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Methylation Pattern of Repetitive Elements in Correlation with Genome Wide Global DNA Methylation in the Prognosis of Paediatric Tuberculosis

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A B S T R A C T

Epigenetic modulation plays a vital role in regulating the transcriptional machinery of human genome in response to infection. Differential Methylation pattern of the genome especially the retro transposon repetitive elements (RE) which constitutes more than 45% of the genome had been studied to trace the impact of RE methylation in the disease severity. The correlation of the levels of genome wide DNA methylation and the methylation levels of LINE1 (Long interspersed transposable elements), SINE- Alu (Short interspersed transposable elements) repetitive elements (RE) showed the influence of repetitive elements in the prognosis of *Mycobacterium tuberculosis* among pediatric population. In comparison with healthy controls in this study we have investigated the association of methylation status altering the transcriptome involved in eliciting immune response. Investigating the levels of methylation of the repetitive elements will be an index to analyze the genome wide methylation which implicates the severity of the disease. Genome wide Methylation profiling could also be a potential biomarker to correlate with the prognosis of tuberculosis. The global methylation status of children infected with TB was found to be significantly differentiated when compared with healthy children. The methylation pattern of the repetitive elements LINE1&SINE (Alu) were also found to be significantly differentiated with that of controls. Significant genome wide hypomethylation was observed in children infected with TB (9% (IQR, 7%-10%) in comparison with healthy children (22 % (IQR, 18%-24%) (P<0.001). The repetitive elements LINE1 and SINE (Alu) in accordance with global methylation of the genome were found to be hypomethylated in children infected with TB. The percentage of LINE1 Methylation was 22% (IQR, 20%-26%) in cases and 67% (IQR 62-70%) in controls(P<0.0001) respectively. Similarly, remarkable difference in the levels of SINE(Alu) Methylation was found between cases (27% (IQR 24-30%) and controls 65% (IQR 58-68%) (P <0.0001)

Introduction

Tuberculosis (TB) is a disease with significant global threat. According to WHO report 2020Tuberculosis is a leading cause of death from a single infectious agent and ranks above HIV/AIDS as a key cause of death globally. Among 10.0 million people (1.0 million children) infected with TB, around 1.3 million people die worldwide. Though the

mortality rate of TB has decreased from 1.8 since 2000, still there is an alarming need to eradicate TB(TB WHO, 2020.). TB burden in children is huge and often grabs less attention. Around 69% of children <5years infected with TB have been ignored from treatment due to poor management in diagnosis and reporting. Among 1 million infected children (aged 0-14), 233000 children died in 2017 according to WHO report 2020. Unfortunately 96% of the pediatric deaths were observed in children who did not access TB treatment and 80% were less than 5yrs of age(WHO Childhood TB, 2018). Children becoming succumbed to tuberculosis disease could be due to the variation in the underlying epigenome with respect to immunological function. Upon infection the host immune system particularly the innate immune molecules undergoes massive transcriptional sequel of reactions to activate the genes involved in establishing first line defense and subsequent cellular immunity(Bierne *et al.*, 2012; Jenner and Young, 2005).

On the other hand, the organism adopts mechanisms to evade host immunity by deregulating the transcriptional machinery of host involved in establishing immunity. However specific activation and silencing of genes in response to infection does not only depends on the transcriptional factors but the epigenetic modulators to trigger a gene (Bierne *et al.*, 2012). Epigenetic modifications are the crucial modifications which implicate the expression of a gene to regulate various cellular processes in response to external stimuli, while the sequence of the genome remains intact. Epigenetic factors like DNA Methylation, Histone modification, chromatin associated complexes, non-coding RNAs, Splicing RNAs can impact the activation of immune molecules which would either lead to successful establishment of immunity or diseased state. Some of the cellular processes where epigenetic regulations have a key role are in maintaining chromosomal integrity, genome imprinting, x-chromosome inactivation, embryonic development, etc (Yadav *et al.*, 2016; Robertson KD, 2005). DNA methylation is the most prominent epigenetic regulation than the other epigenetic phenomenon as it directly obstructs the expression of a gene while the other plays much of post translational silencing. DNA methylation is a reversible chemical change occurs when DNA methyltransferases(DNMTs) shifts a methyl group covalently to the cytosine ring of a DNA at the C-5 position using S-adenosylmethionine as co-factor(Edwards *et al.*, 2017; Jin *et al.*, 2011).In mammals DNA methylation is carried out by DNA methyl transferases and methyl CpG binding proteins (MBDs) to maintain and to read the methylation marks respectively. It has been found that DNA methylation happens predominantly at the cytosines of a genome in a sequence context of 5'CpG 3' and in response to stress and infection over 98% of DNA methylation was observed to happen in CpG dinucleotides of somatic cells(Bird, 2002; Lister *et al.*, 2009; Robertson, 2002). In general, CpG islands overlap the 5' end of a gene possessing the promoter and an initial exon, almost 40% of promoters and exon regions of a genome are linked with CpG islands. CpGs are found to be presented in unmethylated state in somatic cells in normal condition.

However a well-established and maintained DNA methylation pattern of the genome is much essential to maintain the homeostasis of the body (Bird, 2002; Takai and Jones, 2002). Hypomethylated state of Repetitive Elements (RE) which constitutes more than 45% of the whole genome depicting the global DNA methylation will result in destabilizing the chromosomal integrity and thereby paves way for onset of many diseases. RE are presented in 2 major types, one of which is satellite or

tandem repeats likely to be presented in adjacent forms while the other type shows interspread repeats which are the repeated sequences spread throughout the genome. Interspread repeats are also termed as mobile DNAs or transposons. Majority of such transposon activity in human is carried out by LINE1 and SINE (Alu) and are typically in hypermethylated state in a balanced niche. LINE1 in hypomethylated state could rearrange the genome as transposon insertion sequence and thereby influence the transcription of many functional genes(Baba *et al.,* 2018; Consortium, 2001; Elbarbary *et al.,* 2016; Robertson, 2005). Recent studies suggest that hypomethylated state of LINE1 and Alu repeats could be a trigger for diseases development and can also be a potential biomarker to detect the prognosis of a disease(Aparicio *et al.,* 2009; Baba *et al.,* 2018; Huen *et al.,* 2016; Marques-Rocha *et al.,* 2016; Wright *et al.,* 2010).

In context to *Mycobacterium tuberculosis* infection histone monomethylated state of Alu repeats were reported to have widespread control over the transcriptional factors of SP1,p53, c-MYC, ANRIL, NF- kB,MEF2 and ATF families, and nuclear receptors LXR and RAR which plays vital role in macrophage differentiation and elicitation in response to infection and activating innate immunity (Bouttier *et al.,* 2016).

In spite of studies evaluating the genome wide methylation status in metabolic disorders were reported, with respect to TB infection, it's still an area to be explored. Enumerating the pattern of DNA methylation in the repetitive elements LINE1 and SINE(Alu), subsequently comparing with the percentage of genome wide 5- methyl Cytosine (5-mC) content will provide an insight on the impact of genome wide DNA methylation in TB prognosis (Fig. 1-6).

Materials and Methods

Study participants

To correlate the levels of genome wide DNA methylation with the severity of tuberculosis disease, Paediatric patients infected with TB (Cases) aged 2-14yrs of both genders and healthy children (Controls) were recruited for the study. Ethical clearance was obtained from the Institutional Ethics committee. The subjects aged above 7 and the parents/ LAR were explained about the purpose of sample collection. Written informed consent was obtained from the study participants (cases and controls). Children showing clinical signs of infection with TB, abnormal Chest X-ray indicating TB, positive TST, AFB smear positive, culture positive, positive Cartridge based nucleic acid amplification test were included in the study. Both pulmonary and extra-pulmonary tuberculosis cases were included in the study. In comparison healthy controls were recruited matching age and gender with that of study subjects. Healthy controls were children aged 2-14 with no history/signs of TB or any other lung infection/disease. A total of 40 cases and 40 controls were included in the study.

Genomic DNA was isolated from the whole blood samples collected from children infected with TB and healthy controls using Qiagen QIAamp DNA Blood mini kit following the manufacture's guidelines. Nanodrop spectrophotometer 2000 (Thermo Scientific USA) was used to measure the concentration and purity of the isolated genomic DNA.

Genome wide DNA Methylation- 5mC Quantification

The quantity of genome wide 5- methyl cytosine (5-mC) concentration was detected using 5-mC DNA ELISA kit (Zymo

Research, USA). Following the manufacture's guideline approximately 100ng of genomic DNA was used for the assay. 100ng of genomic DNA was made to a total volume of 100µL using 5-mC coating buffer. Subsequently denaturation of the DNA samples was done by heating at 98°C for 5 min in a thermocycler (Eppendorf Master cycler, Germany) and the samples were immediately shifted to ice for 10 min. The samples were then added to a pre-coated 96 welled ELISA microtiter plate to detect the methylation level of genomic DNA. After 1h of incubation at 37°C the wells were washed thrice with wash buffer and 200µL of 5mC buffer was added to each well and incubated for 30 min at 37°C. The direct conjugant of 5-methylcytosine and the secondary antibody were stocked in a ratio of 1:2 using ELISA buffer (2000µL). 100µL of this conjugants stock were added to each well and incubated at 37°C for 1h. Following the washing step thrice (200µL of wash buffer) after incubation to remove the unbound substrate- antibody conjugates, the wells were loaded with 100µL of enhancer solution to enhance the activity of horseradish peroxidase conjugated with the secondary antibody. The activity of horseradish peroxidase with the substrate results in development of color. The intensity of color development varies in each well directly proportional to the concentration of 5-mC content of the DNA sample. Thereupon 1hr of incubation at room temperature the plate was subjected to measure absorbance at 450nm to quantify the 5- methyl cytosine content in a genome. The results were validated by performing in triplicates of each sample.

Standard concentrations of methylated and unmethylated DNA controls were prepared at the concentrations at 0%, 5%, 10%, 25%, 50%, 75% and 100% to plot a standard curve for comparison (Table 1–5). 100% methylated and 100% unmethylated strands supplied with the kit was used as positive and negative control respectively.

Methylation status of Repetitive Elements-LINE1 and SINE (Alu)

Genomic DNA extraction and Bisulphite Conversion

Genomic DNA from whole blood as described earlier was isolated to detect the methylation levels of the repetitive elements LINE1 and SINE (Alu). Approximately 500ng of genomic DNA was taken and subjected to bisulphite conversion using EZ-DNA Methylation – Startup Kit and direct Kit (Zymo Research, USA). Following the treatment with proteinase K, 130µLof bisulphite conversion reagent was added to the samples and placed in a thermocycler with the following temperature settings.

1. 98°C for 8 minutes
2. 64°C for 3.5 hours

The samples were then transferred to a column treated with 600 µL of binding buffer. After a brief centrifugation for 30 secs at > 10,000xg, 100 µl of wash buffer was added to the column and Centrifuged at > 10,000xg for 30 seconds. Subsequently 200 µl of Desulphonation Buffer was added to the column and let stand at room temperature (20-30°C) for 15-20 minutes. After incubation the column was centrifuged at > 10,000xg for 30 seconds. 200 µl of Wash Buffer was then added to the column with a brief centrifugation at > 10,000xg for 30 seconds. The wash step was repeated to remove the unbound particles. Finally, 10 µl of Elution Buffer was added directly to the column matrix and centrifuged for 30 seconds at > 10,000xg, to elute the DNA. The volume of elution buffer can vary according to the concentration of the DNA required.

Methylation Specific -PCR (MSP)

After bisulphite treatment the methylated cytosines (M) in the genome will remain as cytosines and the unmethylated cytosines (U) would have been converted to uracil. Methylation specific PCR is exclusively to detect the methylation levels of a gene; however, the chance of a gene remaining in unmethylated state is also possible. Considering the fact that the gene of interest may exist in Methylated or unmethylated state, the primers designed were specifically for methylated as well as unmethylated sequence with the respective complementarity.

The promotor sequence retrieved with the transcription start site have been subjected as input sequence in methprimer tool (http://www.urogene.org/methprimer) (*MethPrimer | Tools and Databases | The Li Lab., 2019*) to design primer for methylated and unmethylated sequences respectively. The CpG islands were detected using the same software so that to trace the methylation in CpG consisting transcription factors or TSS would hinder the transcription abruptly.

MS-PCR was performed twice for each gene (SINE(Alu) and LINE1) considering the possibilities of presence of both M and U sequences. One of the reaction mixtures will have primers for methylated sequence and the other will have primers of unmethylated strands to detect the amplification respectively. A reaction volume of 25µl was made to perform MS-PCR with the following temperature settings

Bisulfite Converted DNA	- 1µl	Initial Denaturation	95°C for 10 min
Forward Primer	- 1µl	Denaturation	95°C for 30 sec (35 cycles)
Reverse primer	- 1µl	Annealing	57°C for 35 sec (35 cycles)
Master Mix	- 10µl	Extension	72°C for 30 sec (35 cycles)
Nuclease Free Water	- 12µl	Final Extension	72°C for 10 min

The amplicons of methylated and unmethylated reaction from MS- PCR were then detected in 1.5% agarose gel electrophoresis. Universal methylated human DNA standards supplied with the kit was used as control DNA. The percentage of methylation was calculated according to the Rf intensity of the product by comparing with fully methylated control DNA strands using ImageQuant LAS 500 (GE Healthcare, UK).

Results and Discussion

Genome wide global DNA methylation

Global DNA methylation levels were analyzed from the genomic DNA extracted from the blood samples of all the cases and controls enrolled in the study. Standard graph was constructed from the absorbance ratio of standard controls with known concentrations. The levels of genome wide global DNA methylation were estimated by extrapolating the absorbance ratio of all the cases and controls with that of the standard plot.

Significant difference in percentage of genome wide global DNA methylation level was observed between children infected with TB (cases) and healthy children (controls). The difference in percentage of methylation between the groups was expressed in Median IQR. Distinct hypomethylation was seen in children infected with TB while healthy controls presented higher methylation levels when compared to that of controls. As proven by many studies, hypomethylated state of a genome implies that the physiological niche is in a stimulus of the underlying disease pathogenesis and prognosis. Thus, hypomethylated state of the genome in cases is more likely related with the pathogenesis of tuberculosis. The percentage of global DNA methylation level in cases was 9% (IQR, 7%-10%) and in healthy controls percentage of global DNA methylation level was 22% (IQR,

18%-24%) P<0.0001. The median distribution of percentage of global methylation among different forms of TB showed similar percentage of methylation levels among Pulmonary (9% IQR (7-10) %), and Extra Pulmonary TB (9% (7-10) %). However, children presenting multiorgan involved disseminated form of TB (6 % (5.25-6) %) showed distinct difference in percentage of methylation with that of Pulmonary and Extra pulmonary TB (P = 0.017). Further analysis was carried out to differentiate the levels of global methylation with respect to gender and age. No significant difference in the percentage of genome wide DNA methylation was found between male and female children. Similarly, the levels of methylation did not vary across different age group of children.

Genome wide DNA methylation Analysis of LINE1 and SINE(Alu) Repetitive Elements (REs)

Methylation pattern of the repetitive elements LINE 1 and SINE(Alu) have been studied to figure out the association of methylation pattern in response to disease prognosis and severity.

Methylation Specific PCR was performed with respective primers to analyze the methylation status of LINE1 and SINE(Alu). Subsequently the PCR products were run in gel electrophoresis and the image was documented. Based on the intensity of the band comparing with that of 100% methylated and 100% unmethylated controls the percentage methylation was calculated.

Significant difference in the percentage of methylation of LINE1 and SINE(Alu) was observed between TB cases and controls. The difference in percentage of methylation was expressed in median IQR. The median percentage of LINE1 methylation among cases was 22% with an IQR ranging between 20-26% and healthy controls showed median of 67% (IQR 62-70%) LINE1 methylation. The median percentage of SINE(Alu) methylation in cases was 27% (IQR 24-30%) while controls presented a median of 65% (IQR 58-68%) SINE Methylation. The methylation pattern of Repetitive Elements among cases and controls showed distinct difference. As anticipated the children infected with TB showed hypomethylated pattern while healthy controls showed a prominent hypermethylated pattern of repetitive elements.

Further analysis was carried out to determine the difference in the levels of LINE1 and SINE(Alu) methylation among children presented pulmonary tuberculosis, extra pulmonary tuberculosis and multiorgan involved disseminated tuberculosis. Similarly difference in methylation between gender and different age group were analyzed.

The pattern of methylation in LINE1 and SINE(Alu) repetitive elements were further analyzed between children infected with different forms of TB. Similar pattern of distribution was observed between children presenting pulmonary, extrapulmonary and multiorgan involved disseminated form of Tuberculosis. Median LINE1 methylation among children with pulmonary TB was 23% (19-25)%, while extrapulmonary TB 21 % (20-30)% and disseminated TB 21 % (19-24)% on comparison no significant difference was inferred P=0.77.

Median SINE(Alu) methylation among children with pulmonary TB was 26%(24-29)%, extrapulmonary TB 28%(25-30)% and disseminated TB 27 % (24-31)%. On comparison no significant difference was observed between the groups P=0.53. Children falling in different age groups does not show difference in the levels of LINE1 and SINE methylation [LINE1, P= 0.44 and

SINE(Alu), P=0.47]. Indistinguishable difference of methylation levels was recorded between male and female children infected with TB [LINE1, P= 0.19and SINE(Alu), P=0.48].

Table.1 Concentration of Positive and Negative Controls

Percentage of 5-mC standards	Negative Control (100ng/µL)	Positive Control (100ng/µL)
0 %	10.0 µL	0µL
5 %	9.5µL	5 µL
10 %	9.0µL	1.0µL
25 %	7.5µL	2.5µL
50 %	5.0µL	5.0µL
75 %	2.5µL	7.5µL
100 %	0.0µL	10.0µL
Total volume of each standard is 10µL at a concentration of 100 ng/µL		

Table.2 LINE1 and SINE(Alu) MS-PCR Primers

Gene	Primer Type		Tm (°C)	Primer Sequence (5'-3')	Bp
LINE1	M	F	56	TATTAGGGAGTGTTAGATAGTGGGC	164
		R		ATAACCCGATTTTCCAAATACGT	
	U	F	58	TAGGGAGTGTTAGATAGTGGGTGT	164
		R		AAAATAACCCAATTTTCCAAATACAT	
SINE (Alu)	M	F	53	CGGATTATTTGAGGTTAGGAGTTC	203
		R		CCAAACTAAAATACAATAACGCGAT	
	U	F	58.5	GTGGATTATTTGAGGTTAGGAGTTT	204
		R		CCAAACTAAAATACAATAACACAAT	

M- Methylation Specific Primer, U-unmethylation Specific Primer, Tm-Melting temperature, F-Forward Primer, R-Reverse Primer, bp-base pair in length

Table.3 Relationship of Global DNA Methylation levels between different forms of TB, Age and Gender

Type of TB	No. of Cases	Median (IQR)	P value
Pulmonary Tuberculosis	33	9% (7-10)%	0.017
Extra Pulmonary TB	23	9 % (7-10)%	
Disseminated (Multiorgan)	4	6 % (5.25-6)%	
Gender	**No. of Cases**	**Median (IQR)**	**P value**
Male	31	9% (7-10)%	0.88
Female	29	9% (6.5-10)%	
Age	**No. of Cases**	**Median (IQR)**	**P value**
2 to 7yrs	30	8%(6.75-9)%	0.11
8 to14 yrs	30	9% (7-10)%	

Table.4 Methylation pattern of repetitive elements among cases and controls

Gene	Methylation	Case	Control	P value
LINE 1	Methylated	22% (IQR 20-26%)	67% (IQR 62-70%)	P<0.0001
SINE(Alu)	Methylated	27% (IQR 24-30%)	65% (IQR 58-68%)	P<0.0001

Table.5 Methylation pattern of repetitive elements among cases with different forms of TB, age and gender

Name of the Repetitive Element	Type of TB	No. of Cases	Median (IQR)	P value
LINE1	Pulmonary Tuberculosis	33	23% (19-25)%	0.77
	Extra Pulmonary TB	23	21 % (20-30)%	
	Disseminated (Multiorgan)	4	21 % (19-24)%	
SINE(Alu)	Pulmonary Tuberculosis	33	26% (24-29)%	0.53
	Extra Pulmonary TB	23	28 % (25-30)%	
	Disseminated (Multiorgan)	4	27 % (24-31)%	
Name of the Repetitive Element	**Gender**	**No. of Cases**	**Median (IQR)**	**P value**
LINE1	Male	31	23% (20-28)%	0.19
	Female	29	21% (19-25)%	
SINE(Alu)	Male	31	28% (25-30)%	0.48
	Female	29	26% (24-30)%	
Name of the Repetitive Element	**Age**	**No. of Cases**	**Median (IQR)**	**P value**
LINE1	2 to 7yrs	30	22%(20-25)%	0.44
	8 to14 yrs	30	22% (19-25)%	
SINE(Alu)	2 to 7yrs	30	27% (25-30)%	0.47
	8 to14 yrs	30	26% (23-31)%	

Fig.1 Standard graph of Methylated DNA controls

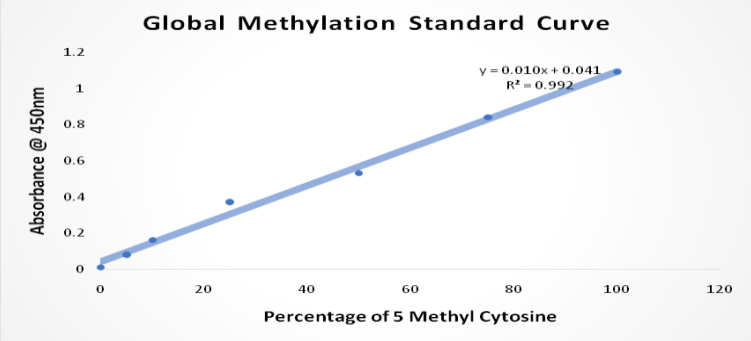


Fig.2 Global DNA Methylation status among cases and controls

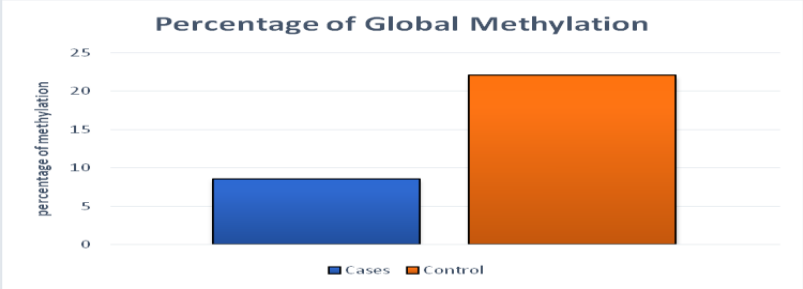


Fig.3 Distribution of Global DNA Methylation levels among cases and controls

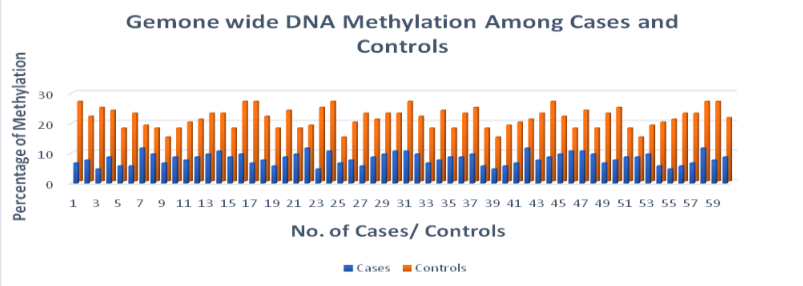


Fig.4 Distribution of Global DNA Methylation levels among different forms of TB

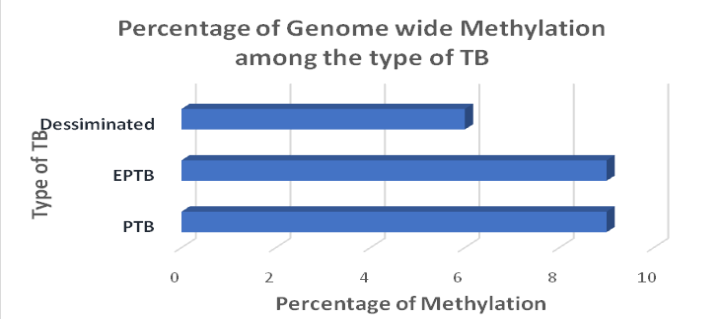


Fig.5 Box plot Representing percentage of methylation LINE1 and SINE Repetitive Elements among cases and controls

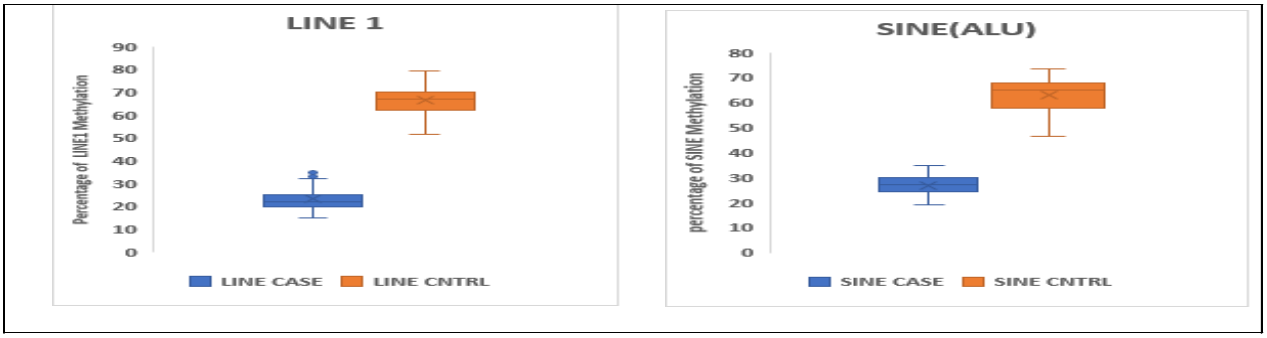
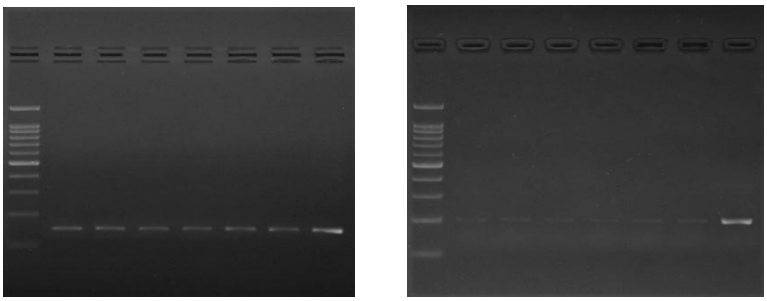


Fig.6 Gel Pics depicting the hypomethylated LINE1 (164bp) and SINE(Alu) (204bp) among cases



LINE1 Methylation SINE(Alu) Methylation

8th lane contains 100% methylated control DNA

Characterization of genome-wide DNA hypomethylation in childhood TB

The host immune cells endure massive transformations upon microbial invasion in the transcriptional cascade involved in a prime biological process like establishing immunity (Jenner and Young, 2005). On the other hand, the organism adapts unique survival strategies to alter the regulation of host immune genes. Infectious pathogens initially target the transcription factors of the host and modify their functions. Studies have shown that bacterial infections can alter the signaling pathways that are involved in triggering immunity by directly impeding the transcription or through posttranslational deregulation (Bhavsar *et al.,* 2007; Perrett *et al.,* 2011; Ribet and Cossart, 2010). This selective regulation of particular genes is carried out not only by transcriptional factors but also by epigenetic factors.

In spite of decades of tedious research and discoveries, tuberculosis is still a major concern of public health. Many studies were aimed to enumerate the role and effectiveness of immune response with respect to tuberculosis infection(Lefford, 1975; North, 1973; Orme and Collins, 1983). Particularly, the activation of innate immune molecules on encountering *Mtb* infection plays a vital role in combating the organism(Liu *et al.,* 2017; Van Crevel and Ottenhoff, 2002). Epigenetic regulations involved in prompt activation of immune molecules such as cytokines, macrophages, DCs, NKs etc with regard to *Mtb* infection determines the prognosis of the disease (Esterhuyse *et al.,* 2015; Marimani *et al.,* 2018; Reiner, 2005; Singh *et al.,* 2018).

We found that the children infected with *Mycobacterium tuberculosis* presented a distinct genome-wide DNA hypomethylation status when compared to that of healthy control children. No distinct difference was observed between children infected with pulmonary and extra pulmonary TB however, children presented with multiorgan involved disseminated form of Tuberculosis showed a significant difference in the pattern of hypomethylation.

The prominent difference of genome-wide DNA methylation observed among cases, controls and with regard to severity of the disease indicates that the genome of host undergoes a genome wide methylation transition upon infection. This transition of global methylation status during the onset of TB infection would have control over the activation of innate immune molecules by regulating the underlying corresponding gene

expression involved in triggering and establishing immunity. Some of the previous studies proved the effect of DNA methylation in regulating the immune cells(Esterhuyse *et al.,* 2012; Fernández-Morera *et al.,* 2010; Minárovits, 2009; Reiner, 2005; Wilson *et al.,* 2009) and further studies on genome-wide methylation supports the notion that globally hypomethylated DNA aids disease progression during infection(Marr *et al.,* 2014; Scharer *et al.,* 2013; Zhang *et al.,* 2016). Numerous studies on cancer epigenetics supports the same phenomenon that hypomethylation aids in disease progression(Kanai *et al.,* 2001; Stirzaker *et al.,* 2014; Toyota *et al.,* 1999). Thus genome-wide hypomethylation in children infected with TB, would either activate the pathways involved in disease progression (Esterhuyse *et al.,* 2012b; Kim *et al.,* 2012) or hamper the transcription of genes involved in eliciting protective immunity (Chen *et al.,* 2013; Esterhuyse *et al.,* 2012).

Although there was no significant difference observed between children infected with extrapulmonary (9% IQR (7-10) %) and pulmonary TB (9% IQR (7-10) %), marked difference was observed in cases presented with multiorgan involved disseminated form of TB (6 % (5.25-6) %) which can further be inferred as the levels of methylation varies according to the severity of the disease. Healthy children on the control arm showed significantly higher percentage of methylation 22% (IQR, 18%-24%) when compared to cases 9% (IQR, 7%-10%).

No significant difference of global methylation was observed among cases with different age group and gender. Thus, genome-wide 5-mc hypomethylation status in children infected with tuberculosis could act as a potential trigger to deregulate the host transcriptional mechanism to support disease progression.

Characterization of genome-wide DNA methylation analysis of LINE1 and SINE (Alu) Repetitive Elements (REs)

Repetitive Elements (RE) LINE and SINE constitutes > 45% of the whole genome. REs are often in hypermethylated state to maintain healthy genomic integrity and homeostasis of the body. Hypomethylated state of REs could hinder the transcription of adjacent functional gene by its retrotransposon activity. Some of the transcriptional factor binding sites and nuclear receptors are hampered when the REs are in hypomethylated state. Hence, methylation pattern of the repetitive elements LINE1 and SINE(Alu) have been studied to figure out the association of methylation pattern in response to TB infection.

Remarkable hypomethylated pattern of the repetitive elements LINE1 (22% (IQR 20-26%) and SINE(Alu) (27% (IQR 24-30%) were documented in children infected with TB, while healthy controls were documented with hypermethylation of LINE1 (67% (IQR 62-70%) and SINE(Alu) (65% (IQR 58-68%). The distinguished hypomethylated state of REs in children infected with TB was found in concurrence with hypomethylated state of genome-wide DNA Methylation. It furthers supports the hypothesis that hypomethylated state repetitive elements contributing to the methylation status of global DNA assists disease progression. However, no evidence of change in pattern of methylation was observed with respect to disease severity, age and gender. Children infected with Pulmonary, Extrapulmonary and disseminated form of multiorgan involved TB showed no difference in the percentage of LINE1 and SINE (Alu) Methylation.

Majority of transposon activity in human is carried out by LINE1 and SINE (Alu) and are typically in hypermethylated state in a balanced niche. LINE1 in hypomethylated

state could rearrange the genome as transposon insertion sequence and thereby influence the transcription of many functional immune genes(Baba *et al.*, 2018; Consortium, 2001; Elbarbary *et al.*, 2016; Robertson, 2005). Recent studies suggest that hypomethylated state of LINE1 and SINE(Alu) repeats could be a trigger for diseases development and can also be a potential biomarker to detect the prognosis of a disease(Aparicio *et al.*, 2009; Baba *et al.*, 2018; Huen *et al.*, 2016; Marques-Rocha *et al.*, 2016; Wright *et al.*, 2010). In our study similar trend of hypomethylated state was observed in children infected with TB disease. In context to *Mycobacterium tuberculosis* infection histone monomethylated H3K4 state of SINE(Alu) repeats were reported to have widespread control over the transcriptional factors of SP1, p53, c-MYC, ANRIL, NF- kB, MEF2 and ATF families, and nuclear receptors LXR and RAR which plays vital role in macrophage differentiation and elicitation in response to infection and activating innate immunity (Bouttier *et al.*, 2016). Thus, from the supporting evidence from our study, it is inferred that hypomethylated state of repetitive elements LINE1 and SINE(Alu) contributing to the overall hypomethylated state of the genome globally could have negatively influenced the activation of essential innate immune molecules and thereby facilitated TB disease progression.

References

Aparicio, A., North, B., Barske, L., Wang, X., Bollati, V., Weisenberger, D., Yoo, C., Tannir, N., Horne, E., and Groshen, S. (2009). LINE-1 methylation in plasma DNA as a biomarker of activity of DNA methylation inhibitors in patients with solid tumors. *Epigenetics*, *4*(3), 176–184.

Baba, Y., Yagi, T., Sawayama, H., Hiyoshi, Y., Ishimoto, T., Iwatsuki, M., Miyamoto, Y., Yoshida, N., and Baba, H. (2018). Long Interspersed Element-1 Methylation Level as a Prognostic Biomarker in Gastrointestinal Cancers. *Digestion*, *97*(1), 26–30. https://doi.org/10.1159/000484104

Bhavsar, A. P., Guttman, J. A., and Finlay, B. B. (2007). Manipulation of host-cell pathways by bacterial pathogens. *Nature*, *449*(7164), 827–834. https://doi.org/10.1038/nature06247

Bierne, H., Hamon, M., &Cossart, P. (2012). Epigenetics and Bacterial Infections. *Cold Spring Harbor Perspectives in Medicine*, *2*(12), a010272–a010272. https://doi.org/10.1101/cshperspect.a010272

Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes and Development*, *16*(1), 6–21. https://doi.org/10.1101/gad.947102

Bouttier, M., Laperriere, D., Memari, B., Mangiapane, J., Fiore, A., Mitchell, E., Verway, M., Behr, M. A., Sladek, R., Barreiro, L. B., Mader, S., and White, J. H. (2016). Alu repeats as transcriptional regulatory platforms in macrophage responses to *M. tuberculosis* infection. *Nucleic Acids Research*, *44*(22), 10571–10587. https://doi.org/10.1093/nar/gkw782

Chen, C., Pan, D., Deng, A.-M., Huang, F., Sun, B.-L., and Yang, R.-G. (2013). DNA methyltransferases 1 and 3B are required for hepatitis C virus infection in cell culture. *Virology*, *441*(1), 57–65. https://doi.org/10.1016/j.virol.2013.03.005

Consortium, I. H. G. S. (2001). Initial sequencing and analysis of the human genome. *Nature*, *409*(6822), 860.

Edwards, J. R., Yarychkivska, O., Boulard, M., &Bestor, T. H. (2017). DNA methylation and DNA

methyltransferases. *Epigenetics and Chromatin*, *10*(1). https://doi.org/10.1186/s13072-017-0130-8

Elbarbary, R. A., Lucas, B. A., &Maquat, L. E. (2016). Retrotransposons as regulators of gene expression. *Science*, *351*(6274), aac7247–aac7247. https://doi.org/10.1126/science.aac7247

Esterhuyse, M. M., Linhart, H. G., and Kaufmann, S. H. E. (2012a). Can the battle against tuberculosis gain from epigenetic research? *Trends in Microbiology*, *20*(5), 220–226. https://doi.org/10.1016/j.tim.2012.03.002

Esterhuyse, M. M., Weiner, J., Caron, E., Loxton, A. G., Iannaccone, M., Wagman, C., Saikali, P., Stanley, K., Wolski, W. E., Mollenkopf, H.-J., Schick, M., Aebersold, R., Linhart, H., Walzl, G., and Kaufmann, S. H. E. (2015). Epigenetics and Proteomics Join Transcriptomics in the Quest for Tuberculosis Biomarkers. *MBio*, *6*(5), e01187-15. https://doi.org/10.1128/mBio.01187-15

Fernández-Morera, J. L., Calvanese, V., Rodríguez-Rodero, S., Menéndez-Torre, E., and Fraga, M. F. (2010). Epigenetic regulation of the immune system in health and disease. *Tissue Antigens*, *76*(6), 431–439. https://doi.org/10.1111/j.1399-0039.2010.01587.x

Huen, K., Harley, K., Kogut, K., Rauch, S., Eskenazi, B., and Holland, N. (2016). DNA methylation of LINE-1 and Alu repetitive elements in relation to sex hormones and pubertal timing in Mexican-American children. *Pediatric Research*, *79*(6), 855–862. https://doi.org/10.1038/pr.2016.31

Jenner, R. G., and Young, R. A. (2005a). Insights into host responses against pathogens from transcriptional profiling. *Nature Reviews Microbiology*, *3*(4), 281–294. https://doi.org/10.1038/nrmicro1126

Jin, B., Li, Y., and Robertson, K. D. (2011). DNA Methylation: Superior or Subordinate in the Epigenetic Hierarchy? *Genes and Cancer*, *2*(6), 607–617. https://doi.org/10.1177/1947601910393957

Kanai Y, Ushijima S, Kondo Y, Nakanishi Y, Hirohashi S. DNA methyltransferase expression and DNA methylation of CPG islands and peri-centromeric satellite regions in human colorectal and stomach cancers. Int J Cancer. 2001 Jan 15; 91(2): 205-12. doi: 10.1002/1097-0215(200002)9999:9999<::aid-ijc1040>3.0.co;2-2. PMID: 11146446

Kim, K. H., An, D. R., Song, J., Yoon, J. Y., Kim, H. S., Yoon, H. J., Im, H. N., Kim, J., Kim, D. J., Lee, S. J., Kim, K.-H., Lee, H.-M., Kim, H.-J., Jo, E.-K., Lee, J. Y., and Suh, S. W. (2012). Mycobacterium tuberculosis Eis protein initiates suppression of host immune responses by acetylation of DUSP16/MKP-7. *Proceedings of the National Academy of Sciences*, *109*(20), 7729–7734. https://doi.org/10.1073/pnas.1120251109

Lefford, M. J. (1975). Transfer of Adoptive Immunity to Tuberculosis in Mice. *Infection and Immunity*, *11*(6), 1174–1181. https://doi.org/10.1128/IAI.11.6.1174-1181.1975

Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., Nery, J. R., Lee, L., Ye, Z., Ngo, Q.-M., Edsall, L., Antosiewicz-Bourget, J., Stewart, R.,

Ruotti, V., Millar, A. H., Thomson, J. A., Ren, B., and Ecker, J. R. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*, *462*(7271), 315–322. https://doi.org/10.1038/nature08514

Liu, C. H., Liu, H., and Ge, B. (2017). Innate immunity in tuberculosis: Host defense vs pathogen evasion. *Cellular and Molecular Immunology*, *14*(12), 963–975. https://doi.org/10.1038/cmi.2017.88

Marimani, M., Ahmad, A., and Duse, A. (2018). The role of epigenetics, bacterial and host factors in progression of Mycobacterium tuberculosis infection. *Tuberculosis*, *113*, 200–214. https://doi.org/10.1016/j.tube.2018.10.009

Marques-Rocha, J. L., Milagro, F. I., Mansego, M. L., Mourão, D. M., Martínez, J. A., and Bressan, J. (2016). *LINE-1* methylation is positively associated with healthier lifestyle but inversely related to body fat mass in healthy young individuals. *Epigenetics*, *11*(1), 49–60. https://doi.org/10.1080/15592294.2015.1135286

Marr, A. K., MacIsaac, J. L., Jiang, R., Airo, A. M., Kobor, M. S., and McMaster, W. R. (2014). *Leishmania donovani* Infection Causes Distinct Epigenetic DNA Methylation Changes in Host Macrophages. *PLoS Pathogens*, *10*(10), e1004419. https://doi.org/10.1371/journal.ppat.1004419

MethPrimer | Tools and Databases | The Li Lab. (n.d.). Retrieved March 26, 2019, from http://www.urogene.org/methprimer/

Minárovits, J. (2009). Microbe-induced epigenetic alterations in host cells: The coming era of patho-epigenetics of microbial infections: A review. *Acta Microbiologica et Immunologica Hungarica*, *56*(1), 1–19. https://doi.org/10.1556/AMicr.56.2009.1.1

North, R. J. (1973). Importance of thymus-derived lymphocytes in cell-mediated immunity to infection. *Cellular Immunology*, *7*(1), 166–176. https://doi.org/10.1016/0008-8749(73)90193-7

Organization, W. H. O (2018). *Roadmap towards ending TB in children and adolescents*.

Orme, I. M., and Collins, F. M. (1983). Protection against Mycobacterium tuberculosis infection by adoptive immunotherapy. Requirement for T cell-deficient recipients. *Journal of Experimental Medicine*, *158*(1), 74–83. https://doi.org/10.1084/jem.158.1.74

Perrett, C. A., Lin, D. Y.-W., and Zhou, D. (2011). Interactions of Bacterial Proteins with Host Eukaryotic Ubiquitin Pathways. *Frontiers in Microbiology*, *2*. https://doi.org/10.3389/fmicb.2011.00143

Reiner, S. L. (2005). Epigenetic control in the immune response. *Human Molecular Genetics*, *14*(suppl_1), R41–R46. https://doi.org/10.1093/hmg/ddi115

Ribet, D., &Cossart, P. (2010). SUMOylation and bacterial pathogens. *Virulence*, *1*(6), 532–534. https://doi.org/10.4161/viru.1.6.13449

Robertson, K. D. (2002). DNA methylation and chromatin – unraveling the tangled web. *Oncogene*, *21*(35), 5361–5379. https://doi.org/10.1038/sj.onc.1205609

Robertson, K. D. (2005). DNA methylation and human disease. *Nature Reviews Genetics*, *6*(8), 597–610. https://doi.org/10.1038/nrg1655

Scharer, C. D., Barwick, B. G., Youngblood, B. A., Ahmed, R., and Boss, J. M. (2013). Global DNA Methylation Remodeling Accompanies CD8 T Cell Effector Function. *The Journal of Immunology*, *191*(6), 3419–3429. https://doi.org/10.4049/jimmunol.1301395

Singh, M., Yadav, V., and Das, G. (2018). Host Epigenetic Modifications in Mycobacterium tuberculosis Infection: A Boon or Bane. In *The Value of BCG and TNF in Autoimmunity* (pp. 39–55). Elsevier. https://doi.org/10.1016/B978-0-12-814603-3.00004-5

Stirzaker, C., Taberlay, P. C., Statham, A. L., and Clark, S. J. (2014). Mining cancer methylomes: Prospects and challenges. *Trends in Genetics*, *30*(2), 75–84. https://doi.org/10.1016/j.tig.2013.11.004

Takai, D., and Jones, P. A. (2002). Comprehensive analysis of CpG islands in human chromosomes 21 and 22. *Proceedings of the National Academy of Sciences*, *99*(6), 3740–3745. https://doi.org/10.1073/pnas.052410099

Toyota, M., Ahuja, N., Ohe-Toyota, M., Herman, J. G., Baylin, S. B., and Issa, J.-P. J. (1999). CpG island methylator phenotype in colorectal cancer. *Proc. Natl. Acad. Sci. USA*, 6.

Van Crevel, R., and Ottenhoff, T. H. M. (2002). Innate Immunity to Mycobacterium tuberculosis. *CLIN. MICROBIOL. REV.*, *15*, 16.

World Health Organization TB Report 2020.

Wilson, C. B., Rowell, E., &Sekimata, M. (2009). Epigenetic control of T-helper-cell differentiation. *Nature Reviews Immunology*, *9*(2), 91–105. https://doi.org/10.1038/nri2487

Wright, R. O., Schwartz, J., Wright, R. J., Bollati, V., Tarantini, L., Park, S. K., Hu, H., Sparrow, D., Vokonas, P., and Baccarelli, A. (2010). Biomarkers of Lead Exposure and DNA Methylation within Retrotransposons. *Environmental Health Perspectives*, *118*(6), 790–795. https://doi.org/10.1289/ehp.0901429

Yadav, V., Dwivedi, V. P., Bhattacharya, D., Mittal, A., and Moodley, P. (2015). Understanding the Host Epigenetics in Mycobacterium tuberculosis. *Infection. J Genet Genome Res*, *2*, 016.

Zhang, X., Justice, A. C., Hu, Y., Wang, Z., Zhao, H., Wang, G., Johnson, E. O., Emu, B., Sutton, R. E., Krystal, J. H., and Xu, K. (2016). Epigenome-wide differential DNA methylation between HIV-infected and uninfected individuals. *Epigenetics*, *11*(10), 750–760. https://doi.org/10.1080/15592294.2016.1221569

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