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Title: CLINICAL SIGNIFICANCE OF MICROBIOME DIVERSITY ANALYSIS IN SUBCLINICAL AND CLINICAL RHEUMATIC HEART DISEASE AMONG SCHOOL CHILDREN IN INDIA: A PRELIMINARY REPORT

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Background & Aims: Microbiome plays an important role in the health of the host and its dysbiosis is one of the causes of as well as the result of host disease state. Host genetics plays an important role in susceptibility to Group A Streptococcus and pathophysiology of rheumatic fever and RHD. Role of host microbiome has not been well studied. To identify throat microbiome diversity and its clinical significance in subclinical and clinical RHD and to compare with matched controls we performed a study

Methods: We performed school based epidemiological study on echocardiographic evaluation of 3000 school children aged 10-15 years in northwest India and found 118 cases of subclinical RHD. All subclinical RHD cases were given prophylaxis and followed up for 7-years. Further we studied throat microbiota from randomly selected 61 participants (Clinical RHD 20, subclinical 22 at 7-year follow-up, controls 19). Quality check was done using FASTQC toolkit and low-quality sequence data were excluded. Merging and pre-processing were performed using standard guidelines. Sequences having a similarity of 97% were grouped together under a single operational taxonomic unit (OTU) for data classification. Shotgun Metagenomic studies were performed using Illumina NovaSeq 6000 and NEB ultra-DNA kit. We performed >5 million reads per sample and generated >5 Gb data per sample.

Results: We found relative abundance of Firmicutes (42%) was highest in healthy cases (n=19) followed by Proteobacteria (25%), bacteroidetes (16%), Actinobacteria (13%) and Fusobacteria (5%). The clinically confirmed RHD cases (n=20) had highest relative abundance of Proteobacteria (34%) followed by Firmicutes (27%), Bacteroidetes (19%), Actinobacteria (14%), and Fusobacteria (6%). It is clearly evident in case of RHD the ratio of Firmicutes to Proteobacteria (F/P ratio) is more important than the F/B ratio. The alpha diversity index (Chao I) and beta diversity index (NMDS) based on the Genus/species composition indicated two well-separated clusters of RHD and controls. Subclinical cases were distributed across the two clusters. However, analyzing beta diversity of

subclinical group alone indicated two clusters, which might indicate the status or severity of RHD. Viriome diversity as well as abundance was reduced in RHD cases compared to controls or subclinical cases. Even the Streptococcus genus abundance was reduced in subclinical and RHD cases probably due to prophylactic use of antibiotics. Analysis of Antibiotic Resistance Genes (ARGs) indicated that Beta-lactam and Macrolide resistance conferring genes were enriched in RHD cases compared to healthy controls, while multidrug resistance conferring genes were present uniformly in all groups. The occurrence of ARGs even in healthy controls indicates the all pervasiveness of Antibiotic resistance in the general population making infectious disease management a public health nightmare for the clinicians. The functional analysis is underway, which might allow further finer differentiation of the sub groups. Host genotype is also being analysed to look for host factors.

Conclusions: Throat microbiome analysis can differentiate RHD from subclinical RHD and normal controls using alpha diversity. It could become a diagnostic criterion to differentiate subclinical cases that would progress to RHD. Beta diversity analysis of subclinical cases alone has shown two definite clusters hinting at the presence of unknown host related confounders for microbiome composition. The microbiome as well as host genotype study could potentially identify protective/pathogenic characteristics which may explain why some subclinical (echocardiographically) RHD revert back to normal, some remain static and some progressed. Functional diversity analysis of the throat microbiome, especially of the emm genes is underway.