kesarkar^{*}, Sweta Kothari, Abhay Chowdhary
Department of Virology and Immunology, Haffkine Institute for Training Research and Testing, Mumbai- 400012, India
E-mail: rohanrk27@gmail.com

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Background: Conventional anti retroviral treatment (ART) has been a core treatment for HIV over the past three decades. Despite the effectiveness of these drugs, most of them abide significant drawbacks such as poor bioavailability and undesirable side effects; thus reducing patient compliance and rendering treatment regime ineffective. This research work is an attempt to develop a dual pronged carrier system using gold nanoparticles (GNP) for increasing effectiveness and sustained drug release of saquinavir.

Methods: Leaf extract of *Azadirachta indica* was used as an efficient sink for synthesis of GNPs by microwave assistance. Saquinavir (Saq) was attached onto GNPs that were pre functionalized by capping polypeptide linkers of *A.indica* and characterized by FTIR. Percentage drug loading efficiency (%DLE) and release kinetics were studied by statistical model dependent method. Biocompatibility of drug nanoparticle conjugate was studied by MTT assay. Antiviral efficacy was assessed by quantifying aspartic protease of HIV by fluorescence (FRET) method.

Results: Mono dispersed GNPs (30-40 nm) were synthesized and found to carry high payloads of saquinavir with %DLE of 93.6% as analyzed by Transmission electron microscopy and UV Vis spectroscopy respectively. GNP-saq conjugate demonstrated 16.7% increase in anti viral efficacy as compared to saquinavir alone. The conjugate showed sustained drug release and followed 1st order release kinetics at physiological pH (7.2).

Conclusion: Biogenic GNPs serve as a potent candidate for ferrying high payloads of anti retroviral drugs with increased efficacy and reduced side effects. This approach can help reduce dosage frequency and improvise treatment strategy against HIV.

E42

Citric acid coated magnetite nanoparticles to overcome intrinsic rifampicin resistance in *Mycobacterium smegmatis*

Priyanka Padwal, Rajdip Bandyopadhyaya, Sarika Mehra^{*}
Department of Chemical Engineering, Indian Institute of Technology Bombay, Mumbai, India
E-mail: sarika@che.iitb.ac.in

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Background: The major problem faced in current tuberculosis (TB) therapy is intrinsic drug resistance of mycobacterium. Hence, there is a need to develop ways to overcome these resistance mechanisms. In the current work, we have investigated the effect of citric acid coated magnetite nanoparticles in combination with rifampicin against a wild type strain of *Mycobacterium smegmatis*.

Methods: We have studied the effect of rifampicin on growth of cells, with and without the nanoparticles. Further, cellular uptake of nanoparticles was studied by transmission electron microscopy. Since, permeability barrier and drug efflux are responsible for intrinsic drug resistance, we have performed accumulation and efflux studies on common efflux pump substrate ethidium bromide (EtBr) by semi automated fluorometric method in the presence and absence of nanoparticles.

Results: Citric acid coated magnetite nanoparticles exhibited a significant growth inhibition when used in combination with rifampicin. However, nanoparticles alone did not have any effect on mycobacterial growth. Enhanced growth inhibition was seen in the presence of a combination of rifampicin and nanoparticles, which was even more than rifampicin alone

at the same concentration. Uptake studies revealed that nanoparticles were internalized by *M. smegmatis*. Transport studies on EtBr demonstrate that, in presence of nanoparticles, the net EtBr accumulation inside the cells was increased.

Conclusion: Citric acid coated magnetite nanoparticles enhanced the antibacterial effect of rifampicin when used in combination with the drug. Increased accumulation of EtBr in presence of nanoparticles indicates that, one of the possible reasons for this enhanced effect is enhanced intracellular drug accumulation.

E43

Anti-HIV-1 activity of *Sargassum swartzii* a marine brown alga

Dinesh Subramaniam¹, Thangam Menon^{1*}, Hanna Luke Elizabeth², Soumya Swaminathan²

¹Department of Microbiology, Dr ALM PG IBMS, University of Madras, Chennai, India; ²Department of Clinical Research, National Institute for research in Tuberculosis (NIRT), Chennai, India

E-mail: thangam56@gmail.com

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Background: Currently available antiretrovirals are relatively expensive and have side effects. Hence, there is need to develop novel drugs against HIV. The present study was planned to investigate the anti-HIV activity of extracts of the marine brown algae *Sargassum swartzii*.

Methods: The HIV inhibitory activity of aqueous and methanolic extracts of *S. swartzii* was determined on laboratory adapted strains of HIV-1 clades C and A by indirectly measuring reduction in HIV-1 gag p24 antigen levels and inhibition of HIV-1 RT activity in supernatants of virus infected donor peripheral blood mononuclear cell (PBMC) cultures grown in varying concentrations of the extracts. Phytochemical analysis and cytotoxicity of the extracts was also undertaken.

Results: Results showed that both aqueous and methanolic extracts of *S. swartzii* had an inhibitory effect on HIV-1 clade C, and the response was dose dependent. At a concentration of 0.39 µg/ml, aqueous and methanolic extracts of *S. swartzii* demonstrated 71.1±2% and 74.7±2.9% inhibition of virus production, respectively. A similar response was observed with a HIV-1 clade A primary isolate. The extracts also showed inhibition of RT activity, and no cytotoxicity on human PBMC. Phytochemical analysis revealed that the aqueous extract of *S. swartzii* contained sulfate and uronic acid, while the methanolic extract contained hexadecanoic acid. These compounds could be responsible for the anti-HIV-1 activity.

Conclusion: *S. swartzii* extracts significantly inhibited HIV-1 virus suggesting that it may be useful in therapeutics. This study is the first report to show that *S. swartzii* possesses compounds with anti HIV-1 activity.

E44

Virulence determinants of MRSA causing skin and soft tissue infections among HIV patients from Chennai

Padma Krishnan^{1*}, A Nagarajan^{1,2}, J Suriakumar^{3,4}

¹Department of Microbiology, Dr. ALM PGIBMS, University of Madras, Chennai, India; ²Food Analysis Laboratory, TNFSDA, Madurai, India; ³Govt. Hospital of Thoracic Medicine, Tambaram, Chennai, India; ⁴Institute of Microbiology, Madurai Medical College, Madurai, India

E-mail: padma.abpkn@gmail.com

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Background: MRSA is a well armed pathogen with an array of virulence factors, which causes skin and soft tissue infections (SSTIs) among healthy individuals in community. Although there are few reports on MRSA causing SSTIs among HIV patients, there are no studies on their virulence determinants. Hence, the current study was done to detect the virulence determinants of MRSA causing SSTIs among HIV patients.

Methods: A total of 70 clinical isolates of *Staphylococcus aureus* collected from HIV patients with SSTIs were screened for MRSA using cefoxitin disc (30 µg). Detection of *pvl*, *femA*, *mecA* of the isolates was done by triplex PCR. The enterotoxins A-E, J, K, P and *egc* cluster, exfoliatins A,B and D, hemolysins α , β , γ , δ & γ_v , leucocidins *lukDE* & *lukM* and innate immune evasions were detected by using multiplex PCRs using suitable *S. aureus* controls.

Results: Of the 70, 16 (22.85%) of the *S. aureus* isolates causing SSTIs among HIV patients were found to be MRSA. *pvl* was detected in 15/16

(93.75%) of MRSA isolates. 14/22 (58.33%) virulence determinants tested were detected among the MRSA isolates. Among enterotoxins, *sea* and *egc* gene cluster were found in all MRSA isolates tested. 11/16 (68.75%) and 10/16 (62.50%) MRSA isolates were found to be positive for *chp* and *lukD-E*, respectively.

Conclusion: Although the MRSA percentage among HIV patients was low, prevalence of *pvl* and other virulence determinants was found to be high among MRSA isolates causing SSTIs in HIV patients.

E45

Feasibility of a Short Message Service based intervention to improve adherence among HIV-positive youth in Uganda: a focus group study

Yashodhara Rana^{1*}, Barbara Mukasa², Andrew Kambugu³, Peter Wabukala², Crystal Huang¹, Glenn J Wagner¹, Sebastian Linnemayr¹

¹RAND Corporation, Santa Monica, CA, USA; ²MILDWAY Uganda, Kampala, Uganda; ³Infectious Diseases Institute, Kampala, Uganda

E-mail: yashodharana@gmail.com

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Background: More than half of those newly infected with HIV are young people yet youths have lower medication adherence than adults. Short message service (SMS) based interventions have demonstrated their effectiveness in improving adherence among adults in resource limited settings, but their efficacy with youths has not been studied. This study is one of the first qualitative studies to explore youths view on strategies for best implementing SMS based interventions.

Methods: Six focus groups were conducted with 20 male and 19 female HIV-positive youths in Kampala, Uganda. Participants discussed barriers to adherence, availability and acceptability of SMS messages and strategies for implementation. Verbatim transcripts were managed in ATLAS.ti and analyzed using a framework approach.

Results: We found that major barriers to adherence were forgetfulness, stigma, poor social support, inadequate food supply and stressful working conditions. Participants reasoned that SMS based interventions would improve adherence by providing them with much needed reminders and social support. However, youths noted that the success of such interventions hinged on: i) ensuring HIV status anonymity given that text messages are vulnerable to security threats ii) developing effective content and having appropriate delivery times so that youth remain engaged iii) increasing accessibility to include youth without phones iv) addressing challenges related to phone sharing and restrictions on the use of phones by younger adolescents.

Conclusion: SMS based interventions have the potential to promote adherence among youths. The primary recommendation of this study is to ensure confidentiality and increase accessibility.

E46

mLearning- a cost effective initiative for stigma reduction in health care settings

Sree T Sucharitha^{1*}, Janani Rajagopal¹, Ravi Siriki²

¹Department of Community Medicine, Tagore Medical College Hospital, Chennai, India; ²Independent Data Consultant, Hyderabad, India

E-mail: sucharithat2@gmail.com

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Background: The total number of people living with HIV (PLHIV) in India estimated to be around 20.9 lakhs in 2011. In Indian hospitals, stigma and discrimination is widespread and has unfavorable impact on treatment outcomes for PLHIV and on battle against HIV epidemic. mLearning is a type of mHealth for educating and increasing the capacity of health care providers. The objective of this study was to assess the effect of mLearning intervention as improvement in knowledge and attitudes in medical undergraduates towards reducing stigma in health care settings.

Methods: This observational cohort study involved mLearning intervention using the free version of WAY2SMS with thirty short messages (SMS) being sent to thirty medical undergraduates of Tagore Medical College, Chennai during August-October 2013.

Results: Fear of contagion (33%) and poor awareness (23%) among care providers is identified as reasons for stigma. Improved knowledge about definition of stigma as 100%, 97% for Quality, Quantity of HIV virus and

Route of transmission (QOR) determining the risk of HIV transmission, 97% for identifying scheduling consultancies till last and charging high for PLHIV surgeries as stigma, was seen in mLearning group. Positive attitudinal shift was seen as 100% participants mentioning they would befriend and would not be ashamed being care providers for PLHIV, 47% realizing the importance of client informed, voluntary testing for HIV, and 60% favored informed consent prior to HIV testing.

Conclusion: mLearning is a cost effective method of reducing stigma in health care settings by improving knowledge and attitudes of care providers.

E47

Evaluations of in-house PCR based diagnostic assay using *pfoB* gene for diagnosis of *Trichomonas vaginalis* among symptomatic women with vaginal discharge

SC Sonkar¹, PK Mishra¹, P Mittal², A Kumar², D Saluja^{1*}

¹Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi-110007, India; ²Department of Obstetrics & Gynecology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi-110029, India
E-mail: dsalujach59@gmail.com

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Background: *Trichomonas vaginalis* is one of the most prevalent non viral sexually transmitted diseases (STDs) in developing countries. Although several PCR based diagnostic tests are available, the performance, accuracy and sensitivity of these assays vary. Therefore, we decided to compare the performance of various diagnostic gene targets (*18S rRNA*, *β tublin*, *pROS21*) of *T. vaginalis* under similar conditions and also compared an in-house developed PCR assay targeting *pfoB* gene with these methods.

Methods: Samples were obtained from Department of Obstetrics & Gynecology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi. The DNA so extracted was used as template for PCR amplification using primers targeting *pfoB*, *18S rRNA*, *β tublin* and *pROS21* gene.

Results: The performance (sensitivity, specificity, positive predictive value and negative predictive value) of different PCR assays was compared using DNA isolated from clinical samples. The *pfoB* PCR showed highest sensitivity (92.31%) and specificity (99.53%) respectively. Our results suggest that though most of PCR based diagnostic assays are highly sensitive and specific, the clinical performance of different primer pairs varies. The positive and negative likelihood ratios were also calculated. The lowest negative likelihood ratio was observed for *pfoB* PCR.

Conclusion: In the present study we evaluated the diagnostic performance of an in-house developed PCR assay by comparing it with already established PCR methods used for diagnosis of *T. vaginalis*. Therefore, we propose that, it can be used as a reliable diagnostic test for screening *T. vaginalis* infection.

ORAL PRESENTATIONS

O1

Development of subtype specific RealTime PCR based growth competition assay for assessing the replication fitness of drug resistant viruses of Indian HIV-1 clade C

Nitin K Hingankar^{1*}, Ramesh S Paranjape¹, Srikanth P Tripathy²

¹National AIDS Research Institute, Pune, Maharashtra, India; ²National JALMA Institute of Leprosy and other Mycobacterial Diseases, Agra, India
E-mail: nitinhingankar@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O1

Background: The knowledge of replication fitness of drug resistant viruses is essential for designing the rational treatment regimens. We report development of HIV-1 Indian Clade C specific assay for studying the replication fitness of drug resistant viruses.

Methods: HIV-1 Subtype C molecular infectious clone plndieC (Genebank AB023804) was used to construct two experimental vectors, plndieC conslnC RTWILD and plndieC conslnC RTSYNM, which are identical except for the presence of four synonymous nucleotides differences in Integrase. Standard curve was prepared for each vector using linear plasmid DNA template. The K103N, V106A, V106M, Y181C resistance mutations were

placed in the plndieC conslnC RTWILD and replication fitness of each mutant was compared with that of wild type in a head to head growth competition assay. Virus supernatant harvested at day 0, 4, 8, 12 for RealTime quantitative PCR assessment. Relative fitness was estimated using online tool <http://bis.urmc.rochester.edu/vFitness> and significant differences in relative fitness between wild and mutant were determined with Mann Whitney test.

Results: The slopes of standard curves for the vectors showed similar reaction efficiencies. Relative fitness of RTSYNM when competed with RTWILD was 1.01 demonstrating similar replication fitness and utility in competition assay. Three mutants showed decreased relative fitness compared to the wild type, with borderline significant difference in two, resulting in fitness order wild type>181C>K103N>V106M, whereas V106A mutant showed drastic decrease as almost non infectious.

Conclusion: The growth competition assay developed is able to accurately measure differences in replication fitness as impact of single drug resistance mutation in Reverse Transcriptase.

O2

The frequencies of naturally occurring protease inhibitor resistance mutations in HIV proviral sequences of drug naïve sex workers in Nairobi, Kenya and their correlation with host immune response driven positively selected mutations in HIV-1

Raghavan Sampathkumar^{1,3}, Elnaz Shadabi^{1,3}, David La³, John Ho³, Binhua Liang^{1,3}, Joshua Kimani^{1,2}, Francis A Plummer^{1,3}, Ma Luo^{1,3*}

¹Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada; ²Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya; ³National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada
E-mail: Ma.Luo@phac-aspc.gc.ca

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Background: Sub Saharan Africa accounts for 69% of the people living with HIV globally. Antiretroviral therapy (ART) has saved 9 million life years in Sub Saharan Africa. However, drug resistance mutations reduce the effectiveness of ART, and need to be monitored for effective ART. Naturally occurring primary antiretroviral drug resistance mutations have not been well analyzed in ART naïve HIV+ patients from Kenya.

Methods: We examined protease inhibitor (PI) resistance mutations in ART naïve HIV-1 seropositive women from Pumwani sex worker cohort, established in Nairobi, Kenya, wherein HIV-1 infection is predominantly caused by subtypes A and D viruses. We have analyzed consensus sequences of HIV protease from 234 drug naïve patients, as a part of HIV-1 whole genome sequencing using 454 sequencing methodology.

Results: Analysis using HIVdb program revealed a prevalence of 0.56% of PI resistance major mutations (1/178; D30N) and 19.1% PI resistance minor mutations (34/178) among the study subjects. D30N mutation, which occurred along with minor mutations G48K and G73S, is known to confer high level resistance to nelfinavir. Several minor mutations were found at five different drug resistance sites. Positive selection analysis and correlation with disease progression revealed L10I, a PI resistance minor mutation and a positively selected mutation driven by host immune response, to be detrimental to host.

Conclusion: This study provides valuable data on primary drug resistance in Kenyan HIV-1 infected patients before ART became available as well as HLA mediated immune pressure over HIV-1 protease.

O3

Drug resistance patterns in new and previously treated patients with tuberculosis presenting to a tertiary care center in southern India

Anto J Udaykumar¹, Jayashree R², Baijayanti Mishra², Rosario Vivek¹, John Kenneth^{1*}

¹Division of Infectious Diseases, St. John's Research Institute, Bangalore 560034, India; ²Department of Microbiology, St. John's Medical College Bangalore 560034, India
E-mail: johnkenneth@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O3

Background: Tuberculosis (TB) caused by *Mycobacterium tuberculosis* remains a major global health problem. It is the second leading cause of

death worldwide. Drug resistance threatens global TB control with the emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) TB. The burden of MDR TB and XDR TB in India is not available as continuous surveillance for drug resistance is not carried out. This study was done to determine the drug resistance patterns to first and second line drugs among new and previously treated patients with TB.

Methods: Sputum samples were collected from 100 suspected TB patients from a tertiary care referral hospital. After digestion and decontamination, samples were stained for microscopy and cultured in liquid (MGIT) and Löwenstein Jensen (LJ) medium to isolate *M. tuberculosis*. Drug susceptibility testing (DST) was done against first line (streptomycin, isoniazid, rifampin and ethambutol) and second line (kanamycin, capreomycin, ofloxacin and ethionamide) drugs.

Results: Among the 100 samples, 56 were smear positives and *M. tuberculosis* was isolated from 61 samples cultured in MGIT and 46 in LJ medium. Of which 20 (32.7%) were MDR strains. Among the MDR strains, 9 (45%) were XDR strains. Two strains were totally resistant to all the 8 drugs. The proportion of MDR and XDR was highest among the previously treated patient.

Conclusion: This study underscores the need for DST in all TB patients particularly in the previously treated patients. New drugs, novel treatment strategies and adherence to treatment are needed to effectively treat and control drug resistant TB.

O4

Analysis of the HIV resistance related HLA dRB1 restricted CD4+ T cell epitopes among Indian HIV patients

H Jemmy Christy*, D Alex Anand

Department of Bioinformatics, Sathyabama University, Chennai-600119, India
E-mail: jemmyjacob@gmail.com

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Background: Host genetic factors are the major determining factor for variation in response to HIV infection and are related to resistance among HIV patients. HLA dRB1*1501, DRB1*1502, DRB1*1301, DRB1*1302, DRB1*1101-05 are widely reported alleles associated with low viraemia in infected individuals. Computational predictions have made it possible to identify potentially strong peptide binders to HLA molecules that so that can be then tested *in vitro* and *in vivo* as putative epitopes for peptide based vaccines. The present study investigated the consequences of sequence variations within HLA dRB1 alleles in selecting the epitopes and their interactions.

Methods: V3 loop based epitope prediction with their binding values restricted to HLA dRB1 alleles were generated using the IEDB, ANN prediction server and Propred II, a Quantitative matrix based method. Population coverage was calculated for selected DRB1 alleles by the Population coverage tool on the IEDB server. We modeled consensus v3 loop based epitopes Pepstr a peptide modeling tool and HLA dRB1*1501, DRB1*1502, DRB1*1301, DRB1*1302, DRB1*1101-05 using I tasser, an ab initio modeller, interaction studied between HLA alleles and epitopes using docking simulation models generated by Flexpepdock.

Results: Out of 189 predicted low percentile ranked epitope, TRKSIRIGPGQTFYA, NNRKRSIRIGPGQTF, YATGDIIGDIRQAHC, NTRKSIRIGPGQTFY were observed with consensus and were found interacting strongly with the HIV resistance related HLA alleles and visualized in Discovery studio visualize 3.5.

Conclusion: The epitope specific to HLA dRB1 alleles have been identified by different methods indicating their potential role as candidate epitope for an effective HIV vaccine.

O5

Placental Hofbauer cells assemble and sequester HIV-1 in tetraspanin and DC SIGN positive compartments that are accessible to neutralizing antibodies

Erica L Johnson^{1,2*}, Hin Chu^{1,2}, Siddappa N Byreddy³, Paul Spearman^{1,2}, Rana Chakraborty^{1,2}

¹Department of Pediatrics; Emory University; Atlanta, Georgia, 30322, USA;

²Children's Center for Immunology and Vaccines; Children's Healthcare of Atlanta, USA; ³Department of Pathology & Laboratory Medicine, Emory

University School of Medicine, & Emory Vaccine Center, Atlanta, Georgia, 30322, USA

E-mail: eljohns@emory.edu

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Background: Within monocyte derived macrophages, HIV-1 accumulates in virus containing compartments (VCCs) that are largely inaccessible to the external environment, which implicate these cells as HIV-1 reservoirs. During mother to child transmission, placental macrophages (Hofbauer Cells [HCs]) are viral targets, and have shown to be infected *in vivo* and sustain low levels of viral replication *in vitro*, however, the risk of *in utero* transmission is less than 7%. The role of these primary macrophages as viral reservoirs is largely undefined.

Methods: With consent, term placentas from 20 HIV-1 seronegative women were obtained following caesarian section. VCCs were evaluated with 3D confocal microscopy and correlated with electron microscopy. Co localization R values (Pearson's correlation) were quantified with co localization module of Volocity5.2.1. Replication kinetics following siRNA and neutralization studies were evaluated using p24 ELISA.

Results: We demonstrate primary HCs assemble and sequester HIV-1 in VCCs with localization specific to intracellular vesicles and the plasma membrane. These compartments are enriched in endosomal/lysosomal markers, including CD9, CD81, CD63 and LAMP 1, along with DC SIGN. Following internalization, we observed HIV-1 accumulation in acidified compartments. Remarkably, these compartments are accessible via the cell surface and can be targeted by exogenously applied small molecules and HIV-1 specific neutralizing antibodies (NAbs). In addition, broadly NAbs (4E10 and VRC01) neutralized HIV-1 infected HCs and involve the participation of FcγRI for inhibition.

Conclusion: These findings suggest placental HCs possess intrinsic adaptations facilitating sequestration of HIV-1, and may serve as a protective viral reservoir to permit viral degradation, neutralization and antiretroviral drug entry *in utero*.

O6

HIV-1 infection and circulating peripheral blood B cell subpopulations

Ravinder Singh^{1*}, Neema Negi², Bimal Kumar Das², Rakesh Lodha¹, SK Kabra¹, Madhu Vajpayee²

¹Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India; ²Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India

E-mail: ravinder.rathore.singh@gmail.com

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Background: Progression of HIV-1 infection can be monitored by studying the frequency of B cell subpopulations which could serve as a better surrogate marker. The study evaluated the distribution as well as the frequency of B cell subsets in peripheral blood of HIV-1 infected Indian individuals.

Methods: In HIV infected, ART naïve adults and healthy controls, frequency of B cell subpopulations were measured by flow cytometry. Difference between groups was compared using Student t test and p value of <0.05 was considered significant.

Results: In HIV infected adults, a significant reduction in non-switched memory B cells (CD19+IgD+CD27+) was observed, compared to healthy controls (p=0.046). With ongoing viral replication and reduced CD4 count, an expansion in CD21^{lo}CD27⁺ (tissue like memory) population was observed and the correlation was statistically significant (p=0.0004). The mean frequency of CD21^{hi}CD27^{hi} (resting memory) was significantly higher in controls (p=0.0002) compared to HIV-1 infected adults, while tissue like memory were highly expanded in HIV infected adults compared to controls (p=0.0001). B cells in HIV-1 infected adults expressed higher frequency of CD95 compared to healthy controls (p=0.0004).

Conclusions: HIV mediated alteration in B cell development and differentiation may result in loss of switched and non-switched memory B cells. Moreover, association of persistent viremia with expansion of CD21^{lo} tissue like memory cells suggests loss of CD21 expression as a marker of ongoing HIV replication. B cells in HIV-1 infected adults are more prone to Fas mediated apoptosis compared to healthy controls.

07

Effect of ART on poly functional profile of CD8 responses to Gag and Nef antigen in HIV infected Indian individuals

Sanjay Mendiratta^{1,2*}, Ravinder Singh¹, Madhu Vajpayee¹

¹All India Institute of Medical Sciences, New Delhi, India; ²National Institute of Biologicals, Noida, India

E-mail: Sanjaymendiratta@gmail.com

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Background: Poly functional CD8+ T cells have been described most competent in controlling viral replication in late stage of HIV infection.

Methods: Multiparametric flow cytometry and intracellular cytokine staining was performed to study the effect of HAART on poly functional responses of CD8+ T lymphocytes to HIV Gag and Nef peptides and polyclonal stimuli in 40 ART naïve and 10 ART treated HIV infected individuals to compare and examine the degree of immune restoration after ART. We analyzed the CD8+ T cell responses (IFN- γ , IL-2, TNF- α , CD107a) and generated fifteen unique subsets using Boolean gating (Flowjo, SPICE-PESTLE software).

Results: Bulk response of HIV-1-specific CD8+ T cells directed against the identified immunodominant peptides in the treated group decreased (average $\pm$ SD: Gag: 2.1% $\pm$ 2.1% to 1.17% $\pm$ 1.17%, $p = 0.14$; Nef: 2.95 $\pm$ 3.23 to 1.33% $\pm$ 1.04%, $p = 0.14$; Polyclonal Activator: 15.16% $\pm$ 8.74% to 5.93% $\pm$ 8.29%, $p = 0.007$). The responses were predominantly mono functional in ART naïve individuals, as also shown by their level of responses and contribution towards global responses. Dual function responses were increased in ART treated individuals as compared to naïve study subjects.

We also observed slight increase in IL2 and TNF α secreting CD8+ T cell subsets in ART treated individuals.

Conclusion: Our data indicates that the functional impairment of antigen specific CD8+ T cells in HIV infection is dynamic and can be partially restored with ART. However the data needs to be substantiated on large sample size.

08

DC SIGNR silencing reduces HIV-1 infection in Dendritic cells

Omkar Chaudhary¹, Sanjeev Kumar¹, Heena Aggarwal¹, Muzamil A Makhdoom¹, Jasbir Singh^{2,3}, Anjali Hazarika³, Kalpana Luthra^{1*}

¹Dept. of Biochemistry, All India Institute of Medical Sciences, New Delhi, India; ²Dept. of Biochemistry, Kurukshetra University, Kurukshetra, India;

³Blood Transfusion Services, Cardiothoracic and Neurosciences Center, All India Institute of Medical Sciences, New Delhi, India

E-mail: kalpanaluthra@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O8

Background: Dendritic cells (DCs) capture HIV-1 from periphery via DC SIGN/R receptors and transfer to T cells. It has been reported that siRNA directed against DC SIGN significantly inhibits HIV infection of DCs. In this study, the expression of DC SIGN/R and its role in HIV-1 infectivity of DCs were assessed.

Methods: DCs were cultured from monocytes from healthy donors and infected with HIV-1 Indian clade C virus. DC SIGN/R expression on DCs was determined by RealTime PCR. The effect of down regulation of this receptor in DCs using DC SIGN/R siRNA on the infection with HIV-1 was assessed. Statistical significance was calculated by Student's t test.

Results: High expression of DC SIGN/R was observed on DCs infected with HIV-1 ($p = 0.01$). DC siGNR expression in DCs was maximally downregulated by siRNA at 24 hrs ($p = 0.002$) and was associated with significant reduction in expression of CD40 ($p = 0.003$), CD80 ($p = 0.008$), CD86 ($p = 0.007$) and P38 MAPK ($p = 0.005$). Transfection of DC siGNR on DCs at 24 hours followed by infection with clade C HIV-1 demonstrated lower levels of p24 compared to that in untransfected DCs ($p = 0.0008$).

Conclusion: Data demonstrates that down regulation of DC SIGNR expression on DCs decreases activation that in turn may inhibit DC T cell interactions needed for progression of HIV-1 infection. Reduced p24 antigen production may be mediated by P38 MAPK inhibition. Our finding suggests that blocking DC SIGNR expression on DCs may prevent initial binding of HIV-1 and serve as a first step in prevention of HIV-1 infection.

09

Expression of the human microRNA miR 29a in an Indian cohort of HIV patients

Rakesh Dey¹, Kartik Soni¹, Shanmugam Saravanan², Pachamuthu Balakrishnan², Vikram Kumar¹, Vinod Scaria¹, Beena Pillai^{1*}, Suniti Solomon², Samir K Brahmachari¹

¹CSIR institute of Genomics and Integrative Biology, Mathura Road, Delhi 110020, India; ²YR Gaitonde Centre for AIDS Research and Education, VHS Campus, Rajiv Gandhi Road, Taramani, Chennai 600113, India

E-mail: beena@igib.in

BMC Infectious Diseases 2014, **14**(Suppl 3):O9

Background: The events during host virus interaction pave the way to differential outcomes in HIV-1 disease progression rates. We have earlier shown that HIV-1Nef gene harbors target sites for the human microRNA, miRNA 29a/b. Over expression of miR 29a/b could reduce virus levels by targeting the Nef gene. We hypothesized that differential expression of the microRNA maybe a protective factor in AIDS progression.

Methods: In a cohort of 75 HIV-1 infected individuals, who show differential disease progression, classified as (1) Long Term Non Progressors (LTNPs), (2) Regular Progressors and (3) Rapid Progressors. We isolated RNA and DNA from PBMCs collected from these individuals and healthy controls. We quantified miR 29a expression through Real Time PCR and performed statistical correlation of the miRNA levels with disease progression rate. We further analyzed miR 29a promoter and coding region from selected patients.

Results: Patients, including various sub classes like LTNPs, Regular Progressors and Rapid progressors, had higher miR 29a levels than healthy controls. A small subset of patients had unusually high levels of miR 29a. Three of these individuals were LTNPs while others were classified as regular progressors.

Conclusion: The absence of known genetic causes of non progression like the CCR5 delta mutation, in this patient cohort suggests that there are novel mutations that confer protection. The absence of healthy control individuals with high miR 29a expression suggests that the induction of miRNA maybe happen in response to infection. However, larger sample sizes are required to confirm this. We conclude that miR 29a expression maybe one of the factors that in certain backgrounds provides a better prognosis.

010

Toll like Receptors (TLRs) expression in HIV children

M Madeshwaran^{1*}, V Gupta¹, J Chakrabarty², G Narayan³, A Kumar¹

¹Department of Pediatrics, IMS, BHU, Varanasi, India; ²Department of Medicine, IMS, BHU, Varanasi, India; ³Department of Molecular and Human Genetics, Faculty of Science, BHU, Varanasi, India

E-mail: madhes_kapv@sify.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O10

Background: Immune activation plays vital role in HIV infection and further progression to AIDS, with TLRs primarily involved in this mechanism.

Methods: Twenty eight children with HIV in age group of 18 months-14 years attending anti retroviral clinic and 17 healthy children as controls were included in the study. WHO clinical staging, CD4+ count and Toll like receptors (TLRs) 1, 7, 8, 9 expressions from PBMC (peripheral blood mononuclear cells) were studied and correlated with various parameters.

Results: Mean age of study population was 8.08 $\pm$ 3.41 years. 75% of patients were in WHO clinical stage I and II, whereas 25% in stage III. 29% HIV children had severe immunosuppression. TLR-1 showed neutral expression in majority (96%) of HIV children. For TLR-7, 53.5% HIV children showed upregulation, whereas 32% and 14.5% showed downregulation and neutral expression, respectively. TLR-8 expression was downregulated in majority (93%) of HIV children. TLR-9 was upregulated in 29% of patients, whereas 71% had neutral expression. Of all the TLRs studied, only TLR-9 had correlation with WHO clinical stage 3 and severe immunosuppression. No correlation of TLR expression was found with age, nutritional status and ART status of patients.

Conclusion: Expression pattern of TLR-7 was similar to adult patients with HIV. ART had no effect on expression profile of TLRs. Children with

severe immunosuppression had significant up regulation of TLR-9. Similar correlation was not observed with TLR-1, 7 and 8. Altered expression pattern of TLR might have implications for disease progression in HIV infection.

O11

Application of Real Time loop mediated isothermal amplification (RT LAMP) as a point of care assay using dried blood spots for rapid detection of HIV infection

Lavanya Vanjari¹, Lakshmi Vemu^{1*}, Lalit Dandona², John A Schneider³

¹Department of Microbiology, Nizam's Institute of Medical Science, Hyderabad, India; ²Public Health Foundation of India, New Delhi, India;

³University of Chicago, USA

E-mail: lakshmi57vemu@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O11

Background: Early and rapid diagnosis of HIV is essential to reduce the transmission and to start Anti Retroviral Therapy. The increasingly sophisticated molecular methods for diagnosing acute HIV infection have a direct impact on patient management as they have been developed to overcome the limitations of serological assays. Development of a further methodology, which is technologically simple and equally sensitive, accessible and affordable, is essential to detect the very few copies of viral genome during acute HIV infection. Hence in the present study a Real Time Loop Mediated Isothermal Amplification (RT LAMP) assay on Dried Blood Spots (DBS) for the detection of HIV RNA was developed for an early and specific diagnosis of HIV.

Methods: To evaluate HIV RT LAMP in comparison with real time PCR, 600 DBS samples from high risk groups (300 truck drivers and 300 Men who have sex with men) and 100 healthy and age matched individuals (control group) were included in the study. Informed consent was obtained from all the patients and controls.

Results: The performance characteristics of the HIV RT LAMP in comparison with Real Time PCRs were 100% sensitive and specific with a % CV less than 10.

Conclusion: The HIV RT LAMP on DBS, developed in this study, proved to be a highly sensitive, accurate, simple and affordable assay and easy to be performed in any small laboratory, especially in developing countries with a potential of a point of care assay, costing approximately 5 times lesser than the sophisticated molecular methods.

O12

M. tuberculosis enhances its virulence during replication in blood from HIV patients

Michelle B Ryndak¹, Krishna K Singh¹, Zhengyu Peng², Hualin Li³, Lu Meng², Suman Laal^{1,4*}

¹Veterans Affairs Medical Center, New York Harbor Health Care System, New York, NY, USA; ²Institutes of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai, China; ³Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA; ⁴New York University Langone Medical Center, Departments of Pathology and Microbiology, New York, NY, USA

E-mail: Suman.Laal@nyumc.org

BMC Infectious Diseases 2014, **14**(Suppl 3):O12

Background: *Mycobacterium tuberculosis* and HIV act synergistically to enhance and accelerate the development of tuberculosis and progression of HIV infection to AIDS. Hematogenous dissemination of *M. tuberculosis* leading to extrapulmonary TB, disseminated TB and miliary TB is greatly increased in HIV+ TB patients. We have compared the transcriptome of *M. tuberculosis* replicating in whole blood from immunocompetent and immunodeficient individuals to understand how *M. tuberculosis* adapts to the blood environment during hematogenous dissemination.

Methodology: Whole genome microarray analysis was performed on RNA from *M. tuberculosis* replicating in whole blood from PPD negative HIV- healthy donors and HIV+ donors. Genes with a fold change of ≥ 2 , at a false discovery rate of $< 2\%$, were considered significantly differentially expressed.

Results: *M. tuberculosis* survives and replicates in blood, and enhances its virulence/pathogenic potential during adaptation to the hematogenous

environment. The blood specific transcriptome reflects suppression of dormancy, induction of cell-wall remodeling, alteration in mode of iron acquisition, evasion of immune surveillance and enhanced expression of important virulence factors that drive active infection and dissemination. Compared to replication in HIV blood, these changes are accentuated during replication in blood from HIV patients. The expression of ESAT-6, known to play an important role in dissemination of *M. tuberculosis* from the lungs, is upregulated in *M. tuberculosis* growing in blood, and especially dramatically during growth in HIV+ blood.

Conclusion: *M. tuberculosis* modulates its aggressive progression to disseminated forms of TB by modifying its transcriptome to acquire a phenotype with enhanced virulence that favors active infection and dissemination.

O13

Promoter trap strategy for gene expression analysis under stress conditions of *M. tuberculosis* latency

Anant Yadav, Shivani Sood, Rahul Shrivastava*

Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan-173234, Himachal Pradesh, India
E-mail: rahulmicro@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O13

Background: Tuberculosis, caused by *Mycobacterium tuberculosis*, still remains a significant bacterial killer globally accounting for ~ 2 million deaths annually. The uniqueness of the infection lies in the competence of the etiological agent to remain dormant in the human body for extended periods of time, escaping the body's defense mechanisms. The ineffectiveness of the currently used antimycobacterials against latent mycobacteria warrants for discovery of novel drugs and drug targets. New methods are also required for the identification, analysis and validation of drug targets against latent mycobacteria.

Methods: A promoter trap vector with *lacZ* reporter system was constructed. For validation of the vector, upstream sequences of genes known to be involved in *M. tuberculosis* latency along with suitable positive and negative controls were PCR amplified and cloned into the shuttle vector containing promoterless *lacZ* reporter system, to yield recombinant constructs. The recombinant constructs were then electroporated into *M. smegmatis* to obtain recombinant mycobacterial strains. All such strains were individually subjected to stress conditions associated with *M. tuberculosis* latency.

Results: Validation of the promoter trap vector was done by expression analysis of *lacZ* reporter using a β -galactosidase assay. Genes known to be involved in the latency of *M. tuberculosis* showed appreciable levels of β -galactosidase expression under anaerobiosis as well nutrient starvation conditions in comparison to the negative control strain.

Conclusion: This promoter trap strategy can be exploited for expression analysis and validation of drug targets deduced from various non conclusive strategies such as bioinformatic analysis and microarray. This can also be utilized for determination of new drug targets by construction of genomic library.

O14

A novel *gyrB* gene mutation in fluoroquinolone resistant clinical isolates of *Mycobacterium tuberculosis*

Pooja Singh, Amita Jain*, Pratima Dixit, Shantanu Prakash, Indu Jaiswal,

Vimala Venkatesh, Mastan Singh

Department of Microbiology, King George's Medical University, Lucknow, UP, India

E-mail: amita602002@yahoo.com

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Introduction: Fluoroquinolone (FQ) resistance in *Mycobacterium tuberculosis* can be conferred by mutations in *gyrA* or *gyrB* gene. Recent studies suggest that amino acid substitutions in *gyrB* gene may also play a crucial role in resistance, but genetic studies of these mutations in *M. tuberculosis* are lacking.

Methods: A total of 100 ofloxacin resistant (OFX r) and 100 OFX sensitive (OFX s) isolates of *M. tuberculosis* isolates were consecutively selected from routine TB laboratory during 2012-2013. All the isolates were screened for

phenotypic OFX r (>2µg/ml) by 1% proportion method and tested for minimal inhibitory concentration by absolute concentration method. Quinolone resistance determining region (QRDR) of *gyrA* and *gyrB* genes of 320bp and 428bp respectively were amplified, sequenced and compared with *M.tuberculosis* H37Rv.

Results: Mutations in the *gyrB* gene were observed in 5 of the 100 OFX r isolates. The single nucleotide mutation sites were in codons 538, 500, 539 (in two isolates) and 592. In one isolate, a substitution at codon 592 (Pro592Ser) was found as novel mutation outside QRDR region of *gyrB* gene. Accession nos. of these isolates include; KF509920-KF509922, KC880086 & KC880101. All the isolates showing mutations in *gyrB* gene also had mutations in *gyrA* gene. Mutations in *gyrA* gene were observed in 79% OFX r isolates. No mutation was observed in *gyrB* gene of OFX s isolates.

Conclusion: No OFX r isolates had shown mutation in the *gyrB* gene in the absence of *gyrA* gene mutation. The role of the *gyrB* gene mutation in conferring resistance to OFX in *M.tuberculosis* needs to be studied further.

O15

An in silico study on molecular level interactions of host Siderocalin with siderophores from *Mycobacterium tuberculosis* and other bacterial species

Tanu Goyal, Sharmila Anishetty*

Centre for Biotechnology, Anna University, Chennai, India

E-mail: s_anishetty@annauniv.edu

BMC Infectious Diseases 2014, 14(Suppl 3):O15

Background: Tuberculosis (TB) drug research and development has witnessed resurgence in recent times primarily due to the high mortality rates despite the availability of front line drugs and a prominent BCG vaccine. Synergy of TB with HIV is another factor which has warranted a search for newer therapeutic interventions. Treatment of latent TB infection is also the need of the hour. Iron acquisition is an important virulence mechanism of *Mycobacterium tuberculosis* (MTB). To scavenge iron, bacterial species have developed high affinity, low molecular weight iron chelators termed as siderophores. To counteract the detrimental effect of microbial iron acquisition systems, the host secretes a 21kDa lipocalin protein (Siderocalin) that binds with these iron laden siderophores in an attempt to restrict the growth of MTB within host macrophages.

Methods: In the current study, using molecular docking tools, we assessed the interactions of host Siderocalin with Mycobactin, Parabactin and Cepabactin structurally non similar siderophores from three different bacterial species-MTB, Paracoccus and *Burkholderia cepacia*. A comparison of molecular level interactions of Siderocalin with these siderophores was performed.

Results: Siderocalin forms energetically favourable and stable complexes with parabactin and cepabactin. However the unfavourable positive binding energies for mycobactin- a salicylate derived MTB siderophore indicated that siderocalin does not dock well with mycobactin.

Conclusion: Siderocalin probably fails to disrupt the role of mycobactin in acquiring iron for mycobacteria thereby helping the pathogen to survive leading to progression of disease.

O16

Generation of differentially regulated genes in *Mycobacterium tuberculosis* isolates from cerebrospinal fluid and respiratory secretions using suppression subtractive hybridization

SH Saw^{1,2*}, VC Yong³, YF Ngeow¹

¹Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur-50603, Malaysia; ²Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar Pera-31900, Malaysia; ³School of Biosciences, Taylor's University Lakeside Campus, Subang Jaya, Selangor Darul Ehsan-47500, Malaysia

E-mail: sawsh@utar.edu.my

BMC Infectious Diseases 2014, 14(Suppl 3):O16

Background: Suppression Subtractive Hybridization (SSH) is an established method for separating DNA molecules that distinguish two closely related DNA samples, which has become a powerful method for generating subtracted cDNA libraries. In this study, we postulated that SSH could be

used to identify significant genetic differences between *Mycobacterium tuberculosis* causing meningitis and *M. tuberculosis* causing pulmonary tuberculosis.

Hypothesis: The unique DNA patterns present only in cerebrospinal fluid (CSF) TB isolates might be markers for neurotropism and neurovirulence in some strains of *M. tuberculosis*.

Methods: Bacterial DNAs were isolated and SSH was performed using PCR-Select Bacterial Genome Subtraction Kit (Clontech). Twenty subtracted DNAs were generated from 3 CSF isolates and 3 respiratory isolates. Molecular cloning was done on the subtracted DNAs and the positive isolates were extracted, purified and sequenced for analysis.

Results: So far, 9 of the subtracted DNAs have been successfully cloned into pGEM-T vector, resulting in 21 positive clones. Two preliminary BLASTP results revealed several mycobacterial PE/PPE proteins which play a role in the evasion of host immune responses, possibly via antigenic variation, together with conserved hypothetical proteins with unknown function.

Conclusion: Further studies are needed to determine the role of these subtracted DNAs.

O17

MDR1 gene polymorphisms and HIV therapeutic response in South Indian population

Shruthi Lingaiah^{1*}, Anita Shet², Arun S Shet¹, Ujjwal Neogi¹

¹Hematology Research Unit, Division of Molecular Medicine, St John's Research Institute, Bangalore, India; ²Division of Public health and Infection Disease Epidemiology, St John's Research Institute, Bangalore, India

E-mail: shruthi.lingaiah@gmail.com

BMC Infectious Diseases 2014, 14(Suppl 3):O17

Background: Multi drug resistance (*MDR1*) gene polymorphisms can alter the drug transport function of p-glycoprotein and are often associated with resistance to antiretroviral therapy (ART) in HIV-1 patients. In this study, we investigated the association of single nucleotide polymorphisms in *MDR1* gene (exon-21 and exon-26) with responsiveness to ART in HIV-1 patients.

Methods: Eighty HIV-1 infected drug naive patients who had atleast one viral load <150 copies/mL within six months of therapy and 21 healthy subjects were included. The HIV patients were categorized into viral responders (n=40) and non responders, two consecutive viral load ≥400 copies/mL after six months of ART (n=40). The genotype analysis of G2677T (GG/GT/TT in exon-21) and C3435T (CC/CT/TT in exon-26) was performed using PCR RFLP. Statistical analysis was carried out using chi-square test in SPSSver16.

Results: The proportion of CC, CT and TT-genotype among the healthy individuals was found to be 0.09, 0.52 and 0.38 whereas GT and TT-genotype was 0.61 and 0.38 respectively (No GG-genotype). Among HIV-1 patients, the distribution of CC, CT and TT-genotype between responders and non responders was (0.15 vs 0.07), (0.37 vs 0.50) and (0.50 vs 0.42) and GG, GT and TT was (0.12 vs 0.05), (0.30 vs 0.55) and (0.57 vs 0.40) respectively. Only the GT-genotype was significantly higher in non responders compared to responders (p=0.023).

Conclusion: The GT-genotype appears to predict poorer response to ART compared to other genotypes. Further studies could define more clearly the prognostic value of *MDR1* gene polymorphisms in determining therapeutic response to ART.

O18

Role of myeloid derived suppressor cells in tuberculosis infection and disease

Nathella Pavan Kumar^{1,2*}, Rathinam Sridhar³, Vaithilingam V Banurekha², Mohideen S Jawahar², Thomas B Nutman⁴, Subash Babu^{1,4}

¹National Institutes of Health international Center for Excellence in Research, Chennai, India; ²National Institute for Research in Tuberculosis, Chennai, India; ³Government Stanley Medical Hospital, Chennai, India; ⁴Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

E-mail: pavanmedtech@gmail.com

BMC Infectious Diseases 2014, 14(Suppl 3):O18

Background: *Mycobacterium tuberculosis* infects 2 billion people worldwide, 90% of infected individuals are able to resist overt disease

development and manifest only latent infection. Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of early myeloid progenitors, immature granulocytes, macrophages, and dendritic cells at different stages of differentiation. These cells have the capacity to suppress activities of adaptive immune response mediated by CD4⁺ and CD8⁺ T cells.

Methods: The frequency of MDSC(CD45+CD14+HLA dR-), Granulocyte derived MDSC (CD45+CD33+HLA dR CD14 CD11b+) and Monocyte derived MDSC(CD45+CD33+HLA dR CD14+CD11b+) was examined by using flow cytometry in pulmonary tuberculosis (PTB)(n=115) and compared with extra-pulmonary TB (ETB)(n=106), latent TB (LTB)(n=102) and healthy controls (HC)(n=73)

Results: PTB is characterized by significantly elevated frequencies of MDSC (Geometric mean [GM] of 0.5587% in PTB and 0.3095% in EP; $p=0.0005$); G-MDSC (GM of 0.1660% in PTB and 0.3089% in EP; $p=0.0189$) and M-MDSC (GM of 0.1307% in PTB and 0.0756% in EP; $p=0.0128$) when compared with ETB. On the other hand, there was diminished frequency of MDSC (GM of 0.5587% in PTB and 1.064% in LTB; $p=0.0060$) (GM of 0.5587% in PTB and 1.187% in HC; $p<0.0001$) and G-MDSC (GM of 0.1660% in PTB and 0.4131% in HC; $p<0.0001$) (GM of 0.1660% in PTB and 0.3516% in HC; $p<0.0001$) in PTB group when compared with LTB and HC, respectively.

Conclusions: The data reveals that MDSC were not only induced during active pulmonary TB infection but also in latent TB and healthy controls after recent exposure to *M. tuberculosis*.

O19

Evaluation of the mycobacteriophage amplification assay for detection of physically and physiologically stressed mycobacterial cells *in vitro*

Eddy SG Cheah^{1*}, Yee Thin Tan¹, Yui Han Tay¹, Seow Hoon Saw²

¹Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, Perak, Malaysia; ²Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, Perak, Malaysia
E-mail: cheahsg@utar.edu.my

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Background: Currently used laboratory detection methods for *Mycobacterium tuberculosis* are either slow or low in sensitivity, and therefore there is a need for a more reliable and rapid method. This study was done to evaluate the efficiency of the mycobacteriophage amplification assay in detecting mycobacterial cells that were exposed to the stresses (desiccation, UV radiation, and nutrient starvation) that simulate those in tuberculous droplet nuclei.

Methods: Exponential-phase *Mycobacterium smegmatis* (non-pathogenic surrogate model for *M. tuberculosis*) cells were exposed to a particular stressor and then subjected to the phage and colony-forming unit (CFU) assays; the numbers of plaque-forming unit (PFU) and CFU yielded were then compared to those for the unexposed control. We also investigated the effects of treating stressed cells with culture supernatants (containing resuscitation-promoting factors) on their phage infectivity and culturability on solid medium.

Results: Only desiccation and UV exposure decreased PFU significantly ($\sim 5 \times 10^6$ to 5×10^5 PFU/mL and 7.7×10^6 to 3.4×10^6 PFU/mL, respectively), whereas all three stressors affected culturability by approximately ten folds (10^6 - 10^8 to 10^5 - 10^7 CFU/mL). These suggest that most stressors affect the culturability of mycobacterial cells more than their phage infectivity. With prior culture supernatant treatment, only desiccated cells showed improved PFU and CFU yields (1.5×10^6 to 3.1×10^6 PFU/mL and 4.4×10^6 to 5.8×10^6 CFU/mL, respectively).

Conclusion: Hence, there is preliminary evidence that the phage assay might be superior to culture for detection of stressed mycobacterial cells that are in the non-culturable but viable state, and prior resuscitation of stressed cells could potentially improve the assay sensitivity.

O20

Experimental mouse model of tuberculous uveitis

Sudhanshu Abhishek¹, Amod Gupta², Indu Verma^{1*}

¹Department of Biochemistry, Post Graduate Institute of Medical Education and Research, Chandigarh, India; ²Department of Ophthalmology, Post Graduate Institute of Medical Education and Research, Chandigarh, India
E-mail: induvermabio@gmail.com

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Background: Tuberculous uveitis is one of the emerging forms of extrapulmonary tuberculosis. To understand the many unknown facets of this disease pertaining to diagnosis, treatment and pathogenesis, a mouse model of tuberculous uveitis was established.

Methods: Mice (Balb/c) were infected with *Mycobacterium tuberculosis* (H37Rv) through two different routes, i.v. and i.n. at different doses. Delayed type hypersensitivity (DTH) assay at 24 and 48 hours before sacrifice, CFU enumeration in eyes, RT-PCR, histopathological studies and cytokine analysis were performed in these animals.

Results: The i.v. and i.n. challenged mice showed significant DTH response. The CFU enumeration in eye did not show any bacilli initially by 30th day however tubercle bacilli could be recovered from the eyes of i.v. infected mice by 45th day at the time of death. Histopathology of these eyes showed inflammation with cluster of lymphocytes. The RT-PCR for IS6110 gene from the eyes of i.n. infected animals was positive at different doses whereas i.v. challenged mice showed variability. Furthermore, the cytokine analysis in infected tissue supernatants of both eyes and lung showed high Th1 response (IFN- γ) in comparison to non-infected group.

Conclusion: Overall, this study evaluated the ability of *M. tb* to establish infection in the eyes of mouse model following infection with different routes, doses and at different time points. RT-PCR positivity for IS6110 gene along with high IFN- γ response in the eyes of infected animals as compared to non-infected mice without any bacteriological evidence indicate the establishment of a paucibacillary TB uveitis mouse model, a model mimicking the human TB uveitis condition.

O21

Effects of progestin-only contraceptives on the phenotype and function of female reproductive tract CD4+ and CD8+ T cells

Uma Shanmugasundaram^{1*}, JW Critchfield¹, J Pannell², J Perry², WC Greene^{2,3}, L Giudice², K Smith-McCune², RM Greenblatt², BL Shacklett¹

¹University of California, Davis, USA; ²University of California, San Francisco, USA;

³Gladstone Institute of Virology and Immunology, San Francisco, CA, USA

E-mail: umashanmugasundaram@ucdavis.edu

BMC Infectious Diseases 2014, **14**(Suppl 3):O21

Background: Use of progestin-only contraceptives may enhance the susceptibility of the female reproductive tract (FRT) to HIV infection. We assessed the effects of progestin-only contraceptives, including Depo-Provera (DMPA), and the levonorgestrel intra-uterine system (LNG iUS) on endometrial and endocervical mucosa of premenopausal women.

Methods: Participants using either DMPA (n=15) or the LNG iUS (n=19) for at least 3 months and women using no hormonal contraceptives (n=24) were recruited. Endocervical curettage, endocervical cytobrush, and endometrial biopsy were obtained from all women. Expression of T cell activation markers, memory/effector differentiation markers and HIV co receptors were assessed by multiparameter flow cytometry. CD4+ and CD8+ T cells producing CD107a, IL-10, IL-2, IFN γ , MIP1 β and IL-17 were measured after stimulation with PMA/ionomycin or staphylococcal enterotoxin B (SEB).

Results: Endometrial CD4+ and CD8+ T cells and endocervical CD8+ T cells were highly activated in women using LNG iUS as compared to controls. Compared to DMPA, LNG iUS resulted in increased activation of endocervical curettage CD4+ T cells. CXCR4+CCR5+CD4+ T cells in the endometrium and curettage were increased in women using LNG iUS as compared to controls. The percentage of endocervical central memory CD4+ and CD8+ T cells were decreased in LNG iUS recipients. Following SEB stimulation, endometrial CD4+ T cells responded with production of IL-10, IL-2 and IL-17. CD8+ T cells produced elevated percentages of IL-10 in women using LNG iUS compared to control women.

Conclusion: LNG iUS and DMPA affected endocervical and endometrial T cell phenotype and responsiveness to polyclonal stimulation. Further studies are warranted to clarify the effects of contraceptive products on upper FRT immune cells and HIV susceptibility.

O22

Effectiveness of targeted viral load strategy in a public health HIV/AIDS programme

Rajyasree De¹, Nivedita Dutta¹, Dolanchampa Modak¹, Shantasil Pain², Sripathi Dasmohapatra³, Sukamal Bisoi⁴, Subhasish Kamal Guha^{1*}

¹Centre of Excellence in HIV Care, Calcutta School of Tropical Medicine, Kolkata,

West Bengal, India; ²Department of Medicine, North Bengal Medical College,

Darjeeling, West Bengal, India; ³Care, Support & Treatment Division, West Bengal State AIDS Prevention & Control Society, Kolkata, West Bengal, India; ⁴Department of Community Medicine, RG Kar Medical College, Kolkata, West Bengal, India
E-mail: drskguha@gmail.com
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Background: Targeted viral load (VL) strategy is followed in the ART programme of India. Patients failing antiretroviral therapy, undergo VL testing and switched to 2ndline ART if VL is >5000 copies/ml.

Methods: Data of patients referred to School of Tropical Medicine, Kolkata for suspected treatment failure (TF) was analyzed for the period of December 2008 to November 2013 to ascertain the positive predictive value (PPV) of immunologic and/or clinical parameter for confirming virologic failure (VF) and to observe the short term outcome of secondline treatment. Paired t- test was used for data analysis.

Results: VF was noted in 287(51.3%) of 560 patients (median age 37 years; male-436(77.85%); median CD4 109 cells/mm³ at suspected TF. Number of patients failing by immunologic, clinical and clinicoimmunologic criteria was 414(74%), 11(2%) & 135(24%) respectively with corresponding PPV of 48%, 46% & 63% respectively. PPV of CD4 falling below baseline, >50% drop from on-treatment peak value and failure to reach 100 cells/mm³ after 12 months of ART was 37%, 55% and 25% respectively. Among 216 patients with minimum 6 months follow-up, the median CD4 & VL changed significantly following 6 months of secondline ART (103.5 to 232 cells/mm³; $p < 0.0001$ and 4.04 to 2.6 log₁₀ copies; $p < 0.0001$ respectively). Undetectable VL was achieved in 164(76%). Out of 284 patients starting secondline ART, 25 (8.8%) died within 6 months.

Conclusion: Immunoclinical criteria have low PPV in diagnosing VF. Despite late switch, majority (76%) could achieve undetectable VL at 6 months but the high early mortality (8.8%) is a concern.

O23

Investigation of anti-HIV activity, cytotoxicity and HIV integrase inhibitory activity of polyherbal formulation BH extracts

S Balraj^{1*}, P Selvam², Y Pommier³, M Metifiot³, M Christophe³, C Pannecouque⁴, E De Clercq⁴

¹Hans Rover Herbal Clinic, Perambalur, Tamil Nadu, India; ²Nova College of Pharm. Edu and Research, Jupudi, AP, India; ³Laboratory of Molecular Pharmacology, NCI, Mary Land, USA; ⁴Rega Institute for Medical Research, Leuven, Belgium
E-mail: rareindianherbals@gmail.com

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Background: HIV integrase (IN) plays important roles at several steps, including viral DNA nuclear import, targeting viral DNA to host chromatin and integration. Identification of novel inhibitors of HIV Integrase has emerged as promising antiviral agents for the treatment of HIV/AIDS. Present work is to investigation of anti-HIV activity and HIV integrase inhibitory activity of various extracts of polyherbal formulation BH.

Method: Polyherbal extracts (BH) were tested for anti-HIV activity against HIV-1 and -2 in MT-4 cells and cytotoxicity also tested against uninfected MT-4 cells. BH extracts were investigated for inhibition of HIV integrase enzymatic activity to understand the mechanism of antiviral action.

Results: All extracts exhibited inhibitory activity against HIV-1 integrase (3'P IC₅₀: 8.8-63 µg/mL and ST IC₅₀: 4.9-65 µg/mL). The ethanolic extract (BH-HT) displayed significant inhibitory activity against both step of HIV in enzymatic activity (3'P IC₅₀: 8.8µg/mL and ST IC₅₀: 7.5 µg/mL). The ethanolic extract also inhibits the HIV-1 replication at the concentration of 59.30 µg/mL and cytotoxicity was found to be more than >125 µg/mL.

Conclusion: All the extracts inhibit the HIV integrase activity and ethanolic extract inhibit HIV and Integrase enzyme.

O24

A study of cardiac complications in HIV infected children

S Pushpalatha, K Anitha*

Department of Pediatrics, Bangalore Medical College and Research Institute, Bangalore, India

E-mail: anithakommalur@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O24

Background: Studies have demonstrated the presence of abnormality on echocardiography in HIV infected adults, but there is a paucity of similar

data regarding children in the Indian subcontinent. The objectives of this study were to evaluate the cardiac abnormalities in HIV infected children and to know the metabolic and nutritional risk factors for cardiac disease.

Methods: One hundred consecutive HIV infected children were subjected to history, examination, nutritional assessment, complete hemogram, lipid profile, CD4 count, chest X ray, 12 lead ECG and M mode transthoracic 2D ECHO. Statistical analysis was done using uni variate and multi variate regression analysis.

Results: Out of 100 HIV infected children, 42% [25 males, 17 females] had cardiac abnormalities of which only 6 [14%] were symptomatic. Majority belonged to WHO clinical stage IV, with 13 [28%] having low CD4 count. The most common cardiac abnormality was left ventricular systolic dysfunction (LVSD) in 19% ($p < 0.05$). Other abnormalities were mild tricuspid regurgitation in 14%, pericardial effusion in 6%, pulmonary artery hypertension in 6%, dilated right ventricle, right atrium in 3%, pulmonary regurgitation in 3% and mitral regurgitation in 1%. ECG showed sinus tachycardia in 14%, right ventricular hypertrophy in 6%, irregular rhythm in 3%. Among children with cardiac abnormalities, dyslipidemia was seen in 32 [77%] ($p < 0.05$), anemia in 29 [69%] ($p < 0.05$), and malnutrition in 19 [45%] ($p > 0.05$).

Conclusion: Though the majority of the children were asymptomatic, investigations revealed 42% with subclinical cardiac abnormalities. They had significant dyslipidemia and anemia as compared to children without cardiac abnormalities. The study thus emphasizes the need for routine echocardiographic screening.

O25

Subacute sclerosing pan encephalitis with HIV: two rare case reports

N Hazra^{1*}, R Mani¹, A Desai¹, S Sinha², M Netravathi², AB Taly², P Satishchandra³, V Ravi¹

¹Dept of Neurovirology, NIMHANS, Bangalore, India; ²Dept of Neurology, NIMHANS Bangalore, India; ³Director/Vice Chancellor, NIMHANS Bangalore, India
E-mail: drnhazra@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O25

Background: Subacute Sclerosing Pan Encephalitis (SSPE) is a rare, chronic neurological disease of children and adolescents resulting from persistent measles virus infection of neurons. Not only is the occurrence of SSPE in HIV infection rare, also the natural history has not yet been elucidated.

Case Reports: Two children, a 10 year old boy and an 11 year old girl, both HIV-1 seropositive following perinatally acquired infection, presented with strikingly different clinical symptoms to Neurology OPD, NIMHANS. The boy presented with deterioration in scholastic performance over a four month duration with generalized tonic clonic seizures of 1 month duration. Vision was normal. The girl was found to have evidence of bilateral blindness, altered gait with dysarthric speech and severe truncal ataxia.

Both children were unvaccinated for measles and gave a definitive history of Measles at the age of 8 months and 6 years. The latent period between Measles infection and SSPE was 9 and 5 years respectively. Clinically both were staged at Jabbour Stage II and Gascon stage III. The CD4 counts were 554/mm³ and 130/mm³ respectively. In both patients, progressive cognitive decline, reduction in scholastic performance, regression of milestones and inability to walk with loss of self care occurred in 12 and 4 weeks respectively following onset.

Conclusion: Presence of HIV resulted in a fulminant course with premature manifestation of myoclonic jerks and rapid-onset cognitive decline as well as rapid progressive deterioration of clinical staging in both patients. This report of two rare cases aims to stress the importance of awareness of the possible association of SSPE with HIV, especially with the increased life expectancy that is seen in HIV post HAART and the need to institute a global program to eliminate measles

O26

Factors associated with loss to follow up after entry into care of HIV infected children ineligible for antiretroviral therapy: data from an HIV cohort study in India

Praveen Kumar Naik, Manoranjan Midde, Raghavakalyan Pakam, Gerardo Alvarez-Uria*

Department of Infectious Diseases, Rural Development Trust Hospital, Bathalapalli, AP, India

E-mail: hivbtp@gmail.com

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Background: Half of HIV-infected adults who are not eligible for antiretroviral therapy (ART) are lost to follow up (LTFU). However, data about the attrition from enrolment in care to ART eligibility of HIV infected children are scarce.

Methods: Two hundred and eighty two children ineligible for ART at enrolment in care were followed up until ART eligibility. Multivariable analysis was performed using competing risk regression. The 12-month risk of AIDS was calculated using the age and the absolute CD4 cell count as in the HIV Pediatric Prognostic Markers Collaborative Study.

Results: The cumulative incidence of attrition (mortality and LTFU) was 15.6% (95% confidence interval [CI], 11.3-20.5) after six years of follow-up, and the attrition rate was higher during the first year after enrolment. Children with a 12-month risk of AIDS < 3% had a higher risk of LTFU (subdistribution hazard ratio [SHR] 10.77, 95% CI 1.93-60.07) than those with a 12-month risk of AIDS >4%. Those children whose father had died had a lower risk of LTFU (SHR 0.26, 95% CI 0.09-0.75) than those whose parents were alive and were living in a rented house. Children aged 10-14 had a lower risk of LTFU (SHR 0.12, 95% CI 0.03-0.55) than those aged 5-9 years.

Conclusions: A substantial proportion of children ineligible for ART were LTFU before ART eligibility. These findings can be used by HIV programmes to design interventions aimed at reducing the attrition in pre-ART care of HIV infected children in India.

027

Cluster detection of HIV infection for clients of female sex workers in selected districts of Tamil Nadu, South India

Vasna Joshua^{1*}, K Bhoopathi¹, Ramesh S Paranjape², Thilakavathi Subramanian¹, S Mehendale¹

¹National Institute of Epidemiology, Ayapakkam, Chennai, India; ²National AIDS Research Institute, Pune, India

E-mail: vasnajoshua@yahoo.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O27

Background: The spread of the HIV epidemic is diverse. The risk behaviors of the HIV infected individuals are not distributed uniformly across the population, but tend to cluster in specific high risk groups. The objective of the study is to explore whether HIV infected individuals are geographically clustered using Kulldorff space time scan statistics.

Methods: A large cross sectional Integrated Behavioral and Biological Assessment (IBBA) survey for clients of female sex workers (FSWs) in the three districts (Chennai, Madurai and Salem) of Tamil Nadu, South India was carried out by India AIDS initiative, the Avahan project. The survey collected information from 1217 clients of female sex workers between June and September 2009. The data set was geocoded using Google Earth, Kulldorff Space time Scan Statistics was used for detection of clusters among the clients of FSWs.

Results: Kulldorff Space time Scan Statistics identified a most significant cluster at the location of latitude 13.123°N and longitude of 80.293°E with the spatial dependency of 8.39 km radius.

Conclusions: The study shows initial evidence of geographical clustering of HIV cases. However this observation needs to be substantiated by undertaking similar studies in other states of India. Also, it is important to critically explore biological and behavioral reasoning for such an occurrence. This would also pave a way to build models incorporating clustering effect in the model and might help to design precise and more focused intervention approaches.

028

Toxic epidermal necrolysis in lenalidomide treated patient with HIV

V Mahalakshmi^{*}, M Krishnakanth, S Adikrishnan, Gayathri R Adithya, S Murugan, S Anandan, R Sudha

Department of Dermatology, Venereology and Leprosy, Sri Ramachandra Medical College, Porur, Chennai, India

E-mail: dr.mahalakshmi1969@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):O28

Introduction: Toxic Epidermal Necrolysis (TEN) is a rare and acute life threatening mucocutaneous reaction characterized by extensive necrosis

and detachment of the epidermis, most commonly drug induced. Lenalidomide is a derivative of thalidomide used in treatment of multiple myeloma, myelodysplastic syndromes. We present a case of TEN in lenalidomide treated patient of multiple myeloma with HIV.

Case Report: A 58 year old male, a k/c/o chronic kidney disease and multiple myeloma presented to the OPD with diffuse redness and peeling of the skin of 3 days duration associated with intense burning sensation and erosions in the mouth and genitalia. Patient was started on T. Lenalidomide 16 days ago for multiple myeloma. In view of an episode of herpes zoster in the past HIV testing was done which was found to be positive. O/E- multiple erosions were present over the scalp, face trunk and genitalia. There was approximately 60-80% of epidermal detachment and SCORTEN was 4. Nikolsky's sign positive.

Discussion: Lenalidomide is used as an alternative for thalidomide for its lesser incidence of side effects. HIV infected individuals are deficient in the enzyme glutathione peroxidase which is essential for drug metabolism. Only two cases of SJS, TEN to lenalidomide have been reported so far. The case is presented for its rarity and association with HIV.

029

An empowerment programme to support children in child headed households in resource-poor communities in Soshanguve, South Africa: phase 2 of an intervention study

Julia E Ibebuikwe^{1*}, Corrien van Belkum², Todd MM Maja¹

¹Adelaide Tambo School of Nursing Science, Tshwane University of

Technology, Pretoria, South Africa; ²College of Nursing, King Saud bin

Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

E-mail: juliaibeibuikwe@yahoo.com

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Background: South Africa has been noted to have the fastest growing rate of people living with HIV/AIDS. This has led to an alarming increase in the number of children orphaned by AIDS and the attendant care of the orphaned children by their siblings leading to the formation of child headed households (CHHs). The study was conducted in two phases. Phase 1 identified CHHs in a resource poor community in South Africa, their lived experiences, their needs and the resource poor communities' knowledge and perceptions about these households. Phase 2 of the study aimed at developing, implementing and evaluating an empowerment programme to support the children in CHHs in the resource poor communities.

Methods: The design for the study was a qualitative contextual intervention study and targeted children in CHHs who participated in Phase 1 of the study. Data was collected using in depth interviews, and data analysis was by open coding using Tesch's approach.

Results: The study findings revealed the following, (1) structure for developing an empowerment programme to support children in CHHs in resource poor communities, (2) framework for empowerment programme to support children in CHHs, (3) summary of the implementation of the framework for an empowerment programme to support children in CHHs, (4) evaluation of the implementation and outcome of the empowerment programme.

Conclusion: An empowerment programme was developed, effectively implemented and the children's needs supported.

POSTER PRESENTATIONS

P1

High prevalence of *Entamoeba moshkovskii* infection in HIV seropositive patients of Barak Valley, Assam, India

Joybrato Nath^{1,2}, Sankar K Ghosh², Prithwiraj Bhattacharjee³, Jaishree Paul⁴, Baby Singha^{1*}

¹Department of Zoology, Gurucharan College, Silchar, Assam, India;

²Department of Biotechnology, Assam University, Silchar, Assam, India;

³Department of Medicine, Silchar Medical College and Hospital, Silchar,

Assam, India; ⁴School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

E-mail: babysingha@gmail.com

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Background: It is now well established that *Entamoeba moshkovskii* and *Entamoeba dispar* are two distinct non-pathogenic species, microscopically

indistinguishable from pathogenic *Entamoeba histolytica*. Being endemic, there are no data on the prevalence of these commensal infections from the North Eastern part of India.

Methods: A total of 274 stool samples collected in this cross sectional study from HIV seropositive patients attending the ART centre of SMCH, Assam, India were screened for cyst and/ trophozoite stage of *Entamoeba* using iodine staining technique and then subjected to *SSU* rRNA gene based multiplex PCR assay.

Results: Out of 274 stool samples, multiplex PCR assay of 61 microscopy positive samples showing cyst and/ trophozoite stage of *Entamoeba*, revealed a higher prevalence of *E. moshkovskii* (12.8%; 95% CI= 9.30, 17.28) and lower prevalence of *E. dispar* (6.2%; 95% CI=3.85, 9.77) compared to *E. histolytica* (8.1%; 95% CI= 5.31, 11.91; $p < 0.05$). Of the 61 samples that were microscopically positive, 5 were *E. histolytica*, 8 were *E. dispar*, 25 were *E. moshkovskii*, 17 were mixed infections with *E. histolytica* and 6 were PCR negative which may be non-histolytica/dispar/moshkovskii infection. Thus, only 36% of the 61 microscopy positive stool samples were actually *E. histolytica*, implying that remaining 64% of so called infections were over represented and would have been treated unnecessarily.

Conclusion: This is the first report of *E. moshkovskii* infection from this region of India and its high prevalence signifies an urgent need of amoebiasis diagnosis using advanced molecular tools to avoid overrepresentation and unnecessary chemotherapy.

P2

Nocardia asteroides ocular infection

Coimbatore Subramanian Shobana^{1*}, Haridas Sreekumar¹, Prithiviraj Aiswariya¹, Kanesan Panneer Selvam², Yendrebam Randhir Babu Singh¹, Perumal Gomathi³, Palanisamy Manikandan³

¹Department of Microbiology, Dr. G.R. Damodaran College of Science, Coimbatore – 641 014, India; ²Department of Microbiology, M R Government Arts College, Mannargudi – 614 001, India; ³Department of Microbiology, Aravind Eye Hospital and Post Graduate Institute of Ophthalmology, Coimbatore - 641 014, India
E-mail: shobana.cs@grd.edu.in

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Background: *Nocardia asteroides* can cause ocular infection in immunocompetent and immunocompromised individuals following minor trauma. Hence present study was carried out to isolate *N. asteroides* from the patients attending a tertiary eye care hospital and to analyse the antibiotic susceptibility pattern of the *Nocardia* isolates.

Methods: The ocular samples viz., corneal swab, corneal scraping, aqueous tap and vitreous tap were processed for culture and the isolated *Nocardia* species were further confirmed for speciation by standard microbiological procedures. Antibiotic susceptibility analysis for the confirmed isolates of *N. asteroides* was performed by agar disk diffusion method following the guidelines of Clinical Laboratory Standard Institute (CLSI, 2000).

Results: Out of 280 ocular clinical specimens collected, 25 isolates of *N. asteroides* were obtained. The rest of the pathogens included bacteria, fungi and mixed culture. The age in most of the cases yielding *Nocardia* was below 5 years (12%) and above 60 years (52%) and men (76%) were more infected than women (24%). Precisely, 17 isolates of *N. asteroides* were isolated from corneal ulcer, 5 from corneal swab and 3 from endophthalmitis. Upon analysis of antibiotic susceptibility tests the nocardial isolates were found to be more susceptible to fourth generation fluoroquinolones antibiotic family (75%), amikacin (66%), ampicillin (80%), vancomycin (50%) and norfloxacin (75%).

Conclusion: Ocular nocardiosis remains difficult to recognize, there by leading to misdiagnosis and underestimation of its incidence.

P3

Hepatitis C virus infection contributes to impregnation of markers of immune inhibition: potential preludes underlying viral latency and persistence

Muttiah Barathan¹, Mohamed Rosmawati², Jamuna Vadivelu¹, Marie Larsson³, Vijayakumar Velu⁴, Saeidi Alireza¹, Li Yen Chang¹, Esaki Muthu Shankar^{1*}

¹Tropical Infectious Disease Research and Education Center (TIDREC), Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; ²Department of Medicine, Faculty of

Medicine, University of Malaya, Kuala Lumpur, Malaysia; ³Department of Clinical and Experimental Medicine, Division of Molecular Virology, Laboratory 1, Level 13, Linköping University, 58185 Linköping, Sweden; ⁴Department of Microbiology and Immunology, Emory Vaccine Center, 954 Gatewood Road, Atlanta, Georgia, USA
E-mail: shankarem@um.edu.my

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Background: Hepatitis C virus (HCV) represents one of the persistent viral infections afflicting humankind, and a significant proportion of chronic HCV disease progresses over time through liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC). One potential mechanism underlying the chronic disease is believed to be viral escape from immune surveillance via upregulation of inhibitory molecules on immune cells by HCV. We investigated the diverse expression of various inhibitory molecules in PBMCs of healthy non-HCV controls and chronically HCV infected patients. **Methods:** The expression of inhibitory molecules on PBMCs was investigated in chronic HCV infected patients relative to healthy non-HCV controls using standard immunological and molecular methods. The serum levels of indoleamine 2, 3 deoxygenase (IDO) and cyclooxygenase-2 (COX-2) were also investigated.

Results: The gene expression profile of chronically HCV infected patients was significantly different from control individuals. Our results showed upregulation of TIM-3 ($p \leq 0.01$), PD-1 ($p \leq 0.01$), FOXP-3 ($p \leq 0.01$), BLIMP-1 ($p \leq 0.01$), CD160 ($p \leq 0.01$), CTLA-4 ($p \leq 0.01$), TRAIL ($p \leq 0.01$), BTLA ($p \leq 0.01$) and LAG-3 ($p \leq 0.01$) with fold change of 1.3, 0.4, 14.6, 0.87, 6.6, 0.4, 14.7, 10.9 and 2.5 respectively in chronically HCV infected patients. The plasma IDO and COX-2 levels were significantly higher ($p = 0.001$) in chronically HCV infected subjects relative to healthy control.

Conclusion: The upregulation of inhibitory molecules on PBMCs in chronically HCV infected patients suggest the contribution of these molecules to immune cells impairment in HCV infection. Viral persistence and eventual progression following potential evasion of the host immune armory via viral impregnation of inhibitory immune biosignatures in HCV disease pathogenesis warrants further elucidation.

P4

Prevalence and risk factors associated with infection of major diarrhoeogenic protozoan parasites in HIV patients with ART at Silchar Medical College and Hospital, Assam, India

Joyobrato Nath^{1,2}, Gulzar Hussain¹, Sankar K Ghosh², Prithwiraj Bhattacharjee³, Baby Singha^{1*}

¹Department of Zoology, Gurucharan College, Silchar, Assam, India; ²Department of Biotechnology, Assam University, Silchar, Assam, India; ³Department of Medicine, Silchar Medical College and Hospital, Silchar, Assam, India
E-mail: babysingha@gmail.com

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Background: In developing countries diarrhoeogenic Protozoan parasites mainly *Cryptosporidium parvum*, *Enterocytozoon bienersi*, *Giardia duodenalis* and *Entamoeba histolytica* have been implicated as major contributors to morbidity and mortality in HIV patients.

Methods: In this cross sectional study, 274 stool samples collected from HIV patients with ART at SMCH, Silchar, Assam, India were initially examined for cysts/oocysts stages using iodine and modified ZN staining techniques respectively and subsequently confirmed through PCR assay using specific primers. Data on associated risk factors were obtained by interviewing all 274 patients with ART.

Results: The overall prevalence of the four diarrhoeogenic Protozoan parasitic infections was estimated to be 22.9% (95% CI= 18.40, 28.33) which included *E. bienersi* was the most common (12.4%; 95% CI= 9.02, 16.84) followed by *C. parvum* (9.5%; 95% CI= 6.56, 13.54), *E. histolytica* (8.1%; 95% CI= 5.36, 11.86), and *G. duodenalis* (4.7%; 95% CI= 2.79, 7.94). The overall infection in patients with CD4 count ≤ 200 cells/ μ L was significantly higher as compared with patients with CD4 counts 200-300 and ≥ 300 cells/ μ L ($p < 0.001$). Logistic regression analysis showed close animal contact (OR= 2.42; 95% CI= 1.36, 4.29), river or well as water source (OR= 5.97; 95% CI= 3.22, 11.05), unhygienic toilet (OR= 3.43; 95% CI= 1.91, 6.17) and poor living condition (OR= 2.12; 95% CI= 1.19, 3.74) significantly increase the likelihood of infection ($p < 0.01$).

Conclusion: The study emphasizes the need for routine screening of diarrhoeagenic Protozoan parasite in HIV patients along with well education to patients about practicing personal hygiene.

P5

Prevalence and co-infection study of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* among symptomatic women using PCR assay

SC Sonkar¹, K Wasnik¹, PK Mishra¹, P Mittal², A Kumar², J Suri², D Saluja^{1*}
¹Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi-110007, India; ²Department of Obstetrics & Gynecology Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi-110029, India
E-mail: dsalujach59@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):P5

Background: Sexually transmitted infections (STIs) are one of the major causes of acute illness, infertility, long term disability and death for millions of men, women and infants globally. *Trichomonas vaginalis*, *Neisseria gonorrhoea* and *Chlamydia trachomatis* are well established agents of STIs leading to vaginal discharge in women. However, the prevalence and co infection patterns among symptomatic women is lacking in India. The present study aims to determine the prevalence and co infection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis*, in women with vaginal discharge attending OPD, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi.

Methods: Dry swab samples were obtained and DNA was extracted used as template for PCR amplification using primers targeting *pfoB*, *gyr A* and *orf1* gene for diagnosis of *T. vaginalis*, *C. trachomatis* and *N. gonorrhoeae* respectively to estimate prevalence of co-infection.

Results: A total of 335 women were studied (mean age 23.2 years), out of which 22 women (6.56 %) women were infected with had at least one pathogen. One woman (4.54 %) was co-infected with all three pathogens. Amongst 335 women, 18 women (5.37 %) tested positive for *T. vaginalis*, 02 women (0.59 %) tested positive for *C. trachomatis* and 1 woman (0.29 %) tested for *N. gonorrhoea* by all the PCR assays, whereas 313 women (93.43 %) tested negative for the all three pathogens.

Conclusion: The results demonstrated that it is necessary to test for all three pathogens namely *T. vaginalis*, *N. gonorrhoea* and *C. trachomatis* in women with vaginal discharge.

P6

Identification of putative secretory and membranous proteins of *Chlamydia trachomatis* using bioinformatics tools

Prashant K Mishra¹, Renu Pandey¹, Uma Chaudhry², Daman Saluja^{1*}
¹Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi- 110007, India; ²Bhaskaracharya College of Applied sciences, University of Delhi, Delhi-110075, India
E-mail: dsalujach59@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):P6

Background: Intuitive understanding of hypothetical genes's signal peptide, transmembrane helix (TH) domain and LipoP box of any pathogenic microorganisms is a prerequisite for the rational development of drugs and vaccine candidates. A protein harboring these sequences may serve as receptor and conveyer for various biostimulations generated during host-pathogen interaction. Identification and annotation of these proteins may help in designing of novel drug or developing new vaccine candidates to control CT infections.

Methods: We have retrieved whole sequence of CT serovar D/UW-3/CX from NCBI database. To identify secreted proteins, TH domain and lipoP box, we have exploited the following online softwares: sigpred (http://bmbpcu36.leeds.ac.uk/cgi_bin/sig_pred), siglanIP4.0 (<http://www.cbs.dtu.dk/services/SignalP>), TMPred (http://www.ch.embnet.org/software/TMPRED_form.html), TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>) and LipoP box (<http://www.cbs.dtu.dk/services/LipoP/>).

Results: The present study focused on the 336 HPs of CT to find out signal peptide, TH domain and lipoP box. Out of 336 HPs. Forty proteins have lipoP box and five proteins (CT149, CT605, CT623, CT823 and CT858) have signal peptide as well as TH domain. Additionally, among five, four proteins (CT149, CT623, CT823 and CT858) also have lipoP box. Harboring

TH domain sequences suggests that they may have either membrane spanning, extracellular or intracellular domain which may help in host-pathogen interactions and/or signal transduction. It is also possible that these proteins may be secreted and thereby induces host immune responses but it will need further validation.

Conclusion: We consider that CT149, CT605, CT623, CT823 and CT858 may be secreted outside the pathogen and should be explored further to understand the pathogenesis of CT.

P7

Transcriptional and translational analysis of subsequent infection with *Staphylococcus aureus* and *Proteus mirabilis* in *Caenorhabditis elegans*

Udayakumar Prithika^{*}, Gnanasekaran Jeba Mercy, Krishnaswamy Balamurugan
Department of Biotechnology, Alagappa University, Science Campus, Karaikudi-630004, India
E-mail: prithikaudayakumar@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):P7

Background: *Caenorhabditis elegans* can be effectively used to study the dynamics of polymicrobial infections. *Proteus mirabilis* as an opportunistic pathogen does not cause death in *C. elegans*. Hence, in the present study, the *C. elegans* was pre infected with the pathogen *Staphylococcus aureus* to make the *C. elegans* immuno compromised to study the effect of *P. mirabilis* in the host.

Methods: This study involved in investigation of impact of subsequent infections at physiological, transcriptional and translational levels using *C. elegans*.

Results: Physiological status of *C. elegans* was analyzed using killing assay, CFU count and CLSM analysis. The mRNA expression analysis indicated the regulation of host specific immune regulatory genes, CUB like proteins (*F08G5.6*), neuropeptide like factors (*nlp-29*) and C type lectin genes (*clec-60* and *clec-87*) during subsequent infections. To study which protein is regulated against subsequent infections, SDS-PAGE analysis was performed using the proteins extracted from *C. elegans* exposed with subsequently infected samples for different time intervals. Results of 1D electrophoresis clearly suggested that subsequently infected samples showed significantly more differentially regulated proteins compared to their respective controls.

Conclusion: The physiological assays and molecular studies demonstrated the vulnerability of a host as an integral event during *S. aureus* infections that enables the bacteria to suppress the host immune system, which subsequently lead to the opportunistic pathogen *P. mirabilis* to exert its pathogenicity in a host system. Further studies are needed to check the translational status of the host during subsequent infections.

P8

Response of *Caenorhabditis elegans* during *Klebsiella pneumoniae* pathogenesis

Arumugam Kamaladevi, Krishnaswamy Balamurugan^{*}
Department of Biotechnology, Alagappa University, Science Campus, Karaikudi, India
E-mail: bsuryar@yahoo.com
BMC Infectious Diseases 2014, **14**(Suppl 3):P8

Background: *Caenorhabditis elegans*, owing to its amenability and conserved innate immune system was recognized as an emerging model to dissect the molecular basis of many mammalian infections. *Klebsiella pneumoniae* causes severe infections in immunocompromised patients. Hence, the host response was studied using *C. elegans* during *K. pneumoniae* infection.

Methods: Pathogenicity of *K. pneumoniae* and its LPS were assessed by variety of physiological and biochemical assays. The qPCR was used to analyze the regulation of innate immune genes.

Results: *K. pneumoniae* and its LPS were lethal to *C. elegans* and required 48±5hours and 24±3hours for complete killing, respectively, with cessation of pharyngeal pumping and egg laying. Infection with *K. pneumoniae* increased the bacterial load in the intestine of host upon course of infection, which was measured as 1.5x10³, 2.2x10³, 3.6x10⁴ and 3.7x10⁵ in 4,6,12 and 24hours, respectively. This increased bacterial load subsequently disseminates oxidative stress markers in host. The level of

ROS was measured to increase by 24.36597nM, 35.60517nM, 39.34052nM, and 28.24774 nM/mg of protein in 6, 12, 24, and 36 hours, respectively. Infection by *K. pneumoniae* also increased the protein carbonyls to 25.57nM, 36.14nM, 35.26nM and 38.84nM/mg of protein in 6, 12, 24, and 36 hours, respectively. *K. pneumoniae* and its LPS suppressed the expression of *pmk-1*, to *l-1* and antimicrobial peptides (clec-60, clec-85, clec-87, *lys-1*, and *lys-7*) and thus succumbed the host by upregulated expression of virulent genes such as *uge*, *mpa* and *oxyR*.

Conclusion: The interplay between *K. pneumoniae* and *C.elegans* in the present study further provides more insights into the mechanism of host defense against this pathogen.

P9

A study to determine and compare the knowledge, attitude and compliance of Tuberculosis treatment among HIV seropositive and HIV seronegative TB patients

S Gouda^{1*}, B Peerapur¹, J Rudramma², A Kaleem¹, R Sandhya¹

¹Department of Microbiology, Raichur Institute of Medical Sciences, Raichur, India; ²Department of Community Medicine, Raichur Institute of Medical Sciences, Raichur, India

E-mail: sheetalkgouda@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):P9

Background: Tuberculosis and HIV are major global health problems. Tuberculosis is the most common opportunistic infection, leading to the mortality among HIV patients. This study aims to determine and compare the knowledge, attitude, and compliance regarding tuberculosis amongst HIV seropositive and HIV seronegative TB patients.

Methods: This study was conducted at Raichur district hospital. Data regarding socio demographic profile, knowledge, attitude, and compliance was collected from the subjects with the help of pretested semi structured questionnaire. Data was analyzed using Microsoft Excel and SPSS 15.0 and Chi square test was applied.

Results: A total of 240 subjects aged 19-67 years were enrolled in the study. Out of them, 120 were HIV seropositive, 53% were male, and 68% had primary education. About 55.4% had average knowledge of TB but in depth knowledge about symptoms and the spread was not adequate among HIV seropositive patients. Regarding knowledge on the symptoms and spread of TB, only 35% knew about chest pain and 23% knew about hemoptysis. There were few misconceptions regarding the spread; majority (77%) believed that TB spreads by unprotected sex and 53.5% believed that it occurs through infected blood transfusion. We noticed positive attitude and positive compliance with TB treatment among majority of the respondents, however the compliance was better (92%) among HIV seropositive patients.

Conclusion: Our study revealed that the knowledge regarding Tuberculosis among HIV seropositive patients is inadequate when compared to HIV seronegative patients. This calls for an awareness program targeting the HIV/AIDS infected individuals through advocacy, communication, and social mobilization.

P10

Methodological lacunae in recruitment of HIV positive persons in randomized controlled trials

Arun K Sharma^{1*}, Vikas K Meena¹, OP Rajoura¹, Kuldeep Kumar²

¹Department of Community Medicine, University College of Medical Sciences, Delhi-110095, India; ²Department of Medicine, University College of Medical Sciences, Delhi-110095, India

E-mail: a.sharma@ucms.ac.in

BMC Infectious Diseases 2014, **14**(Suppl 3):P10

Background: Recruitment of patients in an RCT is crucial to the success of the trial. The sample selection and sample size estimation is based on previously reported research studies. We conducted an RCT to assess the effectiveness of mobile text messaging in improving adherence to highly active anti retroviral therapy in an ART unit of a tertiary care hospital. The present study was aimed to examine the impact of recruitment process and sampling on the outcome of the trial.

Methods: The required sample size for recruitment in the RCT was calculated based on a reported defaulter of 27% in a previous Indian

study. In order to attain an effect size of 0.3 for 1 degree of freedom, the required sample size in each arm was 44. Recruited 60 persons in each arm taking into account 20% drop out and 15% mortality rate.

Results: Analysis of data showed that the initial assumptions were not matching. Loss to follow up due to death and attrition were 4.1% and 6.6% respectively. Using person months of observation, corresponding loss was only 2.6% and 1.2% respectively. Default rates in the intervention and control arms were 1.6% and 2.3% respectively (not significant).

Conclusion: The default rates were much lower than the a priori hypothesized values. The study suggests that selection of cases based on data from a different center may result in wrong estimation of sample size. ART defaulter is more likely to be a function of factors which are not influenced by mobile text messaging.

P11

Prevalence of HBV and HIV coinfection from an infectious disease hospital in Mumbai

Jayanthi Shastri^{*}, Nita Gangurde, Manish Pathak, Sandhya Sawant, Sachee Agrawal

Department of Microbiology, TN Medical College and BYL Nair Charitable Hospital, Mumbai, India

E-mail: jsshastri@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):P11

Background: Hepatitis B virus (HBV) and HIV coinfection are pressing health problems in developing countries including India. Concurrent infection with HBV may alter the natural history and treatment of both diseases. Hepatitis B and HIV frequently coexist due to common modes of transmission. HIV and HBV coinfection leads to complex immunopathological disorder heralding poor prognosis and dismal outcomes. The present study was aimed to examine the prevalence of HIV and HBV co- infection among hospitalized patients.

Methods: Two groups of population were studied; group I (n=1000) hospitalized adult patients with clinical jaundice were tested for HBsAg by ELISA. HBsAg positive patients were further tested for HIV infection as per NACO guidelines. Group II (n= 650) hospitalized patients with symptoms like fever > 1month, weight loss >10%, cough >3 weeks and lymphadenopathy were tested for HIV as per NACO guidelines. HIV positive patients were further tested for HBsAg by ELISA.

Results: In group I, 10 % were positive for HBsAg and out of 100 HBsAg positive 6% were co infected with HIV. In group II, 15.38% were positive for HIV and out of 100 HIV positive 25% were co- infected with HBV. Overall co infection is 15.5% with male preponderance and heterosexual mode of transmission being the commonest.

Conclusion: HIV and HBV share common modes of transmission, therefore all HIV positive patients should be screened for HBV coinfection. Guidelines should be modified to include mandatory HBV testing among HIV positive individuals to monitor progress of infection and improve treatment outcomes.

P12

HIV-1 reverse transcriptase inhibitory activity of *Aerva lanata* plant extracts

Rajendra Prasad Gujjeti^{*}, Sainath Namthabab, Estari Mamidala

Infectious Diseases Research Lab, Department of Zoology, Kakatiya University, Warangal, Andhra Pradesh, India

E-mail: prasadzoology@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):P12

Background: HIV-1 reverse transcriptase (HIV-1 RT) is an essential enzyme for the replication cycle of HIV. HIV-1 RT inhibitors have been extensively investigated for their anti-HIV properties. However, emergence of HIV drug resistance and side effects are the main reasons for failure of anti-HIV therapy. The aim of the present study was to evaluate the HIV reverse transcriptase inhibitory activity of *Aerva lanata* plant extracts.

Methods: Extracts were prepared from dried roots in different solvents. Peripheral Blood Mononuclear Cells (PBMCs) were isolated from healthy donors by ficoll-hypaque density gradient centrifugation method. A toxicity study was performed on all crude extracts among PBMCs by

MTT assay. HIV-1 RT inhibition activity of the all solvent extracts of *A. lanata* was determined by a HIV-1 Reverse Transcriptase activity assay.

Results: All the five solvent crude extracts of *A. lanata* were non cytotoxic up to 0.75 mg/mL concentration in PBMCs. Chloroform and methanol extracts shows highest inhibition of recombinant HIV RT (91% and 89% respectively) at 1 mg/mL concentration. This strong inhibitory effect was confirmed by their IC_{50} . More than 50% inhibition of HIV RT shows from 0.03 to 1 mg/mL concentrations of all extracts.

Conclusion: Experimental results thus suggested that the *A. lanata* plant extracts which have been tested in the present study exert their anti-HIV activity via inhibition of HIV Reverse Transcriptase activity. However, in order to assess the usefulness of this herb, it is necessary to isolate the active principle (s) from the crude extracts.

P13

An automated system based on 2 d empirical mode decomposition and k-means clustering for classification of *Plasmodium* species in thin blood smear images

K Manickavasagam, S Sutha, K Kamalanand*

Department of Instrumentation Engineering, MIT Campus, Anna University, Chennai, India

E-mail: kamalanand@mitindia.edu

BMC Infectious Diseases 2014, 14(Suppl 3):P13

Background: In order to control malarial infection, specific anti-malarial drug for the corresponding *Plasmodium* species must be administered. The objective of this work is to extract a system for classification of *Plasmodium* species in thin blood smear images.

Methods: In this work, thin blood smear sub images (n=87) of different *Plasmodium* species were acquired from the Parasite Image Library of the Centers for Disease Control and Prevention Database [http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Malaria_il.htm]. The images were subjected to 2 d Empirical Mode Decomposition and four features namely the mean value of first Intrinsic Mode Function (IMF-1), IMF-2, IMF-3 and residue, were extracted. The significance of the extracted features was analyzed using ANOVA test. Further, the k-means clustering algorithm was used to classify the different *Plasmodium* species using the significant features.

Results: It was found that the features namely the mean of IMF-1 and residue are statistically significant ($p < 0.001$) and the developed classification system was able to classify the *Plasmodium vivax* images with a high accuracy of 100%. Further, the *Plasmodium malariae* images were classified with an accuracy of 83.33%. However, the developed classifier showed lower accuracy in classification of *Plasmodium falciparum* and *Plasmodium ovale* images.

Conclusion: Results demonstrate that the developed system is highly efficient in classification of *P. vivax* and *P. malariae*. This study appears to be of high clinical relevance since the automated classification of malaria parasite is useful for mass screening and drug selection for treatment of the infection.

P14

Comparative evaluation of baseline characteristics of HIV mono infected and HIV/HBV co infected cohort in eastern India

Jayeeta Sarkar^{1*}, Debraj Saha², Bibhuti Saha¹, Bhaswati Bandyopadhyay¹, Runu Chakrabarty², Subhasish Kamal Guha¹

¹Calcutta School of Tropical Medicine, Kolkata, West Bengal, India; ²ICMR

Virus Unit, Kolkata, ID & BG Hospital Campus, Kolkata, West Bengal, India

E-mail: jayee.gjan@gmail.com

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Background: India has the third largest estimated number of HIV infected people and 40 million individuals in India are estimated to be Hepatitis B virus infected. In comparison to HBV mono-infection, the course of chronic HBV infection is more accelerated in HIV/HBV co-infected subjects. We analyzed the baseline characteristics of the HIV/HBV co-infected and HIV mono-infected patients.

Methods: Between July 2011 and January 2013, a total number of 1331 HIV-seropositive treatment naïve individuals, enrolled in the ART Centre of Calcutta School of Tropical Medicine were screened for HBsAg. A number of 1253 HIV mono-infected and 78 HIV/HBV co-infected

patients were characterized. The co-infected patients were evaluated for HBeAg and anti HBe antibody by ELISA. HBV DNA was detected by nested PCR amplification followed by HBV genotype determination.

Results: The median age (35 years) was similar in HIV mono-infected and HIV/HBV co-infected group. The proportion of advanced HIV disease (WHO clinical stage III & IV) was more in HIV/HBV co-infected patients (37.1% vs. 19.9%) than HIV mono-infected subjects. The co-infected subjects had significantly higher serum bilirubin, ALT, alkaline phosphatase, creatinine and ALT/platelet ratio index. CD4 count was non significantly lower in co-infected patients. Majority (61.5%) were HBeAg reactive with higher HBV DNA ($p=0.0001$) and APRI ($p=0.04$) as compared to HBeAg negative (38.5%) patients. Genotype HBV/D (71.7%) was the predominant genotype followed by HBV/A (19.4%) and HBV/C (8.9%) among the co-infected patients from eastern India.

Conclusion: HIV/HBV co-infection warrants long term prospective study on immune recovery, HIV and HBV viral replication, and hepatotoxicity.

P15

Causes of prolonged fever among HIV infected patients- utility of an algorithmic approach - a single centre study from south India

Sudheer Aree Parambil^{1*}, Nitin Hosmelkar², Rajendra Prasad Shivaswamy³

¹Vivekananda Memorial Hospital, Saragur, Mysore, Karnataka, India; ²ART

centre, Hassan Institute of Medical Sciences, Hassan, Karnataka, India; ³JSS

medical college and Hospital, JSS University, Mysore, Karnataka, India

E-mail: apsdhr@gmail.com

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Background: Availability of ART for the last one decade has changed the pattern of morbidity among HIV infected patients. Researchers from Christian Medical College Vellore had developed an algorithm for evaluation of prolonged fever in the pre HAART era in India. The purpose of this study was to assess utility of this algorithm in the current HAART era to identify causes of prolonged fever and correlate with CD4 count, WHO staging and ART status.

Methods: Prospective longitudinal observational study conducted on convenient sample of 90 consecutive HIV infected patients presenting with prolonged fever at a secondary care hospital.

Results: The algorithm developed by CMC, Vellore was useful in diagnosing causes of fever among 91.1% of the patients. TB meningitis was the most common cause of fever accounting for 23.3% followed by Bacterial pneumonia, Pulmonary TB and *Pneumocystis jiroveci* pneumonia accounting for 13.3%, 11.1% and 13.3% respectively. Immune reconstitution inflammatory syndrome and lymphoma were found to be the cause in 5.4% of patients

Conclusion: Tuberculosis continues to be the most common cause of prolonged fever (58.8%) although the prevalence rates seems to be decreased compared to the pre HAART era studies from India (71%). Bacterial pneumonia was found to be increasing among all CD4 groups and regardless ART status pointing to the need for introduction of effective vaccination programs against *Pneumococcus*. CD4 count and WHO staging were found to be the statistically significant factors in determining the cause of fever and ART status was not statistically significant.

P16

Clinical profile and diagnostic challenges of tuberculous meningitis among HIV infected patients - a retrospective study

Sudheer Aree Parambil^{1*}, Mamatha Thamme Gowda¹,

Rajendra Prasad Sivaswamy²

¹Vivekananda Memorial Hospital, Sargur, Mysore, Karnataka, India; ²JSS

Medical College and Hospital, JSS University, Mysore, Karnataka, India

E-mail: apsdhr@gmail.com

BMC Infectious Diseases 2014, 14(Suppl 3):P16

Background: Tuberculosis is the most common opportunistic infection among people living with HIV in India. Tuberculous meningitis is the most serious form of TB with high morbidity and mortality, with diagnostic challenges in resource poor settings. TB meningitis (TBM) presents atypically especially when CD4 is low with fewer signs of meningeal irritation and non specific CSF picture. Widespread availability of ART has changed the clinical presentation of TBM with more patients presenting as unmasking TB

Immune Reconstitution Inflammatory Syndrome (IRIS). In this study we propose to study the clinical profile of HIV infected patients with presumptive diagnosis of TB meningitis and to assess the utility of a diagnostic criteria for diagnosis of TB meningitis validated in South Africa.

Methods: Retrospective descriptive study conducted among 100 consecutive HIV infected patients presumptively diagnosed to have TB meningitis admitted at a secondary care center.

Results: Fever was the only symptom in 20% of patients. 89% of the patients had elevated CSF protein with only 7% having other CSF features suggestive of TBM. Fifty percentage of the patients had TB IRIS, among them 39.19% were unmasking type, and 10% were of paradoxical type of IRIS. In patients with stage I TBM only 35% fulfilled proposed diagnostic criteria, whilst in stage II and III 65% and 66%, respectively fulfilled diagnostic criteria.

Conclusion: The study shows the proposed diagnostic criteria is not useful in early stages of TBM which was statistically significant. The utility of proposed diagnostic criteria was uniform among patients irrespective of duration of ART.

P17

Molecular characterization of *Pseudomonas* sp. isolated from lower respiratory tract infection in HIV and non-HIV population by 16S rDNA and ARDRA

C Anitha^{1,6*}, Sujatha Kabilan², N Rajinith³, A Santhosh Kumar⁴, Padma Krishnan¹, Illaikiam Rasikan², S Senthilkumar⁵, S Vincent⁴, S Senthamarai⁶, S Sivasankari⁶, P Gunasekaran², Rajasekharan Sikhamani⁷, M Pushkala⁸

¹Dept of Microbiology (Faculty of Medicine), Dr. ALM PGIBMS, University of Madras, Taramani, Chennai, India; ²School of Biological Science, Madurai Kamaraj University, Madurai-21, India; ³SRM College, Chennai, India; ⁴LIFE/P.G and Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, India; ⁵Dr.K.M.Cherien Heart Foundation and frontier Lifeline Pvt. Ltd, Elavur, Gummidipoondi-601201, India; ⁶Department of Microbiology, Meenakshi Medical College and Research Institute, Enathur, Kanchipuram, India; ⁷Government Hospital of Thoracic Medicine, Tambaram Sanatorium, Tambaram, Chennai, India; ⁸Department of Immunohaematology, Dr. M.G.R Medical University, Guindy, Chennai, India
E-mail: ani.phd@gmail.com

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Background: *P. aeruginosa* an important pathogen causing lower respiratory tract infections (LRTI) both in HIV and non-HIV population. Molecular characterization of *Pseudomonas* spp. helps in better understanding of their clonal distribution among these patient populations. Our study aims to discriminate and generate highly specific fingerprints using 16S-rDNA PCR and amplified ribosomal DNA restriction analysis (ARDRA) techniques.

Methods: Seventy-two isolates (45-HIV, 24-Non-HIV, 2-environmental, 1-ATCC) of *Pseudomonas* spp. were subjected to 16S rDNA PCR using universal primers and amplicons digested with *Hae* III, *Alu* I and *Rsa* I for ARDRA analysis. Nucleic acid size confirmation of the digested amplicons was done using MultiNA Bioanalyser. Phylogenetic tree was constructed by maximum parsimonious method using MEGA 4.0. Representative isolates from the major clones were sequenced and submitted to genbank for accession numbers and genetic relatedness was identified by UPGMA using NTSYS 1.80 software. Mean genetic distance (GD) and intraspecies mean GD were calculated.

Results: Based on ARDRA banding pattern, 14 groups and 10 clones were obtained from the 72 *Pseudomonas* isolates with the following accession numbers: JF279962-64, JF303639-45. Sequences showed 97-100% similarity with known *P. aeruginosa*, *P. putida*, *P. stutzeri*, *Alcaligenes fecalis* strains by BLASTN analysis. The overall mean GD and intra-species GD of the various species was as follows: *P. aeruginosa* (JF279962, JF279963, JF303643, JF279964, JF303645, JF303644): 4.664, 16.663, 7.536, 0.733, 0.096, 2.402 and 0.221, 3.487, 2.010, 0.029, 2.728, 0.011; *P. putida* (JF303640, JF303639): 1.904, 7.3 and 1.064, 1.371; *P. stutzeri* (JF303641) is 2.732, 0.036; *Alcaligenes fecalis* (JF303642) : 30.248, 5.377 respectively.

Conclusion: The highly variable mean GD values amongst the isolates from different and within the same species indicates high genetic diversity among *Pseudomonas* spp causing LRTI among HIV and Non HIV patients.

P18

LEDGF/p75 IN interaction inhibitors: *in silico* studies of an old target with novel approach

Amit M Pant¹, Rupesh V Chikhale, Sunil S Menghani, Pramod B Khedekar
Department of Pharmaceutical Sciences, RTM Nagpur University, Nagpur, India
E-mail: amitmpant@gmail.com

BMC Infectious Diseases 2014, 14(Suppl 3):P18

Background: Despite development in Anti Retroviral Therapy (ART), reports of HIV infection remains in continuous momentum and a cure seems to be imaginary. Raltegravir, an Integrase (IN) inhibitor, provides some life expectancy to patients on salvage therapy. Nowadays, IN inhibitors reported with resistance and shows cross resistance to other drugs in this class. Human Lens Epithelium Derived Growth Factor (LEDGF)/p75 plays a vital role in the HIV life cycle and its importance has been shown in numerous studies. In the LEDGF/p75 IN complex, LEDGF binds to IN at a region other than the catalytic active site. Thus, we tried computationally to approach these IN-LEDGF interaction sites as a novel target in therapy.

Methods: The computational studies involved protein preparation, ligand preparation and energy minimization, grid generation, docking and analysis of results. A library of 396 molecules were prepared considering a pyrimidine ring as core. These operations were performed using Maestro, Discovery studio and VLife Sciences suites.

Results: It is known that Ile365 establishes a hydrogen bond with backbone carbonyl group of IN Gln168 whereas Asp366 of LEDGF/p75 forms a hydrogen bond with Glu170, on similar basis it was found that AMP_1071 exhibits hydrogen bonding with Gln95 of one monomer and Gln168, His171, and Thr174 of another monomer of IN.

Conclusion: The designed molecule AMP_1071 shows topological similarity to LEDGF/p75 binding surface. Further antiviral activity, pharmacokinetic and tolerability studies are ongoing. The LEDGF binding inhibitors lacks the cross resistance to any class of ART, possibly making this class as add on to highly active anti-retroviral therapy.

P19

Self assembled cationic cell penetrating peptide nanoparticles as efficient cargoes for antimicrobial agents

A Aravind, A Deepu, K Santhosh Kumar
Chemical Biology Lab, Rajiv Gandhi Centre for Biotechnology,
Thiruvananthapuram, India
E-mail: kskumar@rgcb.res.in

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Background: The development of technologies that enable bioactive molecules with low membrane permeability to effectively penetrate biological membranes is one of the greatest challenges in the pharmaceutical field. The HIV-1 transactivating transcriptor (TAT) peptide is one of the most widely used molecular beacons for drug delivery. Cationic antimicrobial peptides have received increasing attention due to their broad spectrum activities and ability to combat multi drug resistant microbes. Our aim is to develop cationic cell penetrating peptide nanoparticles which could efficiently deliver antimicrobial agents against infectious diseases.

Methods: The cationic TAT peptides were synthesized by Fmoc solid phase peptide synthesis and were purified by semi preparative RP-HPLC. The molecular mass of peptides was confirmed by MALDI TOF-MS. The purified peptides were modified into self assembled nanoparticles (micelles) and structural characterization was done by a particle size analyzer and TEM. The antimicrobial activity evaluation was done against both Gram positive and Gram negative bacterial strains.

Results: We have designed and synthesized arginine and lysine rich cationic TAT peptides and modified them into nanoparticles. These nanoparticles were structurally and functionally characterized. Some analogues of these nanoparticles cargoes showed significant antimicrobial activity against Gram positive and negative bacterial strains especially those cause brain infections with low MIC values. These were shown relatively low haemolysis.

Conclusion: The findings of this study revealed the improved antimicrobial activity of modified cationic TAT peptide nanoparticles against different bacterial strains. This could revolutionize in the field of chemotherapy and can be efficiently used to treat brain infections and other diseases.

P20

Breast abscess due to *Listeria* sp: report of a rare case

KV Ramana

Department of Microbiology, Prathima Institute of Medical Sciences,
Karimnagar, Andhrapradesh, India

E-mail: ramana_20021@rediffmail.com

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Background: *Listeria* sp. are facultative intracellular bacterial pathogens, which pose a potential public health problem related to consumption of contaminated food. Human listeriosis is clinically classified as perinatal listeriosis, neonatal listeriosis and adult listeriosis. Human infections caused by *Listeria* sp. present typically as meningitis. Other infections attributed to *Listeria* sp. include endocarditis, myocarditis, arteritis, pneumonia, pleuritis, cholecystitis, peritonitis, arthritis, osteomyelitis, sinusitis, otitis, conjunctivitis and ophthalmitis. We report a case of breast abscess in a young lactating patient.

Case presentation: A lactating 21 year-old lady presented to hospital with complaints of a lump in her right breast. On observation the size of the lump was 3cmX1cm. Gram stain of the aspirated pus revealed short gram positive bacilli, and culture on blood agar grew small, round translucent beta hemolytic colonies with no growth on MacConkey agar. Conventional biochemical reactions showed that the isolated bacteria was motile at 25°C and non-motile at 37° C and cold enrichment at 40 °C, 10% salt (NaCl) tolerance test and CAMP test were positive. Histopathological study of the tissue biopsy revealed extensive inflammation with no signs of granuloma. Empirical therapy including Amoxy clav 1.2 mg TID and 400 mg Ciprofloxacin TID was started. The patient responded well and had an uneventful recovery.

Conclusion: Human infections with *Listeria* sp. remain under reported owing to its unusual clinical presentation and complex physiological and biochemical characters. Clinical microbiologists should be proactive in the laboratory identification of *Listeria* sp. as most isolates of *Listeria* are ignored as laboratory contaminants.

P21

Comparative evaluation of the anti-Hepatitis B virus activity of *Centella asiatica* and *Camellia sinensis* (green tea)

S Thanigaivel¹, H Durgadevi¹, J Balasubramaniam², V Mythily²,
M Elanchezhian^{1*}

¹Department of Microbiology, Dr. ALM PG IBMS, University of Madras,
Chennai, India; ²Blood Bank, Voluntary Health Services (VHS), Taramani,
Chennai, India

E-mail: emanickan@yahoo.com

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Background: Chronic Hepatitis B virus (HBV) infection is a major health problem and available anti-HBV drugs are known to cause side effects in addition to increased incidence of drug resistance. In the current study anti-HBV properties of *Centella asiatica* and *Camellia sinensis* was evaluated.

Methods: For the assay, equal volume of HBV virus was mixed with extract of *C. asiatica* or *C. sinensis* and incubated at 37°C for 5 days. The supernatant was assayed for the presence of bound/unbound HBsAg using ELISA. A dose response analysis was done for each extract and cytotoxicity of the each extract was measured by MTT assay.

Results: Dose response anti HBV revealed that methanolic extract *C. asiatica* indicated that 2.5 mg/mL concentrations was inhibitory to 0.75 pg/mL of HBV. Aqueous and methanolic extract of *C. sinensis* indicated that 0.5 mg/mL and 0.25 concentrations inhibited HBV, respectively. Interestingly, EGCG indicated 1.5 pg/mL HBV was inhibited by 25µg/mL. These concentrations were well tolerated by HepG2 cells and the non toxic concentration was up to 800 µg/mL.

Conclusion: The study showed that EGCG outperformed other extracts and had anti-HBV activity with minimal concentration (25 µg/mL). This is followed by extracts of *C. sinensis* which exhibited medium anti HBV activity. Poorest anti-HBV activity was noticed with *C. asiatica* i.e.2.5 mg. None of the extracts had cytotoxicity. From this study we could conclude that *C. sinensis* extracts are better in inhibiting HBV.

P22

Predominance of HBV genotype D in southern part of India

Kalyanaraman Narayanan¹, L Thayumanavan², Jayalakshmi Mariakuttikan^{1*}

¹Department of Immunology, School of Biological Sciences, Madurai Kamaraj
University, Madurai, India; ²Department of Gastroenterology, Govt Rajaji
Hospital, Madurai, India

E-mail: jayalakshmi@genomicsmku.org

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Background: The clinical outcome of HBV infection is highly heterogeneous which correlates with viral factors such as genotypes, viremia and mutants. Evidences showed that HBV genotypes have a role in prevalence of variants, IFN therapy and disease severity. So far, 10 HBV genotypes (A to J) have been identified with distinct geographical distribution. Hence, knowledge of the genotype infecting an individual may assist a physician making a decision towards better clinical management. The aim of this study is to identify the circulating HBV genotypes (A e) and its correlation with clinical manifestation.

Methods: A cohort of 72 patients (Acute: 11; Asymptomatic: 36; Chronic: 24; and HCC: 1) attending the Govt. Rajaji Hospital, Madurai, was recruited. Clinical categorization was based on biochemical and viral markers. Viral DNA extraction was carried out from serum samples and genotyping was done by multiplex PCR.

Results: Multiplex PCR was optimized with viral reference samples. Each reaction was carried out with five pairs of primers. We could observe only genotype D in this cohort (100%). None of the samples were assigned with other genotypes.

Conclusion: HBV genotype D has a major role in emergence of precore mutants (1896 G>A) leading to diagnostic failure, vaccine escape mutants, and poor clinical response while therapy.

P23

Time trend analysis of HIV infection showing impact of national programs in an ICTC set up in a tertiary care hospital in North India

Gurleen Metha, Amanpreet Kaur, Gurpreet Singh, Sunil K Arora*

Integrated Counseling and Testing Center (ICTC), Department of
Immunopathology, PGIMER, Chandigarh, India

E-mail: skarorain@gmail.com

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Background: The analysis of changing trend in the HIV infection with respect to various factors in a tertiary care hospital of North India in an ICTC (Integrated Counseling and Testing Centre) setting was studied.

Materials and methods: A total of 78,506 clients who visited the ICTC, PGIMER, Chandigarh from January 2003 to October 2013 were included and variables like age, sex, occupation, pattern of risk behavior and HIV sero status were studied. The patients were divided in three phases based on the phases of epidemic as evident from the variability in the positivity.

Results: Variable trend is seen in the HIV infection in last 11 years. The change in positivity rate in terms of age has affected all the age groups. In terms of profession, during third phase there is a decrease in the businessman and truckers group probably due to very effective condom distribution program during NACPII. Least impact is found among students and laborers. The heterosexual route, IDU and blood, and blood products were stable while the homosexual route and parent to child (PPTCT) was still on the higher side.

Conclusion: National intervention programs started during NACPII and continued during NACPIII have greatly impacted in stabilizing or even reducing the epidemic. However, more intensified epidemiological studies should be carried out which will help planning intervention projects to interrupt and control the transmission of HIV/ AIDS.

P24

Prevalence of Tuberculosis and HIV co infection with respect to CD4 counts in Chengalpattu Medical College and Hospital

M Malathi^{1*}, A Vijayalakshmi¹, CP Ramani¹, P Balapriya¹, R Kulothungan²

¹Department of Microbiology, Chengalpattu Medical College, Chengalpattu,
India; ²ART Center, Chengalpattu Medical College, Chengalpattu, India

E-mail: drmalathi13@gmail.com

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Background: Infection with HIV increases the risk of tuberculosis. HIV and TB co infection is associated with increased number of mortality. This study aimed to characterize the patients of HIV with co infection of TB and their CD4 counts.

Methods: This study is a retrospective study by assessment of the patient's medical records. Study period is from January 2013 to November 2013. 575 patients with CD4 count less than 500 were selected as the study population. Among them, 37 patients were identified with HIV and TB co infection. Their clinical presentations were also studied.

Results: The prevalence of HIV and TB co infection is 6.43%. Co infection is more prevalent in men (67.56%) with a mean age of 42.12 years. Co infection in female is 27.02% with a mean age of 40.4 years. Pediatric co infection rate is 5.40%. Pulmonary tuberculosis (70.27%) is the most frequent form of TB followed by tuberculous lymphadenitis (29.72%).

Conclusion: From this retrospective study, it has been confirmed that the prevalence of HIV and TB co infection is more common in patients with CD4 count less than 500. This study reflects the co-ordinate active participation of RNTCP and Integrated Counseling and Testing centre (ICTC) in identifying the HIV patients who are at risk of developing co infection.

P25

Larvicidal and antimicrobial activities of silver nanoparticles synthesized using marine fluorescent pseudomonads

Shanmugaiiah Vellasaamy^{1*}, Hari Krishnan Hariharan¹, TS Venkatesh², Mani Jayaprakashvel³

¹Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai-625021, India; ²Department of Biotechnology, Anna University of Technology, Tiruchirappalli-620024, India; ³Department of Biotechnology, AMET University, Kanathur, Chennai-603112, India
E-mail: shanmugaiiahv@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):P25

Background: Research on nanoparticles opens up newer avenues for unraveling various biological challenges including the control of infectious diseases. The present investigation has been aimed to study larvicidal and antimicrobial activities of silver nanoparticles (AgNP's) synthesized using marine fluorescent pseudomonads.

Methods: In the present study, marine fluorescent pseudomonads were screened against various human pathogenic bacteria and plant fungal pathogens. An autolyzed cell free culture filtrate of selected isolate was used for the synthesis of silver nano particles and characterized using UV, FTIR, XRD and AFM analysis. The antimicrobial efficiency along with larvicidal activity of the synthesized nanoparticles was carried out by observing the lifecycle of mosquito for 96 hours along with appropriate controls.

Results: Atomic microscopic observation showed that the particle size ranges from 10- 100nm. The peaks in UV spectrum clearly indicate that the particles are in the nanoregime. FTIR measurements explained the reduction of silver ions and stabilization of silver nanoparticles. At 96 hours, 60% mortality observed in test, while only 10% mortality observed in the control. AgNP's showed significant activity against tested human pathogens and plant fungal pathogens.

Conclusion: With the suppression of growth of filarial nematode in the larval stage, AgNP's has the potential in eradicating this vector borne disease. It is clear that this isolate can be used to synthesize bioactive nanoparticles efficiently in an eco friendly and cost effective manner besides the fact that it can be used for the control of human and plant pathogenic microbes.

P26

Prevalence of HBV infection in immunocompromised patients on ART nearly equals HBV mono infection in individuals not infected with HIV, Shimla Northern India

Aarti Garg^{*}, Vineeta Sharma, Santwana Verma, Digvijay Singh, Anil Kanga, Balraj Singh

Indira Gandhi Medical College (IGMC), Shimla, Himachal Pradesh, India
E-mail: aartidewan.79@gmail.com

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Background: It is estimated that 5% of the global population is chronically infected with HBV. The rate of HBV-HIV co infection is 10-20% in countries with HBV endemicity. The present study was undertaken to

examine the HBV prevalence and HBV-HIV co infection at tertiary care institute, Northern India.

Methods: This is a retrospective study for the duration of five years, 2008-13. The HBV infection was detected using HBsAg (Hepatitis B surface antigen) and HIV/AIDS cases were screened as per NACO, 2007 criteria.

Results: A total of 10,134 cases were screened for detection of HBV infection and among these 1650(16.3%) were receiving ART (anti retroviral therapy) while HIV status was not divulged in 8484 (83.7%). HBsAg positive in 324/10134 (3.20%) cases, out of these HBV mono infection was seen in 264 (2.6%) and co- infection with HIV in 60 (0.6%). The concomitant HIV infections were seen in 3.64% (95 %confidence interval 2.8-4.7) while in HBV mono infections were in 3.1% (95 % confidence interval 2.8-3.4). There is no statistical difference among the two groups (p value >0.05). Among the pediatric age group 0.95% had concomitant HIV infections as against 5.2% with HBV infection alone. The mean age of presentation was 40.39 years. Males had higher prevalence rate of co infection (4.5:1).

Conclusion: Nearly identical prevalence of HBV infection irrespective of HIV infection strongly indicates HIV screening for Hepatitis B infected patients. The presence of undetected co infection shall result in missed cases, incomplete or partial treatment and suboptimal clinical follow up.

P27

Correlation of molecular and conventional methods of diagnosis in tuberculous lymphadenitis

Bineeta Kashyap^{1*}, Neha Goel¹, Rajneesh Avasthi², Vinod Arora³, Iqbal R Kaur¹

¹Department of Microbiology, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi, India; ²Department of Medicine, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi, India; ³Department of Pathology, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi, India
E-mail: dr_bineetakashyap@yahoo.co.in

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Background: Incidence of tuberculous lymphadenitis, accounting for 30 – 40% of extra-pulmonary tuberculosis, has increased in parallel with increase in the incidence of tuberculosis worldwide. The diagnosis becomes more challenging when clinical presentation is suggestive but bacteriological proof is lacking.

Methods: Sixty lymph node aspirates from suspected cases of tuberculous lymphadenitis were examined for *Mycobacterium tuberculosis* by Ziehl neelsen (ZN) staining, culture on a Lowenstein Jensen medium, and cytological examination for findings suggestive of tubercular lymphadenitis. DNA from all the samples was amplified by conventional PCR targeting 123 bp and 240 bp fragments of *IS6110* and *MPT64* genes respectively and RealTime PCR targeting *MPT64* gene only.

Results: Histopathology, ZN staining, and culture gave positivity in 25, 20 and 16 cases respectively; out of which 6 (10%) were diagnosed by positivity in all the three conventional techniques, whereas 36 (60%) were positive by any of these three techniques. Diagnosis could be made by conventional PCR in 43 cases when compared to 45 by the RealTime PCR method (7.2 vs 7.5%) while targeting the *MPT 64* gene in PCR could diagnose 45 cases unlike *IS6110* gene which could help in diagnosis of 42 cases (7.5 vs 7%). Whereas PCR gave 13 positivities which were negative by any of the conventional methods, it was negative in 2 samples that were positive on cytology only.

Conclusion: Though conventional diagnosis remains the method of choice in tuberculous lymphadenitis alternative diagnostic method such as PCR which are more rapid and reliable looks promising and help well manage patients.

P28

Spectrum and prognostic significance of parasitic infection in ART naïve HIV patients

G Padmjakshi^{*}, S Saini

Department of Microbiology, Rural Medical College, Loni, Maharashtra, India
E-mail: paddu.mib@gmail.com

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Background: It is a well-known fact that HIV/AIDS patients are vulnerable to many diseases which result in serve morbidity and mortality. The

purpose of the study is to assess the prevalence and pattern of intestinal parasites in ART naïve patients.

Methods: Field sampling started from July 2012 to July 2013 which included a total of 163 HIV seropositive patients. After taking an informed consent and recorded CD4 date, stool samples were collected for screening of parasites. Two stool samples were collected in two vials, one with formalin and other with polyvinyl alcohol (PVA). Saline wet mount and Lugol's iodine mount was done by direct microscopy and a formalin ether concentration method to detect ova, trophozoites, cysts, and larvae of intestinal parasites.

Results: A total of 57 (34.9%) patients were found positive from 163 newly registered HIV patients. The prevalence of parasitic infection was more in the age group of 31-40 years in males, where as in females 21-30 years. The most common isolate was Hook worm (29, 50.8%) followed by *Isoospora* (13, 22%) *Cryptosporidium* (9, 15.7%), *E. histolytica* (5, 8.7%), and *Giardia intestinalis* (1, 1.75%). Most of the cases were asymptomatic and the CD4 count was $\leq 350 \text{ mm}^3$. Coccidian parasites always presented diarrhea and had a CD4 count of $<200 \text{ mm}^3$. In this study the most presenting symptoms were constipation followed by weight loss and anemia.

Conclusion: This high spectrum of infection before ART concludes that early screening should be made mandatory and lower CD4 counts mostly associated with presence of coccidian parasites with diarrhea as presenting symptom.

P29

Metal complexes as therapeutic substitute in tuberculosis treatment

C Justin Dhanaraj^{1*}, M Sivasankaran Nair²
¹Department of Chemistry, University College of Engineering Nagercoil, Anna University, Tirunelveli Region, Tamil Nadu, 629004, India; ²Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, 627012, India

E-mail: cjustindhanaraj@gmail.com

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Background: Tuberculosis (TB) is a disease that produces several million deaths annually. With the appearance of multi drug resistant microbial strains of *Mycobacterium tuberculosis*, innovations in TB drug discovery and evolving strategies to bring new agents with best performance is an essential investigation. Taking this into account, there is a pressing need to develop new and more effective anti tubercular agents. The coordination of metal with organic drugs is a promising strategy that has been successful in many cases with different pharmacological activities. The emergence of new cases and the adverse effects of first and second-line antituberculosis drugs have led to renewed research interest in metal drug complexes in the hope of discovering new antituberculosis drugs. The aim of the present study is to assess the antituberculosis activity of Ni (II) and Cu (II) complexes of polymer ligand poly (3-nitrobenzylidene-1-naphthylamine-co-methacrylic acid).

Methods: Antituberculosis activities of the ligand and its complexes were assessed against *M. tuberculosis* H37 Rv strain by microplate alamar blue assay (MABA) method using pyrazinamide as standard.

Results: The minimum inhibitory concentration values of the ligand and its complexes on comparison with standard (pyrazinamide; 4 µg/mL) indicate that the complexes exhibit promising antituberculosis activity. Cu (II) complex shows more activity (10 µg/ mL) followed by Ni (II) complex (20µg/ mL) and ligand (35 µg/ mL).

Conclusion: Among the synthesized compounds, the Cu (II) complex may be used as therapeutic substitute in the treatment of tuberculosis by replacing the routine drugs.

P30

Penicilliosis with lymphadenopathy mimicking tuberculosis

Biswadeep Borthakur^{1*}, Lahari Saikia¹, Dhrubajyoti Borah², Sreemanta Madhab Barua¹, Ratan Kumar Kotokey¹, BB Rewari², Partha patim Das¹, Divya Singh¹

¹Assam Medical College, Dibrugarh, Assam, India; ²National AIDS Control Organization, India

E-mail: biswadeepb@yahoo.co.uk

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Background: In India, although the maximum cases of penicilliosis caused by *Penicillium marneffei* have been reported from Manipur, cases in the

bordering states like Assam and Nagaland are not very rare. From past 2 years we have been receiving significant number of cases at Assam.

Methods: We describe two similar cases of disseminated penicilliosis presenting with fever and weight loss with multiple mesenteric lymphadenopathy and skin lesion, clinically mimicking extrapulmonary tuberculosis. Penicilliosis was diagnosed by usg guided FNAC of mesenteric LN. *P. marneffei* was confirmed by microscopy and cultured, onto sabouraud dextrose agar. The cases were treated with oral itraconazole along followed by HAART two weeks later. However the lymph nodes enlarged (IRIS), so we withheld HAART for 2 weeks continuing itraconazole. After one and half months of treatment the skin lesions healed and mesenteric Lymph-Nodes disappeared completely with significant weight gain.

Conclusion: Many cases of disseminated penicilliosis have been misdiagnosed as tuberculosis, where the fungal disease is prevalent. Both infections have similar symptomology. Perhaps these cases of disseminated penicilliosis with IRIS which responded only with itraconazole without using Amphotericin-B. Itraconazole can be a safer option than amphotericin-B HIV with lymphadenopathy, penicilliosis has to be brought into consideration in endemic area.

P31

Comparison of rapid methods PBP2' detection, ORSAB and mecA for detection of methicillin-resistant Staphylococcus aureus (MRSA) in a tertiary care centre, Chennai, India

Latha Jayaraman^{1*}, Umamaheswari Subburaya²

¹Department of Microbiology, Sri Ramachandra University, Chennai, India;

²Department of Pharmacology, Sri Ramachandra University, Chennai, India

E-mail: lathaj_73@yahoo.co.in

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Background: Early detection of MRSA in clinical specimens is imperative to prevent cross transmission, morbidity, mortality and overall cost of treatment. Additionally, rapid detection of MRSA helps in preventing superficial skin infection to become deep seated.

Method: Fifty isolates of MRSA from different specimens like blood, urine, exudates and respiratory were collected from 2010 to 2011. MRSA screen slide latex agglutination test kit (Denka Seikan, Japan) was used for PBP2' detection and Oxacillin Resistant Screen Agar Base (ORSAB) with supplement (Oxoid Ltd., UK) was used for detection of MRSA from the isolates with ATCC controls. The detection of *mecA* gene in the MRSA isolates was considered as the reference method for determining the sensitivity of each phenotype rapid method studied.

Results: All the 50 isolates of MRSA were positive for *mecA* by PCR. MRSA screen for PBP2' detection and ORSAB had a sensitivity of 93.75% and 95.8%, respectively. Chi square statistical analysis was carried out for the comparison of the rapid methods. Chi square value was found to be 3.0928 (p value=0.07864) and 2.0408 (p value =0.153127) at 5% level of significance. OR (Odds ratio) =7.4421 (95%CI=0.3744-147.9331) and OR=5.2062 (95%CI=0.2436-111.2444), respectively.

Conclusion: In the present study, we found that the difference in sensitivity between PCR for *mecA* and the phenotype rapid methods is statistically not significant. Therefore, we conclude that phenotype rapid methods can be used for the detection of MRSA from clinical isolates in low resource health care settings.

P32

Distribution of *cylA*, *esp*, *asa1*, *hyl* and *gelE* virulence genes among clinical isolates of *Enterococcus faecium* and *Enterococcus faecalis*

Elango Padmasini¹, G Divya¹, M Karkuzhali¹, R Padmaraj², S Srivani Ramesh^{1*}

¹Department of Microbiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai, India;

²Department of Paediatric Nephrology, Institute of Child Health and Government Hospital for Children, Egmore, Chennai, India

E-mail: dr.srmicro@gmail.com

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Background: Enterococci are opportunistic pathogens causing severe urinary tract infections, surgical wound infections, bacteremia and bacterial endocarditis. Aggregation substance (*asa1*), hemolysin/ cytotoxin (*cylA*), gelatinase (*gelE*), enterococcal surface protein (*esp*), hyaluronidase (*hyl*) and

biofilm formation are the major virulence determinants responsible for pathogenicity of enterococci. This study was undertaken to determine virulence factors among clinical *Enterococcus* species by phenotypic and molecular methods.

Methods: A total of 157 enterococcal isolates obtained from various tertiary care hospitals were characterized by standard biochemical methods. Virulence factors such as gelatinase, cytolysin production by plate method and biofilm formation on 96-well microtitre plate were performed for all the isolates. The presence of *cylA*, *esp*, *asa1*, *hyl* and *gelE* genes specific for virulence were analyzed by multiplex PCR with appropriate primers and cycling conditions.

Results: *Enterococcus faecium* (84/157) was the predominant species obtained, followed by *E. faecalis* (73/157). 72/157(45.85%) strains were positive for hemolysin, 61/157(38.85%) gelatinase, 16/157(10.19%) strong and 66/157(42%) were moderate biofilm producers phenotypically. PCR results showed, 37/157(23.56%) *cylA*, 81(51.59%) *gelE*, 10(6.36%) *hyl*, 87 (55.41%) *asa1* and 78(49.68%) isolates were positive for *esp* genes. Only 25/72, 32/61, 6/16 and 7/16 isolates were phenotypically and genotypically positive for *cylA* +hemolysin, *gelE* +gelatinase, *esp* +biofilm and *asa1* +biofilm, respectively. Interestingly, *E. faecalis* carried multiple virulent genes (>4 genes) when compared with *E. faecium* among our study isolates.

Conclusion: Hemolysin and gelatinase were the predominant virulence factors expressed phenotypically whereas, *asa1*>*gelE*>*esp* were the predominant genes observed. Majority of *E. faecalis* isolates were strong and *E. faecium* were moderate biofilm producers.

P33

Proportion and determinants of adherence to antiretroviral therapy among HIV positive people registered under ART centre in South India

V Hiregoudar¹, B Raghavendra^{1*}, K Ramesh¹, ARB Sameena¹, K Hemagiri¹, S Basavaraj¹, T Gangadhara Goud¹, K Aravind², W Khan¹

¹Vijayanagara Institute of Medical Sciences Bellary, Karnataka, India; ²Mysore Medical College, Mysore, Karnataka, India

E-mail: bellararaghu@gmail.com

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Background: As on December 2012, there are about 6 lakh HIV positive people in India and around 83,000 HIV positive people in Karnataka are on ART. Among PLHIV, ART adherence is the second strongest predictor of progression to AIDS and death, after CD4 count. Consistently a very high level of adherence (> 95%) is required for ART to be effective on long term and to prevent the emergence of resistant viral strains and co-morbidities.

Methods: A case series study was undertaken at ART centre, Bellary from June 2012 to May 2013. HIV positive subject aged above 15 years on ART for more than 6 months were included. Non probability purposive sampling was adopted. Data was collected by interviewing; treatment adherence was assessed by one week recall method.

Results: A total of 536 HIV positive people were studied and out of which 67% [95%CI (62.89%- 70.83%)] of them reported >95% adherence to treatment. On univariate analysis factors like marital status, residential area, side effects to drugs, support of friends and family, knowledge regarding ART and tobacco usage had significant association with adherence ($p < 0.05$). On logistic regression marital status (OR=1.591), area of residence (OR=1.939), side effects to drugs (OR=11.415), support of friends and family (OR=9.374), knowledge regarding ART (OR=2.344) and tobacco (OR=0.484) were independent factors influencing adherence.

Conclusion: Socio demographic factors like marital status, residential area, personal factors like support of friends and family, adequate knowledge about ART and not using tobacco were found to show high level of ART adherence.

P34

Identification of *Corynebacterium jeikeium* from a CAPD patient by 16S rDNA PCR – a case report

Gopalakrishnan Sathya Narayanan^{1*}, Radha Vijayaraghavan², Thangam Menon¹

¹Department of Microbiology, Dr.AL Mudaliar Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai, India; ²Department of Nephrology, Southern Railway Hospital, Perambur - ICF, Chennai, India

E-mail: shathyaag@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):P34

Background: Peritonitis is a serious complication in patients who are on continuous ambulatory peritoneal dialysis (CAPD). We report a rare case of peritonitis caused by *Corynebacterium jeikeium*.

Case report: A 51-year-old male was admitted in Southern Railway Hospital, Chennai with complaint of abdominal pain for four days. He had a history of renal failure two years before admission and was started on CAPD for end stage renal disease. Prior to admission he was on ambulatory peritoneal dialysis (APD). Peritoneal dialysis (PD) fluid was collected and transported to laboratory aseptically. Peritoneal fluid was cloudy at the time of collection. It was inoculated into BHIB culture bottles which were incubated at 37° C and inspected every day for turbidity. DNA was extracted and analyzed for presence of 16S rDNA by PCR. The bacterial culture and identification was done as per standard procedures. Antibiotic susceptibility test was performed for the isolate. DNA was extracted from the colonies and analyzed for the presence of 16S rDNA by PCR and amplicons were sequenced (Xcelris Laboratories, Ahmedabad).The 16S rDNA FASTA sequence was subjected to BLAST analysis and it was confirmed as *Corynebacterium jeikeium*. (Genbank Accession No. KF909134). The isolate was found to be multi drug resistant and sensitive only to vancomycin. The patient responded well to therapy.

Conclusion: Precise and rapid identification of these potentially multi drug resistant organisms are important for patient management. 16S rDNA PCR was found to be a useful tool in establishing etiology in cases of infections caused by rare pathogens.

P35

Clinical study of cardiac autonomic dysfunction in HIV patients

Pradheep C Mathew^{*}, Shishir Nagesh Duble

Department of General Medicine, Rajiv Gandhi University, AJ Institute of Medical Sciences, Mangalore, Karnataka, India

E-mail: elroyamat@hotmail.com

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Background: Cardiovascular autonomic nervous dysfunction has been demonstrated to severely debilitate HIV infected patients, namely by postural hypotension and syncope. It has important implication in health care of HIV patients. Presence of autonomic neuropathy signals the need for added precautions when invasive procedures are performed on HIV patients.

Methods: Fifty patients (25 HIV +ve without AIDS and 25 HIV+ with AIDS) who fulfilled the inclusion and exclusion criteria and 50 healthy matched controls were enrolled in the study. All HIV positive/AIDS patients were evaluated according to a detailed proforma with elicitation of history, symptoms, signs and routine and specialized investigations.

Results: In the present study, 16% of HIV +ve with the AIDS had abnormal autonomic dysfunction and 4% of HIV positive without AIDS had abnormal autonomic dysfunction. Reduced heart rate variability is the commonest manifestation of autonomic dysfunction noted in both HIV positive without AIDS and HIV positive with AIDS groups. Diastolic BP response to sustained handgrip has a limited role in discriminating autonomic function in HIV infected patients. There is no statistically significant correlation with the CD4 level and the presence of autonomic nervous system dysfunction in both the groups.

Conclusion: Cardiac autonomic nervous dysfunction is a common and relevant clinical problem affecting both HIV positive without AIDS and HIV positive with AIDS groups. It may provide an alternative explanation for symptoms commonly observed in HIV infected individuals such as bowel and bladder dysfunction, impotence, syncope and sweating abnormalities.

P36

Serological evidence of *Coxiella burnetii* as a causative agent of culture negative infective endocarditis

Angelina Raghavendran^{*}, Venkatasana Naveen Kumar,

Gopalakrishnan Sathya Narayanan, Thangam Menon

Department of Microbiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai, Tamil Nadu, India

E-mail: angeragh@yahoo.co.in

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Background: Culture negative infective endocarditis (CNIE) accounts for 2.5 to 31% of all cases of infective endocarditis and is responsible for delay in diagnosis and treatment with profound impact on clinical outcome. *Coxiella burnetii* is one of the important etiological agents of culture negative infective endocarditis. The present study was undertaken to determine the role of *C. burnetii* as an etiological agent in CNIE in South India.

Methods: Out of a total of 124 patients with CNIE during the period from 2008 to 2012, 46 were selected for the present study and stored serum samples were analysed by a qualitative ELISA to detect the IgG antibodies against *C. burnetii* using ELISA Kit (NovaTec Immundiagnostica GmbH, Germany).

Results: Among the 46 patients included in the study, 26 (57%) were male and 20 (43%) were female with mean age of 30 years in the range of 11-74 years. Out of the 46 patients screened for Phase I IgG class antibodies, 22 patients were found to be positive by ELISA. Among the 22 positive patients, majority of patients (90%) had native valve endocarditis predominantly of the mitral valve (60%). All the 22 patients presented with fever; about 64% of patients had rheumatic heart disease and 68% of them showed vegetation by echocardiography.

Conclusions: Screening of all CNIE patients for *C. burnetii* Phase I IgG antibodies will help in initiating appropriate treatment and in bringing down the case fatality rates in culture negative infective endocarditis in India.

P37

Protective role of Mannose binding Lectin (MBL2) promoter haplotypes on TB infection in South Indian HIV-1 patients

Kalaimani Pandian¹, Stalinrja Maruthamuthu¹, Suresh Madasamy², Jayalakshmi Mariakuttikan¹

¹Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai- 625021, India; ²ART Centre, Govt. Theni Medical College and Hospital, Theni- 625512, India

E-mail: jayalakshmim@genomicsmku.org

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Background: Mannose binding Lectin (MBL) mediates protection against infections by activating the complement system, but certain microorganisms may increase infectivity by exploiting this host defence system. Hence, the purpose of this study is to evaluate MBL genetic variants with the development of TB infection caused by *Mycobacterium tuberculosis* among HIV patients.

Methods: Blood samples from TB+ART+ and TB-ART+ (n=30) were collected. Genomic DNA was extracted from Peripheral Blood Mononuclear Cells (PBMC) using salting out procedure. MBL promoter haplotypes of -550 H/L and -221Y/X associated with high, medium and low (HY, LY and LX) secretion was assessed by PCR-SSP.

Results: The Promoter haplotype (LY/LX) associated with deficient MBL levels conferred a protective role to TB in our study population with a significant difference (Chi-square (χ^2) =4.00; p<0.05).

Conclusion: In this study, we could observe MBL2 promoter haplotypes with low MBL secretion may play a protective role to intracellular mycobacterium infections like TB in HIV seropositive individuals.

P38

The length of stay and the hospital cost for ICU patients with resistant bacterial infections: a pilot study

Asim Priyendu¹, Nagappa Anantha Naik^{1*}, Muralidhar Varma², Kalwaje Eshwara Vandana³, Balakrishnan Rajesh⁴

¹Manipal College of Pharmaceutical Sciences, Manipal University, Karnataka, India; ²Department of Medicine, Kasturba Medical College & Hospital, Manipal University, Karnataka, India; ³Department of Microbiology, Kasturba Medical College, Manipal University, Karnataka, India; ⁴School of Pharmacy, University of Michigan, MI, USA

E-mail: anantha1232000@gmail.com

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Background: Healthcare in India comes mostly as an out of the pocket payment as health insurance is virtually absent for majority of the population. Determination of the typical hospital cost and length of stay (LOS) are of decisive importance for patients, healthcare providers, and

payers who need to make evidence based rational decisions about patient care and the allocation of resources.

Methods: A cross sectional pilot study was performed and the data for LOS and hospital cost was collected from the medicine ICU and the accounts section for 38 patients with resistant bacterial infections who were admitted to intensive care unit. The data was analyzed for average LOS and hospital cost for infections. The data was analyzed using univariate analysis.

Results: Almost 29% of the patients with resistant infections were between 18-40 years of age. The mean cost of hospitalization was found to be INR76082.79 and only 34% of the patients were having any insurance coverage. The median length of stay in the hospital ICU was 8.5 days. 37% of the patients were on ventilator during the hospitalization and 25% of the total patients died.

Conclusion: Resistant bacterial infections may lead to a high LOS in the ICU furthermore adding to the economic burden of the patient, healthcare provider and the payer. These results can be used to study the treatment alternatives in case of resistant infections and making them cost effective at the same time stressing upon the need of health insurance among the population.

P39

Hepatitis C virus seropositivity and genotyping by RealTime PCR in a tertiary care hospital in Southern India

SR Ramya^{*}, M Kulkarni, GS Vijay Kumar

Department of Microbiology, JSS Medical College, Mysore, Karnataka, India

E-mail: ramyasr121186@gmail.com

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Background: Hepatitis C virus is one of the major causes of parenterally acquired hepatitis. HCV genotypes exhibit different profiles of pathogenicity, infectivity and response to antiviral therapy. The study was taken up to detect HCV seropositivity and common genotype prevalence in a tertiary care hospital. The other objectives of the study were to detect probable risk factors and HIV/HBV co infections.

Methods: The duration of study was 2 years. Adult patients clinically suspected of hepatitis, blood donors attending blood bank, pregnant women attending ANC clinic, renal dialysis patients and patients whose blood samples were received for pre-operative evaluation, were the subjects of the study. Serum was subjected to ELISA for anti-HCV antibody detection. Plasma collected from seropositives, genotyped by RealTime PCR. In accordance with prevalence of seropositivity same number of controls were randomly selected and analysis done for association with risk factors using Chi square test.

Results: Of the 3581 serum samples screened, 32 were anti-HCV antibody positive (0.89%). Highest number of HCV seropositivity found between 41-50 years (40.62%). Blood transfusion was found to be highly significant risk factor (p value 0.004). Rate of HCV-HBV co infection was 6.25% (2/32) and HCV-HIV co infection was 3.12% (1/32). No concomitant HCV, HIV and HBV infection was detected. 12 samples were HCV Genotype 1(60%), 8 were Genotype 3 (40%) and 5 were untypeable.

Conclusion: Blood transfusion is a major risk factor for HCV infection and genotype 1 predominates over genotype 3 in this region.

P40

High diagnostic yield of line probe assay as a point-of care test in a TB rural setting

Aarti Kotwal^{*}, Rajiv Kumar Agarwal, Biswaroop Chatterjee, Barnali Kakati, Pronoti Sarkar, Sudhir Singh, Dolly Pokhriyal, Bhupendra Singh Chauhan

Department of Microbiology, Himalayan Institute of Medical Sciences, Jolly Grant, Dehradun, India

E-mail: aartiraghuvanshi@yahoo.co.in

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Background: Tuberculosis is the second leading cause of death due to infectious disease worldwide. Delay in bacilli isolation in culture, cumbersome susceptibility testing methods, lack of methods for differentiation of *Mycobacterium tuberculosis* (MTB) from non tuberculous mycobacteria, are important issues which impinge on the WHO's millennium goal of disease reduction by 2015. Traditional methods of TB detection and anti TB drug

susceptibility assays are time consuming and with a steep rise in number of MDR TB cases the need of the hour is rapid diagnostic methods like direct drug susceptibility testing in liquid medium, molecular hybridization methods and RealTime PCR.

Methods: This study was conducted to assess the efficacy of line probe assay in detecting MTB from smear negative culture negative samples in a tertiary care centre catering to the need of a high burden TB population of Uttarakhand.

Results: Between January 2013 and November 2013 a 22.7% positivity rate of sputum samples by fluorescent staining was reported. Culture by MGIT (Mycobacterium growth indicator tube) of 162 smear negative samples from patients clinically or radiologically pulmonary TB suspects was performed and 78(48.1%) samples grew MTB. Further, 50 samples which were smear negative and MGIT negative from patients with strong suspicion of pulmonary tuberculosis were tested by line probe assay and 34(68%) samples turned out to be positive.

Conclusion: Newer methods offer definite improvements in the ability to detect MTB. However, there is a strong need to deploy these tests in areas where MTB burden is highest.

P41

Serological response to pandemic influenza A/H1N1 2009 and seasonal influenza A/H3N2 among health care workers (HCWs) in JIPMER, Puducherry

G Nandhini, S Sujatha*

Department of Microbiology, JIPMER, Puducherry, India

E-mail: sujathasistla@gmail.com

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Background: Serum antibody titers against influenza viruses are regarded as markers of partial or complete immunoprotection. Antibody titer of ≥ 40 is associated with at least a 50% reduction in risk for infection or disease.

Methods: Serum was collected from 138 HCWs including laboratory personnel, doctors and nurses working at JIPMER, Puducherry during August - October 2013. Details of influenza vaccination and laboratory confirmed influenza infection were noted. Hemagglutination inhibition assay was performed to determine the serum antibody levels against WHO reference antigens - pandemic influenza A (H1N1) A/California/07/2009, seasonal influenza A (H3N2) A/Victoria/361/2011.

Results: Fifty of the HCWs had received pandemic influenza vaccination at least once in the previous three years. All HCWs had antibody titers ≥ 40 against seasonal influenza A/H3N2. Antibody titers against pandemic influenza A/H1N1 2009 ranged from ≤ 20 (16.7%) to 320 with 36.9% showing borderline protective titers (≥ 40). Six of the vaccinated HCWs had non-protective antibody titers while 71 unvaccinated HCWs showed protective titers. Only one HCW developed seasonal influenza A/H3N2 infection despite having borderline protective antibody titer of 40 and his convalescent serum sample after two weeks showed fourfold rise in titers.

Conclusion: This study showed protective antibody levels against pandemic influenza A/H1N1 2009 and seasonal influenza A/H3N2 among a large percentage of HCWs, regardless of vaccination status, which may be the primary reason for the low incidence of influenza cases encountered this year.

P42

Study of treatment outcome of patients of tuberculous cervical lymphadenopathy

Nisha Mathew*, Sunil Jadhav, Ashish Deshmukh, Hafiz Deshmukh,

Shivprasad Kasat, Sandeep Dandin

Mahatma Gandhi Mission's Medical College & Hospital, N-6 CIDCO, Aurangabad, Maharashtra, India

E-mail: unninishamathewcm@gmail.com

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Background: Tuberculosis lymphadenopathy is the most common form of extrapulmonary tuberculosis. The aim of the present study is to detect the duration of treatment required in patients with cervical lymphadenopathy.

Methods: The patients attending the Department of Pulmonary Medicine OPD with cervical lymphadenopathy were screened. Detailed history,

clinical examination, USG neck, FNAC, Mantoux test, biopsy, and chest X ray frontal view were performed and anti tuberculosis treatment initiated.

Results: Total patients examined: 117, in the age group 10-70 years (mean age 40). Females affected - 89 (76.07%), Males - 28 (23.93%). Most common age group affected (M/F): 15-20 years: 37 (31.63%) patients. Biopsy was done in 73 patients (62.39%). Patients received Category-1ATT under RNTCP. After 6 months of therapy, 22 patients (18.81%) stopped treatment in view of non-palpable mass. Out of remaining 95 patients (81.19%), 14 patients' (11.96%) lymph node size increased with slow regression, 17 patients' (14.52%) liquefaction and repeated aspiration was done, 57 patients' (48.72%) lymph node size showed regression less than 50%, 7 patients (5.98%) came up with new group of lymph nodes with size gradually increased. For all 95 patients, therapy was extended (continuation phase) for 2-3 months. 7 patients received ATT for 12 months.

Conclusion: Extrapulmonary tuberculosis like tuberculous lymphadenopathy is benefited with extension of anti tuberculosis treatment.

P43

Rapid speciation of mycobacteria with simultaneous detection of MDR TB

Barnali Kakati*, Rajiv Kumar Agarwal, Biswaroop Chatterjee, Aarti Kotwal,

Pronoti Sarkar, Sudhir Singh, Victor Masih, Moirangthem Ajit Singh

Department of Microbiology, Himalayan Institute of Medical Sciences, Dehradun-248140, India

E-mail: drbarnali05@rediffmail.com

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Background: Multidrug resistance (MDR) TB is on the rise globally and is associated with significant morbidity and mortality. Conventional culture based drug susceptibility testing (DST) for TB drugs is time consuming, leading to diagnostic delays contributing to higher TB morbidity and mortality and exacerbation of ongoing transmission. Moreover most of the NTM(Non tuberculous mycobacteria) that are resistant to common anti TB drugs, can be falsely labeled as MDR TB if not identified correctly. This study was aimed to identify *Mycobacterium* species and to determine the prevalence of multidrug resistant tuberculosis (MDR TB) by line probe assay.

Methods: This study was done on 76 clinical isolates collected from TB patients who were on Anti tuberculous drug treatment since last six months but without any clinical improvement. Line probe assay was performed for detection, identification of species and drug susceptibility status.

Results: Line probe assay detected 49 isolates (96%) of *M. tuberculosis*, 1 isolate (1.96%) of *M. avium* and *M. intracellulare* each. Out of 49 isolates of *M. tuberculosis* 9(18.4%) were Rifampicin resistant and 11(22.4%) INH resistant but what was alarming was that 9 isolates (18.4%) were MDR.

Conclusion: The majority of multidrug resistant TB cases are due to ongoing transmission of multidrug resistant strains. This is most likely the result of diagnostic delay, thereby emphasizing the need for rapid diagnostics and line probe assay can be used for the rapid identification of *Mycobacterium* species and the determination of susceptibility to drugs, which may accelerate the time to effective MDR TB treatment.

P44

Prevalence of virulence genes among VRE genotypes and their association with clinical outcome

Ramamurthi Arularasi Aberna^{1*}, Kesani Prabhakar²

¹Rajah Muthiah Dental College and Hospital, Annamalai University,

Annamalai Nagar, India; ²Rajah Muthiah Medical College and Hospital,

Annamalai University, Annamalai Nagar, India

E-mail: arularasiaberna@gmail.com

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Background: The dramatic upsurge in the incidence of VRE worldwide had contributed serious concerns in early and specific diagnosis, infection control measures and treatment. There exists a paucity of information regarding VRE genotypes and their virulence genes contributing to disease severity in India. This study determines the prevalence of VRE and the role of four virulence genes in their clinical outcome.

Methods: Enterococci were isolated from a total of 2500 clinical specimens and species identification was done based on conventional methods. VRE were confirmed by agar dilution (vancomycin and teicoplanin) and by the

presence of *vanA/vanB* genes. The presence of *esp*, *agg*, *gelE* and *cylA* gene among VRE were detected by PCR.

Results: VRE were isolated from 7.3% of clinical specimens. VR *E. faecalis* and VR *E. faecium* were primarily derived from UTI and BSI (56.7% and 43.3%) respectively. Among the VRE isolates, 80% of *vanA* genes were noted in *E. faecium* and 64% of *vanB* genes were observed in *E. faecalis*. Most of VR *E. faecalis* harboured 2 virulence genes (38.5%) and VR *E. faecium* harboured one virulence gene (62.5%). The ability to cause invasive infections increased with the number of virulence genes harboured by *E. faecalis*. An association was observed between the presence of virulence gene *esp*, *cylA* and *agg* with UTI, BSI and IAP infections respectively among the VRE isolates.

Conclusion: Isolation of VRE harbouring *vanA* and *vanB* gene gains significance as they are transmissible to co infecting/ resident organisms and to implement infection control measures.

P45

Risk assessment studies on HLA association with drug induced hypersensitivity caused by anti tuberculosis drugs – an *in silico* approach

Prashant Saxena*, Jemmy Christy

Department of Bioinformatics, Sathyabama University, Chennai-600119, India

E-mail: prashant347@gmail.com

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Background: Studies have conferred that there is a strong genetic association between HLA alleles and susceptible drugs leading to hypersensitivity. Patients with HIV have the highest risk of developing active Tuberculosis. So here we studied the genetic predisposition to anti-tuberculosis drugs as a model to study the pathological role of HLA alleles in drug hypersensitivity.

Method: Here we aimed to find out the anti-tuberculosis drugs like sulfasalazine, allopurinol, streptomycin and ofloxacin as candidate drugs and their binding sites on HLA alleles which are prevalent among Indian population like HLA-A*02:06, HLA-B*57:01, HLA-A*02:01 and HLA-A*02:03 using docking simulation method via Autodock.

Result: Anti-tuberculosis drugs can bind within the peptide binding grooves of HLA-B*57:01, HLA-A*02:06 and HLA-A*02:03 alleles and thereby alter its specificity. In the docking simulations, the interactions were found between HLA-A*02:01 and sulfasalazine, streptomycin, ofloxacin at Asp54, Ile237, Glu236, Lys267, Glu256, Thr238 and Val255 with -6.43 kcal/mol, -5.86 kcal/mol and -5.83 kcal/mol binding energy. HLA-A*02:06, HLA-B*57:01 and HLA-A*02:03 have interaction with ofloxacin at Arg6, Lys58, Tyr51 and Arg72 with binding energies of -5.72 kcal/mol, -5.79 kcal/mol and -5.15 kcal/mol respectively.

Conclusion: The ultimate translation of this knowledge of how Anti-tuberculosis drugs interact with HLA would be applicable to preclinical drug screening programs to improve the safety and cost effectiveness of drug design and development.

P46

Is PCR better than culture in TB diagnosis: myth or reality?

Ruqaiyah Johar*, Kiran Chawla, Chiranjay Mukhopadhyay

Department of Microbiology, Kasturba Medical College, Manipal University, Manipal, India

E-mail: dr.rukki@gmail.com

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Background: Polymerase chain reaction (PCR) is emerging as a new diagnostic tool for tubercular infections. Studies indicate variable results in relation to its efficacy making it as an investigation which is operator dependant. It still remains a dilemma to choose between PCR and culture as the most dependant and reliable diagnostic test. The purpose of our study was to determine the efficacy of PCR in diagnosis of pulmonary and extra-pulmonary tuberculosis and its comparison with conventional culture.

Methods: In this observational study, 50 pulmonary and 50 extra-pulmonary specimens from suspected tuberculosis patients were included. All samples after initial processing were subjected to microscopy, conventional culture and PCR.

Results: For pulmonary specimens, positivity of smear, culture and PCR were 56%, 72% and 74%, respectively. Sensitivity and specificity of PCR

was found to be 89% and 71% respectively. For extra-pulmonary specimens, positivity of smear, culture and PCR were 36%, 58% and 78%, respectively. Sensitivity and specificity of PCR was found to be 83% and 29% respectively (with culture as gold standard) whereas it was 92% and 67%, respectively (with clinical diagnosis as gold standard). PCR facilitated additional detection of 4% and 20% pulmonary and extra-pulmonary TB cases respectively than by conventional culture. Results of PCR were obtained in 6-8 hours as compared to 6-8 weeks by conventional culture.

Conclusion: The study highlights PCR as a better diagnostic tool in detection of tuberculosis. Early results of PCR help in early institution of anti tubercular treatment and thus controlling the spread of disease.

P47

Anti-Herpes Simplex Viruses activity of *Camellia sinensis*, member of the family Theaceae (green tea)

Govindharaj Deepika, Hariharan Durgadevi, Rohan Narayan,

Munisamy Sudanthira, Elanchezhian Manickan*

Department of Microbiology, Dr. ALM PG IBMS, University of Madras, Chennai, India

E-mail: emanickan@yahoo.com

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Background: Medicinal plants were shown to possess several bioactivities of which *Camellia sinensis*, a popularly used refreshing beverage has been reported to have immune modulating and anti viral activities. From our lab we had already shown that *C. sinensis* extracts possessed anti-HIV and anti-HBV activities. In the current study we evaluated its anti-HSV activities.

Methods: Aqueous and methanolic extracts of *C. sinensis* were tested for their anti-HSV property by CPE reduction assay on Vero cell monolayers. Cytotoxicity of the extract was measured by MTT assay.

Results: Experiments showed that 12 µg/mL concentration of *C. sinensis* completely inhibited HSV-1 and 2 and the drug was found to be non toxic up to 10 mg/mL.

Conclusion: The study showed that *C. sinensis* extracts and EGCG compound are potent inhibitor of both HSV-1 and HSV-2 infection and it was found to be relatively non toxic.

P48

Syphilitic abdominal aortic aneurysm – a rare case report

R Manipriya*, V Sudha

Institute of Venereology, Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai, India

E-mail: rajanmanipriya@hotmail.com

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Background: The incidence of cardiovascular involvement in late untreated syphilis is about 10%. Syphilitic aortic aneurysm is rare since the era of antibiotics.

Case report: A 50 year old male patient presented with abdominal pain radiating to the back for the past 6 months. He had giddiness, paroxysmal nocturnal dyspnoea. General and cardiovascular system examination showed no evidence of aortic regurgitation. Expansile epigastric pulsation with thrill was felt. Bruit was heard. No lesion or scar on genital examination.

The routine parameters were normal. VDRL was reactive in 512 dilutions, TPHA positive, ELISA for HIV 1 and HIV 2 was negative. CT angiogram showed fusiform aneurysmal dilatation of descending aorta of length 11.6 cm with anterior wall irregularity from D11 to L2 vertebra. Coeliac and superior mesenteric arteries were seen arising from the aneurysm. Aortic arch and ascending aorta were normal. The patient was advised bed rest and treated with injection procaine penicillin 1.2 million units deep i.m. after test dose for twenty days under cover of steroids. Follow up showed decreasing VDRL titres. Abdominal aortic aneurysm accounts for less than <5% of all syphilitic aneurysms. It may be misdiagnosed as atherosclerotic aneurysm. Hence serological testing is very helpful in patients diagnosed with aneurysms. CT angiogram helps to define the size and anatomy of aneurysm.

Conclusion: This case is being reported for its rarity in the post antibiotic era and to emphasize the importance of early diagnosis and treatment to prevent complications.

P49

Disseminated cysticercosis in a child: a rare occurrence presenting as meningitis

A Arunkumar*, I Vivian Praveen, HV Prajwala, Asha Benakappa
Department of Pediatrics, Bangalore Medical College and Research Institute, Bangalore, India

E-mail: arun.exl@gmail.com

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Background: Neurocysticercosis, a parasitic disease is one of the commonest causes of focal convulsions. Disseminated cysticercosis (DCC) is amongst its rarest manifestations. Fewer than 50 cases have been described worldwide with less than 10 in children. We report a 9 year old female child with the disease presenting as meningitis.

Case report: A 9 year old female child presented with fever, headache, myalgia for 15 days, vomiting and altered sensorium for 2 days. She was well nourished, disoriented, febrile, had meningeal signs. CSF examination was unremarkable. CT brain was normal. She was treated as viral meningo-encephalitis and improved. Her subsequent admission with similar complaints showed grossly elevated CSF proteins and lymphocytes, MRI brain showed solitary ring enhancing lesion in brain and treated as tuberculous meningitis with tuberculoma. She did not show improvement, presented back with multiple nodules all over the body. Ultra-sonogram revealed them to be subcutaneous and muscular cysticercoses. MRI showed disseminated cysticercoses involving all areas of brain. She was immunocompetent. She was started on oral prednisolone followed by albendazole for a month. She showed improvement, nodules subsided, had convulsions in follow-up visits, and is on regular anticonvulsants.

Conclusion: The main features of DCC include intractable epilepsy, dementia, muscular enlargement, subcutaneous nodules and absence of focal neurological signs. Our case is remarkable because of combination of disseminated neuro and myo-cysticercosis and meningitis. Short duration of complaints with widespread dissemination in an immunocompetent child necessitates further understanding on the disease process and warrants suspicion of cysticercal meningitis in endemic areas.

P50

Peripheral arterial disease in antiretroviral therapy naïve HIV infected patients – an experience from eastern India

Dibbendhu Khanra^{1*}, Arunansu Talukdar¹, Manjari Saha¹, Ashutosh Dey²

¹Department of General Medicine, Medical College and Hospital, Kolkata, India; ²Department of Radiodiagnosis, Medical College and Hospital, Kolkata, India

E-mail: ddk3987@gmail.com

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Background: HIV induced endothelial dysfunction leads to premature atherosclerosis which may manifest as peripheral arterial disease (PAD) of lower limbs. Studies are not available on prevalence of PAD among antiretroviral therapy (ART) naïve HIV infected population. Our objective was to explore the prevalence of PAD among HIV seropositive cases, and to determine relation of PAD with HIV infectivity and its correlation with CD4 count.

Methods: This hospital based cross sectional study included 100 consecutive newly diagnosed HIV seropositive ART naïve cases (age 20-49 years) and 100 age and sex matched HIV seronegative controls. PAD was diagnosed by Doppler study of lower limb arteries.

Result: Prevalence of PAD was significantly more among HIV infected cases (71%) in comparison to HIV negative controls (p <0.001). Among the cases of PAD, 53% (38/71) patients were asymptomatic. Active tuberculosis was present in 25% (18/71) of HIV infected PAD patients. The mean CD4 count among the HIV positive PAD cases was 220 cells/ dl. HIV seropositivity was found to be independently contributing to development of PAD. Male gender, concurrent tuberculosis and low CD4 count came out to be individually contributing to PAD among HIV sero-positive cases in multivariate analysis.

Conclusion: Prevalence of symptomatic and asymptomatic PAD was high in the ART naïve HIV-infected cases of relatively younger age group. HIV infected patients should undergo evaluation for lower limb peripheral arterial diseases in appropriate background. Larger population based

prospective study involving HIV seropositive patients would enlighten the temporal association of PAD and other cardiovascular morbidities.

P51

Prevalence of SCCmec types among Methicillin resistant Coagulase negative Staphylococci isolated from HIV patients in Chennai, South India

Murugesan Saravanan¹, Betsy S Dass¹, Sankar Baby Abirami¹, Jaya Kumar Suriakumar^{2,3}, Padma Krishnan^{1*}

¹Dr. ALM PG IBMS, University of Madras, Taramani, Chennai, India; ²Govt. Hospital of Thoracic Medicine, Tambaram, Chennai, India; ³Institute of Microbiology, Madurai Medical College, Madurai, India

E-mail: padma.abpkn@gmail.com

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Background: Methicillin resistant Coagulase negative staphylococci (MRCoNS) are opportunistic pathogens among HIV-AIDS patients and serve as a large reservoir of Staphylococcal Cassette Chromosome *mec* (SCCmec) that carries *mecA*. Very little is known about the prevalent SCCmec types among MRCoNS from HIV patients and its molecular epidemiology. Hence, this study was aimed at determining the distribution of SCCmec elements amongst MRCoNS isolates from HIV infected patients from Chennai, South India.

Methods: 52 clinically significant MRCoNS isolates from HIV patients were included in this study. Following speciation, isolates were subjected to antibiotic susceptibility testing and screening for methicillin resistance using cefoxitin disc (30µg) and confirmed by *mecA* gene PCR. The presence of SCCmec types (I - V) was determined using multiplex PCR.

Results: Of the 52 isolates, *S. haemolyticus* (n =29, 56%) was predominant followed by *S. epidermidis* (n =13, 25%), *S. capitis* (n=4, 7%), *S. lugdunensis* (n =3, 6%) and *S. hominis*, (n =3, 6%). Highest resistance was shown towards ciprofloxacin (44%) followed by erythromycin (42%) and ofloxacin (39%). SCCmec typing revealed type I (n=26, 50%) to be predominant followed by type V (n=10, 19%), type III (n=7, 14%), type IV (n=4, 7%) and type I & V (n=2, 4%). Three (6%) isolates were nontypeable and type II was absent.

Conclusion: The MRCoNS isolates carried genetically diverse SCCmec elements including types I, V, III, IV and I and V in combination with SCCmec type I being predominant among HIV patients, which may be attributed to majority of patients being hospitalized.

P52

Characterization of TB-HIV co infected cases of Udupi district in coastal Karnataka

Parashuram Rao*, Kiran Chawla, Vishnu Prasad Shenoy, Chiranjay Mukhopadhyay

Department of Microbiology, Kasturba Medical College, Manipal, India

E-mail: parashuram.pise@yahoo.com

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Background: The rapidly increasing HIV epidemic has fuelled the problem of controlling the spread of tuberculosis. There is a lack of data showing the prevalence of HIVTB co infection from coastal areas of Karnataka. This study was planned to look for the TB and HIV co infection in the Udupi district of Coastal Karnataka and to detect the drug resistance in the study population.

Methods: 750 smear positive TB cases from Udupi district was studied for HIV co infection. The sputum samples from these cases were processed with modified Petroff's method and cultured on Lowenstein Jensen media. Drug sensitivity testing for *Mycobacterium tuberculosis* was performed by proportion method.

Results: HIV TB co infection was observed in 6.8% (51/750) of the studied population. Male:female ratio is 7.5:1. Maximum cases 29 (56.8%) were in the age group of 36-50 years. Ratio of cases in Udupi: Kundapura: Karkala Taluks was 18:22:11. 42(82.3%) cases belong to category 1 whereas 9 (17.6%) were of category 2. Multi drug resistance was detected in 3 out of 47 (5.8%) culture positive HIV TB co infected cases, in comparison to 3% of HIV negative but TB positive cases. Mortality of HIV TB co infected cases was observed in 5.8%, as compare to 1.1% in HIV negative but TB positive cases.

Conclusion: This is the cardinal study highlighting TB-HIV co infection in this coastal area. It shows greater prevalence of MDR in TB-HIV co

infected cases thereby stressing the importance of drug sensitivity testing in these patients.

P53

Study of immune reconstitution inflammatory syndrome during antiretroviral therapy in South Indian HIV patients

A Pramod Kumar^{1*}, G Parthasarathi¹, SN Mothi², VHT Swamy², AP Sudheer³, S Rajendra Prasad³

¹JSS College of Pharmacy, Department of Clinical Pharmacy, JSS University, Mysore, India; ²Asha kirana Hospital; Center for AIDS care and Research, Mysore, India; ³Vivekananda Memorial Hospital, Saragur, Mysore, India
E-mail: pramod_pharmacist@yahoo.com

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Background: Immune reconstitution inflammatory syndrome (IRIS) is a common cause of death and even more common cause of morbidity in patients started on highly active antiretroviral therapy (HAART). At present, very limited data is available about IRIS in the local population so the present study was conducted to determine the incidence, clinical manifestations, risk factors and outcomes of IRIS in south Indian HIV/AIDS patients.

Methods: Study was a prospective surveillance method to identify IRIS from two ART centers in Mysore city, India for a period of two years. HIV patients who are initiated with HAART were included for the study and were followed up for a period of six months. Data were analyzed using SPSS version 21.

Results: Out of 798 people followed 82 patients had experienced IRIS with an incidence rate of 10%. Among them 48% were unmasking and rest were paradoxical worsening. Diagnosis included tuberculosis (44%), herpes zoster (18%), cryptococcal meningitis (11%) and other opportunistic infections (27%). The major manifestation of IRIS were fever (44%) followed by lymphadenitis (36%). Statistically important predictors for occurrence of IRIS were male gender and low CD4 count. Two deaths were attributable to IRIS and 54% of them required hospitalization.

Conclusion: Patients with advanced immunodeficiency at HAART initiation are at greatest risk of developing IRIS and should be appropriately screened and monitored. Educational programs on recognizing and treating these conditions should be initiated in ART access programs.

P54

A case of lichenoid photosensitive eczema in a HIV seropositive patient

R Gayathri^{*}, AK Bubna, M Krishnakanth, S Adikrishnan, S Murugan, V Mahalakshmi, S Anandan, R Sudha
Department of Dermatology, Venereology and Leprosy, Sri Ramachandra Medical College, Chennai, India
E-mail: sharwan@gmail.com

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Background: Cutaneous changes in HIV have protean manifestations. We hereby present a case of a lichenoid photosensitive eczema in a HIV positive patient.

Case report: A 32 year old female presented with burning and itching sensation with hyperpigmentation over the sun exposed areas- face, neck, back and arms. No history of any blisters over skin or hypertrichosis. History of weight loss of more than 6 kgs was documented in a month. Patient's husband was a driver with history of multiple unprotected sexual exposures with CSW. No history of any oral or genital ulcers, arthralgia, diarrhoeal episodes, evening rise of temperature and cough with expectoration.

O/E: Erythematous and edematous plaques present over sun exposed areas. Hyperpigmentation with lichenoid hue + over malar region, neck folds, back and arms. Multiple excoriation marks, hyperpigmented papules and plaques present over bilateral arms, legs, and ante cubital fossa. Oral cavity, genital mucosae were normal. No generalised lymphadenopathy. Routine laboratory investigations were normal. Mantoux test, RPR, Chest X- ray, USG abdomen pelvis was normal. Skin biopsy was suggestive of interface dermatitis. HIV (1 and 2) ELISA was reactive which was confirmed by Western blot. The CD4 count was 610/cumm.

Discussion: Lichenoid photosensitive eczema is known to manifest in the terminal stage of the HIV infection in individuals with a low CD4 count. Our patient is a newly diagnosed case of HIV with a relatively high CD4 count manifesting with photosensitive eczema. This case is presented for its rarity.

P55

A study on prevalence of anemia and growth pattern among HIV infected children and adolescents from rural areas attending VCTC and ART center Govt. General Hospital, Vijayawada, A.P., India

Baratha Jyothi Naladi, Sunita Kanikaram^{*}
Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjunanagar, Guntur, Andhra Pradesh, India
E-mail: drsunitamichael@gmail.com

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Background: Globally, the HIV epidemic remains a serious challenge, and continues to take its toll particularly on vulnerable populations such as children and adolescents. However, background co-morbidities compound the problem in affected populations in India. Two such major co-morbidities include anemia and poor nutrition.

Methods: The present article deals with the profile of HIV infected children and adolescents in HAART era and Pre-HAART era who were attending the ART centre, Govt. General Hospital, Vijayawada.

Results: Totally 125 subjects of age group 1-20 years were included in the study. Among 125 HIV+ subjects only 45 subjects were in HAART era, 75 subjects in pre-HAART era and 5 subjects died during the study period. The study patients in HAART era showed a significant increase ($p < 0.001$) in CD4 counts from 174 to 902 cells/cmm, significant decrease ($p < 0.001$) in Hb content from 7.3 to 6.5 gm/dL and significant increase ($p < 0.05$) in BMI level from 18.1 to 22.6 from baseline to follow up treatment after 18 months. The study patients in pre-HAART era showed a significant increase ($p < 0.05$) in mean CD4 counts from 592 to 790 cells/cmm from initial count to count after 18 months. The subjects in pre-HAART era showed low level of Hb content 6.9 gm/dL in male and 6.3 gm/dL in female subjects; the mean BMI level was 20.43 in male and 18.47 in female subjects.

Conclusion: Our study reinforces the finding that anemia, growth failure and malnutrition are major manifestations of HIV infection in Indian children with prognostic significance.

P56

Primary pulmonary miliary tuberculosis presenting as immune thrombocytopenia- a rare case report

Ashalata Gannepalli^{1*}, Sanjay Reddy Podduturi², Bhargavi Krishna Ayinampudi¹, Ramakrishna Angali¹

¹Department of Oral and Maxillofacial Pathology, Panineeya Institute of Dental Sciences and Research Centre, Hyderabad, India; ²Department of Oral Medicine & Radiology, Awadh Dental College and Hospital, Jamshedpur, India

E-mail: ashalatamd@gmail.com

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Background: Tuberculosis (TB) is a multifaceted disease, which may present with a variety of symptoms, sometimes mimicking autoimmune diseases. Immune thrombocytopenic purpura (ITP) is an exceedingly rare manifestation of tuberculous infection.

Case report: A 42 year old female patient presented with petechiae on palate, tongue and extremities, melena and also developed conjunctival hemorrhage, hemoptysis, occult blood in stool. She had a history of evening rise of temperature since one month, non-productive cough since one week. Physical examination revealed no organomegaly, no lymphadenopathy and detailed investigations were done. Chest radiograph showed fine nodular opacities throughout lung field but tuberculin test and early morning sputum AFB examination for three consecutive days was negative. Hb 9.6gm%, PCV28, TLC4100/cumm, platelets-32000/cumm, ESR 52mm in 1st hour and 94mm in 2nd hour. Ultrasound abdomen was normal, HIV ELISA, dengue antibodies was negative, ANA and RA was positive. Diagnosis of pulmonary miliary tuberculosis was considered and patient was given platelet transfusion, IV corticosteroids, and was on anti tubercular therapy for 6 months. The platelet count was normal within ten days, lung lesions resolved and no antibodies were detected after six months. There was no recurrence of TB or ITP and no complain of joint pains after a follow up of three years.

Conclusion: ITP is a diagnosis of exclusion and TB should be considered as one of the possibility in its differential interpretation. Little is known on the clinical significance of auto antibodies in TB but the present case highlights the importance of anti tubercular therapy in such cases.

P57

Trimethoprim sulfamethoxazole drug resistance with co resistance to extended spectrum β -lactam antibiotics among bacterial isolates from HIV patients

Marimuthu Ragavan Rameshkumar¹, Nallusamy Vijaykanth¹, Ramachandran Vignesh², Pachamuthu Balakrishnan², Suniti Solomon², Narasingam Arunagirinathan^{1*}

¹PG & Research Department of Microbiology and Biotechnology, Presidency College (Autonomous), Chennai, India; ²YRG Centre for AIDS Research and Education, Chennai, India

E-mail: n_arunagir@yahoo.co.in

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Background: Trimethoprim-sulfamethoxazole (TMP-SMX) is a broad spectrum antimicrobial agent and also reduces the mortality among adults and children when used as prophylaxis against opportunistic infections in HIV infected patients. Drug resistant to TMP-SMX along with Extended spectrum β -lactamase (ESBL) production among Enterobacteriaceae is creating major therapeutic problem in clinical settings for treating the bacterial infections among HIV individuals.

Methods: TMP-SMX drug resistance among the isolates was identified using Kirby-Bauer disc diffusion method and ESBL production by combination disc method (CDM). Cefotaxime (30 μ g) and cefotaxime/clavulanic acid (30 μ g/10 μ g) discs were placed 20 mm apart on the agar surface. Similarly, the ceftazidime (30 μ g) and ceftazidime/clavulanic acid (30 μ g/10 μ g) discs were also placed. After incubating overnight at 37°C, a $\geq$ 5mm increase in the zone diameter was interpreted as positive for ESBL production. Statistical analysis was done using SPSS software version 15.0.

Results: A total of 103(40 *Escherichia coli*, 15 *Klebsiella pneumoniae*, 13 *Pseudomonas aeruginosa*, 10 *Klebsiella oxytoca*, 8 *Proteus mirabilis*, 2 *Proteus vulgaris*, 11 *Staphylococcus aureus*, 3 *Staphylococcus epidermidis* and 1 *Streptococcus* sp.) bacterial strains were isolated from HIV patients. Among these 65(63.10%; $p=0.008$) isolates were resistance to TMP-SMX and only 40(38.83%; $p=0.023$) isolates were resistant to extended spectrum β -lactam antibiotics. Twenty nine ESBL producers from HIV patients were found to be co resistant to TMP-SMX. All ESBL producing isolates showed resistance to ceftazidime and also for ceftazidime/clavulanic acid combination.

Conclusion: A rapid increase in the use of prophylactic TMP-SMX might be responsible for the TMP-SMX drug resistance among opportunistic bacterial infections in HIV patients.

P58

Diagnosis of mycobacterial drug resistance in HIV reactive patients by phenotypic and genotypic assay - a comparative study

S Umamaheshwari¹, Sumana M Neelambike¹, K Anuradha², B Sumangala³

¹Department of Microbiology, JSS Medical College, Mysore, Karnataka, India;

²Department of Microbiology, Mysore Medical College and Research Centre, Mysore, Karnataka India; ³Department of Microbiology, Mandya Institute of Medical Sciences, Mandya, Karnataka, India

E-mail: mnsamana12@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):P58

Background: Emergence of drug resistance has complicated the treatment of Tuberculosis (TB). WHO reports India as one among 27 "high burden" multidrug resistant (MDR) TB countries. The study was carried out to detect drug resistance of mycobacterial isolates in HIV patients.

Methods: The clinical specimens collected from HIV seropositive AFB smear negative patients (Group A) with features of TB, AFB smear positive, HIV reactive (Group B) and AFB smear positive, HIV negative (Group C) were processed as per standard protocol. They were inoculated onto Lowenstein Jensen media and the isolates obtained were subjected to drug susceptibility test (DST) by proportion method and Genotype MTBDR plus assay.

Results: In Group A out of 162 patients, 443 clinical samples were collected and 76 mycobacterial strains were obtained from 67 (41%) patients. In this 69 (91%) were *Mycobacterium tuberculosis* complex and 7 (9%) were *Mycobacterium avium* complex. As per phenotypic method, of 76 isolates, 50 (65.8%) were sensitive to all drugs and 26 (34.2%) resistant to one or more drugs. The MDR and resistance to at least single

drug of Group A, B and C were [5 (6.6%), 2 (6.7%), 4 (8%)] and [26 (34.2%, 3(10%), 8 (16%)] respectively. MTBDR assay showed similar results except one discrepancy i.e., one isolate was resistant to rifampicin that was sensitive in proportion method.

Conclusion: The increase of MDRTB in high TB burden settings stresses the need for DST. This helps in providing proper treatment regimens, improve prognosis and prevent the spread of drug resistant TB.

P59

Clinical significance of circulatory chlamydial heat shock protein-60 antibodies in spondyloarthropathy patients

Praveen Kumar¹, Darshan Singh Bhakuni², Sangita Rastogi^{1*}

¹Microbiology Laboratory, National Institute of Pathology (ICMR), Safdarjung

Hospital campus, New Delhi, India; ²Department of Rheumatology and

Clinical Immunology, Army Hospital (Research & Referral), New Delhi, India

E-mail: rastogi_sangita@rediffmail.com

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Background: *Chlamydia trachomatis* is a multifaceted, asymptomatic, most prevalent sexually transmitted pathogen worldwide. Due to lack of specific diagnostic methods for *C. trachomatis* in Spondyloarthropathy (SpA) patients, this pathogen is under diagnosed and under estimated in various countries including India. We aimed to estimate circulatory antibodies to chlamydial heat shock protein-60 (chsp60) IgG/ anti *C. trachomatis* IgA and evaluate their clinical significance in SpA patients.

Methods: In this case control study, serum from 60 arthritic patients comprising of Reactive Arthritis (ReA), undifferentiated SpA, Rheumatoid Arthritis (RA) and Osteoarthritis (OA) was collected for detection of anti chsp60 IgG and anti *C. trachomatis* IgA antibodies by using commercial ELISA kits. Cut-off indices (COI) of infected SpA patients were correlated with clinical data.

Results: Overall, 12/ 30 (40.0%) SpA patients, viz.: ReA/uSpA, were found positive for either circulatory chsp60 IgG or anti *C. trachomatis* IgA antibody (p value < 0.009 versus RA/ OA). 23.3% (7/ 30) SpA patients and 3.3 % (1/30) RA/OA control patients were found to be positive for circulatory chsp60 IgG antibodies. 16.6% (5/ 30) patients found positive for anti *C. trachomatis* IgA antibodies; however, none of the controls was infected. COI, age and disease duration in chsp60-positive SpA patients showed significant difference in median values (Kruskal-Wallis test; p value < 0.0001) while there was no such relation to anti *C. trachomatis* IgA.

Conclusion: Overall findings indicate an active/chronic *C. trachomatis* infection in SpA patients. The study also suggests usefulness of chsp60 serology to facilitate better clinical management of patients.

P60

Culture filtrate proteome analysis of aminoglycoside resistant clinical isolates of *Mycobacterium tuberculosis*

Divakar Sharma^{1,2}, Hari Shankar², Manju Lata^{1,2}, Beenu Joshi²,

Krishnamurthy Venkatesan^{1,2}, Deepa Bisht^{1,2}

¹Department of Biochemistry, National JALMA Institute for Leprosy and

Other Mycobacterial Diseases (ICMR), Tajganj, Agra, India; ²Department of

Immunology, National JALMA Institute for Leprosy and Other Mycobacterial

Diseases (ICMR), Tajganj, Agra - 282004, India

E-mail: divakarsharma88@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):P60

Background: Aminoglycosides are commonly used in tuberculosis treatment and are drugs of choice especially for MDR patients. They inhibit protein synthesis in susceptible bacteria by interacting with steps of translation. Several explanations have been put forward to explain the mechanism of aminoglycosides resistance but still our knowledge is fragmentary. Many culture filtrate proteins of pathogen represent potential targets for drugs, diagnostic probes and vaccine components; analysis of mycobacterial culture filtrate proteome in relation to aminoglycoside drug resistance is urgently required.

Methods: *M. tuberculosis* sensitive and resistant clinical isolates were cultured in Sauton's medium. After four weeks, cells were removed by centrifugation; supernatant was filtered by 0.45 & 0.22 μ filters and precipitated using SDS -TCA precipitation method. Culture filtrate proteins were resolved by two dimensional gel electrophoresis and

differentially expressed proteins (more than two fold) were selected for identification by matrix assisted LASER desorption/ionization time of flight mass spectrometry (MALDI TOF/MS) and characterization by bioinformatic tools (drug-protein docking).

Results: On comparing the protein profile of isolates, twelve proteins were found differentially expressed which were identified by MALDI TOF/MS. These proteins were not only involved in various metabolic pathways (site for drug targets) but also involved in survival of mycobacteria (various heat shock proteins).

Conclusion: These results might help in new drug development through provision of new drug targets and in further revision of therapeutics & vaccines against aminoglycoside drug resistant tuberculosis.

P61

Disentangling the HIV-1 human protein interaction networks and their implications in the dynamics of viral replication and pathogenesis

Devi Priyanka Maripuri, Daniel Alex Anand¹

Department of Bioinformatics, Sathyabama University, Chennai-600119, India

E-mail: danielalexanand@gmail.com

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Background: Network biology has broadened our view and changed our perspective in understanding disease. A systems perspective is imperative to the understanding of the dynamics of viral pathogenesis and host interactions. Probing the complex web of HIV-1 - host protein interactions divulged biologically meaningful results.

Methods: Global and protein specific networks were constructed using the HIV-1, Human Protein Interaction Database. The networks were visualized and analyzed using cytoscape and its plug ins. Functional annotation and enrichment analysis were performed using DAVID. Protein Sub cellular locations were determined using the LOCATE database.

Results: Sixteen HIV-1 proteins interacted with 2545 human proteins, of which 3668 (34.53%) were direct and 6340 (65.46%), regulatory interactions. Seventy seven different types of interactions with T cell surface glycoprotein CD4 isoform 1 precursor (276/10008) and DNA dC->dU editing enzyme APOBEC-3G(109) being the top two interacting proteins. The top interacting HIV-1 proteins were *tat*, p14 (28%) followed by gp120 and *nef* p27. Functional annotation returned 312 clusters with highest enrichment scores of 30.71 for positive regulation of apoptosis (*p* value 7.9E-32). Toll like receptor and the Jak STAT signaling pathways were most crucial to host response. LOCATE identified 491(10.7%) nuclear proteins, 419(9.15%) cytoplasmic, 295 (6.44%) membrane and 190(4.15%) extracellular proteins.

Conclusion: Network theory and application is critical in understanding host - viral dynamics. Integration of the interaction networks, expression data, cell type, disease stage and other factors are bound to enable phenomenal insights that would accelerate the development of highly effective therapeutic interventions.

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Molecular characterization of VIM1 isolates of *Pseudomonas aeruginosa* isolated from cases of sinusitis

S Sasikala^{1*}, S Niren Andrew², F Antony Irudhayaraj³, T Sundararaj⁴

¹PG & Research Department of Microbiology & Biotechnology, Presidency College, Chennai, India; ²Department of Microbiology, Madras Christian College, Chennai, India; ³Department of Ear, Nose & Throat, Rajiv Gandhi Memorial Hospital, Chennai, India; ⁴Jasmin Education & Research Foundation, Chennai-600096, India

E-mail: ssasikala@yahoo.co.in

E-mail: ssasikala@yahoo.co.in

E-mail: ssasikala@yahoo.co.in

E-mail: ssasikala@yahoo.co.in

BMC Infectious Diseases 2014, **14**(Suppl 3):P62

Background: Acquired metallo-β-lactamases (MBLs) are emerging resistance determinants in clinically relevant Gram negative species. These enzymes confer broad spectrum β lactam resistance, including resistance to carbapenems. Their potential for rapid and wide dissemination makes them of great concern.

Methods: This study describes the molecular characterization of 15 *Pseudomonas aeruginosa* strains, isolated from endoscopic pus specimens, obtained from sinusitis cases. Susceptibility testing was performed by disk diffusion assay. The MIC of ceftazidime was determined by E test.

Ceftazidime (CDM) - EDTA combined disc test was used to screen for class B β lactamase production. Plasmid DNA extraction was performed by alkali lysis method. The carriage of the MBL gene, *blaVIM* and IMP1 gene were screened by PCR. PCR products of 7 positive strains for MBL were sequenced and analysed using bioinformatics tools.

Results: Metallo-beta-lactamase was present in 9 strains. These results correlated with the presence of VIM1 gene by PCR. IMP1 gene was absent in all the 15 strains. Plasmid was present in 10 strains only. The nucleotide and deduced protein sequences were analyzed with the software available over the Internet. The nucleotide sequences reported in the study has been submitted to the Genbank and accession numbers were obtained (KF975367, KF975368, KF975369, KF975370, KF975371, KF975372, KF975373). A Phylogenetic tree was constructed and it consisted of 2 clades. Both the clades had evolved from the common ancestor VIM-28.

Conclusion: Direct detection of MBL genes may circumvent the problems of the phenotypic detection of carbapenemase producing microorganisms especially those exhibiting low carbapenem MICs.

P63

Fluoroquinolone resistance among CTX-M producing uropathogenic *Escherichia coli* from HIV and non-HIV patients in South India

Kesavaram Padmavathy^{1,2*}, Padma Krishnan², S Rajasekaran³

¹Sree Balaji Dental College and Hospital, Bharath University, Chennai, India;

²Dept of Microbiology, Dr. ALM PGIBMS, University of Madras, Chennai, India;

³Government Hospital of Thoracic Medicine, Chennai, India

E-mail: padmabakianath@gmail.com

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Background: Extended spectrum β-lactamase (ESBL) especially, the CTX-M producing *Escherichia coli* has emerged world wide as the leading cause of community onset UTI in the era of antibiotic resistance. The CTX-M producers often exhibit co-resistance towards fluoroquinolones and are a growing challenge to patient care. The purpose of this study was to determine the incidence of fluoroquinolone resistance among CTX-M producing uropathogenic *E.coli* (UPEC).

Methods: UPEC isolated from HIV (n=76) and non-HIV antenatal patients (n=42) were screened for ESBL production as per CLSI guidelines. *bla*_{CTX-M} was detected by PCR. Susceptibility to ciprofloxacin was assessed as per CLSI guidelines. Fisher's exact test (two tailed) was employed to analyze the statistical significance of the results.

Results: ESBL producers were more common among the UPEC isolates from HIV compared to those from non-HIV patients (75% vs 52.4%, *p*=0.015, OR=2.7273). Significant difference was observed in the incidence of *bla*_{CTX-M} among the ESBL producers from HIV and non-HIV patients (70.2% vs 31.8%, *p*=0.002, OR=5.042). Compared to the CTX-M non-producers, majority of the CTX-M producers were resistant to ciprofloxacin in both the groups (HIV, 92.5% Vs 58.8%, *p*=0.0037, OR=8.6333, and non-HIV, 71.4% vs 20%, *p*= 0.5235, OR=10).

Conclusion: Fluoroquinolones are the most common non-β lactam antibiotic used in the treatment of infections caused by ESBL producing organisms. The results of our study suggest the possible emergence of plasmid mediated fluoroquinolone resistance among the CTX-M producing *E. coli*. The co-resistance exhibited by the CTX-M producers is a cause of concern, as it might facilitate the co-selection process.

P64

Antibacterial activity of gold nanoparticles and their toxicity assessment

K Umamaheswari^{1*}, R Baskar¹, K Chandru³, N Rajendiran², S Chandrasekar²

¹Department of Biotechnology, University of Madras, Guindy, Chennai, India;

²Department of Polymer Science, University of Madras, Guindy, Chennai, India;

³Centre for Biotechnology, Anna University, Guindy, Chennai, India

E-mail: rk_uma@yahoo.com

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Background: Several classes of antimicrobial nanoparticles (NPs) and nanosized carriers for antibiotics delivery have proved their effectiveness as alternative agents to combat the antimicrobial resistance experienced with conventional drugs. Gold nanostructures find extensive applications in nano electronics and nanomedicine.

Methods: The present study involved the synthesis of gold nanoparticles using a sodium cholate, as both reducing and capping agent and characterization by UV-Visible spectroscopy and High Resolution Transmission Electron Microscopy (HRTEM). The antibacterial activity on significant bacterial species was evaluated by micro broth dilution method. The mechanism for antibacterial action of the gold nanoparticles were studied by reactive oxygen species (ROS) generation that causes oxidative stress to microbial cells and release of intracellular enzyme lactate dehydrogenase into extracellular medium (LDH assay) indicative of loss of cell membrane integrity.

Results: The synthesized gold nanoparticles showed characteristic peak at 541 nm by UV-Visible spectroscopy with particle size of 10nm as confirmed by HRTEM. The minimum inhibitory concentration (MIC₈₀) of the nanoparticles on *E. coli*, *S. typhi*, *P. aeruginosa*, *K. pneumoniae*, ranged from 20 to 40 µg/mL. The ROS generation was directly dependent on the concentration of the nanoparticles and no detectable enzyme leakage was recorded.

Conclusion: The results showed appreciable antibacterial activity of gold nanoparticles against the tested species and the possible mechanism of antibacterial activity may be due to increased intracellular ROS generation causing oxidative stress to the bacterial cells while LDH assay indicated that nanoparticles caused no damage to the cell membrane integrity.

P65

Cloning, expression and purification of Chikungunya virus E2 recombinant protein in *E.coli*

Anil Kr Verma, Anmol Chande, Murali-Krishna Kaja, Arockiasamy Arulandu, Pratima Ray*

Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India

E-mail: pratimaray.aiims@gmail.com

BMC Infectious Diseases 2014, 14(Suppl 3):P65

Background: Chikungunya virus infection is an emerging arbovirus disease. While many infected individuals recover after primary illness, others suffer from persistent debilitating arthralgia that can last for months to years. Currently, there are no immune correlates, vaccines, therapies or robust diagnostics that can affectively prevent, treat or diagnose Chikungunya. Therefore, this study focuses on development of a better diagnostic assay for Chikungunya infection.

Methods: We have initiated this study by cloning a functional form of Chikungunya virus E2 envelope protein. Sequence specific primers were designed based on the Chikungunya virus (African strain) E2 mRNA sequence obtained from NCBI (AF369024). This 846 base pair fragment was cloned in pET28b expression vector that added a His tag at the N terminus. E2 protein that was expressed in *E.coli* BL21 (DE3) strain and purified with Ni nTA affinity chromatography. The purified rHis e2 protein was characterized by SDS - PAGE and western blotting using an anti-His monoclonal antibody.

Results: The E2 protein of the Chikv virus was successfully cloned and characterized as a 37KD protein. This purified protein worked at concentration of 2.5 µg/mL and detected both IgG and IgM antibodies from the plasma of Chikungunya patients and did not show non specific reactivity to normal (healthy) control plasma.

Conclusion: We have successfully cloned the ChikvE2 protein and we are currently in the process of evaluating its use and efficiency in functional assays such as B cell ELISPOT assay and ELISA to be used for confirmatory diagnosis of Chikungunya.

P66

Human cytomegalovirus glycoprotein N polymorphisms among renal transplant recipients in India

A Raj Kumar Patro^{1,4*}, Lalit Dar¹, Sunil K Pati¹, Sanjay K Agarwal², Sandeep Guleria³, Shobha Broor¹, Suresh B Boppana^{5,6}

¹Departments of Microbiology, All India Institute of Medical Sciences, New Delhi, India;

²Departments of Nephrology, All India Institute of Medical Sciences, New Delhi, India;

³Departments of Surgery, All India Institute of Medical Sciences, New Delhi, India;

⁴Department of Microbiology, All India Institute of Medical Sciences, Bhubaneswar, India;

⁵Departments of Pediatrics, University of Alabama at Birmingham, Birmingham, USA;

⁶Departments of Microbiology, University of Alabama at Birmingham, Birmingham, USA

E-mail: rkpatro117@gmail.com

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Background: Human Cytomegalovirus (HCMV) is one of the most common infectious complications in renal transplant recipients. Extensive genetic polymorphisms in envelope glycoproteins of HCMV have been demonstrated among clinical HCMV isolates. Some previous studies have proposed the potential linkage between HCMV gN genotype to the frequency of symptomatic infection and clinical outcome in renal transplant recipients. No data available on circulating HCMV strains among renal transplant recipients in India.

Objective: To determine the cytomegalovirus strain diversity in renal transplant recipients.

Methods: One hundred thirty two renal transplant recipients were included in this study. DNA extracted from blood samples by Qiagen kit method. Specimen DNA was amplified by PCR using glycoprotein N (gN) gene primers. Genotyping was carried out by RFLP and results were confirmed by direct and/or cloning followed by nucleotide sequencing of the plasmid DNA of selected strains.

Results: HCMV detected by PCR for gN gene in the 83(63%) samples and sixty seven of these samples were typed by RFLP. Among these gN1 was identified in 17(25.3 %) cases. None of the samples contained gN2 genotype, gN3 genotype detected in 9(13.4%) cases, while gN4 strain was identified in 23 cases (34.3%), respectively. Infection with multiple gN type was seen in 18(27 %) of the samples.

Conclusion: The results of our study showed that gN4 was the commonest gN genotype found followed by gN1. This study was supported by the Indian Council of Medical Research, Government of India.

P67

Homozygous KIR2DL3 confers susceptibility to tuberculosis infection in HIV seropositive individuals

Stalinraja Maruthamuthu¹, Suresh Madasamy², Jayalakshmi Mariakuttikan^{1*}

¹Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai- 625021, India; ²ART Centre, Govt. Theni Medical College and Hospital, Theni, India

E-mail: jayalakshimim@genomicsmku.org

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Background: Tuberculosis (TB) remains an important cause of death among patients infected with HIV. Genetic variation influences differential development of TB particularly in immunosuppressed patients. Natural Killer cells play an important role in the innate immune system by providing the first line of defence against viral infections and tumors. Their activity is partially controlled by distinct inhibitory and activating killer Ig like receptors (KIR) that recognize specific ligands on potential target cells. Objective of this study is to determine the association between KIR and HIV co-infection.

Methods: This nested case control study comprised of 68 HIV+ individuals (34 treatment naive HIV-1 slow progressors and 34 on ART patients with TB co-infection). Genomic DNA was extracted from peripheral blood samples by salting out procedure and KIR genotyping was performed for 15 KIR genes (Activating and Inhibitory) by Multiplex PCR sequence specific primer method.

Results: All 15 KIR genes were observed in this study cohort. We could observe more number (>4 aKIR) of activating KIR genes in HIV+ TB- individuals (88%) when compared with HIV+ TB+ patients (74%). Homozygosity for KIR2DL3 was more frequent in HIV+ TB+ patients (64%) than HIV+ TB- individuals (32%) which may confer susceptibility to co-infection.

Conclusion: NK cell receptor KIRs limits HIV disease progression. In the present study, HIV positive individuals who were homozygous for inhibitory KIR2DL3 are at higher risk of acquiring TB co-infection and disease progression with decreased CD4 cells.

P68

Auto immune haemolytic anaemia as part of Nevirapine induced DRESS syndrome

Aravind Reghukumar^{1*}, Athul Gurudas¹, VS Kiran Kumar¹, Ranjani Ravi²

¹Department of Infectious Diseases, Medical College Hospital, Thiruvananthapuram, India; ²Cosmopolitan Hospital, Thiruvananthapuram, India

E-mail: drkeetu13@yahoo.co.in

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Background: Drug rash with eosinophilia and systemic symptoms (DRESS) syndrome is an idiosyncratic hypersensitivity reaction to drugs. The diagnosis involves 3 criteria; 1) Drug induced skin eruption 2) Absolute eosinophil count (AEC) >1500/dL or atypical lymphocytosis 3) One of the following enlarged lymph nodes at least 2cm in diameter, hepatitis, interstitial nephritis, interstitial lung disease or myocarditis. Nevirapine and abacavir are known to produce DRESS syndrome. Autoimmune hemolytic anemia [AIHA] as a part of nevirapine induced DRESS syndrome is extremely rare. To the best of our knowledge there are no cases reported so far. We are reporting two cases of the same.

Case report: 45 year old male, with CD4 190 was started on zidovudine, lamivudine, nevirapine and cotrimoxazole. Two weeks later, he presented with fever, generalized maculopapular pruritic rash and was found to have pallor, icterus, multiple cervical lymph nodes and hepatosplenomegaly. All drugs were withheld suspecting hypersensitivity. Routine investigations showed progressive anaemia, eosinophilia, indirect hyperbilirubinemia with transaminitis and interstitial nephritis. Corrected reticulocyte count was 3%. Serum LDH was 620. Peripheral smear was suggestive of extravascular haemolysis. Direct Coombs test was positive. The patient was diagnosed to have DRESS syndrome with AIHA. Steroids were started with which he showed dramatic improvement. The patient tolerated zidovudine, lamivudine and cotrimoxazole on reintroduction thereby identifying the culprit drug as nevirapine.

A second case involved a 36 year old lady with CD4 110 who presented similarly, 3 weeks following initiation of HAART. She also improved with steroids. Nevirapine was again identified as the causative drug.

P69

Erythema nodosum leprosum presenting as pyrexia of unknown origin

Aravind Reghukumar^{1*}, Athul Gurudas¹, VS Kiran Kumar¹, Ranjani Ravi²

¹Department of Infectious Diseases, Medical College Hospital,

Thiruvananthapuram, India; ²Cosmopolitan Hospital, Thiruvananthapuram, India

E-mail: drkeetu13@yahoo.co.in

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Background: Erythema nodosum leprosum (ENL) or Type 2 lepra reaction is an immune complex mediated hypersensitivity reaction. We report a rare scenario where ENL presented as pyrexia of unknown origin (PUO).

Case report: A 63 year old lady presented to a peripheral centre polyarthralgia, multiple tender and erythematous nodules over her face, elbows, back and legs and continuous fever of 2 months duration. Routine investigations being negative, she was referred for further evaluation of PUO. On presentation, the patient was febrile. Apart from the nodules, examination revealed bilaterally thickened and tender greater auricular, ulnar and common peroneal nerves. Her left hand was swollen and tender due to ulnar neuritis. Bilateral symmetric sensory neuropathy was present up to the ankles. Few of the lesions over the face were found to be hypoaesthetic. Laboratory investigations showed mild transaminitis and an ESR of 64. Biopsy of a nodule showed septal panniculitis suggestive of erythema nodosum. ANA profile, cANCA and pANCA were negative. Split skin smears were positive for acid fast bacilli (AFB) with bacillary index between 2+ to 4+. Skin biopsy showed ill defined granuloma formation with occasional multinucleate giant cells. Fite's stain revealed numerous AFB. A diagnosis of multibacillary leprosy with ENL was made. She was treated with prednisolone 1mg/kg/day followed by multidrug therapy comprising of dapsone, clofazimine and rifampicin. fever, neuritis and polyarthritides dramatically subsided after steroid initiation. Steroids were tapered and stopped over the next 2 months. This case is being presented because of rarity of presentation of ENL as PUO.

P70

Emerging MDR producing ESBL among Diarrhoeogenic *Escherichia coli* from paediatric patients

Ashita A Pinto*, Yaser Siahbalaee, Arif Ali

Gene Expression Laboratory, Department of Biosciences, Jamia Millia Islamia, New Delhi, India

E-mail: apintodelhi@gmail.com

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Background: The beta lactam antibiotics are predominant therapeutic agents against a number of infectious diseases. The production of the enzyme beta lactamase by microbes which hydrolyses the beta lactam antibiotics is an important mechanism of resistance. This study aimed to determine prevalence, phenotypic patterns, and ESBL-production status both phenotypic and genotypic for the CTX -M type ESBL in *Escherichia coli* (*E.coli*) isolated from Diarrhoeic children.

Methods: Two hundred thirty non repetitive stool samples were collected from 2 hospitals, one private and other government hospital in Delhi, NCR. *E.coli* was confirmed by 16S rRNA analyses. Antibiotic susceptibility and phenotypic ESBL production were studied by disc diffusion and double disk synergy tests according to Clinical Laboratory Standards Institute guidelines. PCR was performed for the molecular detection of the CTX-M type ESBL.

Results: Out of 230, 215 were confirmed to be *E.coli*. Phenotypically 72.5% (156) showed the presence of ESBL of which 75% (79) in private hospital samples and 70% (77) in government hospital samples. PCR based molecular detection confirmed the presence of the CTX-M gene among 65.6% (141) of the samples.

Conclusion: The high trends in the ESBL presence are alarming and urge means to cope with such ESBL *E.coli* strains. It being a commensal raises concern of the resistance gene transfer from pathogenic bacteria, especially in children due to common diarrhoeal episodes among them. The paediatric population is at a higher risk in case of multidrug resistance due to further limitation in therapeutic options and constrains in prescription of harsher antibiotics.

P71

A rare case of disseminated cysticercosis

S Praneetha¹, R Priyanka, D Srilekha

Melmaruvathur Adhiparasakthi Institute of Medical Sciences & Research,

Melmaruvathur-603319, India

E-mail: praneetha_99@yahoo.com

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Background: Neurocysticercosis is a common tropical parasitic disease of the CNS. But the disseminated form is an uncommon manifestation of this disease. Here we report a case of disseminated cysticercosis.

Case report: A 10-year-old girl was brought to the hospital with complaints of fever, headache and vomiting for 10 days duration before a month. Fever was high grade, associated with chills. There was no history of seizures. Immunized upto date with normal development. On examination, the child was dull looking with regular pulse rate of 100/min. CNS examination showed mild neck stiffness, otherwise unremarkable. Peripheral smear study showed microcytic hypochromic anemia with mild eosinophilia. Fundus examination was normal. Child was admitted and administered antibiotics after which she recovered. Fifteen days after discharge she presented with history of diplopia and headache. There was history of beef intake for 3 years and the recent intake was before 2 months. Eye examination showed well defined white lesion of 0.5cm in the anterior chamber of eye that moved with head movement. Fundus examination showed mild papilledema. Non contrast CT brain was near normal. MRI brain findings showed multiple hyperintense lesions of less than 3mm on T2WI which appears hypointense on T1WI in the gangliocapsular, thalamus and subcortical regions. A diagnosis of cysticercosis was made and the ocular cyst had to be surgically removed and treatment with prednisolone 1-2mg/day had to be started 2-3 days prior and continued with cysticidal therapy - Albendazole 15mg/kg/day for 5 to 28 days. Patient asked to follow up after cyst removal for management and is asymptomatic presently.

Conclusion: Maintaining personal hygiene and avoiding undercooked meat could limit cysticercosis.

P72

IRIS manifesting as chronic inflammatory demyelinating polyneuropathy in HIV

Aravind Reghukumar^{1*}, Athul Gurudas¹, VS Kiran Kumar¹, Ranjani Ravi²

¹Department of Infectious Diseases, Medical College Hospital,

Thiruvananthapuram, India; ²Cosmopolitan Hospital, Thiruvananthapuram, India

E-mail: drkeetu13@yahoo.co.in

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Background: CIDP manifesting as part of immune response inflammatory syndrome (IRIS) in HIV has not been reported till date.

Case report: 45 year old female was diagnosed to have HIV with a CD4 count of 86. At the time of diagnosis, she was very much emaciated with a BMI of 16, had oral candidiasis and sensory neuropathy with numbness upto both ankles. After ruling out other opportunistic infections, she was started on tenofovir, lamivudine and nevirapine. Three months later, she presented to us with difficulty in walking and dribbling of saliva. History was suggestive of gradual worsening of peripheral neuropathy and progressive ascending limb weakness after initiation of anti retroviral therapy. Bowel and bladder involvement were not present. Clinical examination revealed areflexic quadriparesis with bilateral LMN facial palsy. Evidence of both small and large fiber neuropathy was present in both upper and lower limbs. Digital clawing was noted in both upper limbs in both ulnar and median nerve distribution. CSF study was suggestive of lymphocytic pleocytosis with mild protein elevation. MRI brain and spine were normal. Electromyographic nerve conduction studies were consistent with a primary demyelinating polyneuropathy involving all nerves in upper and lower limbs. CSF PCR for Cytomegalovirus, Epstein barr virus, herpes and mycobacterium tuberculosis were negative. Since duration of symptoms was more than 2 months, a diagnosis of CIDP was made. She was given inj methylprednisolone 1 gm for 5 days followed by tapering doses of prednisolone. There was dramatic improvement in muscle power after initiation of steroids and after 2 weeks she was able to walk without support, facial palsy and digital clawing had recovered.

Conclusion: The probable explanation is that CIDP in this case occurred as IRIS as evidenced by the improved BMI (19) and CD4 count (170) at the time of presentation.

P73

The microbiological profile of nosocomial infections in an intensive care unit

P Madhurima*, G Sateesh, Y Saritha, M Padma
Sentini Hospitals, Vijayawada, India
E-mail: chinnupreddy@rediffmail.com
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Background: Nosocomial infections are responsible for morbidity and mortality in hospitalized patients. They also increase cost of treatment and prolong hospitalization. The aim was to study the nosocomial infections in intensive care unit (ICU) at tertiary care hospital.

Methods: This study was done at a tertiary care hospital from June 2012 to June 2013. Patients who developed infection after 48 hours of stay in ICU were included in the study. Clinical samples were processed for bacteriological culture and susceptibility was tested using Kirby bauer disc diffusion method.

Results: Of 2450 patients admitted to ICU, 237 (9.6%) suspected nosocomial infections were studied prospectively and total number of 302 samples were collected from all clinically suspected cases of nosocomial infections.

Samples included urine 91, blood 70, IV catheters 41, ET aspirations 61, ET tips 24, sputum 10 and Foley catheter tips 5. Out of these UTI, 96 (31.71%) was the most common nosocomial infection followed by VAP 84 (27.1%), BSI 55 (23.1%) and catheter related infections 29 (13.5%). Most common organism isolated was *K.pneumoniae* 78 (32.8%), *E.coli* 53 (19.6%), *A. baumannii* 44 (18.5%), *S.aureus* 27 (11.3%), *P.aeruginosa* 23 (9.7%), *S. maltophilia* 12 (5.06%). 41% of *S. aureus* were MRSA and all were sensitive to vancomycin.

Conclusion: Strict infection control measures and antibiotic policy could reduce incidence of nosocomial infections.

P74

A pilot study of varicella zoster infection among patients with fever and rash in a tertiary care centre

Siddhartha Ojah*, Ramya Barani, Padma Srikanth
Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai, India
E-mail: siddhartha.ojah@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):P74

Background: Varicella zoster virus (VZV) causes mild self limiting illness in children. Older children and adults may present with atypical manifestations. At present VZV vaccine is not included in national immunization schedule. The study was undertaken to determine the presence of VZV infection in patients with acute febrile illness with rash.

Methods: Blood samples were collected from patients (n=36) with acute febrile illness with rash in a tertiary care hospital. IgM ELISA was performed according to manufacturer's instructions (IBL International, Germany). Kit standards were used to obtain a standard curve. The results of the samples were interpreted from the standard curve.

Results: All enrolled patients had fever with rash without evidence of vesicles. Majority, (n=29) were adults and 18 were male. Of the 14 (38.9%) that were positive by IgM ELISA for VZV, 5 (35.7%) belonged to the age group of 19-30 years, 4 (28.5%) were < 18 years and 2 (14.3%) in age group 51-60 years. All patients had normal platelet count except 9, who had mildly deranged platelet count (>1.5 to < 2 lakh /cumm). All sera that were tested were negative for Dengue NS1 antigen, IgM and IgG antibody capture ELISA (Panbio, Australia).

Conclusion: VZV may present with atypical manifestation. Non progression of rash to vesicle may warrant testing for VZV and other viruses in the absence of positive Dengue serology.

P75

Profile of opportunistic infections in AIDS patients in relation to CD4 count in a tertiary care hospital

AR Gnananjali, MN Sumana*
Department of Microbiology, JSS Medical College, Mysore, Karnataka, India
E-mail: mnsumana12@gmail.com
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Background: HIV/AIDS is a global pandemic with cases reported from virtually every country. The CD4 cells are the primary target cells for HIV. The hallmark of HIV disease is a profound immunodeficiency resulting from a progressive quantitative and qualitative deficiency of CD4+ T cells. As CD4 count decreases, opportunistic infections (OIs) manifest. HIV infection progressing to AIDS is associated with many OIs.

Methods: One hundred seropositive HIV patients were selected, depending upon clinical presentation and physical examination, appropriate laboratory diagnostic methods were employed for detection of OIs. CD4 count was done by FACS. The occurrence of various OIs and CD4 counts were correlated.

Results: This study involved a descriptive study and tuberculosis (43.9 %) was found to be most common OI seen in patients with mean CD4 count 360 ± 56 cells/ μ L, followed by candidiasis (14.6 %) with mean CD4 count 324 ± 42 cells/ μ L, diarrhea due to coccidian parasite infection (8.5%) with mean CD4 count 97 ± 15 cells/ μ L, cryptococcal meningitis (3.65 %) and *Pneumocystis jirovecii* pneumonia (3.65 %) with mean CD4 count 92 ± 6 cells/ μ L. CD4 count of 20 patients were followed up 6 months after initiating ART and it was observed that CD4 count in 16 patients had increased.

Conclusion: Tuberculosis and candidiasis was seen in patients with CD4 count above 300 cells/ μ L. Diarrhea due to coccidian parasites, cryptococcal meningitis and PCP was seen in patients with CD4 count below 100 cells/ μ L. OIs cause substantial morbidity and hospitalization, and shortens survival of HIV infected patients.

P76

Effect of anti retroviral therapy on mucocutaneous manifestations among HIV infected patients in a tertiary care centre in India

N Prabhakaran*, TJ Jaisankar, Abdoul Hamide, M Malathi, DM Thappa
Department of Dermatology, Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER), Puducherry, India
E-mail: dr.prabhakaran88@gmail.com
BMC Infectious Diseases 2014, **14**(Suppl 3):P76

Background: A panorama of mucocutaneous manifestations is produced by HIV infection which correlates with the degree of immunodeficiency. Viral, bacterial and fungal infections, as well as inflammatory skin diseases, have all been reported to increase as CD4 T cells are depleted. Since the introduction of HAART, there has been a dramatic decrease in

the incidence of HIV associated dermatoses. However, initiation of ART itself causes various cutaneous adverse effects. Our goal was to study the various mucocutaneous manifestations encountered in treatment naïve HIV infected and those on ART.

Methods: This study involved a cross sectional assessment of 85 HIV seropositive patients attending STD and ART clinic. Of which 16 patients were not on HAART and the rest 69 were on HAART.

Results: The mean baseline CD4 count of those not on HAART was 571.94 ± 278.44 and mean baseline CD4 count of those on HAART was 281.88 ± 231.02 and the difference was significant ($p < 0.0001$). Six months post HAART, the mean CD4 count had significantly increased to 515.44 ± 245.54 ($p < 0.001$). The incidence of mucocutaneous manifestations were similar in both the groups ($p = 0.4891$). The most common infections in those not on HAART were fungal (25%), viral (12.5%), bacterial (6%). And among those on HAART were fungal (43%), viral (25.5%) and bacterial (2.8%). The various adverse cutaneous reactions to HAART observed in our study were lipodystrophy (14%), maculopapular rash (7.2%) lichenoid eruptions (5.2%).

Conclusion: These findings indicate that HAART often reduces the incidence of infectious skin diseases in patients with HIV, but can itself be the cause of drug eruptions.

P77

Prevalence of cryptococcal meningitis and lymphadenitis among PLHA

MV Narasimham¹, Pritilata Panda

PG Department of Microbiology, MKCG Medical College & Hospital, Berhampur, India

E-mail: mayurdr9.prajay@yahoo.in

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Background: Cryptococcosis has been a common cause of morbidity and mortality in immunocompromised patients. This study was conducted to estimate the prevalence of cryptococcosis in PLHA cases presenting mainly with meningitis and lymphadenitis.

Methods: An ongoing prospective study conducted from June 2012-October 2013 in 90 HIV seropositive patients presenting with various complaints. CSF and lymphnode aspirates were subjected to preliminary microscopic examination, c (Giemsa staining, mucicarmine staining, negative staining and Gram's staining). Latex agglutination test was done for CSF samples. Samples inoculated on SDA at 37°C and 25°C followed by Bird seed agar (BSA). Identification was done based on demonstration of encapsulated budding yeast cells in microscopy, negative staining with Nigrosin, red capsule on mucicarmine, yeast like creamy mucoid colony on SDA at 37°C, brown to black colony on BSA, inositol assimilation and positive urease test.

Results: Maximum cases were between 25-40 years of age. 4 meningitis & 2 lymphadenitis cases showed positive cryptococcal growth on microscopy and culture. Both CSF and lymph node aspirate were found to be positive in 1 (1.11%) case. 2 cases of disseminated cryptococcosis were seen with one involving multiple lymph nodes & other was a case of meningitis & lymph node involvement. All the positive cryptococcal cases had a CD4 count < 200 cells/ μ l.

Conclusion: Cryptococcal infections without treatment have a high morbidity rate and appropriate systemic antifungal therapy can significantly improve the outcome. A high index of clinical suspicion and mycological surveillance is required to help in an early diagnosis & treatment.

P78

Exploring the protein-protein interface of integrin-galectin complex for drug development against HIV infection- a bioinformatics study

M Xavier Suresh¹, R Barani Kumar, V Sravani, P Suma Priya

Department of Bioinformatics, Sathyabama University, Chennai – 600119, India

E-mail: xaviersuresh@gmail.com

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Background: The formation of protein-protein complexes of the transmembrane receptor integrin and galactoside binding protein galectin assist in adhesion by bridging between cells or cells and the extracellular matrix and form adhesive networks. When HIV is present the galectin bridges between the CD4 co receptor and gp120 ligands thus

facilitating HIV infection of the T cell. The main aim of this study is to investigate the interaction of galectin-3 with glycoligands, integrin, natural compound curcumin and its analogs.

Methods: The structures of proteins were obtained from PDB whereas natural compound curcumin and their analogs have been fetched from PubChem and Drug Bank databases. ZDOCK, FireDock algorithms were used to predict the protein-protein complex and molecular docking studies were carried out using Hex8 and Discovery Studio.

Results: Analysis of the results reveals that the glycoligand 3- α -Gal-LacNAc was found to bind with the CRD of galectin-3 showing interactions with amino acids GLN150, LYS176 and TRP181. The curcumin analog 11 was found to bind with amino acids like ARG144, ARG162, SER237, GLU184 and ASN119 which forms part of CRD of galectin-3. Analysis of the docking poses of protein-protein complexes reveals that the CRD of galectin-3 is the most probable binding interface for integrin molecule.

Conclusion: The knowledge of the mechanism of interaction between integrin and galectin may provide important therapeutic opportunities and the proposed compounds may act as a scaffold for drug design against HIV infection.

P79

Vasculitis with lichenoid eruptions in Hepatitis B infection – case report

Murugan Sundaram^{1*}, R Sudha¹, V Mahalaksmi¹, S Adikrishnan¹,

M Krishnakanth¹, M Sundaram²

¹Department of Dermatology, Sri Ramachandra Medical College Medical

College & Research Institute, Chennai, India; ²Department of Dermatology, Sri Devi Multispecialty Hospital, Chennai, India

E-mail: murug1972@gmail.com

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Background: Hepatitis B virus infection is characterized by asymptomatic to fulminant liver disease, chronic hepatitis, carrier state and liver failure. HBV is known to induce varied skin manifestations, one such presentation as vasculitis with lichenoid eruptions is being reported.

Case Report: A 50 years old male patient, under treatment for Hepatitis B infection, presented with skin lesions of 4 years duration, starting as erythematous patches slowly becoming thickened and scaly, violaceous papules and patches. Lesions were moderately itchy, with tenderness and gradual ulceration in the lesions. Lesions involved hands, legs, lower abdomen and trunk. Palms and soles were spared, hair and nails normal. No mucosal involvement. Patient's hematological and biochemical investigations were within limits. Ultrasound abdomen was normal. ELISA for HIV 1 and 2 were negative. Histopathology of the skin lesion suggestive of lichenoid changes with lymphocytic vasculitis. Patient was managed in conjunction with the gastroenterologist with good improvement.

Conclusion: Vasculitis with lichenoid eruptions needs to be recognized in the context of Hepatitis B Infection and distinguished from the other manifestations in view of their clinical rarity.

P80

Comparison of plasma and salivary HIV loads determined via a coupling of the Abbott HIV detection system with the DNA Genotek OMNIgene™ DISCOVER (OM-505) kits

David J Speicher^{1,2*}, S Saravanan³, N Kumarasamy³, K Ranganathan⁴, NW Johnson^{1,2,4}

¹Molecular Basis of Disease Research Program, Griffith Health Institute,

Griffith University, Queensland, Australia; ²Population & Social Health

Research Program, Griffith Health Institute, Griffith University, Queensland,

Australia; ³YR Gaitonde Centre for AIDS Research and Education, Chennai,

India; ⁴Ragas Dental College, Chennai, India

E-mail: d.speicher@griffith.edu.au

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Background: The DNA Genotek OMNIgene™ DISCOVER (OM-505) kits are designed to collect and store saliva at room temperature before the extraction and detection of DNA and RNA. Utilizing the OM-505 we determined the HIV salivary viral loads (SVL), which were compared with plasma viral loads (PVL).

Methods: SVL and PVL were determined on 40 HIV-positive ART naïve patients presenting at YRG CARE. Saliva was collected with the OM-505,

incubated at 50°C for 1 hour. Prior to extraction 70 mL isopropanol was mixed with 800mL OM-505. From OM-505 and plasma, RNA was extracted automatically on the Abbott m2000sp. HIV loads were determined with the Abbott m2000rt system.

Results: A calibration curve produced by 10-fold dilutions of HIV virion in HIV negative saliva collected in the OM-505 was linear ($R^2=0.9951$) from 57,273 to 621 HIV copies/mL. In clinical isolates, PVL averaged 431,865 HIV copies/mL (range: 62 to 7,604,620 HIV copies/mL) whilst SVL averaged 23,267 HIV copies/mL (range: 153 to 220,104 HIV copies/mL). SVL was not detected in 15 samples and could not be determined in 5 samples due to viscosity and cellular debris causing problems during extraction. In 12/17 patients SVL was lower than PVL.

Conclusion: HIV, if present, can be detected accurately in saliva down to 621 HIV copies/mL. SVL does not correlate with PVL and thus cannot be used to accurately determine HIV carriage, but in most cases HIV shedding is low or nonexistent.

P81

Functional characterization of HIV-1 *Tat* exon-1 variants from North India and their implications on HIV-1 transactivation and TAR interaction

Larance Ronsard^{1,2*}, VG Ramachandran², Akhil C Banerjee¹

¹Virology lab - II, National Institute of Immunology, New Delhi, India;

²Department of Microbiology, UCMS and GTB Hospital, New Delhi, India

E-mail: laraphds@gmail.com

BMC Infectious Diseases 2014, **14**(Suppl 3):P81

Background: HIV-1 virus is a rapidly evolving virus due to genetic variability through its rapid replication, mutation and recombination potential which is a major hurdle in vaccine development. One of the effective ways to modulate HIV-1 infection is to target viral proteins; among the viral proteins, *Tat* plays a major role in HIV-1 pathogenesis. It activates viral gene expression through TAR interaction. The aim of this study was to characterize genetic and functional variants of *Tat* exon-1 from HIV-1 patients from North India.

Methods: DNA was isolated from PBMCs and *Tat* exon-1 was PCR amplified with specific primers followed by cloning, sequencing and sequence analysis using bioinformatics tools for predicting subtypes, recombination events, conservation of domains and phosphorylation sites. Unique *Tat* exon-1 variants were functionally characterized for LTR transactivation, TAR interaction and cell death.

Results: Genetic analysis of *Tat* exon-1 variants revealed 90% subtype C and 10% B/C recombinants, and the functional characterization showed varying levels of LTR transactivation, TAR interaction and cell death. A single mutation (S46F) in *Tat* exon-1 variants showed enhanced LTR transactivation through strong interaction with TAR.

Conclusion: Possible role of *Tat* exon-1 variants in shaping the current HIV-1 epidemic in North India is discernible. Natural substitutions across

the conserved functional domains were observed. There is evidence for the emergence of B/C recombinants within *Tat* exon-1. The impact of genetic variations in *Tat* exon-1 on its pivotal functions is apparent. These are likely to have implications for HIV-1 pathogenesis and strategies of vaccine formulations.

P82

Knowledge, attitude and practice of HIV/AIDS among employees of a medical line factory in Kamunting, Malaysia

Navinder Kaur, Nik Ahmad Izani, Vivekanandan Gopalakrishnan*

UNIKL RCMP, 3 Jalan Greentown, Ipoh, Malaysia

E-mail: gopalakrishnan@rcmp.unikl.edu.my

BMC Infectious Diseases 2014, **14**(Suppl 3):P82

Background: Adults aged between 20 and 39 years constituted for more than half of the new HIV cases in Malaysia. Even though there was a high level of awareness regarding HIV/AIDS among the Malaysian public, there were still misconceptions.

Methods: It is a cross sectional study conducted at the Latex Manufacturing Sdn.Bhd. Questionnaire was distributed to employees, questions in regard to demographic profiling, knowledge, attitude and practice related to HIV/AIDS were included.

Results: The study population (n= 314) showed knowledge level with median score of 13/16. However, 46.8% of them could not differentiate between HIV and AIDS, 30.9% believed HIV was transmittable by mosquito bites, 30.6% believed condom usage would not lower HIV risk and 39.5% believed showering or washing genitals after sexual intercourse could lower the risk. Respondents showed good attitude towards people with HIV/AIDS with median attitude score of 5/6. Higher level of education showed higher knowledge level and better ($p<0.05$). About 29% did not use condom during sexual intercourse. Twenty percent had multiple partners and 8% did not use condom regularly with their multiple partners. Twenty seven (8.6%) of male respondents had sexual relationship with other men. About 10% of them were willing to have blood transfusions with blood products of unknown HIV status. High knowledge level did not show better practices related to HIV/AIDS.

Conclusion: Employees had modest knowledge in regard to HIV/AIDS. Nevertheless, misconceptions and stigmatization in regard to the illness was present, showing that their understanding and concept about the infection was still not satisfactory.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Kaur *et al.*: Knowledge, attitude and practice of HIV/AIDS among employees of a medical line factory in Kamunting, Malaysia. BMC Infectious Diseases 2014, **14**(Suppl 3):P82