Original Research Article

Assesment gene mutations in isoniazid hydrochloride mono resistant mycobacterium tuberculosis

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Abstract

Background: The aim of the study is to detect the of genes in isoniazid resistant mycobacterium tuberculosis and to correlate with the genes in multi drug resistant mycobacteria.To describe the INH resistance and level of INH resistance based on LPA in Government General Hospital/ Government Medical College**. Meterials and Methods:** This study is a retrospective observational study, carried out among INH drug resistant Tuberculosis patients 200 in the department of pulmonary medicine, Government General Hospital/ Government Medical college, Ongole, Andhra Pradesh during the period ie Jan 2018 to Dec2020. A total of 200 patients found to be resistant to INH were enrolled for this study to know the resistant genes. **Results:** The mean age of study population was 45.56+12.39 .There were 155 (77.5%)males,45(22.5%)were females.Out of 200drug resistant patients 190 (95%)were INH mono resistant,while 10(5%)wereresistant to both INH and Rifampicin drugs.The most common genetic mutations observed in INH monoresistance 115 patients was gene IhA(55.5%) followed by KatG gene observed in 86 patients (43%),where as 3(1.5%) patients have both inhA and KatG genes.

Key words: InhA,KatG,mutations,INH

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Introduction

Tuberculosis (TB) is a leading cause of death worldwide which is caused by bacteria called Mycobacterium tuberculosis which mainly affect the lungs.GOI announced that the new aim with regard to Tuberculosis in India was elimination of Tuberculosis by 2025 with funding over rupees 12,000 crores to ensure a proper diagnosis, treatment which mean it would be 1 case in million population. (1)TB is one of the leading causes of death among the infectious diseases. The no of TB cases each year in world is still growing and the rate of growth is slowing. One of the worst elements of TB pandemic in world was spread of drug resistance of TB. The spread of multi drug resistance TB (MDR TB) and extensively drug resistance TB (XDR-TB) have diminished all enthusiasm in controlling and eliminating TB world-wide. It is defined by TB caused by bacilli showing resistance to H and R with or without resistance to other Anti TB drugs.[10]In 2018,an estimated 10 million people developed TB and 1.5 million died from the diseases [3,6]. At least 1 million children become ill with TB every year.In 2018, an estimated 205000 children died ofTB.About 500,000 new cases of multidrug [4,7] and rifampicin – resistant tuberculosis {MDR/RR} are estimated to emerge annually but only one in three cases were reported by countries to have been diagnosed and treated in 2018[4].Ending the global TB epidemic will only be achievable if there is intensive action by all countries that have endorsed the end TB strategy and its ambitious targets[5,8].

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WHO commissioned a systematic review of all the available data in 2019.the results were assessed during a meeting of an independent WHO-convened guidelines Development Group (GDG) on 3-6December 2019[2].Detailed recommendations will be published as part of updated WHO consolidated Guidelines on TB diagnosis in 2020.This rapid communications aims to inform national TB program and other stakeholders about key implications of the least evidence on the use of specific molecular assays as initial diagnostic tests of pulmonary and extrapulmonary TB[4] and RR-TB in adults and children. The updated 2020 WHO Consolidated guidelines will also incorporate recent WHO recommendations on other rapid tests including line probe assays, urine lipoarabinomannan lateral flow assays and molecular loop-mediated isothermal amplification assays [5].

Classification of Dr TB

Primary

When drug resistance occurs to patient who has never received Anti TB treatment previously

Acquired

Resistance results due to specific previous treatment of Anti TB drugs due to Previous treatment of TB is the important factor for development of MDR-TB, Bacilli which initially has resistance to one drug may develop resistance to two drugs and many drugs and Inadequate drug treatment .Poor training,No monitoring of treatment,Poor quality,Unavailability of certain drugs ,Lack of Information ,Lack of money[9]

Mutation of mycobacterial genes of specific drugs

INH= enoyl -acyl carrier protein (ACP) reductase (InhA)
Catalase-peroxidase (KatG)
Alkyl hydroperoxide reductase (AhpC)
Oxidative stress regulator (oxyR)
β-ketoacyl-acyl carrier protein synthase (KasA)
RIF = RNA polymerase sub unit B (rpo B)
Pyrazinamide = pyrazinamide (pncA)
Streptomycin = Ribosomal protein sub unit 12 (rpsL)
Ibs ribosomal RNA (irs)
Aminoglycoside phosphotransferase gene (strA)

Capreomycin = Hemolysin (HlyA)
Ethambutol = Arabino Syl phosphotransferase (embA, embB and embC)
Fluroquinolone = DNA gyrase (gyr A and gyr B)
Other, bacterial virulence factors i.e. W-Beijing genotype and host genetic factors may alsocontribute to drug resistance.The presence of HCA-DRB, DQBI*0503 and DQBI* 0502 alleles were found in MDR-TB cause fordevelopment. Other was wide spread of second line drugs.

Aim and objectives

Aim

The aim of the study is to detect the of genes in isoniazid resistant mycobacterium tuberculosis and to correlate with the genes in multi drug resistant mycobacteria.

Objectives

To describe the INH resistance and level of INH resistance based on LPA in Government General Hospital/ Government Medical College.
To determine the prevalence of INH resistance.
To determine the prevalence of resistance in age groups and gender.
To determine the incidence of INH resistant genes in new cases.

Methodology

This study is a retrospective observational study, carried out among 200 INH drug resistant Tuberculosis patients in the department of pulmonary medicine, Government General Hospital/ Government Medical college, Ongole, Andhra Pradesh during the period from Jan 2018 to Dec 2020.
STUDY PROCEDURE:A quantitative research approach was adopted with a descriptive retrospective observational study. The study will be conducted at Government General Hospital / Government medical college, pulmonology department, Ongole. The samples were collected and tested by using CBNAAT- gene expert machine ,cephid company to detect TB and for testing rifampicin resistance , its procedure include addition of 2:1 sample reagent to the fresh sputum , shake well and stand it for 10 mins , fill it in the pipette up to mark and transfer into cartridge, then the cartridge is scanned for TB and rifampicin resistance, if the samples were identified positive for TB , further same samples were sent to Nellore for detecting INH resistance by line probe assay method(LPA) which use PCR and reverse hybridization for rapid detections of mutations associated with drug resistance of rifampicin and isoniazid made available under Revised National Tuberculosis Control Program(RNTCP)/NTEP.Data was collected in both records of district TB Centre and excel sheet in TB ward of pulmonology department in Government General Hospital, Ongole.

Inclusion criteria

All age groups of TB patients.
All MTB detected cases are sent to line probe assay.
With all comorbid conditions including pregnant and lactating women.

Exclusion criteria

All MTB non detected cases in CBNAAT.
Resistance to Other than MDR & INH drug resistant cases

Statistical method

The association between the presence of katG and inhA will be determined using Pearson's chi-squared and fisher's exact test.

Results

In our study we have collected 200 patients' consecutive samples found to have either INH mono resistant (or) MDR strains were included and were analyzed.

Gender wise distribution(INH)

Males about 77.5% had experienced higher percentage of INH resistance compared to females 22.5% is shown in the table 1.0

Table 1:Demographic variables and INH resistant

S.no	Demographic variables	H resistant (INH)	
		N	%
1	Male	155	77.5
2	Female	45	22.5

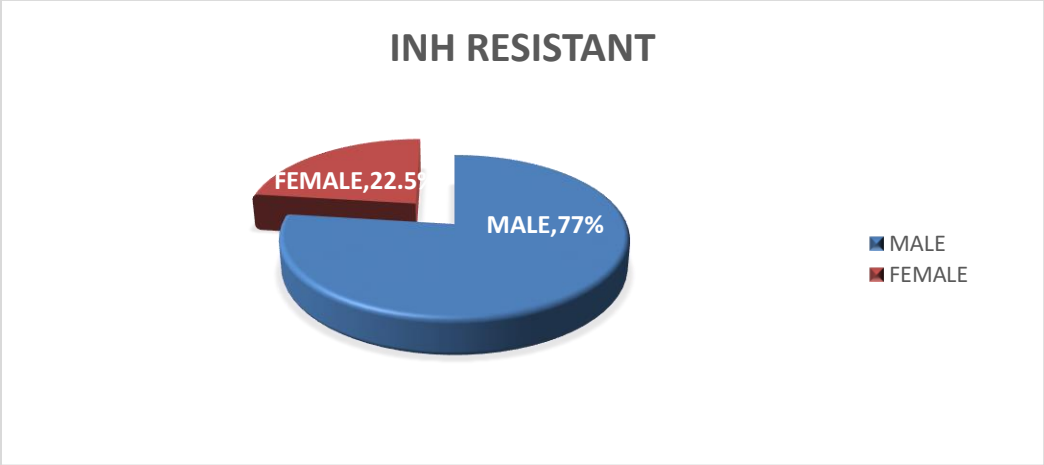

Fig 1:Male and Female percentage

Age wise distribution(INH)

Considering the socio demographic INH resistant is majorly observed among the people with age group of 15-30 years (19.0%) followed by people with age group of 31-45 years (27.0%) 46-60 years (36.0%) >60 years (18.0%) is shown in the table2.0 (p-value -o.00054 significant, <0.05)

Table 2:Age wise distribution

S.no	Demographic Variables (Age in Years)	H Resistant (INH)	
		N	%
1	15-30	38	19
2	31-45	54	27
3	46-60	72	36
4	>60	36	18

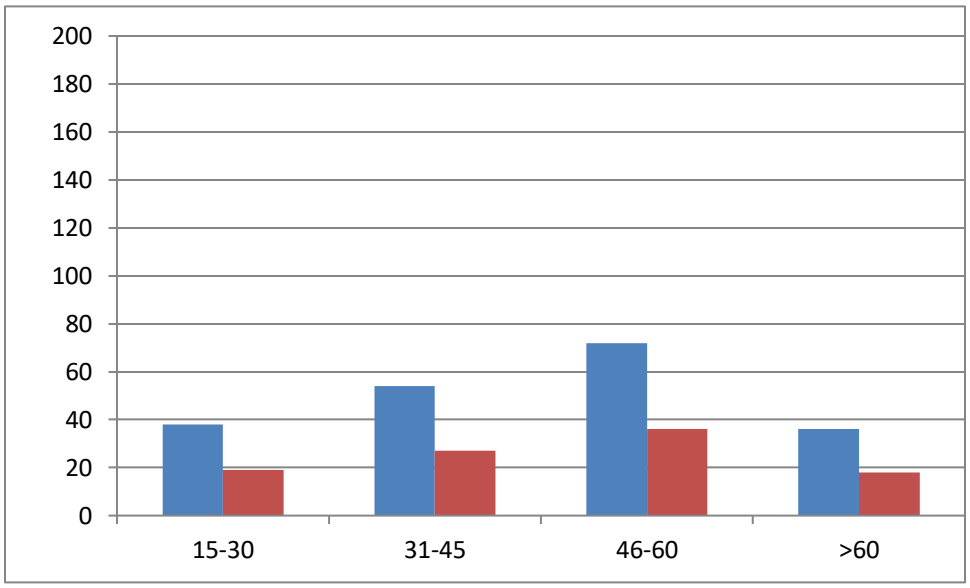

Fig 2:Age wise distribution

Gender wise distribution (MDR tuberculosis):Males about 70% experienced higher percentage of multi drug resistance compared to females 30% is shown in table 3.0 (p-value 0.560084, not significant)

Table 3 : Gender wise distribution

S.no	Demographic variables	H & R Resistance (MDR)	
		N	%
1	Male	7	70
2	Female	3	30

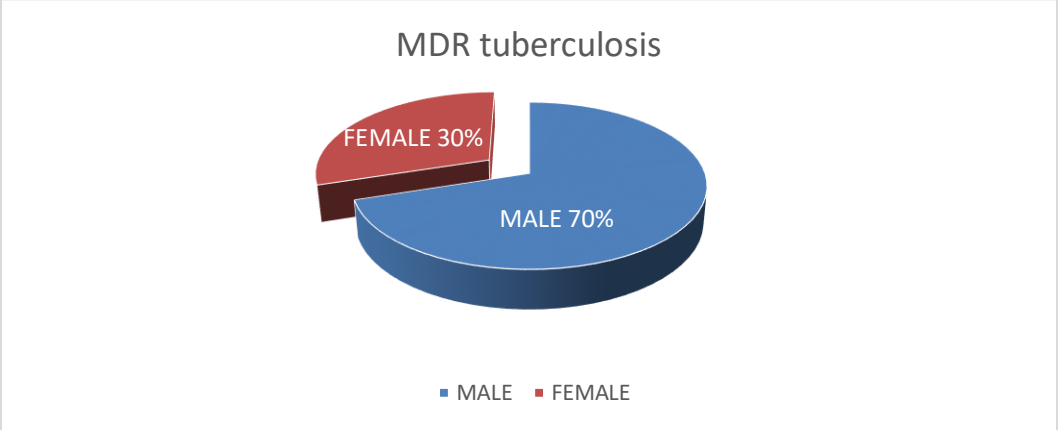

Fig 3: Male and Female distribution

Age wise distribution (MDR): Considering the socio demographic Both INH and Rifampicin (or) MDR is majorly observed mostly among the people with age group 15-30 Years (20%) followed by people with age group 31-45 Years (20%) 46-60 Years (30%) >60 Years is shown the table 4.0.

Table 4: Age wise distribution (MDR)

S.no	Demographic Variables (Age in Years)	H & R Resistance (MDR)	
		N	%
1	15-30	2	20
2	31-45	2	20
3	46-60	3	30
4	>60	3	30

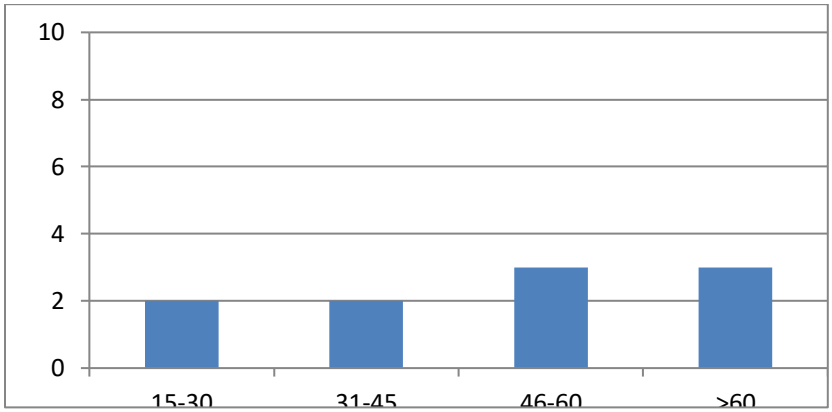

Fig 4. Age wise distribution (MDR)

Table 5: Type of gene resistance in INH Resistance Mycobacterium TB Strains

A total of 200 consecutive patients, HA gene was observed in 111 patients (55.5%), HG gene was observed in 86 patients (43%) and Both HA and HG gene was observed in 3 patients (1.5&%).

HA n(%)	HG n(%)	Both n (%)
111(55.5%)	86(43%)	3(1.5%)

Table 6:Gender wise distribution for type of gene resistance

Sex	HA	HG	Both
Male	89	77	3
Female	22	9	0

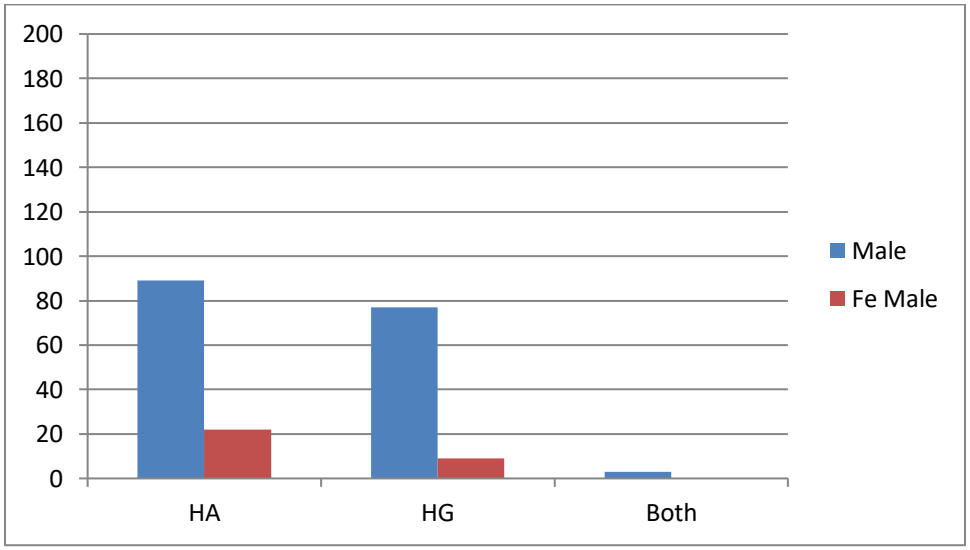

Fig 5:Gender wise distribution for type of gene resistance

Discussion

In the present study of 200 patients, the mean age of the study population was 45.56+15.39. ninety-two (46%) of 200 patients were in a age group of 15-45 years. Seventy-two (36%) patients were in age group of 45-60 years and thirty-six (18%) patients were in a age group of above 60 years, respectively. The mean age reported in other studies .Ashok singh et.al was 40.27+13.82[11] and erdenegel narmandakh et.al was 25-34 years [13]. Thus, all these studies revealed similar age distribution among drug resistant patients and clearly suggest that drug resistant TB effects younger and economically productive age group.In the present study most of the patients were male 155(77.5%) ad 45 (22.5%) were female among 200 patients. The male to female ratio was 5:1. Similar gender distribution was reported in Ashok Singh charan et al(11),(5:1) among 298 patients 250 were males and 48 were females.

Of 200 drug resistant isolates, 197 (98%) were INH monoresistant, while 3 (1.5%) isolates were resistant to both INH and Rifampicin. The INH monoresistant group there were 3 (100%) males and no females were present.

Our study revealed that the inhA mutations accounted for the majority of genetic mutation associated with INH resistance in mycobacterium tuberculosis. In the present study, we observed that the most common mutation in INH monoresistance 115 patients was inh A gene (55.5%) followed by Kat G gene which was observed in 86 patients (43%), where as in 3 (1.5%) patients have both InhA and Kat G mutations .Almost similar results were reported by erdenegerel narmandalch et.al they observed that from total of 409 isolates,294 (71.9%) had inh A mutations and 115 (28.1%) had Kat G mutations

A study by V.R. Bollela at.el observed that InhA mutations were observed higher in brazil those in Mozambique (40.9% vs 10.5%) and KATG mutations was higher in Mozambique than in Brazilian isolates (84.2% vs 54.5%) and there also state that reports from different region of the world show high variations in the proportion of KATG and inhA mutations and a study by seifert et al.Reported that the WHO American region had the highest frequency of inhA mutations (24%) , while In the African region , the frequency tends to be lower

Conclusion

This study concludes that the most common mutation in INH monoresistance is inh A (55.5%) as compared to katG (43%) and both inh A and katG (1.5%).But in most of studies katG mutation seen as predominant mutation in INH drug resistance in other parts of country and other countries. So further studies are necessary for therapeutic regimen which may be decided by mutations and gene patterns.

Conflict of Interest: Nil

Source of support: Nil

Ethical approval

Approval has been taken from the ethical committee, Government medical college, Ongole.

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