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## Detection and subtype identification of Blastocystis in a hospital setting from southeasternIndia



- <sup>1</sup> Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, Puducherry, India
- <sup>2</sup> Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India
- <sup>3</sup> Universidad Nacional Agraria La Molina, Lima 12, Lima, Peru
- <sup>4</sup> National Centre for Cell Science, Pune, India
- <sup>5</sup> Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, Puducherry, India

**Background**: *Blastocystis* was identified almost 100 years back but its clinical significance is controversial. An estimate suggests that *Blastocystis* inhabit large intestine of more than 1 billion human worldwide. Based on the ribosomal lineages different species of *Blastocystis* are designated as various subtypes (ST) with extensive inter and intra subtype genetic diversity. Due to its polymorphic nature identification by microscopy is obscure. However, in India, data pertaining to *Blastocystis* were chiefly derived from direct stool microscopy. In this study we employed microscopy, culture and PCR for the detection of *Blastocystis* from stool samples. Further, subtyping of representative samples were carried out to identify the subtypes available in this region.

**Methods & Materials**: It is a cross sectional analytical study approved by JIPMER Institute Ethics Committee. All the stool samples were screened by routine microscopic investigations and they were subjected to *in vitro* propagation in Jones' medium. Fecal DNA was extracted by using QIAamp DNA stool mini kit (Qiagen, Germany) following manufacturer's instructions and stored at -20°C. Further, extracted DNA was quantified and subjected to PCR, which targets initial 600 bp barcoding region of 18SSU rDNA of *Blastocystis*. PCR products were visualized on 1.5% agarose gel and representative positive amplicons were sequenced for subtype analysis. Sequence results obtained from both the strands were assembled and subtype analysis was performed by using following database http://www.pubmlst.org/blastocystis/

**Results**: A total of 173 stool samples were screened for *Blastocystis*. PCR detected the maximum number of *Blastocystis* (n = 77, 44%) followed by culture (n = 48, 28%) and Microscopy (n = 25, 14%). The Sequencing results of the representative PCR amplicons confirmed the presence of *Blastocystis* ST3 allele 34 (n = 9) and ST1 allele 4 in (n = 5).



**Conclusion**: In comparison with stool microscopy and culture, *Blastocystis* specific PCR is an excellent diagnostic tool in terms of sensitivity, specificity and subtype identification. However, in resource poor settings Jones' medium (xenic culture) could be used as an alternative diagnostic modality for the detection of *Blastocystis* from stool. Subtyping results indicate ST3 predominance. However, large number of samples needs to be subtyped to reveal the association of particular ST with particular clinical manifestations.

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# MicroRNA mediated immune regulation of T helper cell differentiation and plasticity during visceral leishmaniasis infection: A computational approach



R. Pandey<sup>1,\*</sup>, V.K. Prajapati<sup>2</sup>, S. Sundar<sup>3</sup>

- <sup>1</sup> Central University of Rajasthan, Ajmer, India
- <sup>2</sup> Central University of Rajasthan, Ajmer, Rajasthan, India
- <sup>3</sup> Institute of Medical Sciences, Varanasi, India

**Background:** Visceral leishmaniasis (VL) is a tropical neglected disease caused by *Leishmania donovani*, results in significant mortality in Indian subcontinent. The protective immune response to *Leishmania* parasites is mediated by proliferation and differentiation of IFN- $\gamma$  secreting CD4<sup>+</sup> T helper (Th1) cells while IL-4 dependent CD4<sup>+</sup> T helper (Th2) cell leads to aggravate VL pathogenesis. The plasticity of T cell proliferation and differentiation depends on microRNA mediated gene regulation which leads Th1/Th2 or Th17/Treg type of immune response during human VL.

**Methods & Materials**: MicroRNAs participates in T cell proliferation and differentiation in human VL. This study depicts the identification of target immune signaling molecule and transcription factors, which play role in T-cell proliferation and differentiation followed by the identification of miRNA controlling their gene expression using three web servers viz., TargetScan, mirPath and miRDB.

**Results**: The present study provides the *in silico* evidences that seed region present in the microRNAs miR-29-a, miR-29b and miR29c have the putative binding site in the 3'-UTR region of TBX21 transcription factor of CD4<sup>+</sup> T helper (Th1), which may suppress the Th1 specific protective immune response. Development of Th2 type specific immune response can be suppressed by binding of miR-135 microRNA over the 3'-UTR region of GATA-3 transcription factor of Th2 specific CD4<sup>+</sup> T helper cells. Interestingly, miR-21 and miR-24

can inhibit the Th1 immune response and simultaneously activate the Th2 immune response by stimulating T helper cell proliferation and differentiation. We are indicating that miR-135, miR-155 and miR-1272 and miR-223 suppress Th2 specific immune response and maintain the plasticity by activating Th1 specific CD4<sup>+</sup> T helper cells.

**Conclusion:** This study indicates that microRNAs have capacity to regulate immune signaling, cytokine production and immune cell migration to control the VL infection in human. This observation warrants further investigation for the development of microRNA based therapy controlling T cell differentiation in human VL.

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# The epidemiologic considerations about visceral leishmaniasis in Albania 2010-2014



R. Petrela<sup>1,\*</sup>, E. Foto<sup>2</sup>, G. Kuli<sup>3</sup>, H. Hoxha<sup>2</sup>, F. Zavalani<sup>4</sup>, B. Neza<sup>5</sup>

- <sup>1</sup> Faculty of Medicine, Albania, Tirana, Albania
- <sup>2</sup> University hospital center "Mother Theresa", Tirana, Albania, Albania
- <sup>3</sup> University hospital center "Mother Thearesa", Tirana, Albania
- <sup>4</sup> University Hospital Centre "Mother Theresa", Tirana, AL, Albania
- <sup>5</sup> University Hospital Center "Mother Theresa", Tirana, Albania

**Background**: The aim of this study was to analyze some epidemiologic features of visceral Leishmaniasis in Albanian children.

**Methods & Materials**: There were included 194 children aged 0-14 years in this study, all admitted and treated for visceral Leishmaniasis since 1010-2014 in Pediatric Infectious Disease Service. We studied the distribution of the disease according to annual incidence, age, gender, living area.

**Results**: The results are shown in the following table.

YEAR	NEW CASES	GENDER		LIVING AREA		AGE (in years)			
		Female	Male	Rural	Urban	0-1	1-4	4-10	10-14
2010	46	25(54%)	21(46%)	10(22%)	36(78%)	5(11%)	30(65%)	9(20%)	2(4%)
2011	33	14(42%)	19(58%)	4(12%)	29(88%)	5(15%)	20(61%)	8(24%)	0(0%)
2012	47	17(36%)	30(64%)	11(33%)	36(77%)	7(15%)	30(64%)	5(11%)	5(11%)
2013	39	17(44%)	22(56%)	14(36%)	25(64%)	13(33%)	18(46%)	8(20%)	0(0%)
2014	29	11(38%)	18(62%)	6(21%)	23(79%)	3(10%)	19(65%)	6(21%)	1(3%)
TOTAL	194	84	110	45	149	33	117	36	8

**Conclusion:** Visceral Leishmaniasis is a frequent disease in Albania presented with a considerable number of cases per year. The most affected age group is from 1-4 years old, the male gender is the most affected and urban areas are also predominant over rural ones.

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## Cervical cytology as a diagnostic tool for genital schistosomiasis and cervical squamous cell atypia among young women from schistosoma and HIV endemic populations in South Africa



P. Pillay <sup>1,\*</sup>, M. Taylor<sup>2</sup>, H.N. Galappathi-Arachchige <sup>3</sup>, S.G. Zulu <sup>2</sup>, B. Roald <sup>4</sup>, E.F. Kjetland <sup>4</sup>

- <sup>1</sup> DURBAN UNIVERSITY OF TECHNOLOGY, Durban, South Africa
- <sup>2</sup> University of KwaZulu-Natal, Durban, South Africa
- <sup>3</sup> University of Oslo, Oslo, Norway
- <sup>4</sup> Oslo University Hospital, Oslo, Norway

**Background**: Globally, Africa has the highest prevalence of HIV, schistosomiasis and another neglected entity: cervical cancer. Genital schistosomiasis manifests in lesions that are hypothesized to link with HIV and cervical atypia.

**Methods & Materials**: This study was conducted among 833 young women aged 16-23 years from rural high schools in KwaZulu-Natal. Risk factors for schistosomiasis and cervical atypia and the association of genital schistosomiasis and squamous atypia of the cervix were investigated using diagnostic cytology, urine microscopy, and questionnaire data.

**Results**: Participants were sexually active from a young age and 523 (63.0%) had at least one child and 742 (89.1%) relied on rivers as their primary water source. The *Schistosoma* prevalence detected cytologically and via urine microscopy was 12 (1.4%) and 178 (21.4%) respectively. Squamous cell atypia was detected among 567 (68%). There was a significant association between the participants who were positive for any squamous cell atypia and those who had *S. haematobium* eggs in Pap smears (OR = 5.6, P = 0.005; 95% CI 1.6-21.0) and for *S. haematobium* eggs in urine OR = 2.9, 95% CI 1.72 - 4.99, P = 0.005).

Conclusion: The specificity of cytology for Schistosoma detection was low using is seen previously, it is possible that an improved detection rate of genital schistosomiasis could be achieved using cervico-vaginal lavage Schistosoma DNA testing. While a significant association exists between urogenital schistosomiasis as detected by cytology and urine microscopy with squamous atypia in this young population, it must be noted that more than half of the young women have cervical atypia that could potentially regress. Cytology was useful in revealing the squamous atypia among this young population who is not routinely screened, a limitation is that it was not feasible to confirm results using histology or other complementary tests. The relationship between schistosomiasis and cervical cancer is complex, while there may be association, it was not possible to prove causality or eliminate all confounders. In communities at risk, health promotion, screening and health care targeting not only HIV and schistosomiasis, but also cervical cancer should be made available in order to reduce the prevalence of these preventable diseases.