

FIMSA 2018

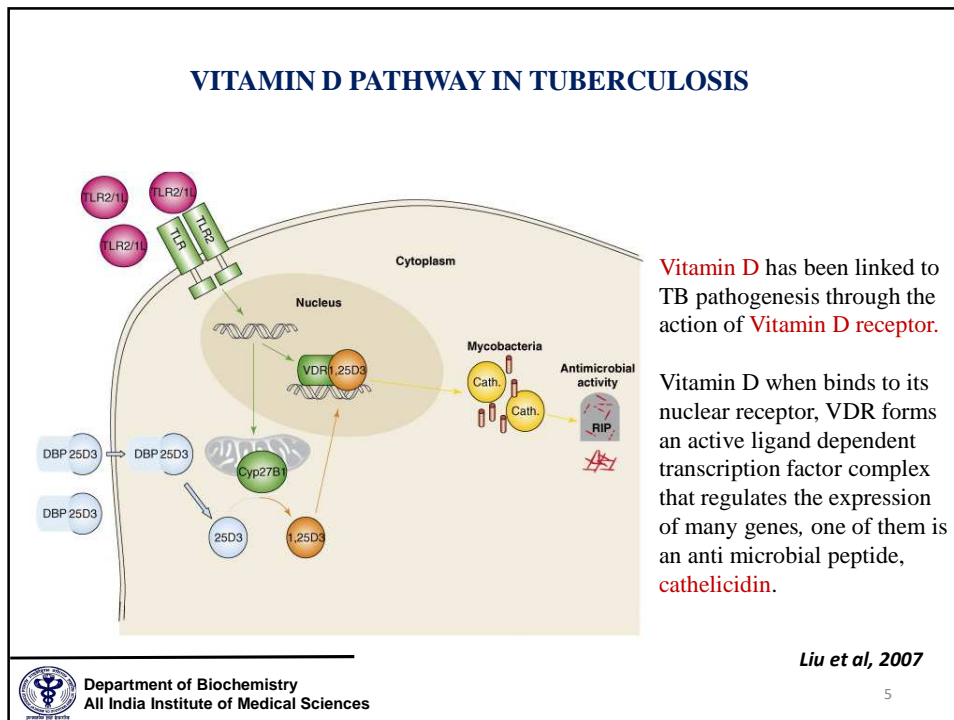
**Role of Vitamin D and its associated molecules
in innate immunity in category I Tuberculosis as
compared to household contacts**

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INTRODUCTION

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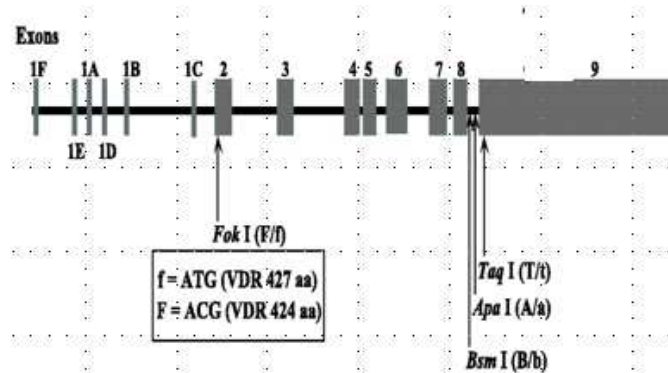
VITAMIN D

- Apart from its classical role, it acts as an immunomodulatory molecule.
- Epidemiologic studies suggest that a poor host vitamin D status is associated with an increased susceptibility to tuberculosis
(Wilkinson et al, 2000)
- Vitamin D supplementation has been linked with sputum clearance in tuberculosis patients
(Nursyam E et al, 2006)

VITAMIN D RECEPTOR (VDR)

- Located on the long (q) arm of [chromosome 12](#) at position 13.11.
- Several VDR polymorphisms are associated with risk of tuberculosis. Some of them are Taq1, Bsm1, Apa1 and **Fok1**.
- Conflicting results regarding association of different VDR polymorphism with susceptibility to tuberculosis

POLYMORPHISM IN VDR GENE



ATG-----ATG (ff, less active form)
 ↓
 ACG-----ATG (FF, more active form)

FF	Wild type
Ff	Heterozygous
ff	Homozygous mutant

Feldman D et al, 2000



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7

CATHELICIDIN

- Molecular weight- 4.5kDa.
- Found in lysosome of macrophages and polymorphonuclear leukocytes.
- Rapidly destroys the lipoprotein membranes of microbes enveloped in phagosome after fusion with lysosome in macrophages.
- Exogenous addition of cathelicidin or endogenous overexpression of cathelicidin in macrophages significantly reduced the intracellular survival of mycobacteria relative to control cells.

Sonawane A et al, 2011



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8

RATIONALE OF THE STUDY

- Paucity of data on association of host and environmental factors (anti microbial peptide cathelicidin and Vitamin D) in pathogenesis of tuberculosis.
- Host genetic factor such as VDR polymorphism with susceptibility to tuberculosis.
- Interaction of above host factors in active disease compared to household contacts.
- Utility of cathelicidin as predictive marker for active disease development in household contacts



AIMS AND OBJECTIVES



AIM AND OBJECTIVES

AIM:

To study the role of Vitamin D and its associated molecules in innate immunity in category I Tuberculosis as compared to household contacts

Objectives:

- To study the association of fok1 VDR polymorphism with tuberculosis.
- To study the expression of vitamin D receptor and vitamin D binding protein in tuberculosis patients and household contacts as compared to controls.
- To assess the level of cathelicidin and vitamin D in household contacts and active tuberculosis patients compared to healthy controls.



MATERIAL AND METHODS

Setting:

- Study was carried out at Department of Biochemistry and Department of Medicine at AIIMS, New Delhi-110029
- Patients were recruited from Medicine Outpatient Department (OPD) after obtaining the approval from institutional ethical committee and the written informed consent from study subjects

Type of study: Cross sectional study.



SUBJECTS:

- Newly diagnosed cases of category I pulmonary tuberculosis coming to medicine OPD/ward.

Sample size: A total of 280 individuals were recruited under these groups.

- 100 Active TB patient
- 80 Household contacts
- 100 Healthy controls

**Active TB patients****Inclusion criteria:**

- Male or female patients with tuberculosis aged > 18 years.
- Patients with newly diagnosed laboratory confirmed pulmonary tuberculosis, should not have received more than 2 week of antituberculosis therapy prior to enrollment.
- A case of pulmonary tuberculosis defined as a clinically diagnosed case of tuberculosis affecting the lungs, having symptoms of fever or cough and a sputum smear that showed acid-fast bacilli or a chest X-ray +ve for TB as well as gene xpert.

Exclusion Criteria:

- Patients with prior anti-microbial drug treatment of tuberculosis for longer than 2 week were excluded.
- Patients with disease like HIV, Diabetes Mellitus, hypertension and osteoporosis were excluded.
- Pregnant or lactating women.
- Patients with disorders like atopic dermatitis (eczema) and hematologic malignancies were excluded.



Household contacts:

Inclusion criteria:

- Age matched apparently healthy household contacts of active tuberculosis patients with no signs and symptoms of active tuberculosis.
- Household contacts should spend at least 6h per day for atleast 2 months with the patient.
- Mantoux positive or negative, AFB and CXR negative for tuberculosis.

Exclusion criteria: Same as Active TB group

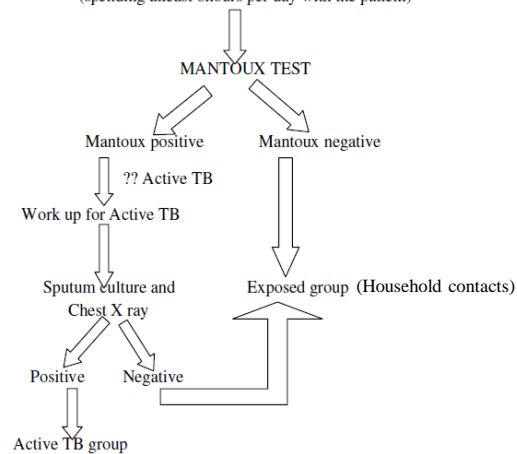
Control: Healthy individuals without any sign and symptoms of tuberculosis



Workup for recruitment of household contact group

APPARENTLY HEALTHY HOUSEHOLD CONTACTS OF TB PATIENTS

(spending atleast 6hours per day with the patient)



Strong Mantoux positive



Weak Mantoux positive



Mantoux negative




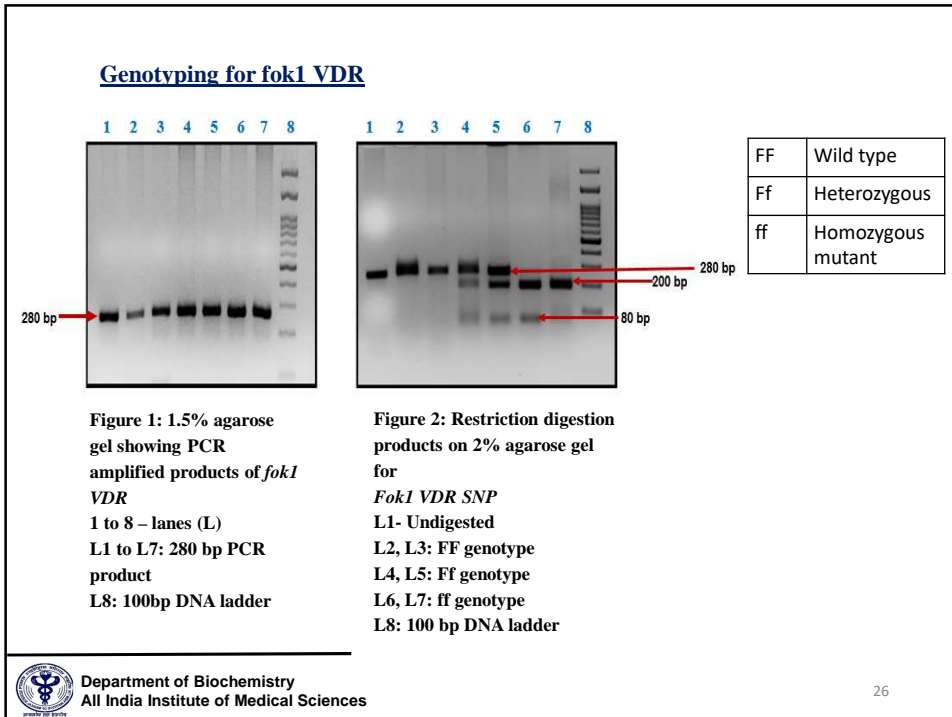
WORK PLAN AND METHODOLOGY



Demographic profile of study groups (Active TB patients and Household contacts)

Demographic characteristics	Active TB patients (n=100)	Household contacts (n=80)	Healthy controls(n=100)
Age (Mean ± SD)	36.2±7.1	35.5±6.9	30.1±5.4
Male	76.47	73.33	86.24
Female	23.53	26.67	13.76
Race	Delhi NCR	Delhi NCR	Delhi NCR
Winter	20.23	16.46	12.67
Spring	31.45	32.34	36.54
Summer	37.32	40.23	38.23
Autumn	11.00	10.97	12.56
Smoking	43.56	30.02	21.82
Non- vegetarian	73.17	63.77	60.26
Vegetarian	26.83	36.32	39.74
Indoor Occupation	32.43	44.23	34.36
Outdoor Occupation	67.57	55.77	65.64
Abnormal chest X ray	96.36	None	None
Cavitary disease on chest X ray	65.25	None	None
Bilateral disease on chest X ray	47.23	None	None
AFB positive smear	72.34	None	None


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Frequency of *FokI* VDR genotypes and alleles in Active TB patients, household contacts and controls

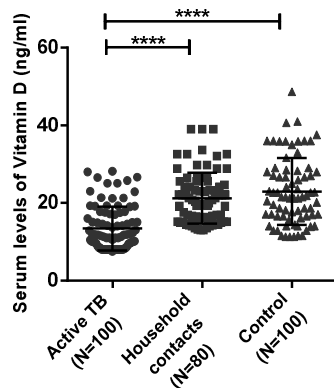
Polymorphism	Genotype	Patients (100) n (%)	Controls (100) n (%)	Household contacts (88) n (%)	χ^2 value	df	p-value
FokI VDR (T/C)	FF (wild type)	39(39)	59(59)	30(34.09)	35.92	4	0.0001
	Ff (heterozygous)	32(32)	37(37)	48(54.54)			
	ff (homozygous mutant)	29(29)	4(4)	10(11.36)			

GENOTYPES	ACTIVE	HOUSEHOLD CONTACTS	CONTROLS	P-Value
F	55	61	79	0.0001
f	45	39	21	



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Serum levels of Vitamin D in different study groups:



Study groups	Vitamin D (ng/ml) Mean±_ SD
Active TB group	11.60±_5.6
Household contacts	20.0±_6.5
Healthy Controls	21.50±_7.1

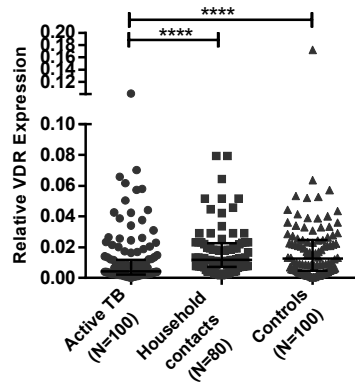
Study groups	p- value
Active vs Household contacts	0.0001****
Active vs controls	0.001****
Household contacts vs controls	0.32 (ns)

Significantly lower levels of vitamin D were seen in active TB patients as compared to household contacts and controls with p- value of 0.0001****



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Expression of Vitamin D receptor in different study groups:



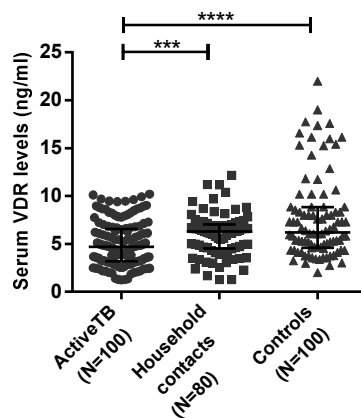
Study groups	p- value
Active vs Household contacts	0.0001****
Active vs controls	0.0001****
Household contacts vs controls	0.74 (ns)

Significantly lower expression of VDR mRNAs was observed in patients as compared to household contacts and healthy controls with p- value of 0.0001****



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Serum levels of Vitamin D receptor in different study groups:



Study groups	Vitamin D receptor (ng/ml) Mean+ SD
Active TB group	4.67+ 2.2
Household contacts	6.28+ 2.2
Healthy Controls	6.19+ 4.1

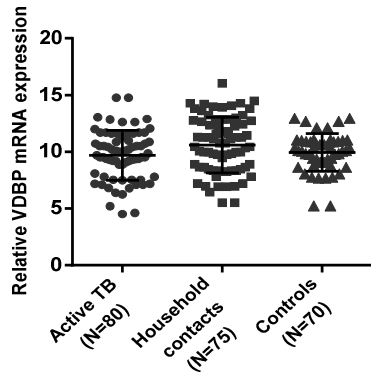
Study groups	p- value
Active vs Household contacts	0.0006****
Active vs controls	0.0001****
Household contacts vs controls	ns

Significantly lower serum VDR levels was found in TB patient as compared to household and controls with p- value of 0.0001****



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Expression of Vitamin D binding protein (VDBP) in different study groups:



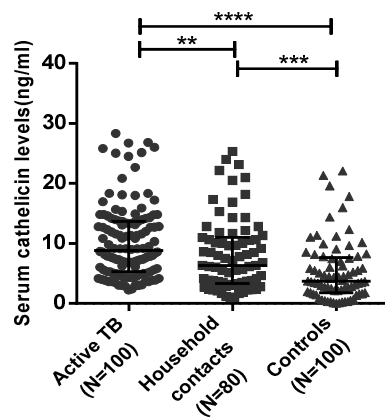
Study groups	p- value
Active vs Household contacts	ns
Active vs controls	ns
Household contacts vs controls	ns

Active TB group had lower expression of VDBP mRNAs compared to household contacts and healthy controls, but the difference was statistically non significant



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Serum levels of Cathelicidin (LL 37) in different study groups:



Study groups	LL-37 (ng/ml) Mean+_SD
Active TB group	8.83+_5.4
Household contacts	6.33+_5.17
Healthy Controls	3.74+_4.2

Study groups	p- value
Active vs household contacts	0.002**
Active vs controls	0.0001****
Household contacts vs controls	0.0006****

Significant difference between serum levels of cathelicidin was observed in all the three study groups with p- value of 0.0006



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SUMMARY AND CONCLUSION:

- Significant association was found between fok1 SNP and susceptibility to tuberculosis.
- Vitamin D levels were significantly lower in active TB patients as compared to household contacts and healthy controls.
- VDR expression was found to be lower in active group, suggesting that the bacteria might try to downregulate the expression of VDR, so that cathelicidin production would be hampered and the bacteria can survive.
- Levels of cathelicidin was higher in active group because of the additional trigger from VDR through Toll like receptor caused by the infection.
- The higher frequency of wild type genotype, increased VDR expression along with increased vitamin D levels in household contacts as compared to active TB group might be responsible for protection against the active disease.



LIMITATION:

- Small sample size
- Cathelicidin at mRNA level needs be studied

FUTURE PROSPECTS:

- The study is still going on for large sample size to validate the results
- The levels of cathelicidin will be analysed in large sample size to find out its utility as predictive biomarker for development of active disease
- Follow up studies of household contacts will be done to assess for levels of cathelicidin, vitamin D, VDR expression and development of active tuberculosis with respect to polymorphic variants of fok1 VDR.



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39

THANK YOU

40