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Details of papers published in the journals notified on UGC – CARE list in the UGC website 2019

S.N	UGC website/ Scopus/ Web of Science/ PubMed	Publication type	Publication title	Author name	Journal name	Year
1.	Scopus	Original article	Poly - Phenolic Study Of South- Indian Nutmeg (MyristicaFragrans)Using Gc-Ms And FTIR	Muthuvel Kanagasabapathy, Nandhakumar Palraj, Rajasankar Srinivasagam	International Journal of Pharmaceutical Research	2019
2.	30544122	Original article	Ameliorative Effect of Withaferin A on Ageing-Mediated Impairment in the Dopamine System and Its Associated Behavior of Wistar Albino Rat	Raziya Banu M, Ibrahim M, Prabhu K, Rajasankar S.	Pharmacology	2019
3.	30468633	Original article	Demethoxycurcumin ameliorates rotenone-induced toxicity in rats	Ramkumar M, Rajasankar S, Swaminathan Johnson WM, Prabu K, Venkatesh Gobi V	Frontiers in bioscience (Elite edition)	2019
4.	30468634	Original article	Antiapoptotic role of Agaricusblazei extract in rodent model of Parkinson's disease	Venkatesh Gobi V, Rajasankar S, Swaminathan Johnson WM, Prabu K, Ramkumar M.	Frontiers in Bioscience-Elite	2019
5.	Scopus	Original article	Neuroprotective efficacy of withaferin a on aging mediated oxidative stress in striatum and Substantia nigra of wistar albino rat	Banu, Mohammad Raziya; Ibrahim, Muhammed; Prabu, K.; Rajasankar, Srinivasagam	Drug Invention Today	2019

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6.	Scopus	Original article	Anti-inflammatory effect of withaferin A on dopaminergic neuron of aged rat	Banu, Mohammad Raziya; Ibrahim, Muhammed; Prabu, K.; Rajasankar, Srinivasagam	Drug Invention Today	2019
7.	Scopus	Original article	Effect of Coccinia indica leaf extract on angiotensin converting enzyme (ACE) inhibitor induced hepatotoxicity in wistar albino rats	G. Vinothkumar , P. Venkataraman, V.M. Vinodhini, R. Lavanya, D. Sathishkumar	Clinical Nutrition Experimental	2019
8.	31572051	Original article	Patient-rated Physicians' Empathy and Its Determinants in Riyadh, Saudi Arabia	Alzayer ZM, Abdulkader RS, Jeyashree K, Alselihem A.	Journal of Family & Community Medicine	2019
9.	30709351	Original article	Are they there yet? Linkage of patients with tuberculosis to services for tobacco cessation and alcohol abuse - a mixed methods study from Karnataka, India.	Navya N, Jeyashree K, Madhukeshwar AK, Anand T, Nirgude AS, Nayarmoole BM, Isaakidis P	BMC Health Services Research	2019
10.	31215476	Original article	Inequity in Catastrophic Costs Among Tuberculosis-Affected Households in China	Xu CH, Jeyashree K, Shewade HD, Xia YY, Wang LX, Liu Y, Zhang H, Wang L	Infectious Diseases of Povert	2019
11.	31134319	Original article	Trends in tobacco consumption in India 1987-2016: impact of the World Health Organization Framework Convention on Tobacco Control	Suliankatchi Abdulkader R, Sinha DN, Jeyashree K, Rath R, Gupta PC, Kannan S, Agarwal N, Venugopal D.	International Journal of Public Health	2019

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12.	30958841	Original article	Attrition and delays before treatment initiation among patients with MDR-TB in China (2006-13): Magnitude and risk factors	Xu C, Li R, Shewade HD, Jeyashree K, Ruan Y, Zhang C, Wang L, Zhang H	PLoS One	2019
13.	SCOPUS, Web of Science	Topical Review	Current treatment of Osteoporosis	N Subramanian	Indian Journal of Rheumatology	2019
14.	30956474	Original article	Complications and Management of Paraovarian Cyst: A Retrospective Analysis	Durairaj A, Gandhiraman K	Journal of obstetrics and gynaecology of India	2019
15.	31000180	Original article	Amlodipine alters hemorheological parameters: Increased efficacy at the cost of edema?	Ravindra RP, Arunkumar S, Puniyani RR, Padgaonkar K, Vadivelu R, Sharma R, Panicker G, Lokhandwala Y.	Indian heart journal.	2019
16.	32035525	Original article	Coronary artery size in North Indian population - Intravascular ultrasound-based study	Reddy S, Kumar S, Kashyap JR, Rao R, Kadiyala V, Reddy H, Kaur N, Ramalingam V, Kaur J	Indian heart journal.	2019
17.	32035520	Original article	Intermediate term outcome after electrogram guided segmental ostial pulmonary vein isolation using an 8 mm tip catheter for paroxysmal atrial fibrillation	Pawar P, Vadivelu R, Bachani N, Jeyashree K, Sharma R, Rathi C, Jadwani J, Bera D, Lokhandwala Y.	Indian heart journal.	2019
18.	31406465	Case Report	Nanosomal docetaxel lipid suspension based chemotherapy in pregnant MBC	Ramaswamy R, Joshi N, Khan MA, Siddhara S	OncoTargets and therapy	2019

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Article Detail

POLY - PHENOLIC STUDY OF SOUTH- INDIAN NUTMEG (MYRISTICA FRAGRANS)USING GC-MS AND FTIR

Author: MUTHUVEL KANAGASABAPATHY, NANDHAKUMAR PALRAJ, RAJASANKAR SRINIVASAGAM

Abstract: Myristica Fragrans (Nutmeg) is one of the most important spices . MF was well-known for its aroma and therapeutic action . In India, Myristica Fragrans (MF) was commonly said to be Jatiphal, Jathikai and Javitri. It belongs to the family myristaceae . Myristica fragrans was used in a lot of industrial applications such as liqueurs, soap production and cosmetics. MF was reported for its improving memory, aphrosidiac ,antioxidant , anti-inflammatory , anticollagenolytic properties . In our study, in MF was found to possess 66.7% polyphenolic content.

Keyword: FTIR, GC-MS, Myristica fragrans (MF), Theaflavin (TF), KBr Potassium Bromide

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Research Article

Pharmacology

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Ameliorative Effect of *Withaferin A* on Ageing-Mediated Impairment in the Dopamine System and Its Associated Behavior of Wistar Albino Rat

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Keywords
Ageing · Dopamine · Withaferin-A · Striatum · Substantia nigra

Abstract
Withaferin A (WA) was evaluated for its neuro-protective efficacy on ageing induced striatal dopamine (DA) and behavioural changes in aged rats. *Wistar albino* rats were divided into group I – young (3 months), Group II – aged (24 months), Group III – aged rats supplemented with WA (50 mg/kg b.w once in a day for 30 days) and Group IV – young rats supplemented with WA (50 mg/kg b.w). The HPLC assay revealed significant decline in the levels of DA and homovanillic acid (HVA) in substantia nigra (SN) and striatum (ST) of aged rat. A marked decline in motor activity of aged rat was observed through open field, beam walking and grid walking motor experiments. These results indicate that ageing reduces nigro-striatal activity as well as nigro-striatal DA levels. Interestingly, the administration of WA (50 mg\kg b.w) resulted in a substantial resurge of DA and HVA in SN and ST and a significant reversal of motor impairment in aged rats. This study is the first report that evidently determines the neuroprotective efficacy of WA on dopaminergic system of SN and ST in aged rats.

Introduction

The nigro-striatal dopaminergic system has received substantial attention in the field of ageing due to its involvement in debilitating motor impairment of Parkinson's disease (PD) [1] in human and similar alterations in different animal species [2, 3]. The activity of rodents tested with different behavioural and motor assessment tests indicates a decline in aged animals [4, 5]. Furthermore, it is evident from studies that age-related alterations in dopaminergic (DA) neurons are similar to pathological changes associated with PD [6]. Oxidative stress seems to be a potential risk factor in ageing-mediated neuronal and neuro-transmitter alterations [7]. This suggests that protection against ageing-mediated oxidative damage in neurons could be a very promising ther-

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apeutic target. The standard drug like Levodopa does not seem to restore motor impairment in the aged subjects [2], and show secondary complications in long-term usage. These issues can be best overcome through the use of medicinal plants and plant products that possess enormous beneficial properties and little or nil side effects.

Withania Somnifera (WS), the medicinal plant had been in use in Ayurvedic medicine for more than 2,500 years for its enormous beneficial effects [8], and referred to as "Rasayana herb" for its anti-ageing property [9]. Its efficacy is notable in oxidative stress-mediated neuro-degenerative disorders like PD [10]. *Withaferin A* (WA), the active withanolide is attributed for the beneficial properties of WS [11]. However, the neuro-protective role of WA has not yet been scientifically validated. The present study was aimed at evaluating the efficacy of WA on neuro-chemical and behavioural impairment associated with nigro-striatal DA system in aged rats, in the absence of overt neurologic disease.

Materials and Methods

Animals

Male *Wistar albino* rats of 3 months (young) and 24 months (aged) were used for this study. The study was approved by the institutional animal Ethics Committee. The quarantine and animal maintenance were as per the guidelines of Canadian Council Guide to the Care and Use of Experimental Animals [12]. Animals were randomly divided into 4 groups (n = 6). The group I (Young) received normal saline containing DMSO 0.1% v/v, group II (Aged), group III (Aged + WA) received WA (50 mg/kg b.w., DMSO 0.1% v/v) and group IV (Young + WA) received WA (50 mg/kg b.w). Withaferin-A (Sigma Aldrich, St. Louis, MO, USA; the purity is ≥94% by HPLC) was administrated once daily for 30 days by gavage.

Behavioural Assessment (Open Field Activity, Narrow Beam Walking Test and Grid Walk Test)

The locomotor and exploratory activities of the rats were recorded with the open-field test as described by Ijomone et al. [13]. Narrow beam walking test was performed to assess the motor coordination requiring balance and equilibrium [14]. The grid-walking test was applied to assess the spontaneous motor deficits and limb movements involved in precise stepping, coordination, and accurate paw placement [15]. These analyses were carried out for 3 consecutive days.

Determination of Neurotransmitter Concentrations

Neurotransmitters were quantified using a C-R8A data processor (Shimadzu, Kyoto) and expressed as nano grams of neurotransmitter per gram of wet weight of brain tissue, as described by Ravindran et al. [16].

Statistical Analysis

All the data were subjected to one-way analysis of variant test and data showing that p value <0.05 was considered statistically significant. Analysis significant was done according to the method of Zar [17].

Results

Behaviour Analysis (Open Field Study, Beam Walking Test and Grid Walking Test)

In an open field study, aged rats exhibited significant reduction in the locomotor activity ($p < 0.001$) with reduced central and peripheral movements (Fig. 1) and increased immobilization time (Fig. 1d), when compared to the young control rats. There was a significant reduction in hiding frequency (Fig. 1c) due to reduced rearing and rearing against the wall (Fig. 1b). Aged rats expressed increased grooming ($p < 0.001$) and defecation ($p < 0.001$; Fig. 1e, f). Notably, the supported rearing (rearing against wall) was relatively higher than the unsupported rearing in both aged and young rats. These impairments significantly ($p < 0.001$) reversed in aged rats treated with WA when compared to aged rats (Fig. 1). These data clearly indicate the efficacy of WA in ameliorating the ageing-induced locomotory impairment.

In aged rats, both beam and grid walking tests showed significant ($p < 0.001$) increase in the time of crossing, number of foot slips, number of footsteps and the foot faults of all limbs with significant ($p < 0.001$) reduction in the velocity, when compared to young rats (Fig. 2). These observations indicate marked alterations in the motor and the sensori-motor coordination in ageing. The aged rat + WA showed significant ($p < 0.001$) restoration in these parameters. (Fig. 2). This signifies the potential of WA towards establishing marked recovery in motor co-ordination and balance in aged rats, probably through striatal striatum (ST) DA resurge.

Determination of Neurotransmitter Concentrations

The ST and midbrain DA and homovanillic acid (HVA) levels were found to be significantly low in aged rats when compared to young rats. This might be due to ageing-mediated imbalance between the synthesis and degradation. The levels of both DA and HVA in ST and midbrain of aged rat + WA were found to be significantly high. There was a slight increase in DA and HVA levels in ST and midbrain of young rat + WA, but this was statistically insignificant when compared to young (Fig. 3). Data clearly demonstrate the neuroprotective role of WA on ageing-mediated ST and substantia nigra (SN) neuro-chemical changes in rat brain.

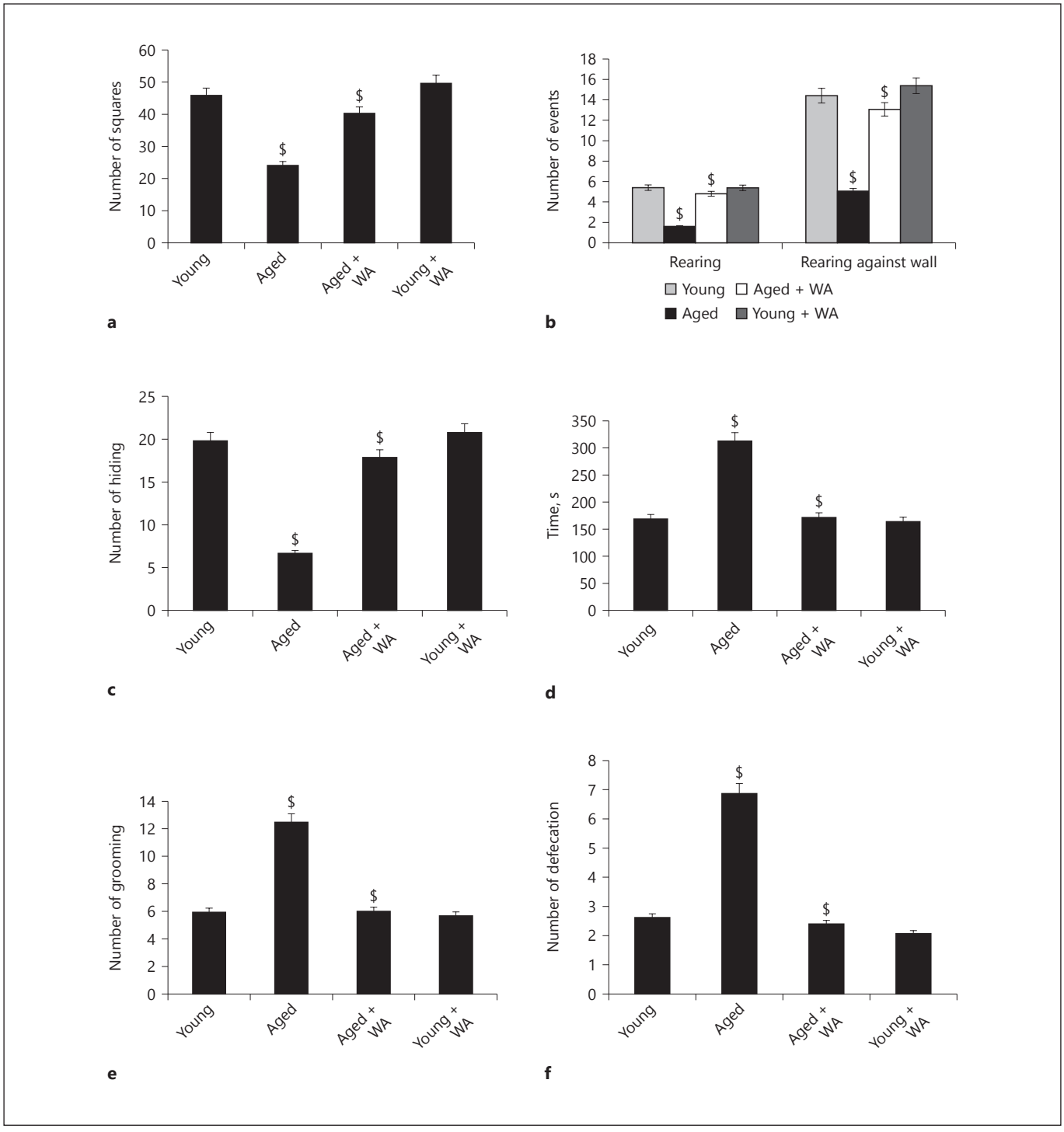

Fig. 1. Shows the effect of WA on open field behaviour in young and various experimental animals. The aged animal showed marked impairment in (**a**) locomotory frequency, (**b**) rearing and rearing against the wall behaviours, (**c**) hiding (calculated by adding the rearing frequency and rearing against the wall), (**d**) immobilaization, (**e**) grooming and (**f**) defecation when compared to the young. This impairment was markedly reversed by the administration of WA. Each bar indicate the mean ± SEM of $n = 6$ of each group. $^{\$}$ $p < 0.001$. WA, Withaferin A.

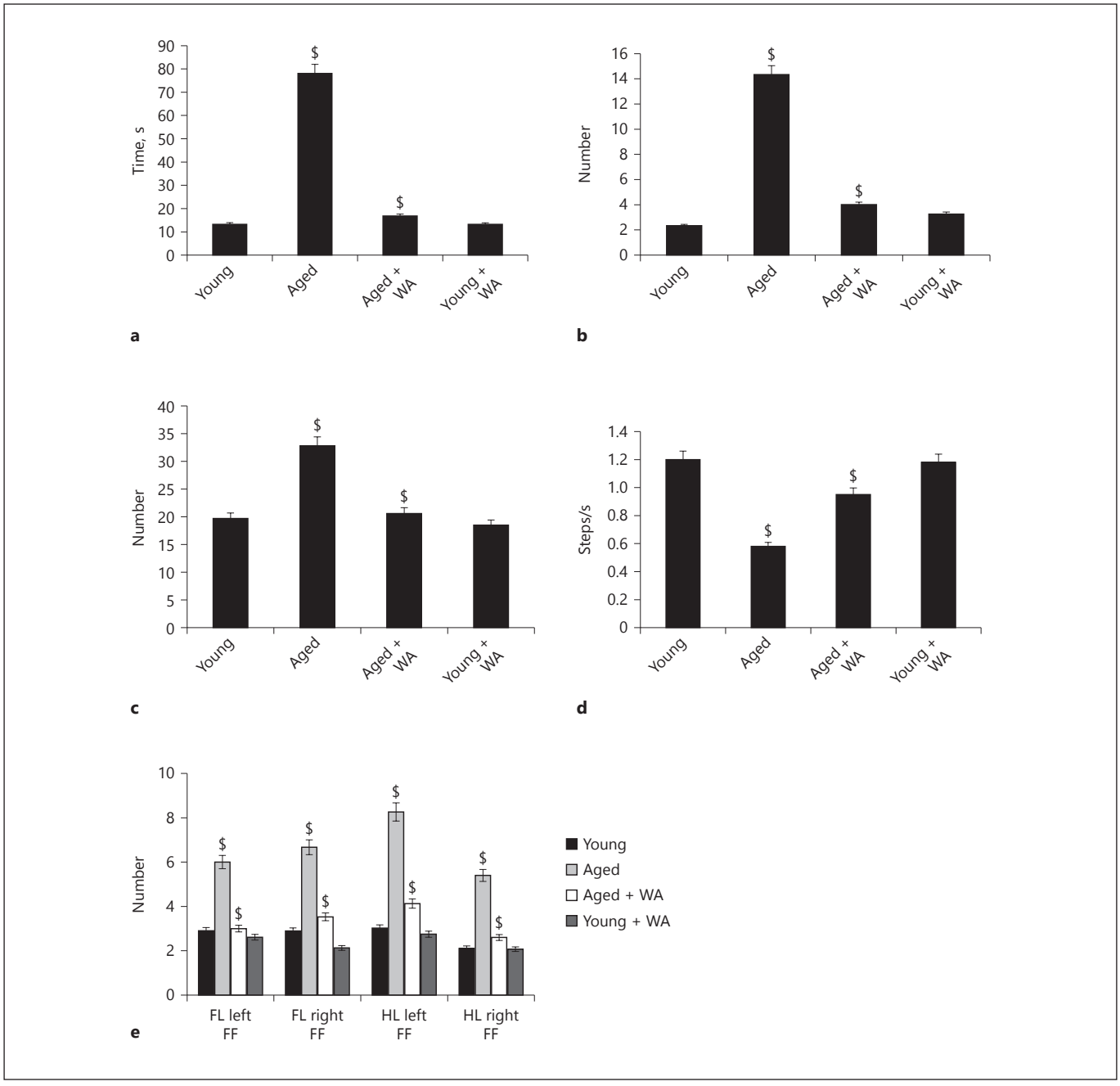

Fig. 2. Shows the effect of WA on motor activity by narrow beam walk analysis of young and various experimental animals. The aged animal showed marked alteration in (**a**) time taken, (**b**) footstep error, (**c**) number of footsteps, (**d**) velocity and (**e**) foot fault when compared to young. This alteration was substantially reversed by the administration of WA. Each bar indicate the mean ± SEM of $n = 6$ of each group. $^{\$}p < 0.001$. WA, Withaferin A; FL, fore limb; HL, hind limb; FF, foot fault.

Discussion

Motor activity is a behavioural biomarker of well-being in middle-aged and older adults [4]. This study has demonstrated a marked decline in the levels of striatal DA in aged rats, a most essential neuro-transmitter that regulates movement. Our data is consistent with the data of earlier studies in rodents [18, 19] and in nonhuman primates [20]. Nevertheless, some studies have reported only small or insignificant changes in DA or SN neurons

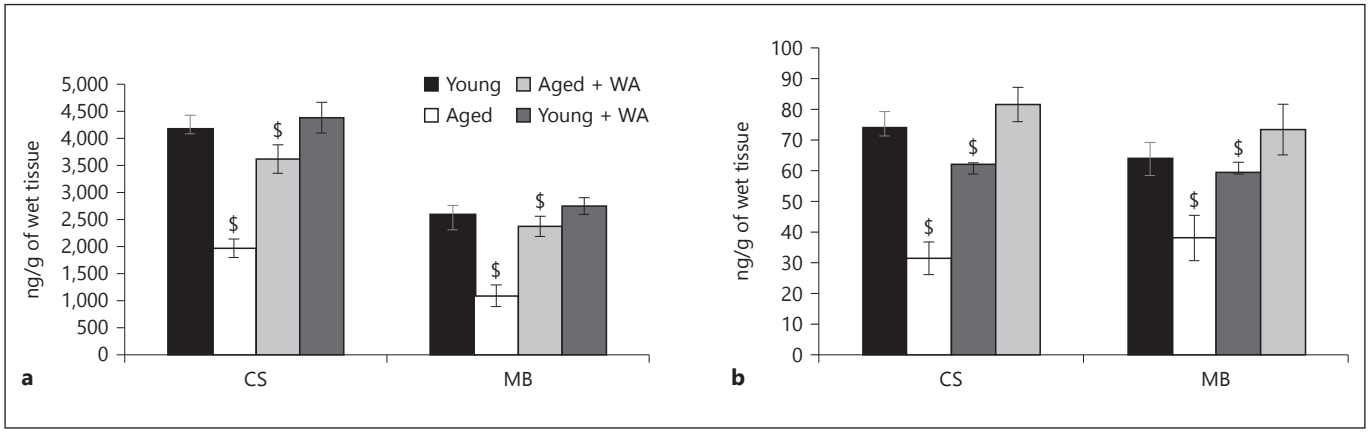

Fig. 3. Shows the effect of WA on levels of dopamine and its metabolites in striatum and mid brain of young and various experimental animals. The aged animal showed reduced levels of (**a**) DA, (**b**) HVA when compared to the young. This reduction was markedly reversed by the administration of WA. Each bar indicates the mean ± SEM of $n = 6$ of each group. $^{\$}$ $p < 0.001$. DA, Dopamine; HVA, homovanillic acid; WA, Withaferin A; CS, corpus striatum; MB, mid-brain.

of aged rodent brain [21]. These differential findings in reduced DA levels in some rodents may be due to the lack of compensatory increase in the size and the activity of remaining neurons to replenish the DA depletion. Interestingly, the administration of WA significantly reversed the DA and HVA levels in aged rats. Our previous study showed similar results after the treatment of PD animal model with WS root extract [22]. These data suggest that WA, an active component of WS, induces catecholamines in aged rats and is therefore able to establish its neuroprotective role.

The markedly reduced motor activity seen in aged rats that also correlated with low DA levels in SN and ST clearly demonstrate the locomotor disability of aged rats. This declined locomotor activity may also be an indicator of high emotionality [23]. Studies often fail to differentiate the types of rearing and attribute rearing to be the marker of motor activity alone. However, recent studies report unsupported rearing to be a vital indicator of emotional state as well. In the present study, both types of rearing were significantly low in aged when compared to young rats indicating reduced motor activity and exploration. The low frequency of unsupported rearing, in correlation with increased defecation and grooming, indicates the increased stress or anxiety in aged rats. This high emotional stress in aged rats can be an outcome of decline in the production of DA in SN, the greatest source consequently causing other DA-associated functions like cognition, emotion and/or reward system. In the beam walking test, the significantly increased time of crossing and foot slipping errors along with marked striatal DA depletion in aged rat as observed in the present study may correlate with bradykinesia of PD [18]. Conversely, the treatment with WA considerably reduced the time of crossing and the foot slips in aged rats. This strongly suggests that WA improved motor balance and co-ordination in aged rats possibly through DA resurge in ST.

The grid walk test is specifically sensitive to DA depletion that assesses limb movements such as precise stepping, coordination and accurate placement of the paw [15]. The DA depletion resulted in significant motor incoordination in aged rats through increased number of footsteps and foot-faults in all limbs and a significant reduction in velocity. After administration of WA, the increase in velocity and the reduction in number of footsteps and foot faults in all limbs strongly suggest the beneficial effect of WA in motor recovery of aged rats. This age-mediated decline in DA and motor activity in aged rat can be due to the specific vulnerability of SN neurons to ageing accumulated reactive oxygen species (ROS) because of their greater pacemaker activity, high levels of lipid peroxidation ingredients like iron and poly-unsaturated fatty acids [24]. Studies have reported that the striata present markers for enhanced susceptibility to age and produce a greater amount of ROS, reduce the mitochondrial membrane potential and mitochondrial Ca2þ overload, followed by a reduction in the anti-apoptotic protein, Bcl-2, resulting in neuronal loss [25].

It can be speculated that WA exerts its anti-oxidant or free radical scavenging potential [19] in DA neuro-protection, thereby replenishing the DA levels and recovery of the motor impairment in aged rat. Our previous reports

[14, 22] have demonstrated the anti-oxidant potential of WS leaf extract through significant reduction of ROS and motor improvement by standard behavioural tests in animal models of PD. The present data distinctly demonstrates the significant DA depletion and concomitant motor functional impairment in aged rats. WA significantly restored the motor activity and replenished the striatal DA levels in aged rats, depicting its neuro-protective potential. To the authors' knowledge, this study is the first report on the beneficial role of WA on neuro-chemical and functional impairment of nigro-striatal DA system in aged rats.

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Sincere thanks are due to Dr. Annamalai Reghupathy (M.S.,), Managing director of Karpaga Vinayaga Medical Institute for providing us with animals and infrastructure facilities to execute this work.

Disclosure Statement

Authors have declared that they have no conflicts of interest to disclose.

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[Frontiers In Bioscience, Elite, 11, 1-11, Jan 1, 2019]

Demethoxycurcumin ameliorates rotenone-induced toxicity in rats

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1. ABSTRACT

Rotenone, an environmental toxin, is used to induce neurodegeneration in both the cellular and animal model of Parkinson's disease. Demethoxycurcumin (DMC), derivative of curcumin has been reported to have antioxidant and anti-inflammatory characteristics in *in vitro* and *in vivo* PD conditions. The present study was aimed to evaluate the efficacy of DMC in the management of neurodegeneration in PD. Male Wistar rats were radomized and divided into control, rotenone, DMC +rotenone and rotenone alone treated animals. Pre-treatment with DMC one hour prior to the rotenone injection, attenuated the motor and non-motor deficits. Western blot analysis indicated that the administration of DMC to PD rats eased the protein expression of dopaminergic and apoptotic indices. These findings showed that DMC effects on ameliorating the PD symptoms induced by rotenone might be associated with the neuroprotective and antioxidant effects of this compound.

2. INTRODUCTION

Neurodegenerative diseases (NDDs) are expected to surpass cancer as the second most cause of death by 2040 worldwide (1). Parkinson's disease (PD) is one of the most common NDDs that mainly affect the movement of aged population. It is characterized clinically by resting tremor, bradykinesia, rigidity and postural instability that primarily arises due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) that synthesize, dopamine (DA), a chemical messenger responsible for transmitting signals to produce smooth and focused muscle activity. Depletion of DA causes the striatal nerve cells to fire out of control, leaving patients unable to direct their normal movements (2). As a result, the amount of DA needed for neurotransmission in the ST is lowered. Parkinsonian signs appear when dopaminergic neuronal death surpasses 70-80% (3). Generally, dopaminergic impairment is assessed by measuring DA level and the expressions of dopaminergic neuronal markers such as tyrosine hydroxylase (TH), a rate-limiting enzyme involved in the DA synthesis (4).

The factors that enhance the risk of PD were pesticide exposure, prior head injury, β-blocker use, agricultural occupation, rural living, well-water drinking. The incidence of PD is more in farmers in rural areas, which could be due to the enhanced pesticides/ herbicides exposure as compared to the general population (5). Rotenone belongs to cytotoxic retinoid family extracted from some plants of Leguminosae family like *Derris mallaccensis*, *Derris elliptica*, *Lonchocarpus utilis* and *Lonchocarpus urucu* (6). It is widely used as pesticide and insecticide. Like MPTP, it is highly lipophilic and can easily cross the BBB followed by accumulation within organelles such as mitochondria, where it inhibits complex I of the ETC (7). Rotenone is reported to cause ROS generation, ATP depletion and cell death in neurons due to its inhibitory action on mitochondrial complex I. Rotenone toxicity mimics many pathological hallmarks of PD, including loss of dopaminergic neurons in SN and formation of Lewy bodies which is presumably due to oxidative damage, mitochondrial dysfunction and disruption of axonal transport (7).

Various pharmacological agents, including MAO-B inhibitors (8), DA agonists, calcium antagonists, NMDA antagonists, glutamate release inhibitors (9), immunosuppressants, nitric oxide syntheses inhibitors, dimethyl thiourea (10), sulfhydryl drugs and other antioxidants (11) along with L-DOPA combination has been shown to protect experimental animals against various PD toxins. Current pharmacological therapies for the PD are inadequate; these are only able to provide symptomatic relief and after long use produce stern side effects and even worsen the condition.

Curcuma longa (turmeric) is used for medical purpose in the history of folk and Ayurvedic medicine. The neuroprotective effect of turmeric attributed due to the presence of curcumin by its antioxidant (12), mitochondrial protective, signal modulating (13), anti-inflammatory (14) and anti-apoptotic functions (15). The ratio of curcumin compounds present in commercially available preparations of curcumin are curcumin: demethoxycurcumin (DMC): bisdemethoxycurcumin (BDMC) in the ratio of about 66:23:11. Both the DMC and BDMC are also used for domestic cooking, food industry and folk medicines (16). Previous studies from our lab demonstrated the neuroprotective role of DMC against rotenone induced motor deficits, neurochemical alteration, oxidative stress and the expression of inflammatory markers in rats (17, 18). However the role of DMC in rotenone induced motor and non-motor symptoms, dopaminergic and apoptosis markers were not investigated.

3. MATERIALS AND METHODS

3.1. Chemicals

Rotenone, DMC, was purchased from Sigma Chemical Company, Bangalore, India. All other reagents used were of analytical grade and were procured locally.Anti-Bcl-2, anti-Bax, Caspase-3, Caspase-6, Caspase- 8, Caspase-9 and TH antibodies were obtained from Cell Signalling (USA) and b-actin antibodies were purchased from Santa Cruz Biotechnology, Inc, (USA). Anti rabbit HRP conjugated secondary antibody (Sigma chemical, USA). All other chemicals were of analytical grade.

3.2. Animals

Male Wistar rats (225-250 g) were procured from the Biogen Laboratory, Bangalore India. They were kept under the ambient conditions and fed with standard pellet and water *ad libitum*. All the experimental protocols met with the National Guidelines on the proper care and use of Animals in Laboratory Research (Indian National Science Academy, New Delhi, 2000) and were approved by the Animal Ethics Committee (IAEC/KMPC/230/2015-2016).

3.3. Experiment

Twenty-four animals were randomised and distributed into four groups (n=6): control (0.5. ml of sunflower oil *i.p.* for 45 days), rotenone (2.5. mg/kg/ day *i.p.* in sunflower oil for 45 days) (19), rotenone as group II + DMC (10 mg/kg b.w. *p.o.*for 45 days) and DMC (10 mg/kg) alone treated. After the end of the experimental period, behaviour tests (open field, akinesia and catalepsy test) were carried out. Then the animals were sacrificed by cervical dislocation and the SN was dissected, rinsed in ice-cold saline

and stored at -80°C for protein expression studies of dopaminergic and apoptotic markers.

3.4. Behavioral analysis

3.4.1. Open field test

The floor of the wooden rectangular open field apparatus (100 x 100 x 40 cm) was covered by rexin cloth with drawn lines that are dividing them into 25 equal squares (20 x 20 cm). Animals were placed individually in the corner of the apparatus and its behavior was observed for 5 min; peripheral locomotor activity- the number of lines crossed in the outer 16 squares with two fore paws, central locomotor activity the number of lines crossed in the inner 9 squares; rearing activity the number of the time rat standing on its fore legs with its hind legs on the ground and grooming activity the number of times the rat licking the fur or washing face or scratching. Between each session the apparatus was thoroughly cleaned with alcohol and dried (20).

3.4.2. Catalepsy

The term implies the inability of an animal to correct an externally imposed posture. The animal is lifted by its tail and is allowed to place its forepaws on a horizontal wooden bar (diameter: 1.2.5 cm; height: 10 cm), which was just above and parallel from the base. Catalepsy was measured as the time elapsing before it climbed down from the bar was recorded. The duration taken for the first movement of paws was measured as cataleptic time. The maximum descent latency for at least 30 s was said to be cataleptic and given one point and maximum time was fixed at 180 s (21).

3.4.3. Akinesia

This tests replicates the difficulty in initiating movement in PD. Akinesia was performed by noting the latency in seconds (s) of the animals to move all four limbs with the test finished within 180 s time frame. Before carrying out each akinesia test rats were acclimatized for 5 min on a wooden elevated (100 cm) platform (100 x 150 cm). Using a stopwatch, the time taken by the animal to move all the four limbs was recorded (22).

3.4.4. Forced Swim Test

In FST, developing an immobile posture, when rodents are exposed to an inescapable situation, resembles depression in humans. The test was conducted in two sessions. First, in the training session, the rats were allowed to swim in water tank (20 x 20 x 40 cm) containing water in room temperature at a depth of 15 cm for 15 min. Twenty-four hours after the training session, the rats were subjected to the forced swimming test for 5 min, for subsequent quantification of immobility time (time required to attain lack of motion of the whole body with only of the necessary movements to keep the animal's head above the water). The water was changed after each animal to avoid the influence of smell (23).

3.4.5. Sucrose preference test

Sucrose intake test is used to measure anhedonia, a decreased ability to experience pleasure, is a core symptom of human depression. The animals were transferred into single housing cages with free access to food. Each rat was provided with two bottles of water, pre-weighed, on the extreme sides of the cage during the 24 h training phase to adapt the rats to drink from two bottles. After training, one bottle was randomly switched to contain 1 % sucrose solution for 1 h, as described previously (24), and 24 h later, the bottles were reversed, and provided for 1 h, to avoid perseveration effects. The sum of water consumption and sucrose solution consumption was defined as the total intake. The percentage of sucrose intake was calculated by using the following equation (% sucrose preference = sucrose intake x 100/total intake). The test was carried out between 9:00 and 11:00 a.m., beginning 1 week prior to the rotenone exposure (to provide baseline values). After the sucrose preference test, all the rats received free access to food and water.

3.4.6. Dissection and homogenization

Animals were sacrificed by decapitation immediately after behavioral assessment and the brain was harvested quickly to procure ST and SN for protein expression studies.

3.4.7. Western Blotting

Tissue samples were homogenized in RIPA buffer and centrifuged at 10,000 rpm for 30 min to isolate the supernatant. Protein amount was estimated according to method of Lowry *et al.* (25), and the sample containing 50 µg protein was loaded onto the polyacrylamide gels. The gel was then transferred onto a nitrocellulose membrane (PALL Corporation, Biotrace). The membranes were incubated with the blocking buffer containing 5 % non-fat dry milk powder or BSA for 2 h to reduce non-specific binding sites and blots were probed with various antibodies: TH, Caspases-3, -6, - 8, -9, Bax, Bcl-2 and β-actin (1: 1000) with gentle shaking overnight at 4ºC. After this, membranes were incubated with their corresponding secondary antibodies (anti-rabbit IgG conjugated to HRP) for 2 h at room temperature. The membrane was washed thrice with TBST for 30 min. Immunoreactive protein was visualized by the chemiluminescence protocol (GenScript ECL kit, USA). Densitometry analysis was performed with a computer using a gel

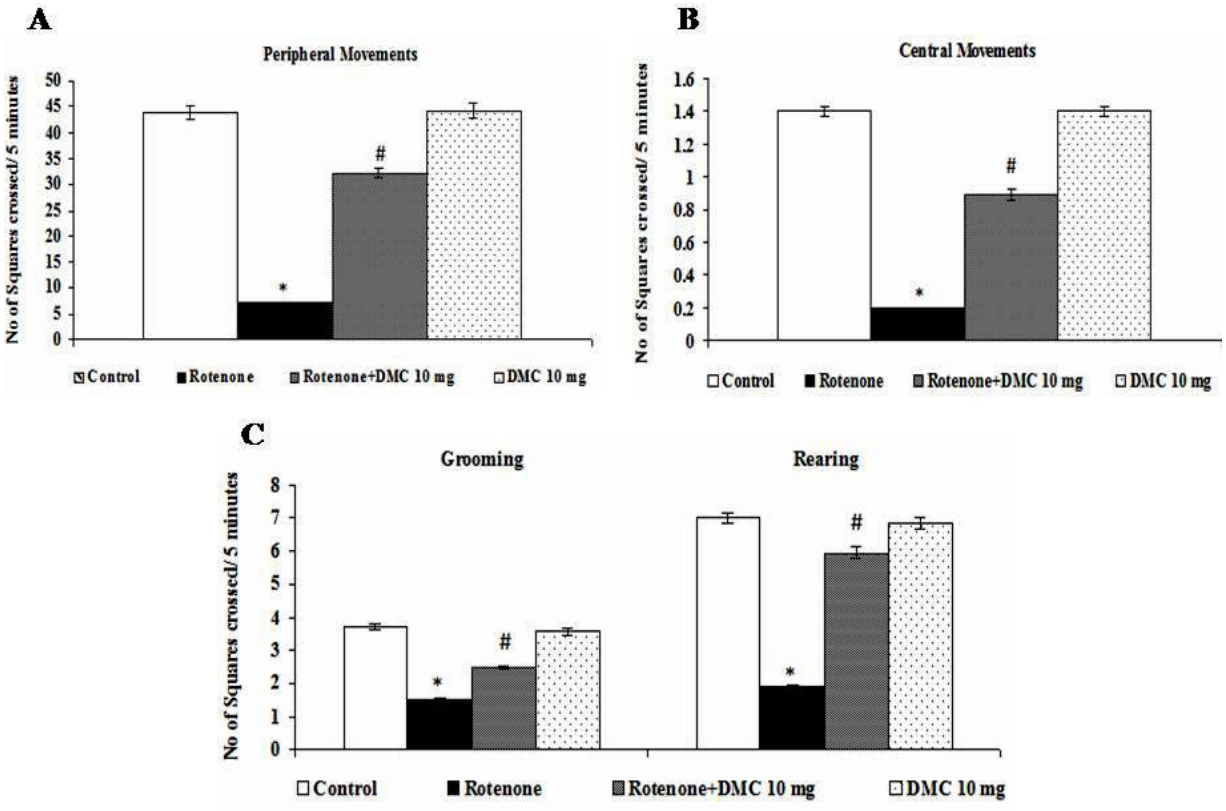

Figure 1. Open field behavior of control and experimental rats: Movements (Peripheral (a) and Central (b)) and activities (Grooming and Rearing (c)) were sign ificantly reduced by rotenone treatment when compared with the control group. However, this reduction in motor activity was attenuated by pre treatment with DMC. Values are given as mean ± SD. #p<0.05 compared to the control, *p<0.05 compared to the rotenone group.

image analysis program. The data were then corrected by background subtraction and normalized against β-actin as an internal control.

3.4.8. Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Science (SPSS) software package version 15.0. All data are expressed as mean ± SD for six rats in each group. Results were considered statistically significant at p<0.0.5.

4. RESULT

4.1. Effects of DMC on rotenone induced locomotion and exploratory activity

Rotenone injection led to a significant reduction in the peripheral and central movements (Figure 1, A, B) along with diminished rearing and grooming activities (Figure 1, C). Co-administration of DMC (10 mg/kg b.w) to rotenone treated rats showed a significant increase in the locomotion and non-locomotion activities as compared to rotenone alone intoxicated rats. However, there was no significant changes were observed in DMC (10 mg/kg b.w) alone treated rats as compared to saline treated control rats.

4.2. Effects of DMC on rotenone induced movement impairments

Impaired coordination in movement was observed by catalepsy test. Chronic administration of rotenone caused impairment in correction of an externally imposed posture (catalepsy) as compared to control rats. Co-administration of DMC to rotenone treated rats significantly attenuated rotenone induced catalepsy. No significant changes were observed between control and DMC alone treated rats (Figure 2).

4.3. Effects of DMC on rotenone induced initial movement impairment

Impairment in initiation of movement was measured by akinesia test. Chronic administration of rotenone caused impaired ability to initiate movement (akinesia) as compared to control rats (p<0.0.5). Oral administration of DMC significantly attenuated rotenone induced akinetic movement however it did not restored completely as that of normal animals (Figure 3). Moreover no significant variations were observed between control and DMC alone treated rats.

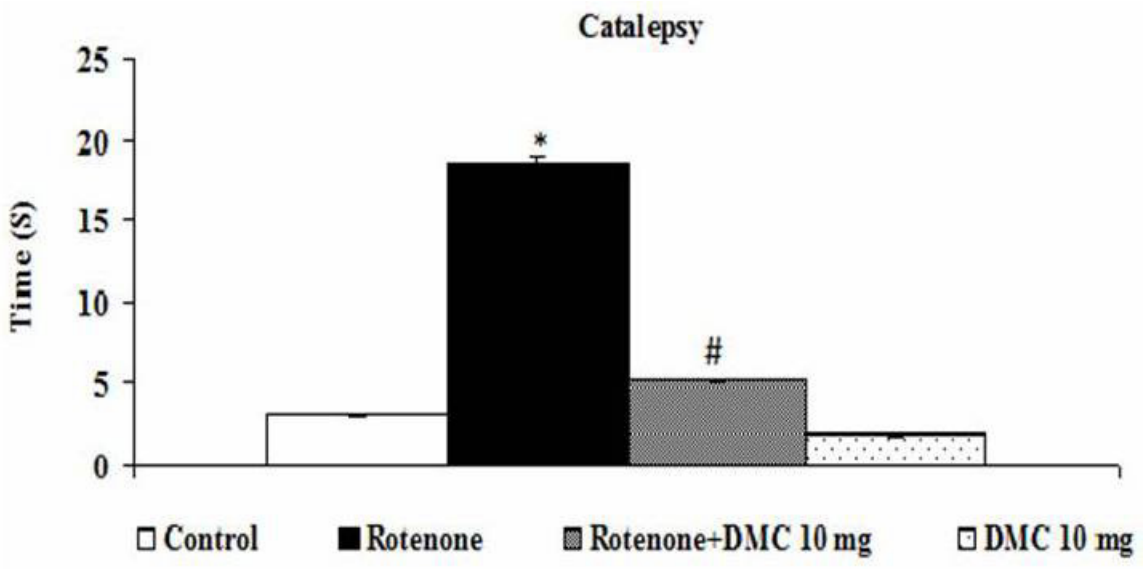

Figure 2. Effect of DMC on rotenone induced control and experimental rats. Rotenone administration caused significantly more latency to move all the four limbs or to correct an externally imposed posture (catalepsy) as compared to the control group. The animals treated with the DMC alone did not show any effect in catalepsy. Data are expressed as mean ± SD. #P <0.05, compared with the control group. *P<0.05, compared with the rotenone group.

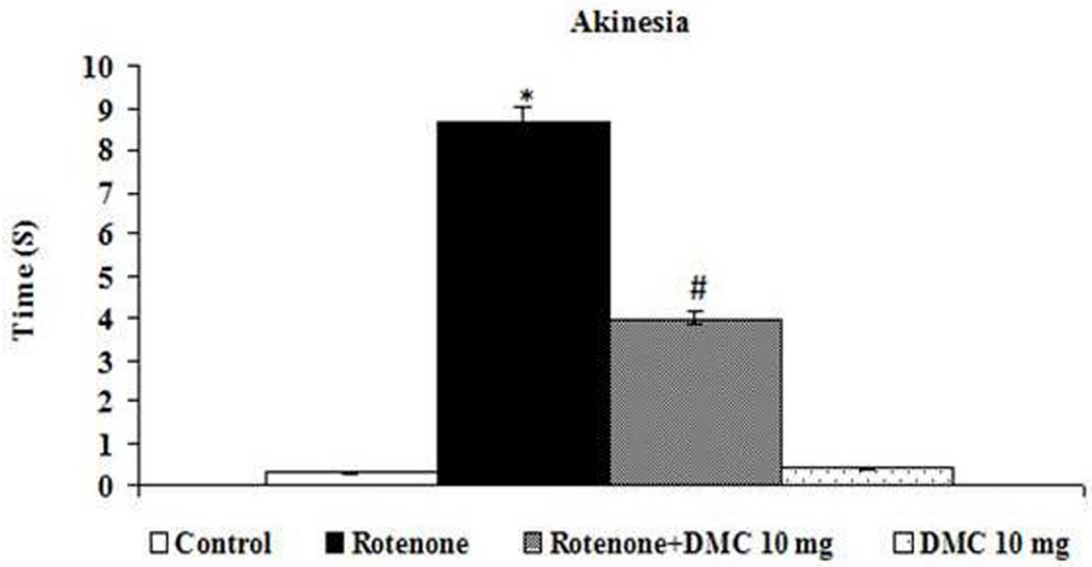

Figure 3. Effect of DMC on rotenone induced control and experimental rats. Rotenone administration caused significantly more latency to move all the four limbs or to correct an externally imposed posture (catalepsy) as compared to the control group. The animals treated with the DMC alone did not show any effect in akinesia. Data are expressed as mean ± SD. #P <0.05, compared with the control group. *P<0.05, compared with the rotenone group.

4.4. Effects of DMC on rotenone induced forced swim test

The forced swim test is one of the most commonly used animal model for assessing antidepressant like behaviour. Rotenone administered rats showed more immobility time in forced swim test as compared to control rats (Figure 4). Rotenone and DMC co-administrated animals had significantly reduced immobility time as compared to rotenone alone treated animals. No significant differences were observed between control and DMC alone treated animals ($p < 0.0.5$).

4.5. Effect of DMC on rotenone induced Sucrose preference test

Depressive like behaviour was indicated by sugar intake test to investigate anhedonia. Rotenone induced animals showed significant decrease in pleasure to drink sweet water (sucrose intake test) as compared to control rats (Figure 5). Co-administration

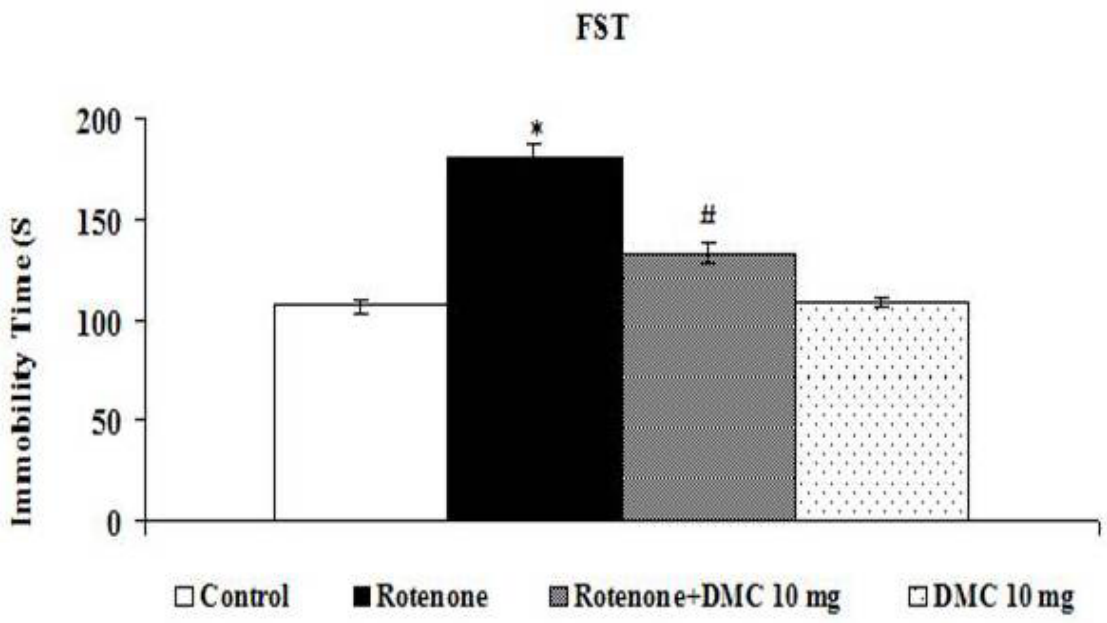

Figure 4. Preventive effect of DMC in rotenone induced swim disability in control and experimental rats. After 3 min, the time spent in immobility was measured, and a difference between the rotenone treated groups and pretreated DMC to rotenone treated rats and no significant change observed in saline treated control group and alone DMC treated group. Data are expressed as mean ± SD. (p0.05). #P <0.05, compared with the control group. *P<0.05, compared with the rotenone group.

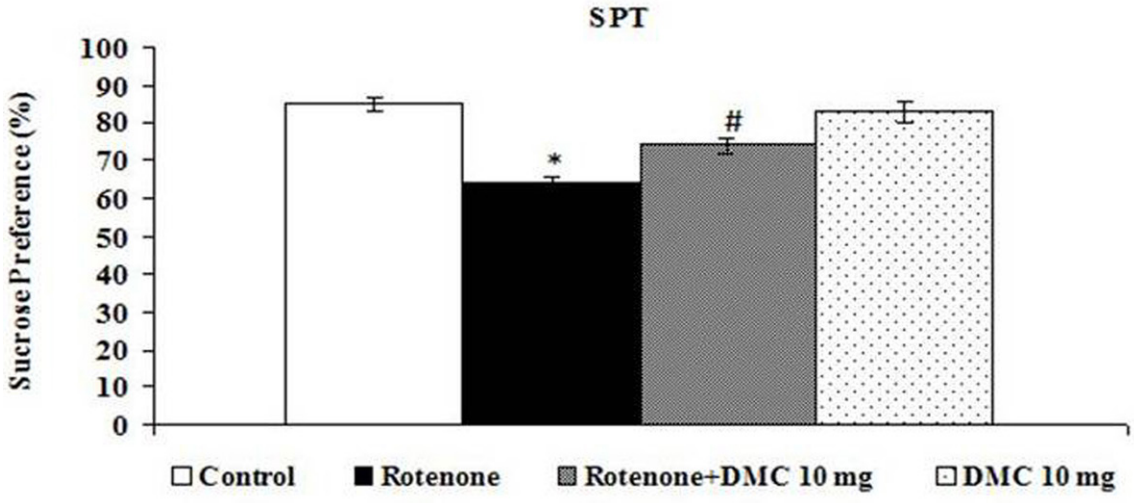

Figure 5. Effect of DMC on rotenone induced depressive like behavior that is indicated by sugar intake in sucrose intake test and immobility time in forced swim test. Data are shown as mean ± SD. #p<0.05 compared to the control rats *p<0.05 compared to the rotenone treated rats.

of DMC significantly improved the sucrose intake. Moreover no significant variations were observed between control and DMC alone treated rats ($p < 0.0.5$).

4.6. Effect of DMC on rotenone induced expression of TH in SN and ST

Rotenone treatment significantly reduced TH expression as compared to control. However, oral administration of DMC (10 mg/kg b.w) significantly enhanced TH expression as compared to rotenone injected rats (Figure 6). Quantification of TH expression showed significant protection in SN and ST by DMC from rotenone induced loss ($p < 0.0.5$). DMC alone treated rats showed no significant changes in the TH expression as compared to control rats ($p < 0.0.5$).

4.7. DMC suppressed rotenone induced apoptosis

As shown in Figure. 7, rotenone significantly enhanced the expression of pro-apoptotic (Bax, Caspase-3, -6, -8 and -9 markers and diminished

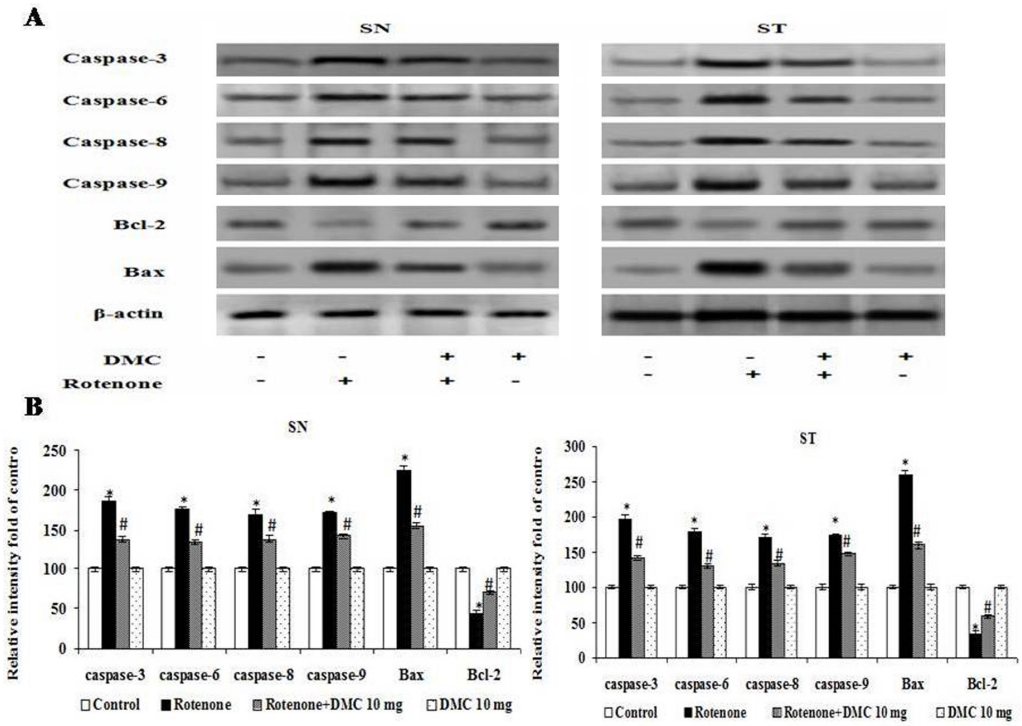

Figure 6. Effect of DMC on expressions of the TH proteins in SN and ST of control and experimental rats. Figure a represents autoradiogram of these protein expressions by using β-actin as an internal control. Figure show the band density was quantified by scanning densitometry. Data are shown as mean ± SD. #p<0.05 compared to the control rats, *p< 0.05 compared to the rotenone treated rats.

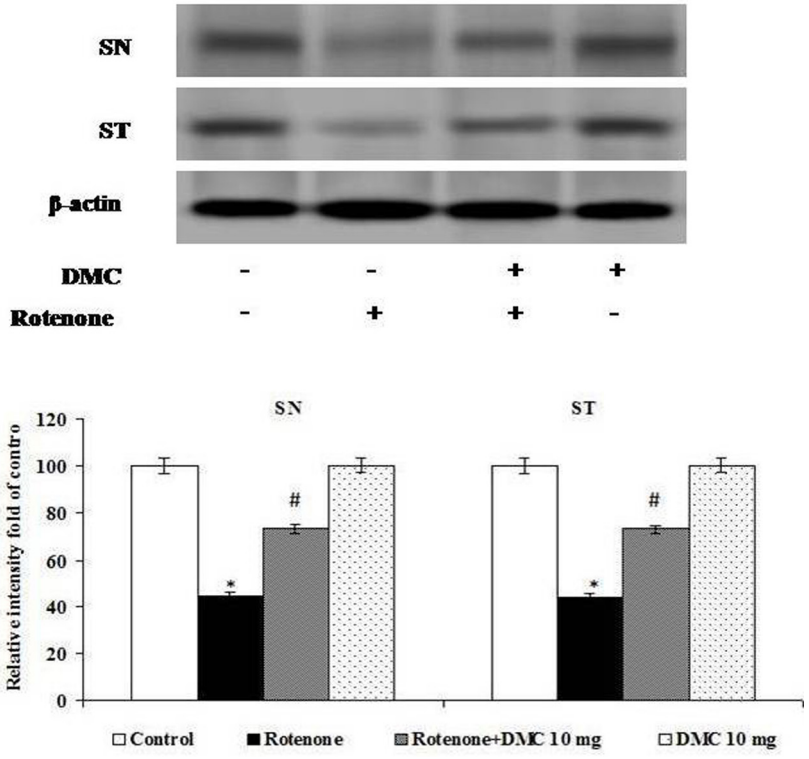

Figure 7. Effect of DMC on rotenone induced changes in the expressions of apoptotic protein markers (Bax, caspase 3, 6, 8 and 9 in control and experimental animals. Figure a represents autoradiogram of these protein expressions by using β-actin as an internal control. Figure A and B show the band density was quantified by scanning densitometry. Data are shown as mean ± SD. #p<0.05 compared to the control rats, *p< 0.05 compared to the rotenone treated rats.

the expression of anti-apoptotic protein Bcl-2 when compared to control ($p < 0.0.5$). Meanwhile, treatment with DMC significantly prevented the expression of pro-apoptotic markers when compared to rotenone group by restoring the expression of Bcl-2 ($p < 0.0.5$).

5. DISCUSSION

Motor function is normally measured by performing a number of behavioural analysis specifically, open field test, hang test, narrow beam walking (4), rotarod performance, stride length (26), swim test, akinesia, catalepsy, pole test (27). Behavior analysis is reported as more sensitive technique to detect functional impairments in PD rodent models and to quantify the therapeutic efficacy that restores dopaminergic function. Baydar *et al.,* (28) reported that the analyses of behavioural changes are more sensitive than neurochemical alterations during neurotoxin exposures. Various behaviour test were performed to measure depression, anxiety and memory impairment (29). As rotenone model mimics both the motor and NMS of PD (30), the neuroprotective effect of DMC against rotenone induced motor and non-motor impairments were assessed in this study.

DA levels are closely associated with the open field activity. In the open field test, peripheral square crossing indicates the general motor performance and acclimatization attempt, whereas central square crossing indicates the exploratory behaviour. Rearing and grooming activities are also indicators of stress. Rearing is known to be highly sensitive to ST or SN lesions (31). Reduced performance of square crossing and activities in rotenone induced animals could be associated with dopaminergic loss (32). In the present study, administration of rotenone exhibited impaired ability to initiate movement (akinesia) and rigidity or inability to correct an externally forced posture (catalepsy). Behavioural assessment of akinesia in rodent models of PD resembles limb akinesia and gait problems of PD patients (33). Rotenone destroyed the dopaminergic neurons selectively and resulting in impaired motor function (34). Depletion of brain DA levels in rotenone treated rats (18) caused behavioural abnormalities as seen in PD patients, whereas enhancement of striatal DA (18) and its regulators including TH in this study, by DMC clearly indicated the neuroprotective efficiency of DMC in protecting dopaminergic neurons and thereby normalizing the behaviour (35).

Rotenone treatment exhibited cognitive impairment such as depression and anxiety as evidenced by sucrose intake and forced swim test. Sucrose preference is frequently used as a measure of anhedonia, another form of depression in rodents. Damages to dopaminergic, serotonergic and noradrenergic systems have been postulated to the prevalence of depression in PD (35). Forced swim test is based on the observation of rodents that were placed in an in escapable and stressful situations develop an immobile posture, after exerting initial escape oriental movements. In a consequent exposure, the commencement of the immobility is faster and more marked. This phenomenon is called "behaviour despair" and is arised due to the animals response to the development of depression process (36). Depletion of monoamine neurotransmitters by rotenone leads to depressive behaviour (37), whereas DMC offers antidepressive action as evidenced by behaviour analysis.

Administration of rotenone induced the loss of TH-immunopositive neurons in SN with significant motor defects. Experimental studies suggested that the decrease in the TH activity or its levels have been due to the underlying pathogenesis of PD (38). In the present study, rotenone treated rats exhibited significant decrease in the expression of TH in SN and ST demonstrated the possible degeneration of dopaminergic neurons. Oxidative stress induces post-translational modification of TH and diminished its catalytic function (38). Furthermore, S-glutathionylation of TH enzyme has been accelerated by ROS (39). It was reported that antioxidants exert a protective effect on TH expression (40). Oral treatment of DMC may enhance TH expression in ST and SN mainly due to its antioxidant property (17,18).

There are several evidences implicating apoptosis and caspase activation in patients as well as in *in vivo* models of NDDs. Mitochondrial dysfunction mediated oxidative damage could further initiate apoptotic neuronal cell death in PD (41). Loss of MMP leads to release of cyt-c into the cytoplasm where it can initiate the activation of caspase cascade by the activation of caspase-9, which leads to the activation of capsase-3, resulting in the morphologic alterations associated with apoptosis (41). On the other hand, activation of FADD leads to the activation of caspase-8, which translocates to mitochondria which, induces cyt-c release and ultimately to caspase-3 activation. Caspase-3, 8, 9 expressions were significantly enhanced in rotenone treated animals as compared to control animals. DMC attenuated rotenone induced apoptosis by inhibiting the release of cyt-c and reducing the expression of caspases (17). These results indicated that DMC treatment had a clear neuroprotective effect against rotenone toxicity. Neurotoxins such as MPTP, rotenone, paraquat and maneb induced cell death by the activation of members of the B cell lymphoma 2 family of proteins (Bcl-2) (42) and Bcl-2 plays an important role in the regulation of mitochondrial mediated apoptosis. Bcl-2 proteins are classified into three groups: those inhibit apoptosis (Bcl-xL, Bcl-2, Bcl-w, Mcl-1, Bcl-10 and Bcl-2 related protein A1); those promote apoptosis (Bax, BAK, Bcl-

rambo, Bcl-xs, BOK/Mtd); and the pro-apoptotic BH3 proteins (Bad, BID, Bik/Nbk, BIM, BLK, Bmf, Hrk/DP5) that regulate the action of anti-apoptotic Bcl-2 proteins. Thus, the balance between Bcl-2 family members performs a vital role in determining cell survival or death. In our study, rotenone administration significantly elevated the expressions of pro-apoptotic Bax and reduced the anti-apoptotic Bcl-2, indicating that rotenone toxicity could be in favour of apoptosis. Bcl-2 act as a frustrating force to reduce apoptotic damage by diminishing lipid peroxidation reactions triggered by cytotoxic agents such as ROS (43). Bcl-2 was also found to prevent the release of cyt-c. In contrast, Bax enhances apoptosis by (i) dimerizing with anti-apoptotic Bcl-2 proteins, (ii) enhancing cyto-c release and subsequent activation of caspase-3 that finally leads to cell death (Ethell and Fei, 2009). However, DMC treatment prevented rotenone induced apoptosis by reducing the expression of Bax, caspases and enhancing the expression of Bcl-2. The multi pharmacological effect of DMC may be responsible for its therapeutic potential and in future DMC may be used alone or alone with present drugs for the treatment of PD. However clinical studies are warranted to support these findings.

6. CONCLUSION

The findings obtained from this and our previous studies showed that DMC offers neuroprotective effect by ameliorating the PD symptoms induced by rotenone might be associated with its antioxidant, mitochondrial protective and anti-inflammatory effects.

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Antiapoptotic role of Agaricus blazei extract in rodent model of Parkinson's disease

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1. ABSTRACT

Rotenone is a pesticide that has been shown to induce the pathological symptoms of Parkinson's disease (PD) in both cellular and animal models. In this study, we investigated the protective effect of Agaricus blazei extract on rotenone-induced dopaminergic degeneration and apoptosis in mice model. A. blazei extract blocked the rotenone-mediated diminution of dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT 2) expression and the downregulation of Bcl-2 and the upregulation of Bax, caspases-3, -6, -8 and caspase-9. Present data suggest that A. blazei extract plays a crucial role in regulation of proteins expression such as DAT and VMAT2 and pro-apoptotic and anti-apoptotic in Parkinsonism. In conclusion, the present study shows that A. blazei extract act as potential neuroprotective agent in the management of Parkinsonism.

2. INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease (AD) that mainly affects the movement in elderly population. PD is characterized by tremor, rigidity, akinesia and postural instability, which arises largely due to the massive loss of dopaminergic (DA-ergic) neurons projecting from the substantia nigra (SN) to the striatum (ST) (1). PD affects about 1% of the population over 60 years of age and its incidence increases to 3% of the population over 80 years (2). In 2005, the estimated number of PD cases worldwide was about 4.4. million (3) and by the year 2030, this number will be expected to get doubled to about 9 million, based on the expected growth of the population over the age of 60.

Though the cause of PD is not known, most of the knowledge about PD pathology is gathered from various *in vivo* and *in vitro* models involving 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat and rotenone (4-6). Rotenone, a naturally occurring plant flavonoid and widely used pesticide, mimicked the symptoms of PD, both *in vivo* and *in vitro* conditions (7-9).

In vitro studies indicated that rotenone can easily cross the biological membranes, due to its

lipophilic nature and could access into the cytoplasm of DA-ergic neurons easily (7). Furthermore it could enter into mitochondria and inhibit mitochondrial complex I activity. It can also induce reactive oxygen species (ROS) generation, mitochondrial membrane potential loss (MMP) and release of cytochrome c (cyt-c) from mitochondria, which in turn activate the caspase cascades and finally leads to apoptosis (10). Tyrosine hydroxylase (TH) catalyzes the conversion of L-tyrosine to DOPA, which is the initial and rate-limiting step in the biosynthesis of DA (11). The loss of ability to optimally synthesize catecholamines is an important step in the progression of PD and other neurodegenerative diseases. Indeed, early loss of TH activity followed by a decline in TH protein is considered to contribute towards DA deficiency, which is widely used as a marker of dopaminergic depletion in PD (12). Presence of pathological inclusions of α-synuclein, a major component of LBs in dopaminergic cells contributes to the intra cellular neuropathological mechanisms in PD (13). Moreover rotenone is transported into dopaminergic neurons through DAT and it can be taken into cytoplasmic vesicles by the action of the VMAT-2. The combined *in vivo* assessment of DAT and VMAT-2 may provide an index of dopaminergic nerve terminal integrity and potential vulnerability of surviving neurons (14).

Symptomatic and effective treatment of PD in modern medicine is the supplementation of the DA in the form of L-dopa (15). However, long term administration typically leads to motor complications, such as L-dopa induced dyskinesia (LIDS) (16). Current pharmacological therapies for the disease are also inadequate. Regrettably, other therapeutic strategies such as neural transplantation, deep brain stimulation and stem cell transplantation remains in the experimental stage. Unfortunately effects to find effective agents that provide protection against neurodegeneration have been unsuccessful. A number of factors have been implicated in the pathogenesis of cell death in PD which includes mitochondrial dysfunction, oxidative stress, proteosome dysfunction, Lewy bodies formation and apoptosis (4), which offers resistant to therapeutic agents. Hence drugs from plant origin with multiple mechanisms of pharmacological actions including antioxidant, anti inflammatory, anti apoptotic and mitochondrial protective properties, may be forward in delaying/protecting neuron from neurotoxicity.

There is a rich history of the use of natural products and their active compounds in the treatment of neurodegenerative diseases, including PD. The mushrooms have been generally considered as functional foods and reported to possess various pharmacological properties due to the presence of the active components such as the β-glucans, terpenes, phenolics, steroids, and nucleosides (17-18). Agaricus blazei Murrill (A. blazei), popularly known as sun mushroom, has been subject of great interest due to its nutritional value and having pharmacological properties against various diseases including cancer, diabetes, atherosclerosis, hypercholesterolaemia, and cardiac diseases. (19) It is rich in various antioxidant compounds including gallic acid, syringic acid, pyrogallol, and also polysaccharides. (20) It is also reported to contain more amounts of nucleosides and nucleotides, adenosine etc., (21) which are able to exert neuroprotective actions. (22) Recently, Soares *et al.* (23) reported that the oral administration of A. blazei extract offered neuroprotection against experimentally induced cerebral malaria and paracetamol injury by virtue of its antioxidant, mitochondrial protective, and anti-inflammatory properties. Thus considering, the increased neuroprotective effect of A. blazei during the progression of PD, we aimed to study the effect of this mushroom extract on dopaminergic protective and antiapoptotic properties against rotenone-induced mice model of PD.

3. MATERIALS AND METHODS

3.1. Chemicals

Rotenone was purchased from Sigma Chemical Company, Bangalore, India. Anti-Bcl-2, anti-Bax, Caspase-3, Caspase- 8, Caspase-9, DAT and VMAT-2 antibodies were obtained from Cell Signalling (USA) and b-actin antibodies were purchased from Santa Cruz Biotechnology, Inc, (USA). Anti rabbit HRP conjugated secondary antibody (Sigma chemical, USA). All other chemicals were of analytical grade.

3.2. Preparation of methanolic extract of A. blazei

Mushrooms were collected and then air dried in an oven at 38°C. For methyl alcohol extraction, 20 g of dried mushroom samples was weighed, ground into a fine powder, and then mixed with 200 ml of methyl alcohol at room temperature at 17 × g for 24 hours. The residue was re-extracted under the same conditions until the extraction solvents became colorless. The extract obtained was filtered on a Whatman no. 1 paper and the filtrate was collected, then methyl alcohol was removed using a rotary evaporator at 38°C to obtain the dry extract. The extract was placed in a plastic bottle and then stored at −80°C.

3.3. Animals and drug treatment

Male Albino mice (25–30 g) aged 10 weeks was procured from the Biogen Laboratory, Bangalore, India. They were kept under ambient conditions and fed with standard pellet and water ad libitum. All the experimental protocols conformed to the National Guidelines on the proper care and use of Animals in Laboratory Research (Indian National Science Academy, New Delhi, India, 2000) and were approved

by the Animal Ethics Committee (SJC/IAEC/2015–2016/01; Dated 05/10/2015).

3.4. Experimental group

Twenty-four animals were randomized and distributed into four groups (n = 6): Group I - control (0.1. ml of sunflower oil i.p. for 45 days), Group II - mice treated with rotenone (1 mg/kg/day i.p. in sunflower oil for 45 days), (24) Group III - mice treated with *A. blazei* extract (100 mg/kg b.w. p.o for 45 days), (24) and rotenone (as group II) and Group IV - mice treated with A. blazei extract alone (100 mg/kg). Then the animals were sacrificed. The striatum and substania nigra were procured and utilized for the protein expression studies of dopaminergic and apoptotic indices.

3.5. Western blotting

Tissue samples were homogenized in RIPA buffer and centrifuged at 10,000 rpm for 30 min to isolate the supernatant. Protein amount was estimated according to method of Lowry *et al.* (25) and the sample containing 50 lg protein was loaded onto the polyacrylamide gels. The gel was then transferred onto a nitrocellulose membrane (PALL Corporation, Biotrace). The membranes were incubated with the blocking buffer containing 5 % non-fat dry milk powder or BSA for 2 h to reduce non-specific binding sites and blots were probed with various antibodies: Caspase-3, Caspase-8, Caspase-9, Bax, Bcl-2, β-actin (1: 2000) and DAT and VMAT 2 (1:1000) with gentle shaking overnight at 4 C. After this, membranes were incubated with their corresponding secondary antibodies (anti-rabbit IgG conjugated to HRP) for 2 h at room temperature. The membrane was washed thrice with TBST for 30 min. Immunoreactive protein was visualized by the chemiluminescence protocol (GenScript ECL kit, USA). Densitometric analysis was performed with a computer using a gel image analysis program. The data were then corrected by background subtraction and normalized against β-actin as an internal control.

3.6. Statistical Analysis

Statistical analysis was performed by one-way analysis of variance followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Science (SPSS) software package version 15.0. All data are expressed as mean ± SD for six rats in each group. Results were considered statistically significant at $p<0.05$.

4. RESULTS

4.1. Effect of A. blazei extracts on DAT and VMAT2 Expressions

To find out the protective effect of A. blazei extract against rotenone induced neurodegeneration, the expression pattern of phenotypic markers (DAT and VMAT2) in SN and ST was analyzed by Western blotting. Rotenone treatment significantly alleviated the expression of DAT and VMAT2 in both SN and ST compared to control group (*$p < 0.05$). Meanwhile, treatment with A. blazei extract reinstated these protein expressions distinctly as compared to rotenone group (#$p < 0.05$). There were, however, no significant changes between control and A. blazei extract treated groups (Figure 1).

4.2. A. blazei extract effect on rotenone induced apoptotic gene expressions

Mice treated with chronic rotenone manifested significant induction in the expression of Bax and depletion in the expressions of Bcl-2, Caspases-3, -6, -8 and -9 in SN as compared to control animals (*$p < 0.05$). Meanwhile, these alterations were significantly attenuated by co-treatment with A. blazei extract when compared to rotenone alone-treated animals (#$p < 0.0.5$). The results also revealed that prolonged treatment of A. blazei extract to mice had no significant changes in the expression of pro- and anti-apoptotic markers as compared to the control mice (Figure 2).

5. DISCUSSION

Corona *et al.* (26) demonstrated that the i.p. administration of rotenone resulted in loss of TH-neurons in SN with significant motor defects. In the experimental PD animals, decrease in the activity of TH and the dramatic drop in the expression of TH have been suggested to be of underlying importance in the pathogenesis of PD (27). It is suggested that the decrease in nigral DA caused by rotenone coincides with the enzymatic inactivation of TH with affecting the actual level of TH protein expression and cell counts. The key aspect of our study was that gavage administration of A. blazei was able to save TH expression in cells during a period when rotenone treatment alone would have abolished it. Furthermore, S-glutathionylation of TH enzyme has been suggested to be accelerated by ROS (28). In fact, it was reported that antioxidants exert a protective effect on TH immunoreactivity (29). Oral treatment of *A. blazei* may enhance TH expression in ST and SN may be due to its antioxidative property (30).

DAT is a critical regulator of DA distribution within the brain and is also a crucial determiner of the neurotoxicity of various toxins (4). Although DAT expression is essential for normal DA neurotransmission, it also prevents the entry of toxin. Degeneration of dopaminergic neurons in PD may lead to decrease in DA storage efficiency due to the decreased DAT population on the depleted dopaminergic neurons (31). The nigrostriatal system has more and heterogeneous DAT distribution and the transporter is found on plasma membranes of

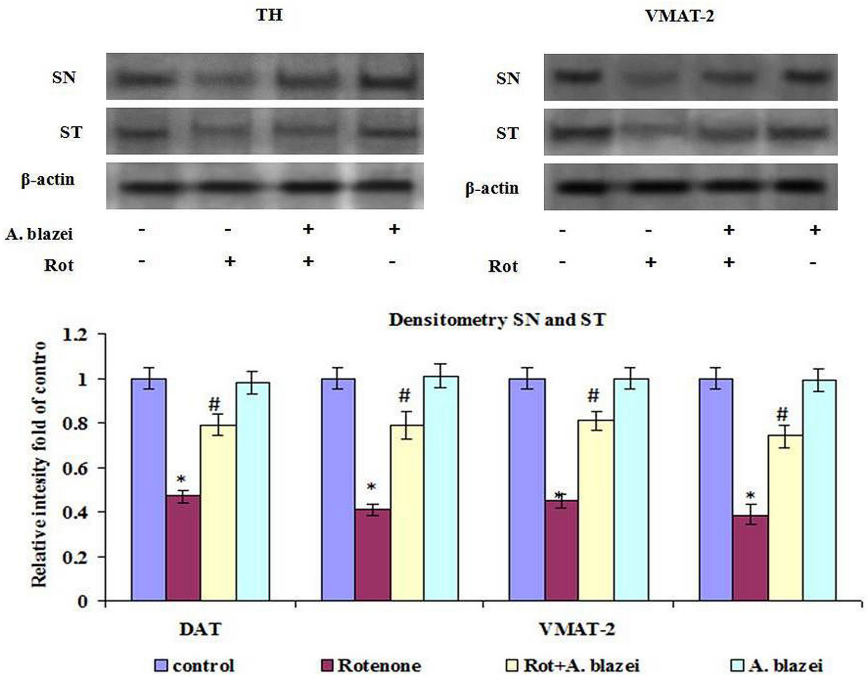

Figure 1. Effect of A. blazei on rotenone induced DAT and VMAT2 in SN and ST of experimental animals. Injection of rotenone significantly reduced DAT, and VMAT2 expressions in SN and ST. Pretreatment with A. blazei significantly increased DAT, and VMAT2 expression. Protein expressions were quantified using β-actin as an internal standard and values are expressed as arbitrary units and given as mean ± SD. P*< 0.0.5 compared to control, #p < 0.0.5 compared to rotenone.

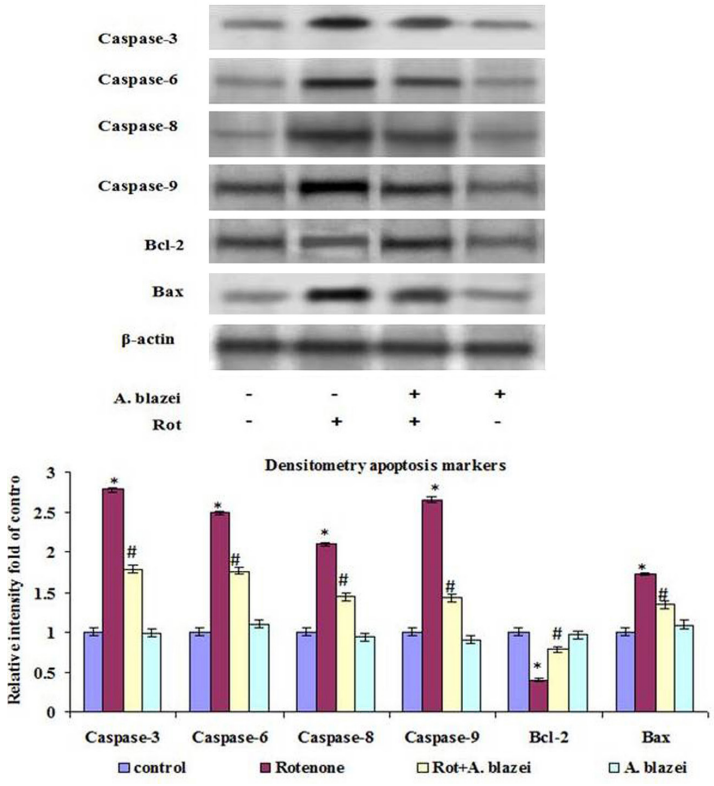

Figure 2. Effect of A. blazei on rotenone induced apoptosis in experimental animals. Administration of rotenone significantly increased the expression of pro-apoptotic proteins Bax, Caspase-3, -6, -8 and -9 and decreased the expression of anti-apoptotic protein Bcl-2. Pretreatment with A. blazei attenuated apoptosis by decreasing the expressions of pro-apoptotic markers. Protein expressions were quantified using β-actin as an internal standard and values are expressed as arbitrary units and given as mean ± SD. *p < 0.0.5 compared to control, #p < 0.0.5 compared to rotenone group.

axon terminals (32). Decline in the DAT expression is due to the loss of dopaminergic cells and fibers as a consequence of rotenone treatment in rats (24). Our western blot analysis indicated that declined expression of DAT in SN of rotenone treated mice, whereas oral administration of A. blazei extract led to increased protein expression levels of DAT in the SN as compared to rotenone alone group, which may be due to its neuroprotective effect.

Analyzing the VMAT-2 protein expression is a reliable indicator of vesicular concentration and provides a more accurate measure of nerve terminal density compared to other phenotypic markers such as DAT and TH protein expression levels (32). VMAT-2 serves as a neuroprotective factor by sequestering neurotoxin into vesicles and a critical regulator of cytoplasmic DA levels and dopaminergic function. Chen *et al.* (33) reported that the loss of VMAT-2 action in dopaminergic neurons may be a precursor to PD. Therapeutic strategies to prevent degradation of VMAT-2 or restore its function may be fruitful areas of investigation in PD research. Our western blot analysis showed the diminution of VMAT-2 expression in SN of rotenone treated mice, however, co-administration of A. blazei significantly attenuated rotenone induced neurotoxicity via enhanced VMAT-2 expression in mice.

α-synuclein is abundant in neuronal cytosolic proteins enriched at presynaptic terminals and are thought to be involved in synaptic function and plasticity (34). It is a major component of LBs and neurites, and present abundantly in LBs (35). The role of α-synuclein in normal cell function and in neurodegeneration have not been elucidated elaborately, but its potential roles in synaptic plasticity (36), neuronal differentiation, the up-regulation of DA release and mitochondrial deficits (37) have been reported. Previous findings imply that rotenone induced α-synuclein aggregation is probably mediated by oxidants generated from ROS generation (38). Because α-synuclein may be selectively and specifically nitrated, and it may link oxidative and nitrative damage to the onset and progression of neurodegenerative synucleinopathy lesions (39). Moreover, it was reported that oxidative stress can drive to α-synuclein aggregation and inclusion formation in cellular models (40). Rotenone leads to upregulation of α-synuclein expression, where as oral administration of A. blazei to PD mice partially rescued the level of α-synuclein.

During normal ageing, the rate of neuronal apoptotic cell death in dopaminergic neurons lies between 0.5. and 0.7.% per year and that the number of dopaminergic neurons is around 3,00,000-4,00,000 at the beginning of degeneration, one expects five to 10 dying neurons every day. In PD brains, the estimated rate of cell death could be around 5% per year and a maximum of 100 apoptotic neurons could be detected in the SN of PD patients (41). Apoptosis has recently been recognized as an important mode of cell death in PD (42). This has mainly been discovered by the identification of key markers of apoptotic cell death including mitochondrial Cyto-C release, alterations in Bax/Bcl-2 ratio, activation of caspases and DNA fragmentation in PD (42). Bcl-2 an antiapoptotic protein, appears to directly or indirectly preserve the integrity of the outer mitochondrial membrane, thus preventing Cyto-C release and mitochondria mediated cell damage initiation, whereas the pro-apoptotic protein, Bax, promotes Cyto-C release from mitochondria (43). Dhanalakshmi *et al.* (24) reported that rotenone treatment increased the expression of Bax protein and decreased Bcl-2 expression, resulting in imbalance between Bcl-2/Bax. This was also in concurrence with our results. Bax is translocated to the mitochondria, which results in increased colloidal osmotic pressure and mitochondrial swelling (the inner membrane cristae unfolded), rupturing of outer mitochondrial membrane and ultimately Cyto-C release (44), thereby leading to the activation of various caspases (45).

Role of caspases in the pathogenesis of PD has been established on the basis of studies in both postmortem brain tissue and animal models (46). Both extrinsic pathway or death receptor pathway and intrinsic pathway or mitochondrial pathways are known to be involved in the pathogenesis of PD. In intrinsic pathway, released Cyto-C could form apoptosomes together with apoptosis-activating factor-1 (Apaf-1) and procaspase-9, leading to the activation of caspase-9 and subsequent activation of caspase-3 (47). Apaf and Cyto-C binds to procaspase 9 to form an apoptosome by activating caspases-9, leading to subsequent proteolytic activation of the executioner caspases -3, -6 and -7, ultimately resulting in apoptosis. In the extrinsic pathway, activation of caspases 8 results in proteolytic activation of the executioner caspases -3, -6 and -7 resulting in apoptosis (48). In the present study, expressions of Caspases -3, -6, -8 and -9 significantly increased in rotenone treated animals when compared to control animals, which indicate that both the intrinsic and extrinsic pathways play important roles in apoptosis. Findings of our study demonstrate that pretreatment with A. blazei to rotenone treated mice suppresses apoptosis not only by decreasing the release of Cyto-C, but also by inhibiting the activation of caspases-9, -8, -6 and -3 and increasing Bcl-2 and decreasing Bax expressions.

5. CONCLUSION

A blazei administration protects rotenone induced dopaminergic cell loss by its anti-apoptotic action. Based on results of our investigation, we speculate that A. blazei might be a promising candidate for the prevention or treatment of PD, but further clinical studies are required.

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Key Words: A. blazei extract, Mitochondrial dysfunction, Apoptosis, Rotenone

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Neuroprotective efficacy of withaferin a on aging mediated oxidative stress in striatum and Substantia nigra of wistar albino rat.

- **Source:** Drug Invention Today . Mar2019, Vol. 12 Issue 3, p425-431. 7p.

- **Author(s):** Banu, Mohammad Raziya; Ibrahim, Muhammed; Prabu, K.; Rajasankar, Srinivasagam

- **Abstract:** Background: The oxidative stress plays a crucial role in aging mediated patho-biology of neuro-degeneration and its associated behavioral impairment. The purpose of this study is to evaluate the efficacy of Withaferin-A (WA) on aging induced oxidative stress in striatum (ST) and substantia nigra (SN) of aged rat brain. Materials and Methods: Animals were randomly separated into group I – young (3 months), group II – aged (24 months), group III – aged rat supplemented with WA (50mg/kg b.w once in a day for 30 days), and group IV – young rat supplemented with WA (50mg/kg b.w). After 30 days, through cervical dislocation, animals were sacrificed and the striatum and substantia nigra (mid brain) were dissected out from brain. Brain tissues were subjected to the estimation of enzymic antioxidants, levels of free radical production and apoptotic analyses (active caspase-3 and DAPI staining). Results: The data demonstrated reduced motor activity (p<0.001), increased Reactive oxygen species (ROS) (p<0.001), reduced enzymic antioxidant defense system (p<0.001), increased active caspase-3 activity (p<0.001) and increased apoptotic nuclear morphology (p<0.001) in aged ST and SN compared to young, which was significantly reversed by the supplementation of WA. Conclusion: The evidence from the present data emphasizes that WA bears beneficial property in the protection of striatal and nigral neuron from aging mediated oxidative stress and its associated apoptosis.

Anti-inflammatory effect of withaferin A on dopaminergic neuron of aged rat.

- **Source:** Drug Invention Today . 10/15/2019, Vol. 12 Issue 10, p2352-2355. 4p. 1 Diagram, 1 Graph.

- **Author(s):** Banu, Mohammad Raziya; Ibrahim, Muhammed; Prabu, K.; Rajasankar, Srinivasagam

- **Abstract:** Objective: The objective of the present study is to evaluate the effect of Withaferin A (WA) on aging induced inflammation in the dopaminergic system of the rat brain. Materials and Methods: Wistar albino rats were divided into Group I - young (3 months), Group II - aged (24 months), Group III - aged rat supplemented with WA (50 mg/kg body weight [b. w.] once in a day for 30 days), and Group IV - young rat supplemented with WA (50 mg/kg b. w). Results: The estimation of nitrate and nitrite (NOx) levels revealed significantly increased levels in the aged animal when compared to young. The estimation of superoxide (O2-) showed significantly increased in the midbrain and striatum of aged rat when compared to young. The estimation of pro- and antiinflammatory cytokines presented significantly increased levels in aged midbrain and striatum when compared to young. The apoptotic study also revealed increased apoptotic nuclear morphology in substantia nigra and striatum of aged rat when compared to young. Interestingly, the WA administration significantly reversed the NOx, O2- levels. Conclusion: The present data clearly demonstrate that the WA potentially repress the aging mediated oxidative stress and inflammation in the dopaminergic neuron, thereby it prevents aging induced neurodegeneration of dopaminergic neurons.

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Effect of *Coccinia indica* leaf extract on angiotensin converting enzyme (ACE) inhibitor induced hepatotoxicity in wistar albino rats

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SUMMARY

Enalapril is angiotensin converting enzyme (ACE) inhibitor have been extensively used to treat cardiovascular disorders, including hypertension. *Coccinia indica* is indigenous plant wildly used in traditional medicinal system including Ayurvedic, Siddha and Unani. The aim of this study was to find out the hepatoprotective activity of *C. indica* leaf extract against enalapril induced toxic effects. The 24 experimental rats were divided into 4 groups. Group 1 was maintained as control. Group 2 and 3 were treated with single dose of enalapril (1.5g/kg of bwt/orally). After treatment, group 2 maintained as enalapril control. The group 3 was treated with leaf extract of *C. indica* (400 mg/kg bwt/orally/day) for 7 days. Separate *C. indica* alone supplementation for 7 days were also maintained. After end of the treatment, animals were sacrificed simultaneously. To determine the serum liver function parameters were estimated. The histophological study of liver tissue was also determined. The changes liver function parameters were observed in enalapril control rats along with histological changes. The *C. indica* leaf extract supplementation restored all the parameters significantly. In this study, the protective effect of *C. indica* leaf

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extract against enalapril induced hepatotoxicity proves, the plant has many medicinal properties.

1. Introduction

Hypertension (HTN) is an important public health problem in both economically developed and developing nations [1]. The world's leading risk factor for global disease burden, is expected to cause more than half of the estimated 17 million deaths per year resulting from cardiovascular disease (CVD) worldwide [2]. Liver is major metabolic organ previous studies shows in drug induced liver injury occur as an unexpected reaction to therapeutic dosages. More than 900 different drugs have been implicated in causing liver injury, with the type of injury and degree of severity highly varied [3], and liver injury accounts to an elevated grade of morbidity and mortality [4–7]. The association of liver injury with antihypertensive agents has been best characterized by angiotensin-converting enzyme inhibitors [8]. Angiotensin converting enzyme (ACE), is responsible for the synthesis of angiotensin II, the final product of the renin angiotensin cascade [9–12]. Enalapril is angiotensin converting enzyme (ACE) inhibitor have been extensively used to treat cardiovascular disorders, including hypertension. These drugs are generally well tolerated [13,14]. However, hepatic injuries, which are rare [15], have been reported in patients treated with enalapril [3,13,16–20], captopril [21–23] and lisinopril [24,25].

Coccinia indica is a type plant belonging to the Cucurbitaceae. It is commonly known as Scarlet-fruited gourd and locally known as 'Kovai,' it is natively found in India, Asia and Central Africa [26]. Indigenous people used various parts of the plant to vegetable and medicinal purpose. The fruit of *C. indica* is used as vegetable when it is green in colour and eaten fresh when ripened into bright scarlet color. The young leaves and shoot tips of *C. indica* are used in Asia for cooking purpose [27,28]. The plant has also been extensively used in Ayurvedic, Unani and Siddha practice in the indian subcontinent [29–31]. *C. indica* is a fast growing perennial vine that grows several meters long. It can form dense mats on lands that readily cover shrubs and small trees. Its leaves are arranged alternately along the stems, the shape of leaves varies from heart to pentagon shaped. The upper surface of the leaf is hairless, whereas the lower is hairy. There are 3–8 glands on the blade near the leaf stalk. Tendrils are simple [32].

C. indica contain important raw material for drug production like bioactive compounds such as secondary metabolite like Aerial part - Heptacosane, Cephalandrol, β -sitosterol, Alkaloids Cephalandrins A and B, Fruits- β- Amyrin Acetate, Lupeol, Cucurbitacin B, Taraxerone, Taraxerol, β-carotene, Lycopene, Cryptoxanthin, Xyloglucan, Carotenoids, β-sitosterol, Stigma-7-en-3-one. Root - Resin, Alkaloids, Starch, Fatty Acids, Carbonic acid, Triterpenoid, Saponin Coccinoside, Flavonoid Glycoside, Lupeol, β-amyrin, β-sitosterol, Taraxerol [33]. Previous scientific research showed that leaf extract of *C. indica* exhibits hepatoprotective [34–36], antioxidant [37,38], anti-inflammatory [39] anti diabetic [40], hypolipidemic [41,42], and anti-bacterial [43] activities. This study investigated how the use of *C. indica* leaf extract could interfere with hepatotoxicity caused by enalapril by means of pharmacological and biochemical approaches.

2. Materials and methods

2.1. Animals

A total of 24 female healthy wistar albino rats with body weight ranging from 150 to 200 g were purchased from Sri Venkateswara Enterprises, Bangalore. The experimental rats were quarantined in animal house for 10 days and were maintained at 12:12 h's light/dark cycle at 20 °C ± 2 for 7 days with

ad libitum of food and sterile reverse osmosis water. All procedures were performed according to the institutional animal ethics committee's approval.

2.2. Drugs and chemicals

All the drug and chemicals were purchased from Hi media and Sigma Chemical Co, USA.

2.3. Collection of plant materials

The leaves of *C. indica* were collected from near Tiruchirappalli, India. The plant leaves were cleaned of extraneous matter, and necrotic parts removed by rinsing in fresh water. The leaves were further repeatedly rinsed thoroughly with running distilled water for further analysis.

2.4. Plant identification

The plant was identified and confirmed as *C. indica* in the RAPINET Herbarium, St. Joseph's college, Tiruchirappalli, India.

2.5. Preparation of diethyl ether extracts

The fresh green leaves of *C. indica* were shade dried and made into powdered manually. The 150 gm of dried powder soaked in the solvent diethyl ether and kept aside for two days. After two days, the ethereal layer was decanted and process repeated till plant material was exhaustively extracted with diethyl ether. Total extract was distilled off and concentrated under reduced pressure and controlled temperature by using rotary evaporator. The extract was green sticky mass (yield 50 gm). The extract was stored in freeze at 4c for further studies. Suspensions of extract were freshly prepared using 5% Tween 20 and 0.9% saline, for experimental use.

2.6. Experimental design

Rats were divided into four groups with six animals each. Group I was maintained as control with food and distilled water for 7 days. Other animals were subdivided and maintained as following groups up to 7 days such as Group II (Enalapril control), Group III (Enalapril + *C. indica* leaf extract therapy), Group IV (*C. indica* leaf extract control). The respective *C. indica* leaf extract were administered simultaneously along with enalapril supplementation to group III animals for 7 days. enalapril were purchased from The Madras Pharmaceuticals, Chennai, Tamil Nadu, India, respectively. The enalapril (1.5 g/kg bwt/p.o) for single dose was selected according to Jurima romet and Huang, (1992) [44]. The *C. indica* leaf extract (400 mg/kg bwt/p.o) for 7 days therapy was selected according to Kumar et al., (2010) [35].

2.7. Sample collection

After end of the treatment, rats were anaesthetized by chloroform, sacrificed and the blood and liver samples were collected from aseptically. Serum was isolated after centrifugation at 3000 rpm for 15 min. The serum aliquots were immediately used to determination of serum protein and liver function parameters and liver sample used histopathological studies.

2.8. Methods

2.8.1. Determinations of Fourier transform infrared (FT-IR) spectroscopy analysis

Shade dried diethyl ether extracts of *C. indica* leaf samples were ground into fine powder using a mortar and pestle. About 2 mg of the sample was mixed with 100 mg potassium bromide (FT-IR Grade) and then compressed to prepare a salt-disc (3 mm diameter). The disc was immediately kept in the

sample holder and FT-IR spectra were recorded in the range of absorption between 400 and 4000 cm-1. All investigations were carried out with a Shimadzu FT-IR spectrometer.

2.8.2. Estimation of liver function parameters (LFT)

Serum liver function parameters (LFT) were determined by standard methods using spectrophotometrically. Total protein estimated by method of Lowry et al., (1951) [45]. The results were expressed as g/dl. Total Bilirubin level determined by method of Van den Bergh and Muller. (1916) [46]. The results were expressed as mg/dl. Serum glutamate oxaloacetate transaminase (sGOT, EC 2.6.1.1) determined by method of Raitman and Frankel. (1957) [47], Serum glutamate pyruvate transaminase (sGPT, EC 2.6.1.2) determined by method of Raitman and Frankel. (1957) [47], Serum alkaline phosphatase (ALP, EC 3.1.3.1) determined by method of King and Armstrong. (1934) [48], Lactate dehydrogenase (LDH, EC 1.1.1.27) and Gamma-glutamyl transferase (GGT, EC 2.3.2.2) were estimated using commercial kits purchased from Crest Biosystems, India. All the liver enzyme results were expressed as IU/L.

2.8.3. Histopathological studies

The same groups were maintained for histopathological study. The animals were perfused transcardially. The whole blood was cleared from the circulation by flushing normal saline till the draining fluid becomes clear and then 10% normal saline was flushed for fixation. The Bouin's fixed tissues were processed for routine paraffin sectioning and stained with Haematoxylin and Eosin (H&E) [49]. Briefly, liver tissues were hydrated, then dehydrated in graded alcohol series, briefly cleared in chloroform and xylene and then embedded in paraffin wax. Liver tissues were sectioned at 5 mm thickness using Rotary microtome and incubated overnight at room temperature. Then the sections were deparaffinized, rehydrated through descending alcohol series (100% alcohol, 90% alcohol, 70% alcohol and 50% alcohol) followed by distilled water. These sections were stained with H & E and then rapidly carried through ascending alcohol series. Morphology of liver tissues was analyzed and recorded by Nikon microscope (400).

2.8.4. Statistical analysis

Data were expressed as mean ± SEM. Statistical significance was evaluated by one way ANOVA using SPSS version 21. In all cases, $p < 0.05$ was considered as statistically significant.

3. Results

FTIR was used to analyze the functional groups of compounds. The absorption spectra of diethyl ether extracts of *C. indica* leaf are shown in Table 1. Diethyl ether extracts of *C. indica* leaf FTIR analysis confirms the presence of amines, alkanes and alcohol at 3412.53/cm peak value. The peak at 2921.54 and 2362.69/cm indicated the presence of alkane. The remaining peak at 2117.22, 1634.74, 1438.98, 1242.57, 1096.46, 665.86 and 601.60/cm represents the presence of Isothiocyanates stretch, Conjugated alkenes stretch, Bend carboxylic acids, Alcohols stretch, Ethers stretch, Alkyl halides stretch and Acid chlorides

Table 1
FTIR peak values and functional groups of *Coccinia indica* leaf extract.

Origin	Peak	Functional groups
N–H	3412.53	Amines stretch
C–H	2921.54	Alkanes stretch
C–H	2362.69	Alkanes stretch
N=C=S	2117.22	Isothiocyanates stretch
C=C	1634.74	Conjugated alkenes stretch
O–H	1438.98	Bend carboxylic acids
C–O	1242.57	Alcohols stretch
C–O–C	1096.46	Ethers stretch
C–Cl	665.86	Alkyl halides stretch
C–Cl	601.60	Acid chlorides stretch

stretch as a functional group, respectively. The major peaks and functional of dynamic compounds groups were analysed and results were compared with standard infrared chart by Coates. (2000) [50].

Histopathological examination of liver section of control animals showed normal cellular architecture with distinct hepatic cells, prominent nucleus, sinusoidal space and central vein. There was no sign of inflammation, fatty change or necrosis in these animals (Fig. 6 A). Liver section of intoxicated with enalapril (1.5 g/kg bwt) treated animals showed severe hepatocyte degeneration and necrosis in the centrizonal area. Inflammatory cells were also observed in the portal triad (Fig. 6 B). Liver section of rat treated with diethyl ether extract of *C. indica* leaf (400 mg/kg bwt) and intoxicated with enalapril (1.5 g/kg bwt) showed reduction of necrosed area and inflammatory infiltrates in the centrizonal area with disappearance of inflammatory infiltrate around portal triad (Fig. 6C). Liver section of rat treated with diethyl ether extract of *C. indica* leaf (400 mg/kg bwt) showed normal cellular architecture with distinct hepatic cells, prominent nucleus, sinusoidal space and central vein. There was no sign of inflammation and necrosis in these animals (Fig. 6 D).

4. Discussion

Liver injury caused by toxins or chemicals is generally associated with acute or chronic damage. Injury by cytotoxins is characterized by necrosis, In this regard, the mechanism by which ACE

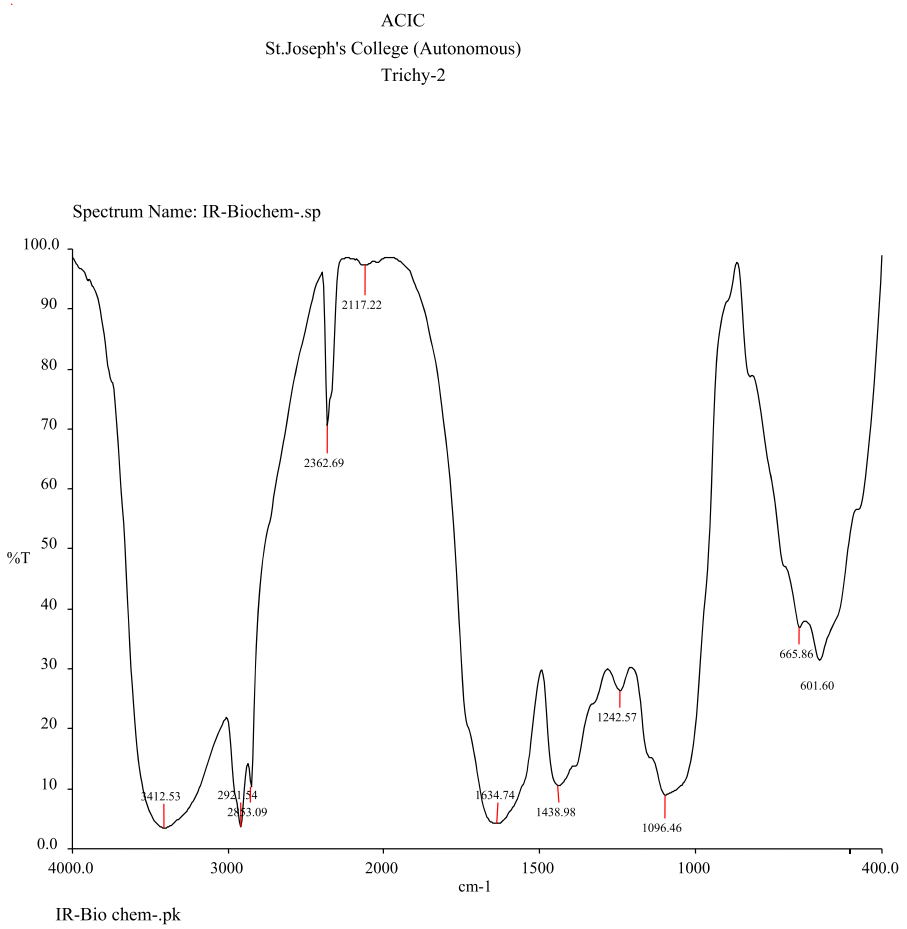

Fig. 1. Fourier transform infrared (FTIR) spectroscopy analysis of *Coccinia indica* leaf extract.

inhibitors cause liver injury remains unclear, mainly because of the lack of a suitable experimental model [51]. Studies also show enalapril treatment and the first signs of liver damage suggest the possibility of a metabolic idiosyncrasy [52]. Based on literature in our present study enalapril was found to cause of hepatotoxicity in rat livers, high doses of enalapril elicited significantly decreased in total protein (Fig. 2) at same trend significantly increased levels of enzymatic biomarkers including serum sGPT, sGOT, GGT, ALP, LDH and total bilirubin of enalapril treated animals compared with normal group (Figs. 3–5). Histopathological changes were also observed in liver tissues in enalapril induced group such as severe hepatocyte degeneration and necrosis in the centrizonal area. Inflammatory cells were also observed in the portal triad (Fig. 6 B). We suggest that the elevated serum liver markers were released from the injured liver upon exposure to high doses of enalapril. In addition, serum enzymatic biomarkers and total bilirubin (Figs. 3–5) levels were markedly increased, especially at single dose of enalapril 1.5g/kg of bwt by orally, and this may be caused by excessive

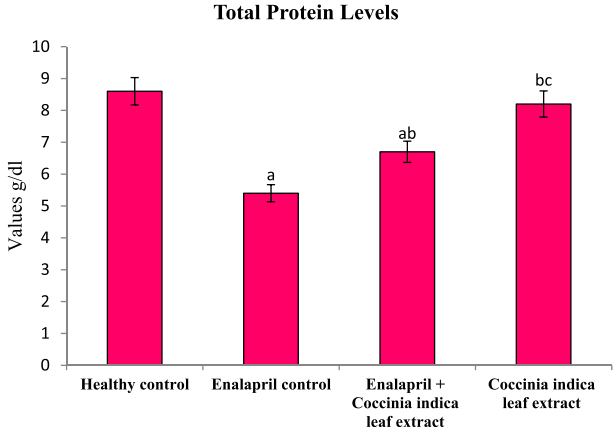

Fig. 2. Total protein values was expressed as mean ± SEM. Letters a, b and c denote the statistical significance of the data at the level of P < 0.05. (a) denote comparison of control Vs. other groups; (b) denote comparison of Enalapril control Vs. Enalapril + *Coccinia indica* leaf extract therapy, (c) denote comparison of Enalapril + *Coccinia indica* leaf extract therapy Vs. *Coccinia indica* leaf extract control.

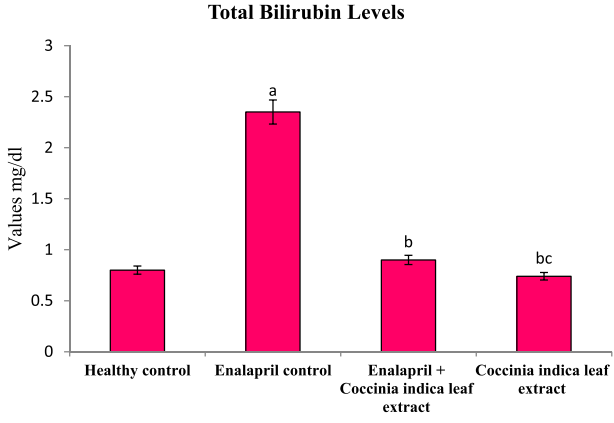

Fig. 3. Total bilirubin values was expressed as mean ± SEM. Letters a, b and c denote the statistical significance of the data at the level of P < 0.05. (a) denote comparison of control Vs. other groups; (b) denote comparison of Enalapril control Vs. Enalapril + *Coccinia indica* leaf extract therapy, (c) denote comparison of Enalapril + *Coccinia indica* leaf extract therapy Vs. *Coccinia indica* leaf extract control.

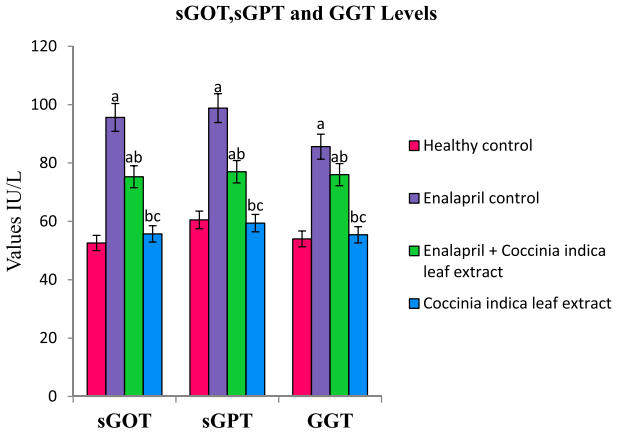

Fig. 4. Serum glutamic oxaloacetic transaminase (sGOT), glutamic pyruvic transaminase (sGPT) and gamma glutamyl transferase (GGT) values were expressed as mean ± SEM. Letters a, b and c denote the statistical significance of the data at the level of $P < 0.05$. (a) denote comparison of control Vs. other groups; (b) denote comparison of Enalapril control Vs. Enalapril + *Coccinia indica* leaf extract therapy, (c) denote comparison of Enalapril + *Coccinia indica* leaf extract therapy Vs. *Coccinia indica* leaf extract control.

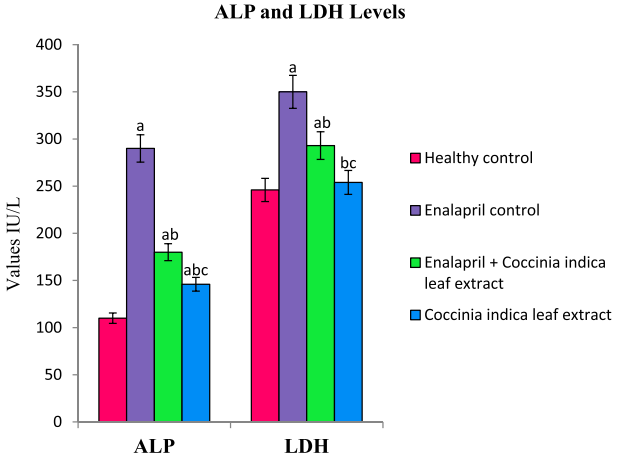

Fig. 5. Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) values were expressed as mean ± SEM. Letters a, b and c denote the statistical significance of the data at the level of $P < 0.05$. (a) denote comparison of control Vs. other groups; (b) denote comparison of Enalapril control Vs. Enalapril + *Coccinia indica* leaf extract therapy, (c) denote comparison of Enalapril + *Coccinia indica* leaf extract therapy Vs. *Coccinia indica* leaf extract control.

accumulation of enalapril in liver tissues. Evidence of enalapril induced liver injury has been demonstrated by various studies [3,13,16–20].

C. indica is traditional medicine system different parts of this plant namely the roots, leaves and fruits have long been considered as valuable sources of medicine for treating variety of diseases and ailments [31]. Recent experimental and clinical studies have shown the importance of *C. indica* leaf extract as a hepatoprotective [34–36], antioxidant [37,38], anti-inflammatory [39] anti diabetic [40], hypolipidemic [41], and anti-bacterial [43] activities. Preliminary phytochemical study showed the presence of Carbohydrates, Glycosides, Alkaloids, Tannins and Flavonoids in the ethereal extract of *C. indica* leaves [35]. The acute toxicity study LD_{50} values suggested that aqueous and diethyl ether leaf extract of *C. indica* is safe and nontoxic in healthy wistar rats and mice up to a dose of 2.00 g/kg of bwt

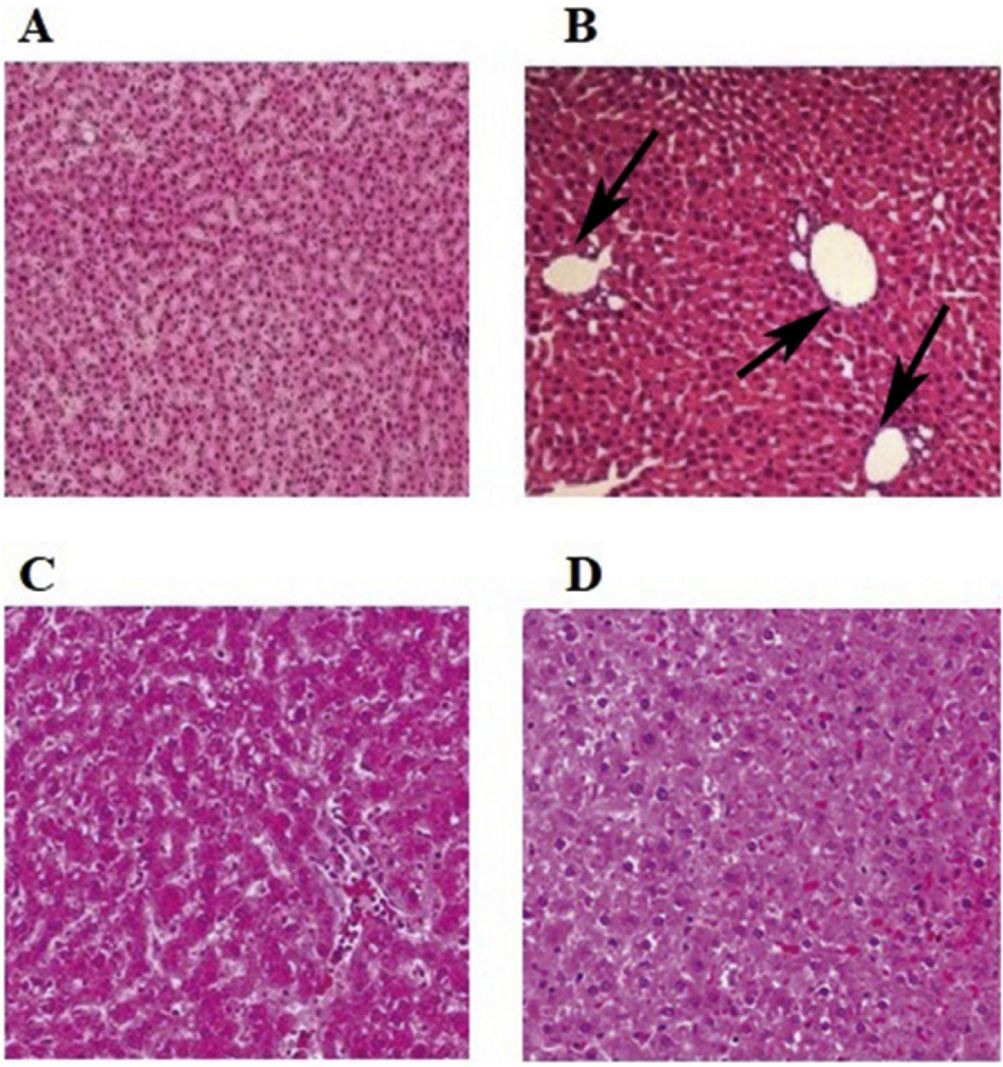

Fig. 6. Histopathological changes occurred in the liver after enalapril intoxication and prevention by the treatment with diethyl ether extract of *Coccinia indica* leaf. A) Normal, B) Enalapril control, C) Enalapril + *Coccinia indica* leaf extract therapy, D) *Coccinia indica* leaf extract control.

[35,53]. In our present hepatoprotective activity study dose (400 mg/kg of bwt) was selected according to Kumar et al., (2010) [35]. The FTIR spectral analysis showed (Fig. 1 & Table 1) the presence of Isothiocyanates stretch, Conjugated alkenes stretch, Bend carboxylic acids, Alcohols stretch, Ethers stretch, Alkyl halides stretch and Acid chlorides stretch as functional group. It indicates the medicinal property of *C. indica* leaf, which can be utilized for various pharmaceutical purposes. In this study, we also observed changes in serum liver function parameters and total protein levels (Figs. 2–5) in enalapril control rats along with histological changes (Fig. 6B) when compared to healthy rats. The *C. indica* leaf extracts supplemented group restored all the serum liver function parameters and total protein levels (Figs. 2–5) significantly. In histopathological studies reduced hepatic damage, lesser necrosis, inflammatory infiltrates, demonstrated a normal architecture of liver and no significant pathological

manifestations (Fig. 6C) like healthy group. *C. indica* leaf extract was found to be toxicologically safe as a potential hepatoprotective agent.

In conclusion based on the results of liver function parameters and histopathological studies, we found that diethyl ether extract of *C. indica* leaf therapy did not show any side effects in liver tissues of rats when compared to enalapril intoxication. Hence, this plant may be clinical usefulness of liver damage. However, further study is required to evaluate using a cardioprotective effect of *C. indica* leaves.

Conflict of interest

None of the authors declares a conflict of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yclnex.2019.01.004.

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Patient-rated physicians' empathy and its determinants in Riyadh, Saudi Arabia

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Abstract:

BACKGROUND: Patients' perception of their physician's empathy influences their compliance with treatment and the resulting quality of life. We aimed to measure the patient-rated empathy of physicians and to determine patient-level factors associated with it.

MATERIALS AND METHODS: This hospital-based cross-sectional study enrolled adult (≥18 years) patients attending the outpatient clinics of the departments of family medicine, internal medicine, and surgery. We measured patients' rating of their physician's empathy using the Jefferson Scale of Patient's Perception of Physician Empathy questionnaire. Data were analyzed using SPSS v 23.0; categorical variables were presented as frequencies and percentages, and all quantitative variables were presented as mean and SD. Associations were explored by Chi-square test and Student's *t*-test. Regression analysis was performed to identify factors significantly associated with the empathy score; $P < 0.05$ was considered statistically significant.

RESULTS: Of a total of 390 patients with a mean (standard deviation [SD]) age of 40.5 (13.6) years, 189 (48.5%) were male. The mean (SD) total patient-rated physician empathy score was 26.6 (6.0). Multilevel linear regression modeling revealed that having a health professional in the family, suffering from an acute illness (as compared to chronic illness), consulting a physician recommended by relatives/friends, trusting the physicians' expertise, shorter (<10 min) waiting time, and perceived adequate consultation time were associated with higher empathy ratings.

CONCLUSIONS: Patients' perception of physicians' empathy is indispensable for the success of a clinical consultation. It is influenced by patient-level social and clinical factors. On-the-job physician training in empathy, effective monitoring, and feedback mechanisms should be an integral component of the quality control of hospital services.

Keywords:

Patient satisfaction, patient rated, physician empathy, Saudi Arabia

Introduction

Empathy is a complex, broad, multidimensional concept comprising four components – the emotive (the ability to imagine patients' emotions), cognitive (the intellectual ability to identify and understand patients' emotions), moral (the internal motivation to empathize), and behavioral (the ability to convey the understanding of those emotions back to the patient).[1,2] Physician's empathy is fundamental to patient–doctor relationship.[3] An empathetic encounter with the physician can have several benefits, such as better reporting of symptoms, improved diagnostic accuracy, increased patient participation in the diagnostic process, improved patient satisfaction, better ability to cope with the prescribed treatment, reduced depression, and improved quality of life. A higher level of empathy has also

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been found to be significantly associated with less clinical burnout of physicians.[4-7]

Empathy expressed by a physician in a physician–patient encounter can be viewed from three perspectives – the physician, the patient, and a third person. Patients' perception of physician empathy is influenced by physician's characteristics such as age, gender, years of experience, academic performance, emotions and emotional control, personal experiences, feelings and attitudes toward patients, and academic and clinical experiences.[8-10] Apart from these, many patient-level factors such as sociodemographic, illness, and consultation-related characteristics also affect their perception. Understanding how and why certain types of patients rate some physicians can be of great importance in planning effective health-care services.

Of the different ways of measuring physician empathy, patients' viewpoint is the most important in terms of maximizing successful clinical outcomes. It is also important to understand patients' expectations from their physicians and the factors that influence their perception because the literature shows that physicians' self-assessment of empathy may not always correlate with patients' assessment.[11] This knowledge can then be utilized to train physicians and improve the quality of health services to produce better health outcomes.

With this aim, the current study was conducted to measure patient-rated physicians' empathy and determine the various factors associated with a higher rating at a tertiary care governmental hospital in Riyadh, Saudi Arabia.

Materials and Methods

A hospital-based cross-sectional study was conducted at the Family Medicine, Internal Medicine, and Surgery outpatient clinics (outpatient department [OPD]) of King Saud Medical City in Riyadh, Saudi Arabia, from September to December 2018. The study hospital caters to a large section of Riyadh's population from diverse socioeconomic backgrounds. Adult patients (18 years old and above) who attended these three clinics were invited to participate in the study. Severely ill patients and patients incapable of giving valid consent due to mental health issues were excluded from the study.

We sampled patients clustered around thirty physicians (ten each from the above mentioned OPDs). The number of patients sampled under each physician was calculated on the following assumptions. An assumed population standard deviation (SD) of 2.5 obtained from a previous study,[12] a confidence level of 95%, an acceptable error of 2, and a nonresponse rate of 20%. The sample size so derived was multiplied by a design effect of 1.5 to account for the clustering, giving us the final sample size of 390 (13 patients interviewed for every physician). These patients were enrolled in a consecutive manner outside each physician's clinic (exit interview) till the required size was achieved.

The Arabic version of the 5-point Likert-type Jefferson Scale of Patient's Perception of Physician Empathy (JSPPPE) questionnaire was used to score the patient's rating of the empathy of the physician consulted. This scale had been validated in previous studies and therefore, deemed appropriate for this study.[13,14] The first author of this paper with another bilingual colleague translated the English scale into Arabic. This Arabic version was then re-translated into English by two different bilingual experts who were not familiar with the original English version. The author's team then compared the two English versions for validity and observed no inconsistencies. Along with the JSPPPE, a patient's questionnaire including sociodemographic details such as age, gender, nationality, educational level, income, and questions related to their clinical history was also administered. Patients were approached as they exited the clinic and asked to participate, and their eligibility was checked by the study staff. After the objectives of the study were explained to the patients, a written informed consent was obtained from them, and they were then asked to complete the self-administered questionnaire.

Data were captured electronically using Epicollect 5 software (Imperial College, London, UK) and were analyzed using SPSS v 23.0 (SPSS Inc., IBM, Armonk, New York, USA). Descriptive analysis was performed: all categorical variables were presented as frequencies and percentages, and all quantitative variables were presented as mean and SD. Associations were explored by Chi-square test and Student's *t*-test. Regression analysis using multilevel general linear method with random intercepts for the consulting physicians around whom the patients were clustered was performed to identify factors significantly associated with the empathy score. The variables to be entered in the model were decided on the basis of $P < 0.2$ in the bivariate analysis. $P < 0.05$ was considered to be statistically significant in the final model.

Ethical approval had been obtained from the Institutional Review Board, and informed written consent was taken from all participants in the study.

Results

The summary measures of the individual items and the total score of the JSPPPE scale are given in Table 1. Of a total of 390 patients who participated in the study,

189 (48.5%) were male. Their mean age (SD) was 40.5 (13.6) years (range: 18–98 years). Three hundred and twenty-nine patients (84.4%) were Saudi nationals, 263 (67.4%) were currently married, and 202 (51.8%) were employed, 48 (23.8%) of whom work in the health sector [Table 2]. The mean (SD) total patient-rated physician's empathy score was 26.6 (6.0) (range of 5.0–35.0). Of the five questions on the JSPPPE scale, patients rated their physician highest on whether their doctor was an "understanding doctor" (6.24 ± 1.1) and lowest on their doctor's concern for them and their family (4.75 ± 1.8) [Table 1].

Table 1: Summary measures of the Jefferson scale of patient's perception of physician empathy in adults attending outpatient clinics in a tertiary care hospital in Riyadh (*n*=390)

	Mean±SD
Can view things from my perspective	5.7±1.3
Asks about what is happening in my daily life	4.8±1.8
Seems concerned about me and my family	4.8±1.8
Understands my emotions, feelings, and concerns	5.1±1.7
Is an understanding doctor	6.2±1.1
Total empathy score rated by client	26.6±6.0

Higher score indicates higher empathy. SD=Standard deviation

In the bivariate analysis, it was observed that patients who were more than 40 years of age rated their physician significantly higher than younger patients (P = 0.04). Patients who were currently married (P = 0.001), those with college degrees (P = 0.008), and those who had a higher monthly family income (>10,000 SR and above) (P = 0.04) rated their physicians higher on empathy than their counterparts [Table 2]. Patients who had a health professional in their family (P = 0.003), who had an acute illness (as compared to those with a chronic illness) (P = 0.008), whose doctor was recommended by friends/relatives (P = 0.01), who trusted their doctors (P < 0.001), who understood

Table 2: Factors (sociodemographic) associated with patient-rated physician empathy by characteristics of adults attending outpatient clinics in a tertiary care hospital in Riyadh (*n*=390)

Characteristics	Number (%)	Empathy score mean±SD	*P*-Value
Age category (years)			
18-40	206 (52.8)	25.99±5.89	0.04
>40	184 (47.2)	27.24±6.10	
Sex			
Female	201 (51.5)	26.15±6.31	0.14
Male	189 (48.5)	27.04±5.66	
Marital status			
Currently married	263 (67.4)	27.13±5.90	0.01
Never married	87 (22.3)	25.05±6.27	
Divorced/widowed/separated	40 (10.3)	26.33±5.73	
Nationality			
Non-Saudi	61 (15.6)	26.62±6.52	0.95
Saudi	329 (84.4)	26.57±5.93	
Educational level			
Can read and write	21 (5.4)	24.52±4.86	0.08
Any school certificate	202 (51.8)	26.78±5.89	
Any college degree	128 (32.8)	27.10±6.07	
Technical training others	39 (10.0)	24.92±6.71	
Employment status			
Employed	202 (51.8)	26.90±6.23	0.34
Retired	22 (5.6)	27.86±5.45	
Student	28 (7.2)	25.43±3.80	
Unemployed	138 (35.4)	26.14±6.13	
Employment in the health sector			
No	342 (87.7)	26.49±5.95	0.41
Yes	48 (12.3)	27.25±6.50	
Total family income (SR)			
<3000	72 (18.5)	25.49±6.72	0.04
3000-5000	62 (15.9)	25.63±6.15	
5000-7000	76 (19.5)	26.25±4.99	
7000-10,000	75 (19.2)	26.53±6.12	
10,000-15,000	65 (16.7)	28.03±6.11	
>15000	40 (10.3)	28.37±5.38	

P value is from Student's *t*-test/ANOVA test, P<0.05 is statistically significant, higher score means more empathetic. SD=Standard deviation

Table 3: Factors (consultation and illness) associated with patient-rated physician empathy by characteristics among adults attending outpatient clinics in a tertiary care hospital in Riyadh (*n*=390)

Characteristics	Number (%)	Empathy score mean±SD	*P*-Value
Any health professional in the family			
No	288 (73.8)	26.03±5.97	0.003
Yes	102 (26.2)	28.12±5.89	
Type of illness			
Acute illness	70 (17.9)	28.30±6.53	0.008
Chronic disease	320 (82.1)	26.20±5.84	
Consulted this doctor before			
No	233 (59.7)	26.57±6.16	0.95
Yes	157 (40.3)	26.60±5.81	
First visit to this doctor for the current illness			
No	174 (44.6)	26.63±5.32	0.89
Yes	216 (55.4)	26.54±6.53	
Consulted another doctor for the current illness			
No	164 (42.1)	27.13±6.46	0.12
Yes	226 (57.9)	26.18±5.66	
Doctor was recommended by relatives, friends or other doctors			
No	347 (89.0)	26.32±6.03	0.01
Yes	43 (11.0))	28.67±5.52	
Trust in doctor's expertise			
No	15 (3.8)	14.67±5.90	<0.001
Yes	375 (96.2)	27.06±5.51	
Have relatives following up with this doctor			
No	364 (93.3)	26.58±6.01	0.99
Yes	26 (6.7)	26.58±6.26	
Understood the doctor's language			
No	13 (3.3)	21.23±7.76	0.001
Yes	377 (96.7)	26.76±5.87	
Waiting time before consultation (min)			
<10	128 (32.8)	28.19±5.79	<0.001
10-30	152 (39.0)	26.20±5.61	
>30	110 (28.2)	25.23±6.43	
Consultation time (min)			
<10	228 (58.5)	26.25±6.32	0.19
10-30	153 (39.2)	26.90±5.63	
>30	9 (2.3)	29.56±3.09	
Perceived adequacy of consultation time			
No	24 (6.2)	18.79±7.51	<0.001
Yes	366 (93.8)	27.09±5.55	
Would recommend this doctor to others			
No	64 (16.4)	21.11±7.03	<0.001
Yes	326 (83.6)	27.65±5.17	

P value is from Student's *t*-test/ANOVA test, *P*<0.05 is statistically significant. SD=Standard deviation

their doctor's language ($P = 0.001$), who had shorter waiting times ($P < 0.001$), and who thought that the consultation time was adequate ($P < 0.001$) gave a significantly higher empathy score. However, the actual duration of the consultation time did not influence the rating. Furthermore, patients who gave a higher score also stated that they would recommend their doctor to others ($P < 0.001$) [Table 3].

Because patients were clustered around their consulting physicians, we performed a multilevel linear regression analysis with random intercepts for the consulting physicians. We found that the presence of a health professional in the family ($b = 1.44$; 95% confidence interval [CI]: 0.33–2.54), an acute illness ($b = 1.66$; 95% CI: 0.24–3.07), a doctor recommended by relatives/friends ($b = 3.04$; 95% CI: 1.47–4.61), confidence in the doctor's expertise ($b = 7.96$; 95% CI: 5.38–10.53), waiting time before consultation of <10 min ($b = 1.72$; 95% CI: 0.46–2.98), and perceived adequate consultation time with their doctor ($b = 6.27$; 95% CI: 4.28–8.26) were the factors significantly associated with higher empathy scores. The

Table 4: Random intercepts model for factors associated with client-rated physician empathy among adults attending outpatient clinics in a tertiary care hospital in Riyadh (*n*=390)

	Coefficient	SE	Lower CI	Upper CI	*P*-Value
Age category (years)					
<40	Reference				
>40	1.15	0.59	−0.003	2.31	0.05
Marital status					
Currently married	Reference				
Never married	−0.55	0.65	−1.83	0.73	0.40
Divorced/widowed/separated	0.28	0.82	−1.32	1.88	0.73
Educational level					
Can read and write	Reference				
Any school degree	1.43	1.14	−0.81	3.66	0.21
Any college degree	1.29	1.28	−1.21	3.79	0.31
Technical training/others	−0.09	1.32	−2.68	2.50	0.95
Total family income (SR)					
<3000	Reference				
3000-5000	0.11	0.81	−1.48	1.71	0.89
5000-7000	0.24	0.81	−1.35	1.83	0.77
7000-10,000	−0.12	0.86	−1.80	1.57	0.89
10,000-15,000	1.32	0.87	−0.39	3.02	0.13
>15,000	0.95	1.03	−1.07	2.97	0.35
Any health professional in the family	1.44	0.56	0.33	2.54	0.01
Type of illness					
Chronic illness	Reference				
Acute illness	1.66	0.72	0.24	3.07	0.02
Doctor was recommended by relatives, friends, or other doctors	3.04	0.80	1.47	4.61	<0.0001
Trust in doctor's expertise	7.96	1.31	5.38	10.53	<0.0001
Understood the doctor's language	2.52	1.34	−0.11	5.14	0.06
Waiting time before consultation (min)					
>30	Reference				
10-30	0.55	0.61	−0.64	1.74	0.37
<10	1.72	0.64	0.46	2.98	0.01
Perceived consultation time as adequate	6.27	1.02	4.28	8.26	<0.0001
Constant	6.86	2.23	2.50	11.23	<0.0001

Wald χ^2 (19)=190.9, Log likelihood=−1154.8, P<0.0001, LR test versus linear model: χ^2 (01)=33.1, P<0.0001. SE=Standard error, CI=Confidence interval, LR=Likelihood ratio

overall model was statistically significant (Wald $\chi^2 = 190.9$, $P < 0.001$) and performed better (log likelihood ratio test: chi-bar square value = 33.1, $P < 0.001$) than a comparable linear regression model [Table 4].

Discussion

This questionnaire-based cross-sectional study of 390 patients presenting at the OPD clinics at a tertiary care hospital in Riyadh city, showed that the presence of a health-care professional in the family, suffering from an acute illness as opposed to a chronic illness, consulting a doctor recommended by someone, confidence in the doctor's expertise, shorter waiting time, and perceived adequate consultation time were associated with a perception of higher physician empathy. The credibility of our findings was enhanced by the fact that the use of a widely validated scale and the statistical analysis done to account for the clustering of patients around physicians led to more robust estimations.

The maximum score that patients could award their physician in the JSPPPE scale was 35. In our study, the average score of physicians was 26.6, indicating that patients perceived their physicians to be empathetic. Of the other studies conducted using the same scale, two studies reported a score higher than that of our study. A study on 535 outpatients of a teaching hospital in the United States reported a mean JSPPPE score of 29.6 ± 7.8, and a study conducted on 945 outpatient patients at a multispecialty hospital in Brazil reported a score of 30.6 ± 5.6.[11,14] However, a study on 225 ambulatory patients in the United States reported a mean score of 23.8 ± 2.5, which is lower than that reported in our study.[15]

In the present study, patients assigned the lowest scores to the following items: "the doctor asks about what is happening in my daily life" and "the doctor seems concerned about me and my family." This was somewhat similar to the study by Borracci *et al*.,[16] in

which the respondents scored their physicians lowest on the following items: "the doctor asks what is happening in my daily life" and "the doctor can view things from my perspective." This is an important area in which physicians were found lacking empathy. In situations where patients and their immediate family members have to make a difficult healthcare-related decision, the physician should have a good understanding of the patient and his/her family to be able to effectively involve him/her in the decision-making and arrive at the best course of action for the patient.[17] It may be necessary to provide training and direction to consulting physicians on this aspect of their interactions with patients to understand them in their microcosm.

Earlier studies using the JSPPPE reported that males, elderly patients, the less educated, and public hospital attendants gave their physicians higher scores.[16] Duberstein *et al*. reported that older patients gave higher ratings compared to younger patients.[18] The mean score given by older patients in our study was also higher than that of their younger counterparts, but this finding was not statistically significant on the multilevel model. Patients who belong to the upper social classes or with higher total family income usually tend to give higher empathy rating because they have a more direct participatory consulting style, characterized by more questioning and more information-giving, leading to more discussions and greater socioemotional partnership with their doctors compared to patients who have lower monthly incomes.[19]

Another finding was that patients who have family members working in health care gave better scores to their physicians. This could be because they have a better understanding of the role of their doctors and are liberal in their scoring. Further, as they have a better understanding of the field of medicine and the "system" of patient–doctor relationships, they understood their doctors' shortcomings and were generally more lenient. This is similar to the situation in which doctors or nurses are patients.[20]

Patients with acute and nondebilitating illnesses were found to give higher scores as they were less likely to suffer from psychological and emotional burnout compared to chronically ill patients who were more likely to be emotionally preoccupied with finding the means of coping with their illness. Patients with chronic illnesses who have lived with the condition for longer periods of time and have had many previous consultations have higher expectations from the physician and have less satisfaction with the expressed empathy. When patients have confidence in their doctors, they are more satisfied and their health outcomes are better.[21] This is the reason why patients who trust their doctors rated their physician's empathy higher than patients who had no trust in them.

Shorter waiting times before seeing the doctor was found to be significantly associated with higher rating. It has previously been reported that a patient's satisfaction is substantially reduced with longer waiting times (5 min or more).[22] This could mean that a patient's perception of physician empathy is also influenced by extraneous system-related factors. Moreover, patients rated their physician's empathy higher if they perceived that adequate time had been spent with them. It is interesting to note that the patients' perception that they had adequate consultation time with the doctor significantly influenced their rating. However, the actual length of time spent by the doctor with the patient was not significant. This finding is important because when physicians are trained for clinical practice, they should be informed that the absolute length of consultation time should be decided jointly by both parties rather than on the doctor's assessment only.[23] Attention to these factors is required for the successful execution of a physician–patient encounter and the achievement of optimal health outcomes for the patient.

One of the most important tasks of every physician, regardless of his/her specialty, is to communicate with patients and their relatives or other caregivers. Studies have reported that patients' perceived empathy was significantly different from their expectations and was associated with their satisfaction with treatment and trust in their health-care providers.[24] Physician's communication skills and the establishment of good rapport were also strongly associated with patient satisfaction.[25,26] Studies conducted in Saudi Arabia showed that patients rated their physicians high on privacy and being respected and the feeling that the staff understood their needs.[27] Similar to other international studies, local studies have also shown significant differences between expectation and perception and their significant influence on patient satisfaction.[28,29]

The main limitation of the study was that the hospital is run by the Ministry of Health and does not provide services for profit. Therefore, the results could be generalized only to similarly administered hospitals. Another limitation is that patients in the OPD setting only were assessed. The perception of empathy by inpatients or postsurgical patients who have had a longer interaction time with their physicians might be different from OPD patients.

Conclusions and recommendations

Physicians were rated higher on empathy by patients with family members in the health-care sector, patients with acute illness, those who saw physicians who had been

recommended, and those who had spent adequate time with the consultant. Here are a few recommendations resulting from the study: orientation of medical students on clinical empathy should be integral to the medical curriculum[30] and on-the-job training of physicians is required to make them more responsive to the changing philosophies of physician–patient relationship and its impact on treatment outcomes. At the level of hospital administration, there should be routine empathy assessment through regular patient feedback mechanisms.

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Conflicts of interest

There are no conflicts of interest.

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RESEARCH ARTICLE Open Access

Are they there yet? Linkage of patients with tuberculosis to services for tobacco cessation and alcohol abuse – a mixed methods study from Karnataka, India

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Abstract

Background: Tobacco use and alcohol abuse are associated with higher risk of tuberculosis (TB) infection, progression to active TB and adverse treatment outcomes among patients with TB. Revised National Tuberculosis Control Programme (RNTCP) treatment guidelines (2016) require the documentation of tobacco and alcohol use among patients with TB and their linkage to tobacco and alcohol abuse treatment services. This study aimed to assess the extent of documentation of tobacco and alcohol usage data in the TB treatment card and to explore in-depth, the operational issues involved in linkage.

Methods: A convergent parallel mixed methods study was conducted. All new TB treatment cards of adult patients registered under RNTCP between January and June 2017 in Dakshina Kannada district were reviewed to assess documentation. Document review was done to understand the process of linkage (directing patients to tobacco and alcohol abuse treatment services). In-depth interview of health care providers ($n = 7$) and patients with TB ($n = 5$) explored into their perspectives on linkage.

Results: Among 413 treatment cards reviewed, tobacco use was documented in 322 (78%), of whom 86 (21%) were documented as current tobacco users. Sixteen (19%) out of these 86 patients were linked to tobacco cessation services. Alcohol usage status was documented in 319 (77%) cards of whom 71(17%) were documented as alcohol users. Eleven (16%) out of these 71 patients were linked to alcohol abuse treatment services. The questions in the treatment card lacked clarity. Guidelines on eliciting history of substance abuse and criteria for linkage were not detailed. Perceived enablers for linkage included family support, will power of the patients and fear of complications. Challenges included patient's lack of motivation, financial and time constraints, inadequate guidelines and lack of co-ordination mechanisms between TB programme and tobacco/alcohol abuse treatment services.

Conclusion: Documentation was good but not universally done. Clear operational guidelines on linkage and treatment guidelines for health care providers to appropriately manage the patients with comorbidities are lacking. Lack of coordination between the TB treatment programme and tobacco cessation as well as alcohol treatment services was considered a major challenge in effective implementation of the linkage services.

Keywords: Tuberculosis, Tobacco use, Alcoholism, Integrated delivery system, Operational research, SORT IT

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Background

Exposure to tobacco smoke increases the risk of tuberculosis (TB) infection, the risk of progression to active TB as well as the risk of relapse and death among TB patients. Moreover, it affects the clinical manifestations, microbiological conversion (sputum smear or culture) and adversely affects the treatment outcomes [1–4]. Lastly, it has been shown that it increases the risk of anti- tubercular drug resistance [5]. Similarly, alcohol abuse increases the risk of acquiring TB infection, causing adverse drug reactions among TB patients undergoing treatment and is associated with adverse treatment outcomes including loss to follow-up, treatment failure and death [6–9].

One fourth of the global burden of TB is from India. Globally, in 2016, an estimated 10.4 million cases and 1.4 million deaths occurred due to TB of which 2.79 million cases and 435,000 deaths had occurred in India [10, 11]. According to Global Adult Tobacco Survey 2 (GATS) in India (2016–17), 28.6% of adults (15 years and above) use tobacco in any form [12]. In 2012, 5.9% of all global deaths or approximately 3.3 million deaths were attributable to consumption of alcohol [13]. In India, the prevalence of heavy episodic alcohol drinking was observed in about 12.9 and 0.7% of men and women above the age of 15 years respectively [13].

There is a formal coordination mechanism between the Technical Working Groups for National TB Programme and Tobacco Control in India [14]. The recent Revised National Tuberculosis Control Programme (RNTCP) treatment guidelines for TB in 2016 included the requirement to document the tobacco use status of TB patients and to integrate brief advice for tobacco cessation in the routine TB care. It also suggested linkage to tobacco cessation services, the Tobacco Cessation Clinics (TCC) or alcohol abuse treatment services and assessment of the tobacco use status at the end of TB treatment [15]. Data on tobacco and alcohol use were added in the new TB treatment card, for the first time since January 2017 in Karnataka and hence it is essential to understand the adequacy of documentation, the process of this linkage and the operational issues in its implementation. Thus, this study aimed to 1) report the extent of documentation of tobacco and alcohol usage data in the TB treatment card 2) explore the process, facilitators and challenges in the linkage of services for tobacco cessation and alcohol abuse from the perspective of health care providers and adult patients with TB under programmatic conditions in Dakshina Kannada district, India.

Methods

Study design

This was a convergent parallel mixed methods study including a quantitative (cohort study) to assess the extent of documentation of linkage to tobacco and alcohol treatment services among patients with TB and a simultaneously conducted qualitative (descriptive study) component to understand the enablers and challenges faced by health care providers and patients with TB in doing so [16].

Setting

General setting

The study was conducted in Dakshina Kannada district, one of the 30 districts in the state of Karnataka, India. Tuberculosis units (TUs) are nodal points for TB control activities in the subdistrict. RNTCP services in a district are delivered through the Designated microscopy centres (DMCs) and peripheral health institutions (PHIs) [15]. The district has one Government treatment centre for tobacco and alcohol abuse.

Study site

Data was collected from all five TUs (as of June 2017) in the study district. Dakshina Kannada District is the field practice area of the institution where the PI is currently employed and has good working relations with the TB program managers. This would help the results to be of immediate and direct relevance in policy implementation.

TB, tobacco and alcohol use: Documentation and linkage

When a patient gets registered under the RNTCP, his/her tobacco use status is enquired and the information is recorded in the new TB treatment card. If the patient is a smoker or tobacco user, he/ she is offered a 'brief advice' to quit tobacco use based on the 5As and 5Rs model [15, 17]. The status of tobacco use is assessed at every follow up visit and at the end of treatment, and recorded in the treatment card. On assessment, if the patient has not quit tobacco then s/he is referred to the nearest Tobacco Cessation Clinic (TCC) or quit line or m-cessation initiative. Information recorded in TB treatment card, is sent through existing Health Management Information System (HMIS) under RNTCP [15].

Similarly, history of alcohol use is documented (in the new TB treatment card) when the patient gets registered under RNTCP and he/she is linked (directed) to alcohol abuse treatment services (referred to as "deaddiction" in the new TB treatment card) whenever indicated.

Study population

Quantitative

The study population included all adult patients with TB registered under RNTCP between 1st January 2017 and 30th June 2017. A total of 413 new TB treatment cards of patients registered between 1st January 2017 to 30th June 2017 were available in all the TUs and all these cards were reviewed for the study.

Qualitative

Medical Officers (MOs) involved in the treatment of patients registered under RNTCP, health care providers (counselor, psychiatrist) involved in providing tobacco cessation and alcohol abuse treatment services, and patients with TB who were registered for treatment between January 1st and June 30th 2017 were selected by using purposive sampling.

Data variables, sources of data and data collection

Quantitative data collection

TB treatment cards of all registered patients in the five tuberculosis units of Dakshina Kannada district were reviewed by the principal investigator (NN). Data of all registered patients with TB (sociodemographic, TB related, history of tobacco and alcohol use, cessation services, usage status at the end of treatment) was extracted from the TB treatment cards into a structured data collection form. However, only cards of patients with drug sensitive TB were reviewed as substance use details of patients with drug resistant TB are not entered in the new TB card. Data validation was carried out by contacting a subset of patients with TB after taking informed consent.

Qualitative data collection

Programmatic factors were explored through document review of the TB treatment cards and the TB treatment guidelines 2016 [15] and one to one interviews with key informants involved in providing TB treatment and tobacco cessation and alcohol treatment services. Registered patients with TB were also interviewed to elicit their experiences and opinions about these services. Seven key informants from TB (Medical officers) and tobacco cessation and alcohol abuse treatment services (Psychiatrist and Counselor) willing to participate were purposively selected and interviewed. The interviews had a mean (range) duration of 12.2(6–32) minutes. The interviews explored the processes, perceived facilitators and challenges faced by them in linkage to tobacco cessation and alcohol treatment services as well as their suggestions to improve it. Five patients, who were willing to participate were purposively selected and interviewed to explore their experiences in accessing care. After 7 interviews with health care providers and 5 interviews with patients with TB no new information was being obtained hence data collection was terminated.

The Principal Investigator (NN) conducted the interviews. She is a faculty member (MBBS, MD in Community Medicine) in a medical college located in the study area, speaks the local languages (Kannada, Tulu), not involved in implementation of RNTCP and is trained in qualitative research methods.

Key informant Interviews (KII) were done at the place, date and time most convenient to the participants. The Principal Investigator (PI) conducted one-to-one interviews using an interview guide (Additional file 1) with open ended questions after obtaining their consent to participate in the study and audio-recorded the interviews. The interview was conducted in a place were only the interviewer and interviewee were present during the interview, and the interviewees were assured that their identification would not be revealed to anybody, thus ensuring anonymity, privacy and confidentiality.

The Principal Investigator conducted telephonic interviews with consenting patients in local language at a time convenient to them. The consent was sought in two stages. The community health worker asked the purposively selected patients whether they consent to be interviewed over the phone and obtained a written informed consent from them. At the second stage, PI called the patients and after obtaining a verbal confirmatory consent for audio recording of the interview, proceeded to the interview using an interview guide with open ended questions. At the end of the interview the participants were debriefed and given opportunity to clarify the same.

The findings of the in-depth interviews were discussed with the co-investigators and questions were modified accordingly for the subsequent interviews. Transcripts prepared were reviewed by the co investigators.

Analysis and statistics

Quantitative

Quantitative data collected was double- entered and, validated using EpiData entry v.3.1 and analyzed using EpiData analysis v. 2.2.2.178 EpiData Association, Odense, Denmark. Key analytic outputs included the number and proportion of patients with TB whose tobacco and/ or alcohol usage status is documented at registration and end of treatment, the number and proportion of TB patients documented as tobacco and alcohol users, the number and proportion of tobacco and alcohol abusers referred to cessation/ treatment services. Association of linkage to tobacco and/ or alcohol abuse treatment services with various socio- demographic and clinical factors was assessed using Relative Risks (RR).

Qualitative

Audio recorded interviews were transcribed in English on the same day of the interviews. Manual thematic analysis was used to analyze the data. The initial coding and theme generation was done by the Principal investigator (NN) and reviewed by a second investigator (JK and PI). Any difference between the two was resolved by discussion. Similar basic themes were grouped as organizing themes and then into a global theme, utilizing a thematic network

analysis method as described by Attride-Stirling [18]. The findings are reported by using 'Consolidated Criteria for Reporting Qualitative Research [19].

Results

Quantitative

Of the total 413 patients included in the study 278 (67.3%) were males, 234 (56.7%) were residing in rural areas and 254 (61.5%) belonged to below the poverty line (BPL- income limit less than Indian Rs 27,000 per annum) income group. The mean age of the study participants was 42.6 years; Pulmonary TB accounted for 335 (81.1%) of the cases and 320 (77.5%) were new TB cases (Table 1).

Tobacco use was documented in 322 (78%) of the TB treatment cards reviewed. Among the 86 (21%) patients documented as current tobacco users, 16 (19%) were linked to tobacco cessation services while for 46 (53.5%) of them linkage was not documented in their treatment cards.

Alcohol use status was documented for 319 (77%) of the TB patients. Among the 71(17%) documented as alcohol abusers, 11 (16%) were linked to alcohol treatment services.

Of the 413 TB patients, 47 (11.4%) were documented as users of both tobacco and alcohol, out of which 7(14.9%) were linked to tobacco cessation and 9 (19%) to alcohol abuse treatment services.

The cascade of patients from registration under RNTCP to the linkage to tobacco and alcohol abuse treatment services (as assessed at the end of TB treatment) are shown in Figs. 1 and 2 respectively.

Factors associated with linkage to tobacco cessation services and alcohol abuse treatment services are presented in (Tables 2 and 3). Patients with pulmonary TB were more likely to be linked to alcohol abuse treatment services than extra pulmonary TB and this difference was found to be statistically significant ($p = 0.01$) (Table 3). Patients linked to alcohol abuse treatment services were more likely to be also linked to tobacco cessation services and this difference was found to be statistically significant ($p = 0.02$). (Table 2).

Qualitative findings

Seven key informant interviews and five patient interviews were conducted to explore the process of documentation of tobacco and alcohol use in the new TB treatment cards and to explore enablers and challenges in linking patients to tobacco cessation and alcohol abuse treatment services. Our document review included the TB treatment guidelines and the TB treatment cards. The details regarding addiction related information in the TB treatment card included if the patient is a current tobacco user or not, if yes type of tobacco use, linked to cessation or not and status of tobacco use at end of treatment which was assessed by the option Quit or Not

Table 1 Clinical and demographic profile of adult patients with TB[a] ($N = 413$) between January and June 2017

Variable	Frequency/Mean	Percentage/SD
Age in years	42.6	14.7
Gender		
Male	278	67.3
Female	130	31.5
Not documented	5	1.2
Area		
Rural	234	56.7
Urban	109	26.4
Not documented	70	16.9
Socio economic status		
Above poverty line	84	20.3
Below poverty line	254	61.5
Not documented	75	18.2
Site of disease		
Pulmonary	335	81.1
Extra pulmonary	78	18.9
Type of Patient		
New	320	77.5
Recurrent	41	9.9
Transferred in	3	0.7
Treatment after loss to follow up	20	4.8
Treatment after failure	10	2.4
Others	16	3.9
Not documented	3	0.7
Regimen		
New	271	65.6
Previously treated	69	16.7
Not documented	73	17.7
HIV status		
Reactive	20	4.8
Non reactive	383	92.7
Not documented	10	2.4
Treatment Outcome		
Cured	168	40.7
Completed treatment	110	26.6
Treatment failed	13	3.1
Lost to follow up	24	5.8
Died	28	6.8
Not evaluated	3	0.7
Not documented	67	16.2

[a]*TB* Tuberculosis, *RNTCP* Revised National Tuberculosis Control Programme, *SD* Standard Deviation

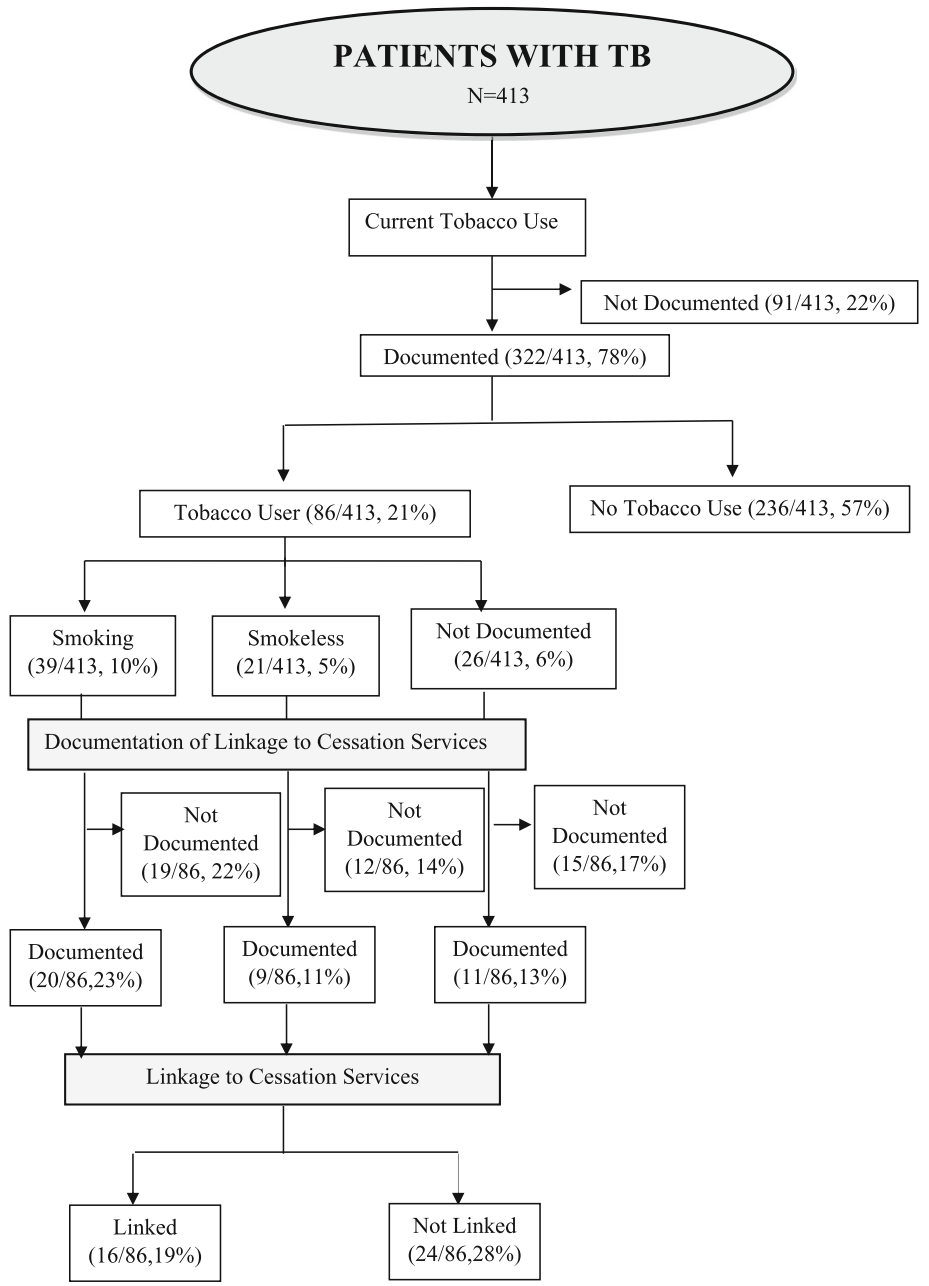

Fig. 1 Documentation of Tobacco Use and linkage to cessation services among adult patients with TB. The extent of documentation of current tobacco use status and linkage to cessation services among adult patients with TB was assessed by document review of the new TB treatment cards which is depicted in the flowchart. Data showed that 78% of the cards had documentation of tobacco use status and 19% of the users were linked to cessation services

Quit. Regarding alcohol abuse, the information includes if there is history of alcohol intake or not, if yes linked to deaddiction or not.

Documentation of substance use

Health care providers overall felt that the inclusion of substance use related information in the new TB card was relevant, simple and beneficial for understanding the magnitude of the problem at the community and individual patient levels.

> "*It's required both to control tuberculosis as well as the addiction problem. So under one umbrella we will be covering two things. It is simple and easier for the health worker as well as the participant also". (*43 year old male medical officer)

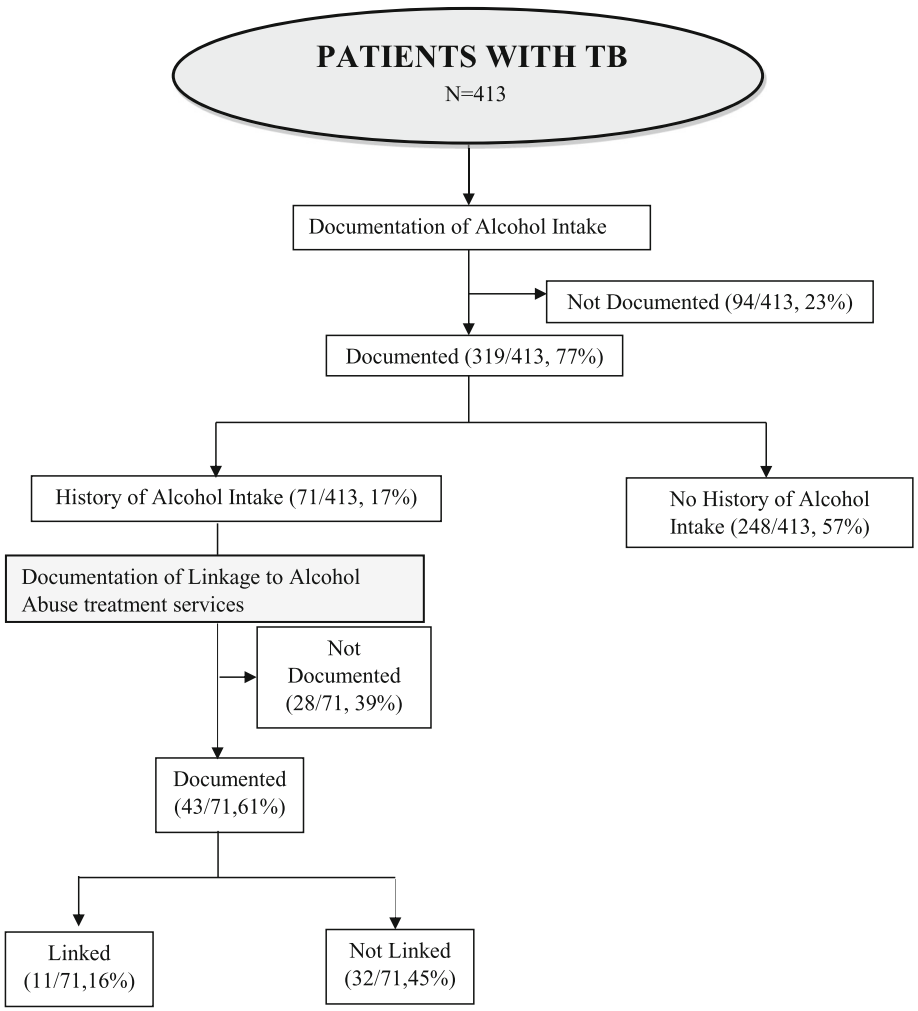

Fig. 2 Documentation of alcohol intake and linkage to alcohol abuse treatment services among adult patients with TB. The extent of documentation of alcohol intake and linkage to alcohol abuse treatment services among adult patients with TB was assessed by document review of the new TB treatment cards which is depicted in the flowchart. Data showed that 77% of the cards had documentation of history of alcohol intake and 16% of the patients with history of alcohol intake were linked to alcohol abuse treatment services

On the other hand they revealed that there are multiple people involved in collecting information in certain places and designated staff in other places. This lack of standardization may affect the completeness and quality of the data collected.

> *"ANM (Auxiliary Nurse Midwife) and I take mainly, because these direct questions are asked by the medical officers. My lab technician also takes"* (39 years old, male, medical officer)

Process of implementing tobacco cessation services

Tobacco cessation services offered by health care providers include assessment of the level of addiction followed by counselling (general and TB specific), and, when indicated, referral to the psychiatric Department of the District Hospital.

Some medical officers mentioned that initially they provide counselling to the patients themselves and if there is no improvement, then they refer to specialists. *"For tobacco, No, we just advice and treat the case" (*43 year old male medical officer*). "We also counsel them as in what happens when you take tobacco and what are the ill effects of tobacco"*(37 years old, male, counsellor, Private centre).

Some practitioners gave examples of the content of their counseling sessions. *"Your lungs are already damaged and tuberculosis is going to eat up your lungs so much and if you further smoke then it will be totally destroyed and living a normal life will become difficult". (*39 years old, male, Medical Officer).

Table 2 Association of various factors with linkage to tobacco cessation services among adult patients with TB

Variables	Linkage to tobacco cessation Services n (%)		
N = 86	Yes	No	
Gender (*N* = 39)			
Male	14 (38.9)	22 (61.1)	*P* = 0.84
Female	1 (33.3)	2 (66.7)	
Area (*N* = 36)			
Rural	11 (35.5)	20 (64.5)	*P* = 0.84
Urban	2 (40)	3 (60)	
Socio economic status(*N* = 32)			
Above poverty line	2 (33.3)	4 (66.7)	*P* = 0.8
Below poverty line	10 (38.5)	16 (61.5)	
Site of disease (*N* = 40)			
Pulmonary	14 (37.8)	23 (62.2)	*P* = 0.32
Extra pulmonary	2 (66.7)	1 (33.3)	
Regimen type (*N* = 37)			
New	11 (42.3)	15 (57.7)	*P* = 0.73
Previously treated	4 (36.4)	7 (63.6)	
Type of Tobacco use (*N* = 29)			
Smoking	9 (45)	11 (55)	*P* = 0.07
Smokeless	1 (11.1)	8 (88.9)	
Tobacco use at end of treatment (*N* = 8)			
Quit	3 (50)	3 (50)	*P* = 0.2
Not Quit	0	2 (100)	
History of Alcohol Intake(*N* = 40)			
Yes	7 (35)	13 (65)	*P* = 0.57
No	7 (41.2)	10 (58.8)	
Not documented	2 (66.7)	1 (33.3)	
If History of Alcohol intake present, linked to alcohol abuse treatment services (*N* = 14)			
Yes	3 (75)	1 (25)	*P* = 0.02
No	1 (10)	9 (90)	

TB Tuberculosis, *RNTCP* Revised National Tuberculosis Control Programme

Referral is either to public or private centers: *"Right now in District Hospital Psychiatry Ward they are doing [smoking cessation]. So we refer there or if they are ok then we refer to private also"* (35 years old, female, medical officer).

Tobacco cessation treatment involves initial assessment followed by treatment in outpatient basis.

> *"During interview, we will try to know if he/she has dependence on tobacco and accordingly we treat with either nicotine gums or tablet Bupropion and counselling. Nicotine gums are available here freely". (*male psychiatrist, 48yrs)

Alcohol abuse treatment services

The review of TB treatment guidelines revealed that there are no clear cut guidelines as to how a patient with TB and alcohol abuse needs to be treated. The process of alcohol abuse treatment services as described by the health care providers include initial assessment, counselling (general and TB specific) and referral when indicated, based on the judgment of the treating physician. The management of TB patients with alcohol abuse does not appear to be standardized and no clear protocols and guidelines are followed. As one 35 year old medical officer described her typical practice; *"For alcoholics I usually send them for liver function tests initially. As this addition of drugs [TB] alter their liver function. Then again we counsel them. Most of them quit as they can't take those [TB] tablets along with alcohol as it causes severe gastritis."* The lack of clear guidance for assessing alcohol abuse and for linking to treatment services was also confirmed during our document review.

The health care providers were of the opinion that alcohol abuse treatment is a stepwise, graded approach and distinctively different from the smoking cessation procedure.

> *"Alcohol is not like cigarette smoking. Cigarette smoking, if you stop one day you need to maintain it. But alcohol is not like that, if he takes today 1 quarter, in another 3 days he has to take 90ml and in another 3 days or 1- 2 weeks he has to take half of 90 ml. So it's a gradual process in treatment."* (Male medical officer, 39yrs)

As is the case with tobacco cessation, TB patients are referred to either public or private alcohol treatment services ("deaddiction" services as they are called in this context) according to the patient's preference. The process of treatment as mentioned in the district hospital included initial assessment of dependence, counselling and detoxification. At both sectors the treatment is offered on in-patient basis.

> *"From the history and assessment we will know if he is dependent on alcohol. If he is dependent on alcohol then we advise based on if he's prepared to stop alcohol or not. If he is prepared to stop alcohol then it's easy or we need to counsel him again regarding the ill effects of alcohol and how it can affect on his existing TB and accordingly we admit in the ward and detoxify them and we start medications."* (48 year old male psychiatrist, district hospital)

Table 3 Association of various factors with Linkage To Alcohol Treatment Services Among Adult Patients with TB

Variables	Linkage to Alcohol Abuse Treatment Services n (%)		
N = 71	Yes	No	
Gender(*N* = 41)			
Male	10 (25.6)	29 (74.4)	*P* = 0.4
Female	0	2 (100)	
Area (*N* = 37)			
Rural	8 (25)	24 (75)	*P* = 0.2
Urban	0	5 (100)	
Socio economic status (*N* = 34)			
Above poverty line	2 (33.3)	4 (66.7)	*P* = 0.5,
Below poverty line	6 (21.4)	22 (78.6)	
Site of disease (*N* = 43)			
Pulmonary	9 (82)	32 (100)	*P* = 0.01
Extra pulmonary	2 (18)	0	
Regimen type (*N* = 33)			
New	5 (20.8)	19 (79.2)	*P* = 0.93
Previously treated	2 (22.2)	7 (77.8)	

TB Tuberculosis, *RNTCP* Revised National Tuberculosis Control Programme

Enablers in providing tobacco cessation and alcohol abuse treatment services

The health care providers identified three broad enablers that facilitated linkage to tobacco cessation and alcohol abuse treatment services among patients. The themes were common for both conditions and included firstly family support, secondly will power and motivation from the side of the patients, and thirdly a perceived fear of complications.

The interviewees perceived that the role of family members in the tobacco cessation and especially the alcohol abuse treatment process was essential and tried to have them involved and engaged.

> *"The family get them here as they think that alcohol and tobacco is also a major problem and the addiction also has to be treated."* (37 years old, male, counsellor, private)

> *"My family members also started irritating me they did so much Kirikiri (local slang for irritation). Then I felt very bad I decided to stop".* (40-year-old male patient)

Some emphasis was given to perceived patient's characteristics and especially what was perceived as "will power". As a 52 years old female medical officer mentioned: *"Firstly as I earlier said will power of the patients. Some of the patients have good will power and will stop but some patients are so addicted and do not have the will power to stop".*

Patient interviews also indicated the role of "will power" as one of the enablers especially with respect to alcohol abuse treatment. As a 40-year-old male patient mentioned *"It was my will power (garva). I just resisted it with the help of my will power".*

The interviewed health providers referred to "fear of complications" as an enabler both among patients who use tobacco and the ones with alcohol abuse, however, it was not clear whether this is what they perceived as patient's "own fears" or whether they felt they had to "enforce or instill" such fear as part of their intervention. The following quotes highlight this

> *"One is the fear factor that because of these addictions they have got TB. So if we say that "Your tuberculosis will get cured only if you stop taking these things" then they might accept."* (45 years old, male, medical officer)

> *"They told me that "if you continue smoking beedi your disease will not get cured". They told me that, "only if you stop smoking beedi it will get cured". So I completely stopped beedi"* (60 year old male patient)

Challenges in implementing tobacco cessation and alcohol abuse treatment services

The challenges in implementing tobacco and alcohol abuse treatment services as perceived by the healthcare providers and the patients could be classified as being related to the patient, the health care provider or to the health system. Figure 3 shows a non-hierarchical model of the organizing and basic themes that emerged from the data.

Patient related challenges

There was one overarching theme about the linkage to tobacco cessation services and this was patient's lack of motivation that was expressed as reluctance to seek cessation services and even refusal to quit tobacco. Interestingly this theme was described by the health care providers while we were not able to elicit such information from the patient interview data (likely due to our inability to interview patients who refused linkage or participating in the interview)

> "*Yeah, some will be chronic patients. They would be like "I cannot quit whatever maybe the treatment". Then we send them to deaddiction centres. Some are reluctant to even go to the deaddiction centres." (*35 years old female, medical officer)

The emerging challenges in linking patients to alcohol abuse treatment services were found to be slightly

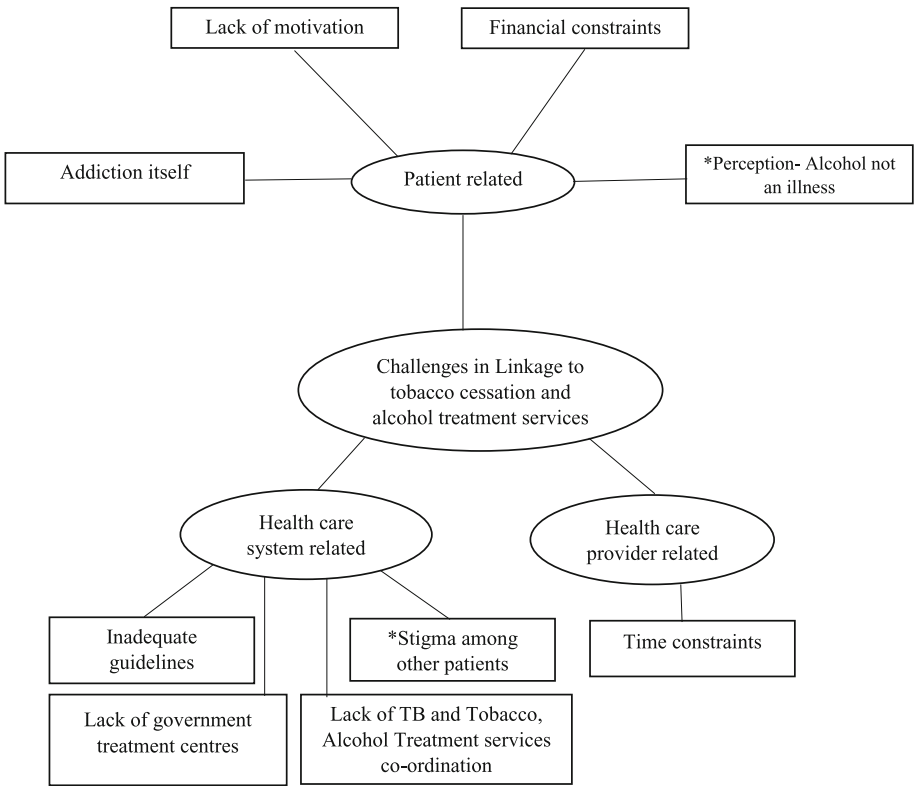

Fig. 3 Perceived challenges in linkage of patients with TB to tobacco cessation and alcohol abuse treatment services. Key informant interviews and interview of patients with TB was conducted to explore the challenges in linkage of patients to the tobacco and alcohol abuse treatment services. Perceived challenges included lack of coordination between the TB treatment programme and tobacco cessation as well as alcohol treatment services, lack of motivation among patients and financial constraints

different. While the perceived patient's lack of motivation was a common theme, in the case of alcohol abuse it was partly attributed to patient's perception of alcoholism not being an illness. "*Sometimes they are in contemplation stage whether to stop alcohol or not or they may feel this is totally a different thing, it's not an illness so it's difficult to convince them."* (48 years old, male, psychiatrist).

Financial constrains emerged as a challenge for both, especially for alcohol abuse requiring hospital admissions *"For deaddiction you have to pay here, it's not free. Hence for people who are of lower socioeconomic status it becomes difficult."* (52 years old, female medical officer, private facility).

Lastly withdrawal symptoms and the addiction itself ("temptation") was found an additional challenge for patients who used alcohol compared to tobacco users. *"Especially in alcohol there is rum fit syndrome, that is if there is high intake of alcohol, even though we try our best they get this withdrawal symptoms and they have to take little amount of alcohol." (*39 year old male medical officer).

A 40 year old male patient mentioned *"Initial two days it was very difficult. My hands were trembling, I was feeling like I need a drink, my head was aching, and morning as soon as I got up too I felt like drinking."*

Health care provider related challenges

Busy OPDs in some primary health centres pose a challenge to medical officers in properly managing patients, delivering quality counseling and advice and linking them to the tobacco cessation or alcohol treatment services.

> "*Yeah sometimes in heavy OPDs (Out- patient department) it is difficult to talk for long".* (35 years old, female, medical officer).

Even though during our document review we were able to locate guidelines regarding linking TB patients who are tobacco users to cessation services we found that there was lack of information among some health care providers, especially the ones working in primary health centres. *"No, right now we do not have any guidelines from Government of India"* (39 years old, male, medical officer).

The health care providers also felt that they lack training in treating patients with alcohol abuse, especially handling withdrawal symptoms.

> "*We have some medications but we do not have so much knowledge to tackle the withdrawal symptoms"* (43 years old, male, medical officer).

Health care system related challenges

The overall lack of formal coordination mechanisms between the TB programme and tobacco cessation services posed a challenge in linking patients between the two. Moreover, the extremely limited number (particularly the public ones) and geographic location of the few existing cessation centers made the navigation of patients difficult and especially for patients who had to travel; *"If they are coming from a distant place, these nicotine gums are not available in Taluka or PHC level, so they may find it difficult to come for follow up"* (48 yr. old male psychiatrist).

The existing integrated, one-stop service between HIV (Human Immunodeficiency Virus) and TB programmes was emerged as a model that juxtaposed the fragmented services offered by the TB programme and smoking cessation clinics; *"For example all TB patients have to do HIV testing and they are directly linked ie TB and ART (Antiretroviral Therapy) and follow up is there in ART centre. But such facility is not available for deaddiction or cessation services in government setup"* 52 yr. old female medical officer.

The existing guidelines do not offer clear and detailed guidance on linkage of TB patient to alcohol treatment services.

> *"They have not mentioned anything in the guidelines about the cessation of alcohol and deaddiction. Only co morbid conditions have to be addressed is mentioned."* (43yr old male medical officer)

There were no established mechanisms for linkage and thus they could not follow up referred patients.

> *"We refer the patients and thus we are not in direct contact with them."* (52 year old female medical officer)

Stigma and discrimination among other admitted patients towards the patients with TB was another challenge.

> *"It's difficult because if other patients come to know about the patients with TB or even HIV they do not talk or mingle with them, and start telling that " I will leave the centre as there are TB patients here""* (37 year old male counsellor, private facility.)

Discussion

This is the first mixed methods study in India that assessed the recently introduced documentation of alcohol and tobacco use among patients with TB treated under RNTCP and explored the enablers and challenges in linking them to tobacco cessation and alcohol abuse treatment services.

We found that almost three out of four TB treatment cards had the status of tobacco and alcohol use documented. Despite this being encouraging for a newly introduced set of data variables to be collected, there is definitely need for improvement. From the document review, it was observed that the addiction related information to be entered in the card is inadequate especially regarding alcohol use, leaving health care providers confused on whom to consider as current smoker or alcohol user. The card does not have the provision to include about brief advice on cessation given which has been mentioned in the TB treatment guidelines [15]. It is not clear from the card regarding who is to be linked to cessation services. The process for improvement should start by providing clear guidance on how to collect such data, what the definitions of the data variables are, who should collect and analyze and who should act upon them. In the case of alcohol abuse, our document review and interview data show that there is a need to immediately provide clinical and referral guidelines [9].

The proportion of tobacco users among the study population was high and consistent with the national figures according to GATS 2 survey, although this assessment was only among TB patients which may be the reason for the higher proportions, however it was lower when compared to other studies [5, 20–22]. About half of those who were tobacco users had documentation of the linkage status and more than one fourth of the tobacco users were not linked to the tobacco cessation services as per the new TB treatment cards. Since there is no documentation of the brief advice being offered as prescribed by the TB treatment guidelines, one cannot comment on whether this proportion is high or adequate.

In the present study males, patients with pulmonary TB, from rural area, belonging to lower socioeconomic status and new patients had higher linkage rates to tobacco cessation and alcohol abuse treatment services. Linkage to tobacco cessation services was higher among patients with TB who are linked to alcohol treatment services as well and this was found to be statistically significant.

Our interview data have shown that the inclusion of the addiction related information in the treatment card was considered to be beneficial for comprehensive management of TB by the health care providers which was also indicated in previous studies [22, 23]. The process of linkage to tobacco cessation services showed disparities as most of them followed different approach from counselling to referral.

We have observed a lack of standardization in managing and referring TB patients to substance use services. We encountered some counseling techniques and messages (as reported by the health providers themselves) that may not follow international standards by virtue of failing to take into account the individual patient sensitivities such as messages that may instill fear or helplessness. Some of the health care providers reported that there were not enough government tobacco cessation or alcohol treatment centres to which they could refer and this posed additional challenges in linkage and added financial constraints to the patients and their families.

Lack of coordination between the TB treatment programme and tobacco cessation and alcohol treatment services was considered a major challenge in effective implementation of the linkage services. A feasibility study on tobacco cessation done in Brazil also indicated similar findings [23]. The successful paradigm and model of the TB/HIV service integration could be considered in the case of substance abuse particularly for setting with high burden of TB and high rates of tobacco and alcohol use.

The solutions provided by the health care providers to improve the linkage system included establishment of government treatment centres for tobacco and alcohol treatment services preferably exclusive for TB patients, better TB treatment and tobacco, alcohol treatment services co- ordination and integrated services. The importance of alcoholism and TB drug's adverse interactions was also stressed upon by the health care providers indicating the importance of alcohol treatment among TB patients. A study done in Poland also reports the association of alcoholism and adverse drug interactions among TB patients [7]. The health care providers also felt the need of training in implementing these services which was also stated in other studies [23, 24].

The present study gave an overview of the programmatic factors and challenges encountered in linking the TB patients to cessation services indicating the need for emphasis on monitoring, evaluation and better co-ordination between TB programme and tobacco and alcohol abuse treatment services. The study implies that there is a need for clear operational guidelines, accountability for the data entered, monitoring action on the linkage of patients with TB and substance use to treatment services, documentation of follow up status and outcome along with inclusion in TB reporting format.

Limitations

All of the new TB treatment cards were not available for data collection as implementation of new TB cards was delayed till the month of April 2017 in few TUs of Dakshina Kannada district. This would have lead to an over or underestimation of the results. The lack of documentation posed a limitation to assess the association of various socio-demographic factors with the linkage services. Data could be validated for only a small subset of the total study population due to challenges in the two step obtaining informed consent.

Conclusions

In conclusion, our study has shown that the documentation of the tobacco and alcohol use status was good but not universally done; hence this needs to be improved. Clear operational guidelines on linkage and treatment guidelines for health care providers to appropriately manage the patients with comorbidities are lacking. Lack of coordination between the TB treatment programme and tobacco cessation as well as alcohol treatment services was considered a major challenge in effective implementation of the linkage services, implying the need of these services to be strengthened and the health care providers and patients navigating between the services supported. Adequate monitoring and evaluation of the performance of linkage services are recommended.

Additional file

Additional file 1: Interview guide. The Additional file 1 contains the interview guide which was used to collect qualitative data by patient interviews and key informant interviews of health care providers. (DOCX 17 kb)

Abbreviations

ANM: Auxiliary Nurse Midwife; ART: Antiretroviral Therapy; BPL: Below Poverty Line; DMC: Designated Microscopy Centre; GATS: Global Adult Tobacco Survey; HIMS: Health Management Information System; HIV: Human Immunodeficiency Virus; KII: Key Informant Interview; MO: Medical Officer; OPD: Out Patient Department; PHI: Peripheral Health Institution; PI: Principal Investigator; RNTCP: Revised National Tuberculosis Control Programme; SD: Standard Deviation; TB: Tuberculosis; TCC: Tobacco Cessation Clinic; TU: Tuberculosis Unit

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Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
NN: Principal Investigator and corresponding author, conception / design of the protocol, acquisition of data, data analysis / interpretation, drafting / critically reviewing the paper, giving approval for the final version to be published. JK: Conception / design of the protocol, data interpretation, drafting/ critically reviewing the paper, giving approval for the final version to be published. AKM: Conception / design of the protocol, data interpretation, critically reviewing the paper, giving approval for the final version to be published. TA: Design of the protocol, development of data capture tool, critically reviewing the paper, giving approval for the final version to be published, ASN: Critically reviewing the paper, giving approval for the final version to be published. BMN: Acquisition of data, critically reviewing the paper, giving approval for the final version to be published. PI: Conception / design of the protocol, data interpretation, drafting/ critically reviewing the paper, giving approval for the final version to be published.

Ethics approval and consent to participate
Ethics approval was obtained from the Institutional Ethics Committee of Yenepoya University, Mangalore, Dakshina Kannada, India, (YUEC512/2017) and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease, Paris, France. The quantitative component of the study, involved review of TB patient treatment cards, hence a waiver for informed consent was obtained from the ethics committees. Written informed consent was obtained from the key informants and patients with TB as described above.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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Infectious Diseases of Poverty

RESEARCH ARTICLE Open Access

Inequity in catastrophic costs among tuberculosis-affected households in China

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Abstract

Background: There are limited nationally representative studies globally in the post-2015 END tuberculosis (TB) era regarding wealth related inequity in the distribution of catastrophic costs due to TB care. Under the Chinese national tuberculosis programme setting, we aimed to assess extent of equity in distribution of total TB care costs (pre-treatment, treatment and overall) and costs as a proportion of annual household income (AHI), and describe and compare equity in distribution of catastrophic costs (pre-treatment, treatment and overall) across population sub-groups.

Methods: Analytical cross-sectional study using data from national TB patient cost survey carried out in 22 counties from six provinces in China in 2017. Drug-susceptible pulmonary TB registered under programme, who had received at least 2 weeks of intensive phase therapy were included. Equity was depicted using concentration curves and concentration indices were compared using dominance test.

Results: Of 1147 patients, the median cost of pre-treatment, treatment and overall care, were USD 283.5, USD 413.1 and USD 965.5, respectively. Richer quintiles incurred significantly higher pre-treatment and treatment costs compared to poorer quintiles. The distribution of costs as a proportion of AHI and catastrophic costs were significantly pro-poor overall as well as during pre-treatment and treatment phase. All the concentration curves for catastrophic costs (due to pre-treatment, treatment and overall care) stratified by region (east, middle and west), area of residence (urban, rural) and type of insurance (new rural co-operative medical system [NCMS], non-NCMS) also exhibited a pro-poor pattern with statistically significant ($P < 0.01$) concentration indices. The pro-poor distribution of the catastrophic costs due to TB treatment was significantly more inequitable among rural, compared to urban patients, and NCMS compared to non-NCMS beneficiaries.

Conclusions: There is inequity in the distribution of catastrophic costs due to TB care. Universal health coverage, social protection strategies complemented by quality TB care is vital to reduce inequitable distribution of catastrophic costs due to TB care in China.

Keywords: Catastrophic health expenditure, tuberculosis, Patient cost, Universal health coverage, Social protection, Equity

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Multilingual abstracts

Please see Additional file 1 for translations of the abstract into the five official working languages of the United Nations.

Background

The World Health Organization (WHO) issued a post-2015 global tuberculosis (TB) strategy that envisaged "a world free of TB" with zero death, disease, and suffering due to TB by 2035. One of its four principles is to ensure protection and promotion of human rights, ethics and equity [1]. This is in line with the policy to move health systems closer to universal health coverage, which is conventionally defined as access to health care without risk of financial hardship due to out-of-pocket health care expenditures [2]. Besides free or affordable TB care, social protection interventions are required that prevent or mitigate other financial risks associated with TB. This is also vital to attain the sustainable development goals [3].

TB is mainly a disease of the poor and marginalized people and communities [4]. TB affects the poorest segment of society disproportionately and thus the impoverishing effects of TB are gravest for those who are already vulnerable [2, 3]. Though it is quite likely that distribution of catastrophic costs is pro-poor, there are limited nationally representative studies globally in the post-2015 period regarding documentation of wealth related inequity in the distribution of catastrophic costs due to TB care. In India (during TB diagnosis in 18 randomly selected districts in 2016–2017) [5] and China (during TB treatment in six counties in 2013) [6], catastrophic costs was disproportionately high among the poorest quintile.

China conducted a nationally representative "TB patient cost survey" in 2017 [4]. This study reports the extent of equity in distribution of TB care costs (pre-treatment, treatment and overall), total costs as a proportion of the annual household income (AHI) and catastrophic costs due to TB, also compared across regions, residence and insurance schemes.

Methods

Design

This was a cross-sectional analytic study involving primary data collection.

Setting

Health financing in China

China is a developing country with a per capita gross national product of USD 7941 in 2016. The total expenditure on health in 2016 was USD 6815 billion, accounting for 6 % of the gross national product [7]. The provinces are divided into east, middle and west region. Three percent of people fall below the poverty line (USD 430) [7]. The health care delivery system is "mixed" with a dominant role for public sector institutions [8].

Public funded health insurance schemes cover more than 95% of the population. There are three basic schemes namely urban employee basic medical insurance (UEBMI), urban resident basic medical insurance (URBMI), and new rural cooperative medical scheme (NCMS). Payroll taxes are the main funding source for UEBMI and government subsidies are the major funding sources for NCMS and URBMI. NCMS funds are pooled at the county level and URBMI and UEBMI are pooled at the prefecture level. The benefit packages and financial protection are not equal within and across the schemes, which is a crucial barrier to achieving universal health coverage in China. The service package of NCMS was smaller and the reimbursement level was 10% lower than URBMI or UEBMI [9].

China national TB Programme (NTP)

The National center for tuberculosis control and prevention, which belongs to China Centre for Disease Control (CDC), is in charge of NTP. TB management units are established at provincial, prefecture and county levels (basic management units [BMU] at county level). TB diagnostic facilities are centralized at the BMU level and rarely available at township level (below county). Diagnosed patients are registered in web-based TB information management system (TBIMS) and initiated on directly observed therapy (DOT) at BMU with assistance from township clinics and village health workers.

Nearly 90% of the patients with TB get treatment within these designated facilities. TB patients are provided free chest radiography, free sputum smear test and free first-line drugs. Additional TB services in the form of other investigation and ancillary drugs are charged.

Study population

Drug susceptible pulmonary TB patients who had received at least 2 weeks of intensive phase therapy at the time of national TB patient cost survey (March to June 2017) were included. Pulmonary TB included pediatric TB and TB with comorbidity. We excluded people who were treated in facilities not under NTP.

Sample size

Assuming the prevalence of catastrophic costs due to TB was 30% [10], relative precision as 0.2 and α error as 0.05, average cluster (defined at county level) size of 50, between-cluster variation of 0.4, design effect of 4.36 and anticipating a non-response rate of 10%, the final sample size was 1086, to be sampled from 22 clusters (see Additional file 2: Annex 1).

Sampling methodology

We adopted multi-stage stratified cluster sampling. There were significant variations in the economy and the TB prevalence across China. The per capita gross national product of the six provinces sampled under the survey is shown in Additional file 2: Annex 2. The main stratifying factors were patient's region and residence (rural/urban one each from each of the east/middle/west provinces - see Additional file 2: Annex 3 for the steps followed in sampling). The 22 counties included in the study are depicted in Additional file 2: Annex 4.

Data collection and management

Data collection

Face to face interview (at BMU in the county) was done by trained investigators (trained university students and staff from China CDC) using a structured questionnaire (see Additional file 2: Annex 5). Costs related information was collected from symptom onset up to the day of interview. Direct medical costs included the costs for outpatient registration, hospitalization, investigations and medicines. Direct non-medical costs included transportation, accommodation and food of the patients and family members. Indirect costs were estimated as the total period of absence from work in hours multiplied by the hourly wage of the absent worker. The investigators directly asked the annual income of the patients.

Data management and analysis

Data were double entered and validated using EpiData 3.1 (EpiData Association, Odense, Denmark) during July to December 2017. The analysis was conducted using STATA 12.1 (copyright 1985–2011, StataCorp LP, Texas USA).

We calculated the average monthly direct medical cost, direct non-medical costs and indirect costs during treatment. This average was used to impute the treatment costs of patients within the county for the remainder of treatment (assuming a total of 6 months for new patients and 8 months for previously treated patients).

The analysis was done separately for the pre-TB treatment phase, treatment phase and TB care overall (pre-TB treatment and treatment phase combined). Costs were described using the median and inter quartile range (IQR). The total costs (direct medical, direct non-medical and indirect costs combined) were defined as catastrophic if they exceeded 20% of pre-TB annual household income [4].

Income quintiles were generated by ranking the households based on monthly income per capita (MIPC). The distribution of total costs due to TB care were summarized across income quintiles as follows: i) absolute total costs, ii) annual total costs as a proportion of pre-TB AHI, and iii) proportion of households experiencing catastrophic costs.

Concentration curves and concentration indices (along with 95% confidence intervals [*CI*]) were used to assess the extent of equity in the distribution of all the above three indicators. The concentration curves plot the cumulative distribution of the health outcome variable in the y axis against cumulatively ranked households (poorest to richest) on the x axis. The values of concentration index ranges from + 1 to − 1; with positive value (concentration curve below the line of equality) suggesting pro-rich and negative value (concentration curve above the line of equality) suggesting a pro-poor distribution [11, 12].

For the indicator 'total costs', we assumed equity if the concentration curve and index revealed significant distribution across the richest quintiles (positive concentration index, 95% *CI* not including zero). For the indicators, 'annual total costs as a proportion of pre-TB AHI' and 'catastrophic costs', we assumed equity if the concentration curve and index revealed equal distribution across the quintiles (concentration curve not significantly different from the line of equality). The statistical significance of the concentration index was interpreted based on whether or not its 95% *CI* included zero.

We also compared the concentration curves across various subgroups (insurance type, residence and region) using dominance tests [11]. For further details on analysis, the readers may refer to these references by Demery L, McIntyre D et al. and O'Donnell O et al. [5, 11, 12].

Results

Demographic and socio-economic profile of patients

Of 1147 TB patients, 811 (70.7%) were male and mean age was 51 years (range 12–89). A total of 422 (36.8%) patients came from east region, 322 (28.1%) from middle region and 403 (35.1%) from west region. Sixty five percent patients resided in rural areas. NCMS covered 864 (75.3%) of the patients. The median (IQR) MIPC was USD 190 (46, 243). The incomes of 223 (19.4%) households were below the poverty line (Table 1).

Equity in the distribution of costs

The median (IQR) costs due to pre-treatment, treatment and overall TB care were USD 283.5 (41.8, 945.7), USD 413.1 (231.9, 927.8) and USD 965.5 (461.8, 2059.3), respectively. Total costs due to pre-treatment and treatment care were significantly ($P < 0.001$) highest among the richest quintile, while the total costs expressed as a proportion of the AHI and catastrophic costs were significantly ($P < 0.001$) higher among the poorest quintile when compared to the richest quintile (Table 2). This pattern was also reflected in the concentration curves and indices (Fig. 1, Table 3).

Table 1 Demographic and socio-economic profile of patients enrolled in China's TB[a] patient cost survey (2017) (n = 1147)

Variable	*n*	(%)
Total	1147	(100)
Age group in years		
< 15	6	(0.5)
15–44	352	(30.7)
45–64	497	(43.3)
≥ 65	292	(25.5)
Gender		
Male	811	(70.7)
Female	336	(29.3)
Region		
East	422	(36.8)
Middle	322	(28.1)
West	403	(35.1)
Residence		
Urban	407	(35.5)
Rural	740	(64.5)
Monthly income per capita in USD (Median [IQR])[b]	190	(46, 243)
Below poverty line (Yes)[c]	223	(19.4)
Insurance		
None	40	(3.5)
Urban employee basic medical insurance	114	(9.9)
Urban residence basic medical insurance	116	(10.1)
New rural cooperative medical scheme	864	(75.3)
Others	13	(1.1)
Direct medical costs	608.7	(286.1, 1301.8)
Costs((Median [IQR])		
Direct non-medical costs	160.5	(74.4, 315.2)
Indirect costs	70.4	(24.6, 296.2)

SD Standard deviation, *IQR* Interquartile range, *USD* United States Dollars, *CDC* Centre for disease control and prevention, *HIV* Human immunodeficiency virus
[a]Drug-susceptible pulmonary tuberculosis
[b]A currency exchange rate of CNY 687 to USD 100 (December 2018)
[c]Poverty line in China is Annual per capita household income less than USD 430

Equity in the distribution of catastrophic costs

All the concentration curves for catastrophic costs stratified by region, area of residence and type of insurance exhibited a pro-poor pattern with statistically significant ($P <$ 0.01) concentration indices (Fig. 2, Table 4). The curve of the middle region exhibited statistically significant dominance over the east and west during pre-TB treatment. For catastrophic costs due to treatment, the rural curve dominated over the urban curve while the NCMS dominated the non-NCMS curve in being significantly more pro-poor (Table 4).

Discussion

Our study revealed that while there is equity in costs due to pre-treatment and treatment care in China, there is inequity in the distribution of catastrophic costs which was also consistently seen across various population sub-groups. Catastrophic costs due to pre-TB treatment were more inequitably shared by the poor in the middle compared to their counterparts in the west and east regions of China. The distribution of the catastrophic costs due to TB treatment was significantly more inequitable among the rural population compared to urban and among those covered under NCMS compared to those covered under non-NCMS insurance schemes.

Interpretation of key findings

Pro-rich distribution of total costs may be due to the nature of facilities and the type of care availed by the rich; these are different from that sought by the poor. Their capacity to pay is naturally higher than the poorer quintiles, who may not be availing services that are beyond their spending capacity and thus spending lesser than the rich. Another reason could be that the poor are availing schemes by virtue of belonging to poorer socio-economic status which offer them subsidized or free services. Thereby, the total costs experienced by the poor are lesser than that of the rich.

The poor, however, bore an unfair share of the burden of the total costs expressed as a proportion of AHI and the catastrophic costs. Though they were spending less in absolute quantities, even that took a toll by robbing a significant proportion of the AHI, leading to a financial catastrophe.

The uniform pro-poor distribution of the catastrophic costs due to TB treatment across all population sub-groups studied was significantly more inequitable in the rural areas compared to the urban areas. Rural populations' access to appropriate, affordable TB services is unsatisfactory compared to that of urban population of China and this difference is exaggerated among the poorer quintiles [13]. Despite the provision of fully subsidized care, patients with TB in China are charged for various reasons like additional investigations and supplements, irrespective of their capacity to pay [14]. Li et al. have reported that a significant proportion of the patients experience catastrophic non-medical expenses [15].

Similarly, the NCMS covered population experienced a more inequitable distribution of catastrophic costs due to treatment compared to those covered by other schemes. This could be a reflection of the rural urban pattern given that the NCMS covers the rural population

Table 2 Distribution of total costs and total costs as a proportion of AHI across income quintiles among TB[a] affected households in China (2017) (n = 1147)

Characteristic	Pre-TB treatment		TB treatment		TB care overall	
	Median	(IQR)	Median	(IQR)	Median	(IQR)
Total costs(USD)						
1st MIPC quintile	219.5	(25.2, 579.9)	254.7	(161.3, 490.3)	588.4	(320.6, 1106.7)
2[nd]MIPC quintile	213.8	(9.6, 669.2)	302.7	(193.9, 624.3)	668.5	(331.8, 1590.1)
3[rd]MIPC quintile	220.4	(37.7, 857.3)	425.5	(270.0, 1016.7)	1093.0	(503.3, 1873.0)
4th MIPC quintile	416.4	(103.4, 1376.2)	567.2	(316.3, 1115.8)	1264.4	(661.8, 2781.2)
5th MIPC quintile	438.7	(65.2, 1436.9)	600.5	(367.3, 1378.3)	1638.5	(658.6, 3077.2)
Overall	283.5	(41.8, 945.7)	413.1	(231.9, 927.8)	965.5	(461.8, 2059.3)
P-value	< 0.001		< 0.001			< 0.001
Total costs as proportion of AHI						
1st MIPC quintile	30.6	(1.6, 83.5)	33.4	(16.3, 80.1)	72.5	(31.7, 189.8)
2nd MIPC quintile	6.8	(0.0, 28.3)	14.1	(8.9, 32.6)	31.7	(13.7, 68.9)
3rd MIPC quintile	7.7	(1.2, 23.6)	10.5	(5.2, 20.9)	23.5	(11.8, 40.0)
4th MIPC quintile	5.4	(0.9, 15.1)	8.4	(4.6, 17.3)	19.5	(8.5, 37.9)
5th MIPC quintile	2.6	(0.4, 8.6)	5	(2.8, 13.0)	13.5	(5.4, 25.1)
Overall	6.3	(0.6, 25.1)	11.8	(5.2, 27.2)	24.7	(11.3, 60.7)
P-value	< 0.001		< 0.001			< 0.001

TB Tuberculosis, *AHI* Annual household income, *MIPC* Monthly income per capita, *IQR* Inter quartile range
[a]Drug susceptible TB patients - China's TB patient cost survey (2017)

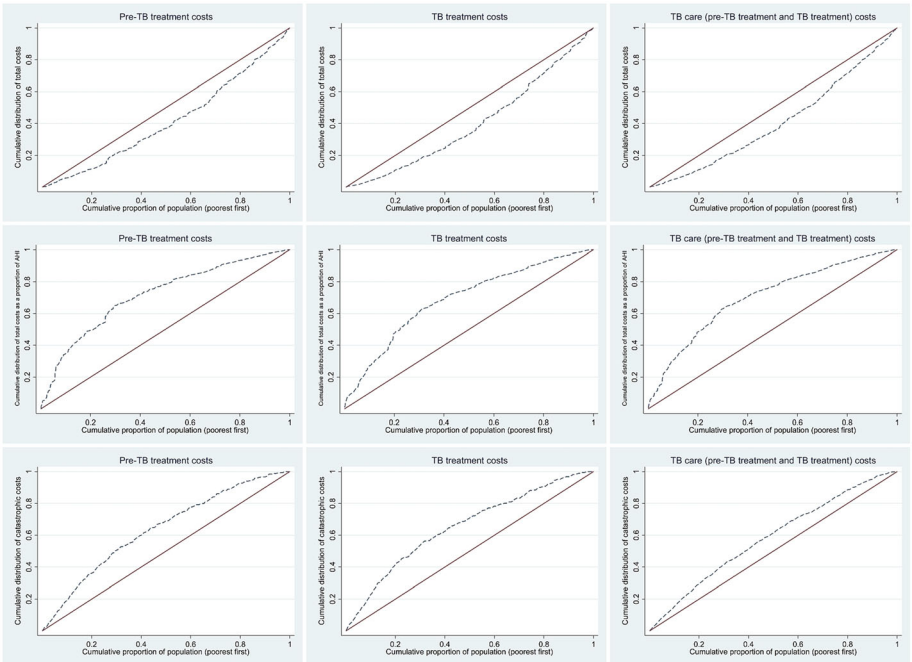

Fig. 1 Concentration curves for total costs, total costs as a proportion of pre-TB annual household income and catastrophic costs due to TB care, among TB* affected households in China (2017) (n = 1147). TB: Tuberculosis; AHI: Pre-TB annual household income. *drug susceptible TB patients - China's TB patient cost survey (2017). **all concentration indices were significantly away from the line of equality (P < 0.001)

Table 3 Concentration indices for total costs, total costs as a proportion of pre-TB annual household income and catastrophic costs due to TB care, among TB[a] affected households in China (2017) (n = 1147)

Costs due to TB care	Concentration index (95% *CI*)*		
	Total costs	Total costs as proportion of AHI	Catastrophic costs
Pre-TB treatment	0.172 (0.113, 0.231)	−0.429 (− 0.528, − 0.331)	− 0.277 (− 0.327, − 0.227)
TB Treatment	0.199 (0.140, 0.259)	− 0.377 (− 0.449, − 0.305)	− 0.306 (− 0.351, − 0.261)
TB care (pre-TB treatment and treatment)	0.186 (0.145–0.228)	−0.402 (− 0.466, − 0.338)	−0.169 (− 0.197, − 0.141)

TB Tuberculosis, *AHI* Pre-TB annual household income
*All concentration indices were significantly away from the line of equality ($P < 0.001$)
[a]Drug susceptible TB patients - China's TB patient cost survey (2017)

of China. It has been previously proven that the NCMS did not do much to remove the inequity in the distribution of the TB care costs [9]. Increase in insurance coverage and the reimbursement of expenses has not been translated into reduction in catastrophic costs due to TB care [16]. Various reasons have been attributed to this including that costs incurred as an outpatient are not covered under the NCMS. TB diagnosis and treatment mostly happens in the out-patient settings, thus leaving the costs uncovered. Further, the risk pooling being at the county level and not above doesn't support high reimbursement rates. Thus, despite over 90% of the rural population being enrolled under NCMS, the benefits drawn by patients with TB are limited.

The middle region showed a significantly more pro-poor distribution of catastrophic costs due to pre-TB treatment care compared to the East and West. This could be due to the differential experience of costs between rich and poor of the respective regions.

Implications for policy and practice

By 2035, even with aggressive expansion of TB services, catastrophic costs would reduce only by 5–20% when compared to 2015 [17]. Therefore, countries need to move towards attaining universal health coverage and social protection. Universal health coverage will reduce the direct medical costs and social protection will reduce direct non-medical and indirect costs [18–22].

Under universal health coverage, the social insurance schemes in China only marginally reduced catastrophic costs with no effect of inequity [6, 9, 23]. Risk pooling at a level higher than the county, raising the "height" of the

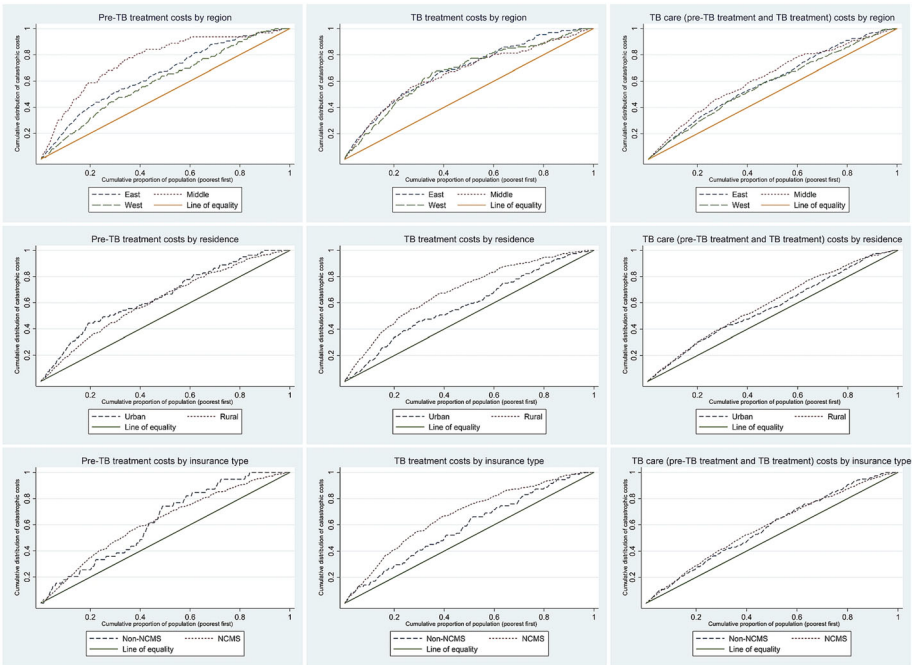

Fig. 2 Comparison of concentration curves for catastrophic costs due to TB care among TB* affected households in China (2017): stratified by region, residence and insurance type (n = 1147). TB: Tuberculosis; NCMS: new cooperative medical scheme; non-NMCS: include urban employee basic medical insurance, urban residence basic medical insurance, public service medical insurance and other private medical insurance. *drug susceptible TB patients - China's TB patient cost survey (2017)

Table 4 Comparison of concentration indices for catastrophic costs due to TB care among TB[a] affected households in China (2017): stratified by region, residence and insurance type (n = 1147)

Variable	Pre- TB treatment		TB Treatment		TB care (pre-TB treatment and treatment)	
	Concentration index (95% *CI*)	Dominance test	Concentration index (95% *CI*)	Dominance test	Concentration index (95% *CI*)	Dominance test
Region						
East	−0.281 (− 0.351, − 0.21)	East versus west – non-dominance	−0.37 (− 0.443, − 0.297)	Non-dominance	−0.182 (− 0.224, − 0.141)	Non-dominance
Middle	− 0.439 (− 0.559, − 0.32)	Middle dominates east as well as west[b]	−0.32 (− 0.393, − 0.247)		−0.228 (− 0.284,-0.172)	
West	−0.197 (− 0.281, − 0.113)		−0.355 (− 0.438, − 0.272)		−0.164 (− 0.212, − 0.114)	
Residence						
Urban	−0.301 (− 0.410, − 0.191)	Non-dominance	−0.174 (− 0.257, − 0.09)	Rural dominates urban[b]	−0.131 (− 0.184, − 0.078)	Non-dominance
Rural	− 0.237 (− 0.292, − 0.181)		−0.383 (− 0.435, − 0.331)		−0.179 (− 0.212, − 0.147)	
Insurance						
NCMS	−0.244 (− 0.297, − 0.192)	Non-dominance	−0.343 (− 0.392, − 0.294)	NCMS dominates non-NCMS[b]	−0.168 (− 0.199, − 0.138)	Non-dominance
Non-NCMS	− 0.25 (− 0.415, − 0.085)		−0.168 (− 0.280, − 0.055)		−0.162 (− 0.233, − 0.091)	

TB Tuberculosis, *CI* Confidence interval, *NCMS* New cooperative medical scheme, *Non-NMCS* include urban employee basic medical insurance, urban residence basic medical insurance, public service medical insurance and other private medical insurance

[a]Drug susceptible TB patients - China's TB patient cost survey (2017)

[b]Statistically significant

NCMS by modifying the benefit package and alternate provider payment mechanisms are recommended [6, 9]. Regulation of unnecessary prescription of additional medications like supplements may also cut costs. The pre-treatment catastrophic costs could be controlled by adhering to standardized diagnosis and treatment algorithms for all forms of TB. This would cut down unnecessary consultations, investigations and associated indirect costs for a patient before she/he is initiated on treatment.

For social protection, TB-specific approach (cash transfers for households with a confirmed case of TB) are expected to be more effective and affordable than a TB-sensitive approach (cash transfers for households with high TB risk to strengthen their economic resilience) [24]. India has started direct benefit transfer of about USD 8 per month up to treatment completion for all patients notified with TB (TB-specific approach) [25, 26].

Strengths and limitations

To the best of our knowledge, this is the first nationally representative study reporting a detailed analysis of inequity in pre-TB treatment; treatment and overall TB care costs globally. The nationally representative patient level data was collected using the WHO recommended TB patient cost survey guidelines [4]. This equity analysis can be readily adopted to similar nationwide exercises in the world (Viet Nam, Ghana and Indonesia) [27–29].

There were some limitations. Some patients may not have accurately remembered the exact amount they spent for seeking TB care. We attempted to minimize recall limitation by surveying patients still on treatment and imputing costs to the entire episode assuming that all patients complete treatment. However, this might overestimate the costs considering some patients might have failed treatment or been lost to follow up. On the other hand, as we did not include multi-drug resistant TB patients, costs could be an underestimate. Data on service utilization, service quality and outcome were not collected and beyond the scope of this study. The lower costs among poorest quintile may also be due to non-receipt of care.

Conclusions

We found inequity in distribution of catastrophic costs due to TB care, including pre-treatment and treatment care, in China. This inequity was consistently seen across various population sub-groups. However, inequity was significantly high during treatment phase in rural areas that are covered by NCMS and during the pre-treatment phase in middle region of China. Attainment of universal health coverage and social protection ably complemented by quality TB care is the need of the hour to reduce inequitable distribution of catastrophic costs due to TB care in China.

Additional files

Additional file 1: Multilingual abstracts in the five official working languages of the United Nations. (PDF 571 kb)

Additional file 2: Annex 1. The parameters used for sample size calculation under in China's TB* patient cost survey (2017). Annex 2. The per capita gross national product (GNP) of six provinces sampled in China's TB* patient cost survey (2017). Annex 3. Multi-stage stratified cluster sampling adopted in China's TB* patient cost survey (2017). Annex 4. The 22 counties sampled in China's TB* patient cost survey (2017). Annex 5. The questionnaire used in China's TB* patient cost survey (2017). (DOCX 82 kb)

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Authors' contributions

CX was a major contributor in study design, data collection and manuscript writing. YL and YX was great contributor to the data collection and analysis. HDS, JK, LW, HZ and LXW designed the study, interpreted the data, modified and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset and codebook used in this study are available on request from the corresponding author (zhanghui@chinacdc.cn, huizhang1974@126.com).

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Chinese Center for Disease Control and Prevention (No. 201625) and Ethics Advisory Group of The Union, Paris, France (EAG number 22/18). The written informed consent process and the provision USD 15 to patients (as a reimbursement for costs related to travel to BMU office for the interview) was approved by the ethics committee.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Trends in tobacco consumption in India 1987–2016: impact of the World Health Organization Framework Convention on Tobacco Control

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Abstract

Objectives We describe national and subnational trends in tobacco use over three decades in India, assess the impact of the World Health Organization's Framework Convention on Tobacco Control (FCTC) on them and draw inferences for regional tobacco control policy.

Methods Data from nine cross-sectional surveys conducted between 1987 and 2016 were analysed. Time trends in gender- and state-wise prevalence were derived for different forms of tobacco. To assess Framework Convention's impact, relative changes in tobacco prevalence before and after its implementation were estimated. Progress towards global noncommunicable diseases target was also measured.

Results Post-implementation of the FCTC, smoking and smokeless tobacco use declined by 52.9% and 17.6%, respectively. The tobacco product mix (exclusive smokeless/exclusive smoked/dual) underwent a reversal from 37:52:11 in 1987 to 65:22:13 in 2016. Having achieved 20.5% relative reduction since 2009, India is en route to achieving the global noncommunicable diseases target.

Conclusions Steep declines in tobacco use have followed the implementation of FCTC in India. However, the impact has been unequal on smokeless and smoked forms. Tobacco-control policies in high smokeless burden countries should take cognizance of this pattern and design comprehensive and flexible policies.

Keywords Framework Convention on Tobacco Control · Smokeless tobacco · Tobacco smoking · Trends · Tobacco control · India

Introduction

India is the second largest producer of tobacco in the world with an estimated annual production of 800 million kilograms (Food and Agriculture Organization of the United

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Nations 2003). It is also among the top three highest burden countries in terms of the number of users (GBD 2016 Risk Factors Collaborators 2017). In 2016, the global adult tobacco survey (GATS) estimated the prevalence of smokeless and smoked tobacco use in India among those aged $\geq$ 15 years to be 21.4% and 10.7%, respectively (Ministry of Health and Family Welfare 2017). Consequently, India is one of the four countries that shared more than 50% of the 6.3 million deaths and 155 million disability-adjusted life years (DALY) attributable to smoking, worldwide in 2016 (GBD 2016 Risk Factors Collaborators 2017). Besides smoking, South East Asian (SEA) countries, including India, suffer the additional burden of smokeless tobacco (SLT). SEA is home to more than 80% of the world's smokeless tobacco users (290 million) who outnumber the smokers (National Cancer Institute and Centers for Disease Control and Prevention 2014). Globally, in 2010, 652 494 deaths and 46 million DALYs were attributable to SLT use, of which over 80% was borne by SEA countries (Sinha et al. 2016).

Worldwide, tobacco control efforts have historically focussed most of the attention on cigarette smoking and accorded lesser importance to SLT control due to various reasons (National Cancer Institute and Centers for Disease Control and Prevention 2014). In tandem with the global movement, the earliest attempts at tobacco control in India began in 1975 but were limited in their effectiveness due to fragmented implementation, non-coordination between states and overemphasis on cigarettes (Mehrotra et al. 2010). The Indian Government was active in the World Health Organization's Framework Convention on Tobacco Control (WHO FCTC) negotiations, signed it in September 2003 and ratified it in February 2004 (Reddy and Gupta 2004). While becoming a signatory to the FCTC in 2003, India had also enacted the first recognizable federal law to regulate the supply, production, distribution, advertisement and sale of tobacco products—the Cigarette and Other Tobacco Products Act (COTPA) (Ministry of Law and Justice 2003). COTPA enforced five important regulations in sync with the FCTC recommendations, i.e. prohibition of smoking in public places, ban on tobacco advertising and sponsorship, ban on sale to and by minors and within 100 yards of educational institutions, display of pictorial health-warning labels and content regulation of tobacco products (Reddy et al. 2008, 2010). However, specific SLT control strategies (i.e. ban on sale of *gutka* and *paan masala*) were not incorporated under this law until after 2011, setting up the stage for non-uniform impact on the two major types of tobacco consumption. Although a few studies have tried to assess the impact of this law in the past, they have had a narrow approach, only looking at a few provisions at a time and none have assessed the overall, long-term impact on tobacco prevalence making use of all available data at the national and subnational levels.

The global noncommunicable diseases (NCD)-monitoring framework, which followed the United Nations political declaration on NCDs in 2011, targets a 30% reduction in tobacco use from 2010 levels, before 2025 (World Health Organization 2013). To monitor the progress towards this target, an analysis based on robust and comparable survey data across the evaluation period is essential. Despite the availability of such data in India, previous attempts have been limited in scope. Some studies have considered only two or three time points, while others have examined only smoking trends or specific subsets of the population (Gupta and Sankar 2003; Jhanjee 2011; Goel et al. 2014; Bhan et al. 2016). Further, India is an amalgamation of states with variations in socio-economic, developmental and health indicators. This heterogeneity necessitates examination of the state-level trends as being distinct from the national trend. Such monitoring of trends is also a requirement of the World Health Organization—Monitor, Protect, Offer help, Warn, Enforce and Raise taxes (WHO-MPOWER) strategy (World Health Organization 2008).

In keeping with the above, we analysed data from nationally representative surveys spanning over three decades to examine the national-level and state-wise trends in the prevalence of different forms of tobacco use among adults (15–49 years) in India, assess the impact of the FCTC and derive important lessons on tobacco control for other countries in the region undergoing a similar tobacco epidemic.

Methods

We performed a trend analysis based on a series of cross-sectional surveys conducted at different time points at the national and state levels.

Data sources

Three main indicators were selected for trend analysis—current use of any smokeless tobacco, any smoked tobacco and any tobacco. We attempted to identify all available surveys, which were conducted at the national level or state level across all available years. The selected surveys provided data for the above-mentioned indicators for at least one gender from a representative sample of the general population. Surveys that did not provide information on sample weighting or the selected indicators and those that did not allow access to raw data sets were excluded. A total of nine surveys (eight national and one subnational), conducted at different time points between 1987 and 2016,

were found eligible for the analysis. These surveys can be categorized into four types—the national sample surveys (NSSO rounds 43, 50 and 52), national family health surveys (NFHS 2, 3 and 4), district-level household and facility survey (DLHS-4) and the global adult tobacco surveys (GATS 1 and 2). All except DLHS-4 provided national-level data. The methodologies of the individual surveys are described in detail elsewhere (International Institute for Population Sciences 2018a, b; Ministry of Statistics and Program Implementation, Government of India 2018; Centres for Disease Control and Prevention 2018). All surveys used a multistage stratified random sampling but differed on the age groups studied. Among these surveys, NFHS 3 and NFHS 4 were different from the rest as majority of their sample consisted of women.

Excluded surveys

We identified seven other major survey reports, which could not be included in the analysis due to lack of publicly available/accessible raw data sets or sample weighting information. These were the special fertility and mortality survey (1998), the national household survey of drug and alcohol abuse (2000), the world health survey (2003), the sample registration system baseline survey (2004), two WHO STEPwise surveys (2004 and 2007) and the baseline annual health survey (2010).

Data availability and management

The common variables required for analysis across different surveys were mapped and their details recorded in a codebook. Questions on the selected tobacco-use indicators across the different surveys were matched in a standardized manner (Table S1). The availability of the original data sets and their main characteristics are summarized in Table S2.

Statistical analysis

Descriptive analyses were performed, and prevalence was calculated, state and gender wise, for each time point. Since the age groups included were different across the nine surveys, the analysis was restricted to the common age group of 15 to 49 years. Smokeless tobacco referred to those tobacco-containing products that are chewed or applied orally, for example, snus, tobacco tooth powders, snuff, *gutkha, khaini, tambaku, qiwam, dohra, kimam,* tobacco powder, *and mawa* among others. Any smoked tobacco included cigarettes, *bidis*, *hookah*, cigars, cheroots, *chuttas* and pipes. All proportions and their 95% confidence intervals were weighted to account for the complex survey design. For NFHS 3 and NFHS 4 surveys, which had sample size imbalances in sex, overall prevalence was adjusted according to state-wise sex proportions obtained from the nearest census data. The state-wise prevalences were represented in a series of heatmaps. Relative percentage change was calculated for two time intervals 1987–2005 and 2005–2016 to assess the impact of the FCTC. To monitor progress towards the fifth global NCD target, relative change was calculated for the time interval 2009–2016. Slopegraphs were used to visualize the ranks and relative percentage change in the states. Analyses were conducted in Stata 11 and QGIS 2.18.20. The codes and codebook of the statistical analysis are available at https://github.com/sarizwan1986/India-tobacco-trends.git.

Description of the surveys

All the included surveys employed a probability proportional to size, multistage, stratified random sampling technique. Trained interviewers collected data from household members by a face-to-face interview using a pretested structured questionnaire, translated into appropriate local languages. Data quality was maintained through standard quality control measures. The global adult tobacco surveys were focused tobacco surveys, whereas the NSSO surveys collected data on household expenditure patterns and the NFHS and the DLHS surveys dealt with maternal and child health issues. Sample sizes were comparable across the surveys; the GATS had the smallest, and the DLHS 4 had the largest sample sizes. All surveys had been conducted under the patronage of the Government of India.

Results

Time trends in tobacco use

Prevalence of any smokeless tobacco use increased from 15% in 1987 to 24.2% in 2009 and thereafter, declined to 19.3% in 2016. A similar pattern was observed among males and females—but in males the decline began earlier than the females (2005 vs. 2009). Despite this, the 2016 prevalence among females was 10% points lower than their baseline, while in males it was 47% higher than their baseline. On the other hand, prevalence of any smoked tobacco declined from 19.8% in 1987 to 8.6% in 2016; the prevalence among males ranged between 36% and 16%, while among females it never went over 3%. Prevalence of any tobacco use showed a declining trend initially, from 1987 (31.3%) to 1998 (25.9%), after which it briefly peaked to 34.7% in 2005 before falling to 24.6% in 2016. Males and females exhibited similar trends except that the changes in females were lagging behind males; the peaking

Table 1 Trends and relative change in the prevalence of tobacco consumption among adults (15–49 years) in India, 1987–2016

Type of tobacco product	Prevalence (95% confidence intervals)								% Change (2005–2016)
	1987	1993	1995	1998	2005	2009	2015	2016	
Any smokeless tobacco use									
Total	15.0	13.2	14.1	17.2	23.4	24.2	17.6	19.3	− 17.6
	(14.9, 15.2)	(13.1, 13.4)	(13.8, 14.3)	(17.1, 17.4)	(23.1, 23.8)	(23.6, 24.8)	(17.4, 17.9)	(18.7, 19.8)	
Male	19.5	18.4	21.4	25.7	36.9	32.7	29.0	28.7	− 22.2
	(19.2, 19.7)	(18.2, 18.7)	(21.0, 21.8)	(25.4, 26.0)	(36.3, 37.4)	(31.8, 33.7)	(28.6, 29.4)	(27.8, 29.6)	
Female	10.5	7.7	6.3	8.8	9.0	14.9	5.6	9.4	4.3
	(10.3, 10.7)	(7.6, 7.9)	(6.1, 6.5)	(8.6, 9.0)	(8.8, 9.2)	(14.3, 15.6)	(5.5, 5.7)	(8.9, 9.9)	
Any smoked tobacco use									
Total	19.8	17.2	17.6	13.7	18.3	11.9	12.5	8.6	− 52.9
	(19.6, 20.0)	(17.0, 17.4)	(17.4, 17.9)	(13.6, 13.9)	(18.0, 18.6)	(11.5, 12.4)	(12.3, 12.7)	(8.3, 9.0)	
Male	36.3	31.7	32.8	25.9	33.3	21.4	23.5	15.8	− 52.5
	(36.0, 36.6)	(31.4, 32.0)	(32.4, 33.2)	(25.6, 26.2)	(32.9, 33.8)	(20.6, 22.3)	(23.1, 23.9)	(15.2, 16.5)	
Female	2.8	1.9	1.7	1.6	2.2	1.6	0.8	1.0	− 53.8
	(2.7, 2.9)	(1.8, 2.0)	(1.6, 1.8)	(1.5, 1.7)	(2.1, 2.3)	(1.4, 1.9)	(0.7, 0.8)	(0.9, 1.2)	
Any tobacco use									
Total	31.3	27.2	27.9	25.9	34.7	31.0	25.8	24.6	− 29.0
	(31.1, 31.5)	(27.0, 27.4)	(27.6, 28.2)	(25.7, 26.1)	(34.3, 35.1)	(30.4, 31.7)	(25.5, 26.1)	(24.0, 25.2)	
Male	49.2	44.2	47.1	41.8	57.0	45.0	44.3	38.4	− 32.6
	(48.9, 49.5)	(43.9, 44.6)	(46.7, 47.6)	(41.5, 42.1)	(56.5, 57.5)	(43.9, 46)	(43.8, 44.7)	(37.4, 39.4)	
Female	12.8	9.3	7.7	10.0	10.8	15.9	6.2	10.1	− 6.6
	(12.6, 13.1)	(9.1, 9.5)	(7.5, 8.0)	(9.8, 10.2)	(10.6, 11.1)	(15.3, 16.6)	(6.2, 6.3)	(9.6, 10.7)	

2005–2016 refers to the period after the implementation of Framework Convention on Tobacco Control provisions. State-wise, product-wise and gender-wise data are presented in Table S3A-C

happened in 1995 and 2005 in males but in females they occurred in 1998 and 2009 (Table 1, Figure S1).

Impact of the FCTC regulations

In the period following the implementation of the national anti-tobacco law as per FCTC obligations (2005–2016), all forms of tobacco use declined. The highest decline of 52.9% was seen in smoked tobacco use, whereas smokeless tobacco use declined by 17.6% and any tobacco use declined by 29%. Since smokeless tobacco use in females had a delayed peak in 2009, a sight increase of 4.3% was seen, but it had actually reduced by 35% in the interval 2009–2016 (Table 1).

Change in product mix

Overall, there was an inversion in the predominant type of tobacco product used across the study period. The relative contribution of exclusive SLT to exclusive smoking to dual use of 37:52:11 in 1987 switched into a 65:22:13 distribution in 2016. A similar inversion was observed for males but not females (Figure S2).

State-wise trends

State-wise prevalence of tobacco use for 1987, 2005 and 2016 is presented in Fig. 1. In 2016, the top three states with the highest smokeless tobacco burden were the north-eastern states of Manipur (49.2%), Tripura (48.3%) and Nagaland (40.6%). Among these, Manipur and Tripura were yet to reverse the rising trend in smokeless tobacco use since 1987. As of 2016, the smokeless tobacco use among males in these states was twice the national average (28.7%) but among their females, it was almost five times the national average (9.4%). In 2016, Himachal Pradesh (2.8%), Kerala (2.9%) and Jammu & Kashmir (3.4%) had the lowest prevalence of SLT usage overall and among males. Among females in the states of Goa, Haryana, Jammu & Kashmir, Chhattisgarh, Kerala, Punjab and

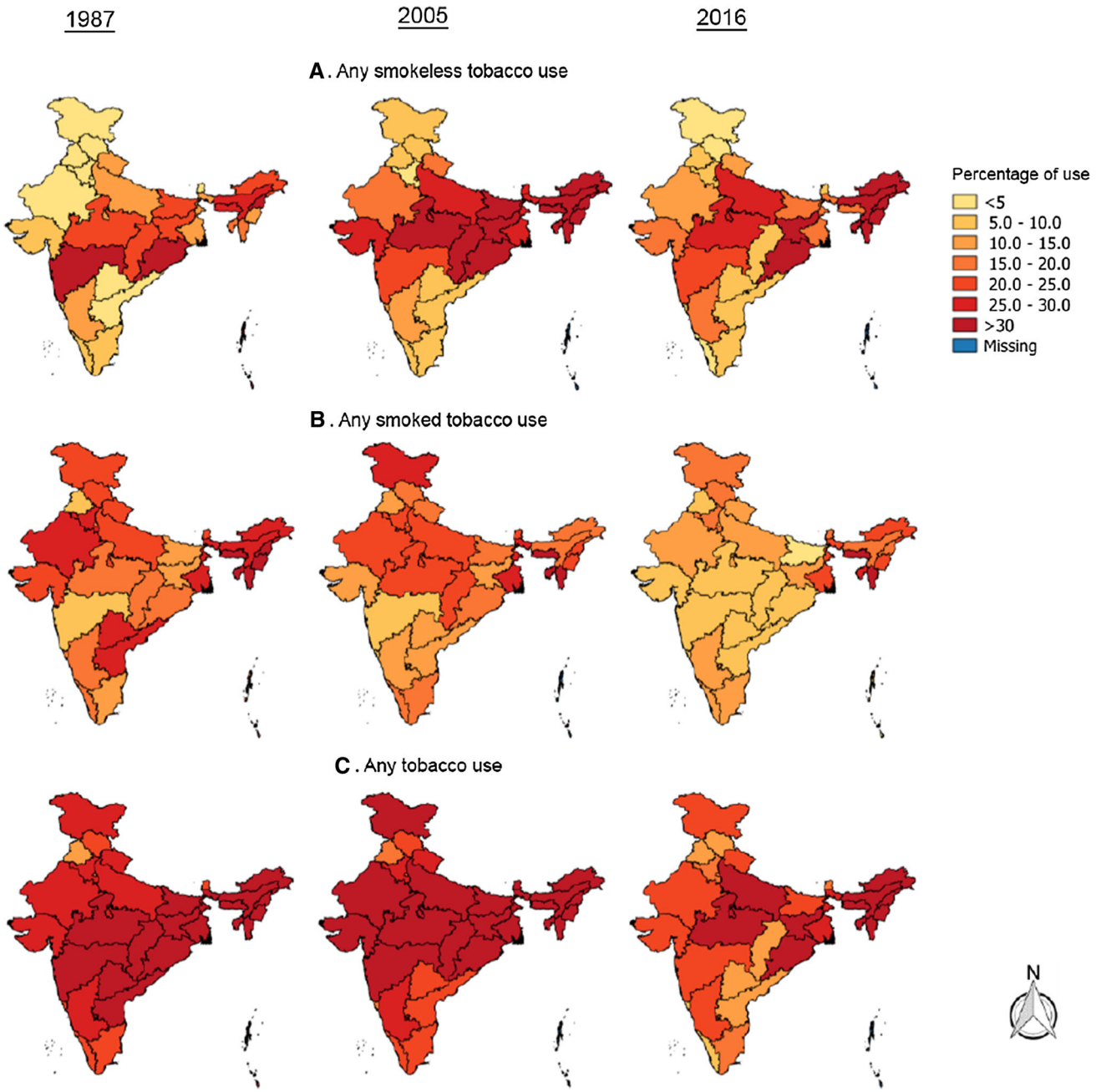


Fig. 1 State-wise trends in the prevalence of tobacco consumption in India, 1987–2016

Himachal Pradesh, prevalence of $\leq 1\%$ was reported. Post-2005, Chhattisgarh (81.1%), Sikkim (67%) and Kerala (65.4%) had achieved the highest relative reductions, whereas Haryana (31.4%) and Karnataka (16.2%) had recorded an increase in SLT use (Figure S3A-C, Table S3A).

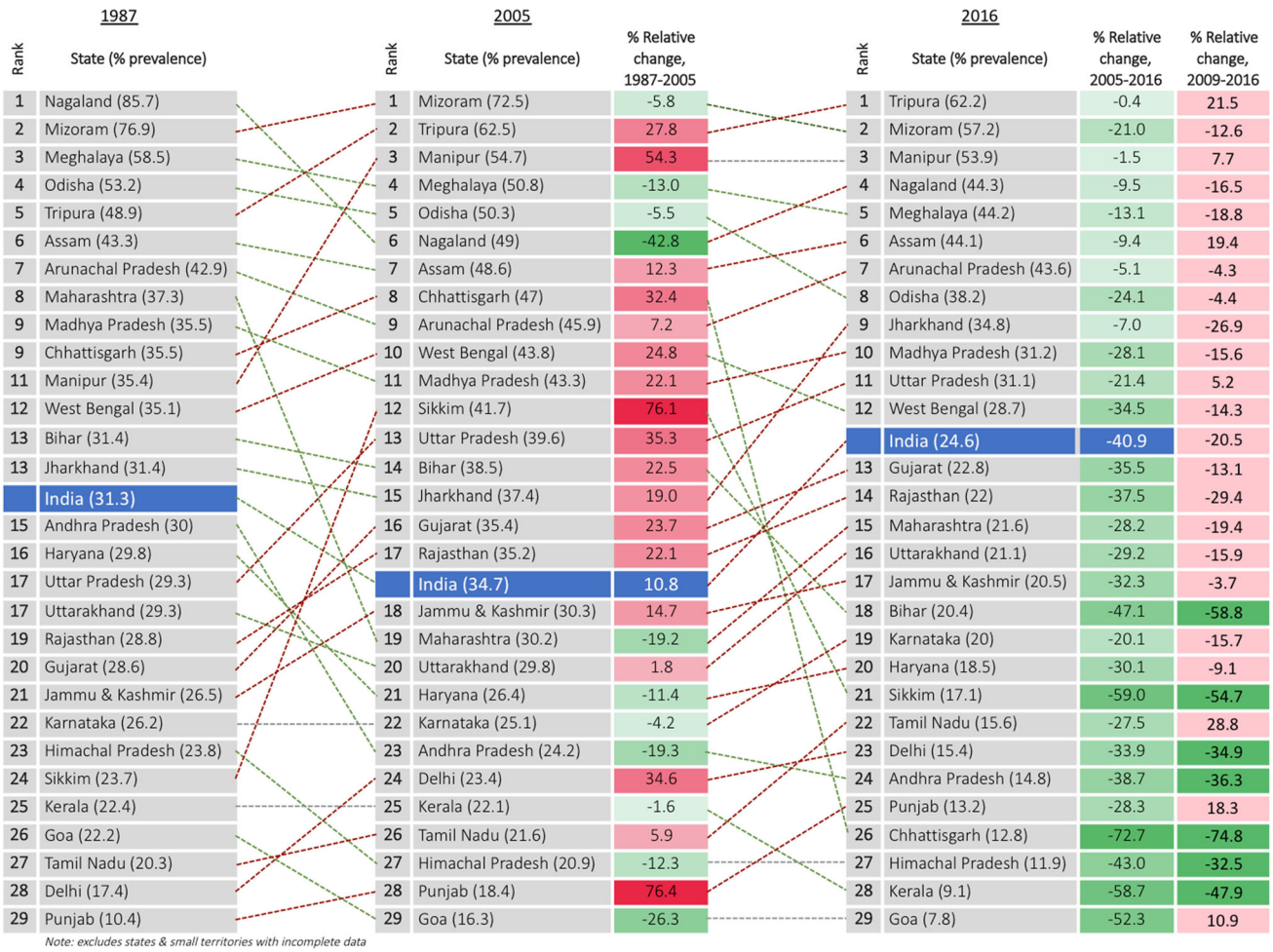
The north-eastern states of Mizoram (33.8%), Meghalaya (30.3%) and Tripura (25.5%) were the top three states with the highest prevalence of tobacco smoking since 2005, despite recording a consistent reduction in the prevalence between 1987 and 2016. Arunachal Pradesh was the only state that registered a relative increase in smoking (22.1%) from 2005 to 2016. Karnataka (67.9%), Andhra Pradesh (172.7%) and Meghalaya (338.6%) reported a relative increase in female smoking between 2005 and 2016. During this period, Goa (2.9%), Bihar (2.9%) and Maharashtra (2.6%) had the lowest prevalence and they also registered a relative decline of $> 60\%$ since 2005. In 2016, Kerala, Odisha, Tamil Nadu and Goa reported zero prevalence of female smoking (Figure S3D-F, Table S3B).

Tripura (62.2%), Mizoram (57.2%) and Manipur (53.9%) had the highest burden of any tobacco use in 2016.

Goa (7.8%), Kerala (9.1%) and Himachal Pradesh (11.9%) had the least burden of any tobacco usage. Post-2005, all states have reported a reduction in any tobacco usage ranging from 0.4% (Tripura) to 52.3% (Goa). Among the states with tobacco-use prevalence higher than the national average, the north-eastern states recorded lesser gender gap (< 17%) compared to the other states where wider gender gap (> 25%) was the norm (Fig. 2, S3G-H, Table S3C).

Progress towards global NCD target number five

India is en route (20.5% reduction since 2009) to achieve the global monitoring framework for NCDs target that requires a 30% reduction in tobacco prevalence since 2010, before 2025, among population aged 15 years and over. At the national level, the target has been achieved only for females (36.5%), while among males (14.6%) the progress is just half way through. Out of the 29 states considered, only seven, namely Chhattisgarh (74.8%), Bihar (58.8%), Sikkim (54.7%), Kerala (47.9%), Andhra Pradesh (36.3%),



1987		2005			2016			
Rank	State (% prevalence)	Rank	State (% prevalence)	% Relative change, 1987-2005	Rank	State (% prevalence)	% Relative change, 2005-2016	% Relative change, 2009-2016
1	Nagaland (85.7)	1	Mizoram (72.5)	-5.8	1	Tripura (62.2)	-0.4	21.5
2	Mizoram (76.9)	2	Tripura (62.5)	27.8	2	Mizoram (57.2)	-21.0	-12.6
3	Meghalaya (58.5)	3	Manipur (54.7)	54.3	3	Manipur (53.9)	-1.5	7.7
4	Odisha (53.2)	4	Meghalaya (50.8)	-13.0	4	Nagaland (44.3)	-9.5	-16.5
5	Tripura (48.9)	5	Odisha (50.3)	-5.5	5	Meghalaya (44.2)	-13.1	-18.8
6	Assam (43.3)	6	Nagaland (49)	-42.8	6	Assam (44.1)	-9.4	19.4
7	Arunachal Pradesh (42.9)	7	Assam (48.6)	12.3	7	Arunachal Pradesh (43.6)	-5.1	-4.3
8	Maharashtra (37.3)	8	Chhattisgarh (47)	32.4	8	Odisha (38.2)	-24.1	-4.4
9	Madhya Pradesh (35.5)	9	Arunachal Pradesh (45.9)	7.2	9	Jharkhand (34.8)	-7.0	-26.9
9	Chhattisgarh (35.5)	10	West Bengal (43.8)	24.8	10	Madhya Pradesh (31.2)	-28.1	-15.6
11	Manipur (35.4)	11	Madhya Pradesh (43.3)	22.1	11	Uttar Pradesh (31.1)	-21.4	5.2
12	West Bengal (35.1)	12	Sikkim (41.7)	76.1	12	West Bengal (28.7)	-34.5	-14.3
13	Bihar (31.4)	13	Uttar Pradesh (39.6)	35.3		India (24.6)	-40.9	-20.5
13	Jharkhand (31.4)	14	Bihar (38.5)	22.5	13	Gujarat (22.8)	-35.5	-13.1
	India (31.3)	15	Jharkhand (37.4)	19.0	14	Rajasthan (22)	-37.5	-29.4
15	Andhra Pradesh (30)	16	Gujarat (35.4)	23.7	15	Maharashtra (21.6)	-28.2	-19.4
16	Haryana (29.8)	17	Rajasthan (35.2)	22.1	16	Uttarakhand (21.1)	-29.2	-15.9
17	Uttar Pradesh (29.3)		India (34.7)	10.8	17	Jammu & Kashmir (20.5)	-32.3	-3.7
17	Uttarakhand (29.3)	18	Jammu & Kashmir (30.3)	14.7	18	Bihar (20.4)	-47.1	-58.8
19	Rajasthan (28.8)	19	Maharashtra (30.2)	-19.2	19	Karnataka (20)	-20.1	-15.7
20	Gujarat (28.6)	20	Uttarakhand (29.8)	1.8	20	Haryana (18.5)	-30.1	-9.1
21	Jammu & Kashmir (26.5)	21	Haryana (26.4)	-11.4	21	Sikkim (17.1)	-59.0	-54.7
22	Karnataka (26.2)	22	Karnataka (25.1)	-4.2	22	Tamil Nadu (15.6)	-27.5	28.8
23	Himachal Pradesh (23.8)	23	Andhra Pradesh (24.2)	-19.3	23	Delhi (15.4)	-33.9	-34.9
24	Sikkim (23.7)	24	Delhi (23.4)	34.6	24	Andhra Pradesh (14.8)	-38.7	-36.3
25	Kerala (22.4)	25	Kerala (22.1)	-1.6	25	Punjab (13.2)	-28.3	18.3
26	Goa (22.2)	26	Tamil Nadu (21.6)	5.9	26	Chhattisgarh (12.8)	-72.7	-74.8
27	Tamil Nadu (20.3)	27	Himachal Pradesh (20.9)	-12.3	27	Himachal Pradesh (11.9)	-43.0	-32.5
28	Delhi (17.4)	28	Punjab (18.4)	76.4	28	Kerala (9.1)	-58.7	-47.9
29	Punjab (10.4)	29	Goa (16.3)	-26.3	29	Goa (7.8)	-52.3	10.9

Note: excludes states & small territories with incomplete data

Fig. 2 Trend in state-wise ranks and relative change in the prevalence of any tobacco use in India, 1987–2016. *Note* In each column, the states are arranged in descending order of prevalence. The change in rank of a state between two time points is tracked by dashed lines (maroon for worsening and green for improvement). The 1987–2005 column refers to the period before and the 2005–2016 column refers to the period after the implementation of the Framework Convention on Tobacco Control. In the columns presenting relative change, a relative increase in prevalence is coloured in shades of red and a relative decline is coloured in shades of green (intensity of colour is proportional to the magnitude of change). The last column, 2009–2016 relative change, depicts achievement of global tobacco control target (green for target achieved and pink for not). For example, let us take the state of Tamil Nadu. Its rank was no. 27 in 1987. It worsened to rank 26 in 2005 (dashed maroon line) with a relative increase in prevalence of 5.9%. It again worsened to rank 22 in 2016 (dashed maroon line), in spite of a relative decline of 27.5% in prevalence. Although the prevalence of tobacco use declined by 27.5% between 2005 and 2016, the rank of Tamil Nadu worsened because there were other states (Delhi, Andhra Pradesh, Punjab, Chhattisgarh, Himachal Pradesh, Kerala, Goa) that performed much better than Tamil Nadu. Finally, it can be seen from the final column that Tamil Nadu has not achieved the NCD target (relative increase of 28.8% from 2009 to 2016, coloured pink) (Colour figure online)

Delhi (34.9%) and Himachal Pradesh (32.5%), have achieved the target. Jharkhand (62.2%), Rajasthan (32.8%), Jammu & Kashmir (35.9%), Karnataka (37.1%) and Andhra Pradesh (45.9%) have achieved the target only for females (Fig. 2, Figure S3G-H, Table S4).

Discussion

We analysed data from nine national/subnational cross-sectional surveys to assess tobacco trends among adults in India. Availability of data across three decades enabled an ecological evaluation of the impact of the FCTC implementation in India. Between 1987 and 2016, smoked tobacco use showed a steeper decline compared to SLT use. There has been a radical switch in the predominant type of tobacco product used after 1995, with chewers outnumbering the smokers. After the implementation of FCTC provisions, all forms of tobacco use declined and India is on track to achieve the global tobacco control target by 2025 but with constituent state at varying levels of achievement.

Tobacco trends in India: Interplay between smoked and smokeless tobacco use

Globally, current smoking has declined by 6.7% since 2000 and by 4.1% since FCTC implementation (World Health Organization 2018). This fall has been observed everywhere except for some countries in the African and Eastern Mediterranean regions (Ng et al. 2014; Bilano et al. 2015; Brathwaite et al. 2015; World Health Organization 2015). Even in China, the nation with the highest number of smokers, current smoking had declined sharply between 1993 and 2003 (Qian et al. 2010). Our analysis demonstrating the decline in smoking in India was in line with the findings of the global burden of disease study and the World Bank (Institute for Health Metrics and Evaluation 2018; The World Bank 2018). Presently, high- and low-income countries find themselves at different phases of the four-stage smoking epidemic (Thun et al. 2012). India, which now lies in stage three may not pass through stage four, when female smoking exceeds male smoking prevalence, because of the marked differences in the epidemic drivers between India and other developed nations.

In contrast to smoking, the global trends in SLT prevalence are not clearly understood. The SLT problem is prevalent only in some regions of the world such as the SEAR and data on SLT are sparse. Nevertheless, its prevalence has been found to be declining in Bangladesh, India, Indonesia, Myanmar and Nepal according to a WHO report (World Health Organization 2015a, b). In India, unlike the smoking trend that declined consistently from 1987, SLT use was on the rise until 2009, after which it began to decline. One reason for this difference could be the differential impact of early anti-tobacco measures that focused primarily on smoking. After the ratification of FCTC in 2004, India introduced a slew of legal anti-tobacco measures, but initially the measures were focussed on controlling smoking (Arora and Madhu 2012). Some of these measures included ban of sale to minors, point-of-sale advertisements, sale near educational institutions, smoking in public places and implementation of tobacco packaging health warnings. Although some of these regulations (advertisements and educational institutions' embargo) applied to SLT products as well, the law specifically banned the manufacture and sale of *gutka* and *paan masala* (the major SLT products) only in 2013 (Ruhil 2018). However, many other diverse SLT products still continue to be sold and consumed freely. Taxation, one of the most effective tobacco control measures, has been very beneficial in reducing smoking prevalence in India (World Health Organization 2015a, b). However, the SLT market has evaded the tax net for far too long. Taxation on cigarettes and other smoked products have continued to rise between 2008 and 2015, but there was no commensurate increase in taxes on SLT products encouraging their unabated use (The International Tobacco Control Evaluation Project 2018).

Besides the lopsided legal restrictions and taxation policies, inadequate sensitization of the population to the SLT hazards may have influenced their use. While SLT users are aware that it is not harmless, the more explicit depiction of the hazards of smoking can be contrasted with the mellowed-down statements of SLT health risks (Kozlowski and Sweanor 2018). Also, cultural acceptance, religious connections, diverse and unregulated nature of the SLT market, the livelihood of local tobacco farmers and the lower socio-economic status of its users make the control of SLT difficult (Moore et al. 2012; Zaatari and Bazzi 2018).

From smoking to chewing—the product switch

As a result of the differential decline between SLT and smoking, a paradigm shift occurred in the major type of tobacco used post-1995, when the SLT prevalence surpassed smoking by a large margin. A complete understanding of the tobacco landscape in India necessitates the simultaneous consideration of the evolution of SLT use and smoking. Although no strong evidence exists for the complementarity of smoked and smokeless tobacco, it is possible that a substitution of smoked forms by SLT in response to price hikes and legal restrictions on smoked forms could have occurred (2015). After all, smokeless tobacco use has been commonly reported among former

smokers (Richardson et al. 2014). Misleading marketing and promoting SLT as a smoking cessation aid may also explain smokers switching over. The manner in which the complex interplay of legal, political, economic and cultural influences and risk communication will affect the product preferences of new tobacco users should be explored in future studies (Perkins and Neumayer 2014). This knowledge could help us to better predict the future directions of the tobacco epidemic.

Over the years, SLT has been strongly implicated in the causation of several cancers. Worryingly, SLT use and its attributable disease burden, unlike cigarettes, have the distinction of disproportionately affecting the vulnerable sections of the population, propagating health inequities (Thakur et al. 2013). The lack of emphatic evidence on effective SLT cessation interventions deters the provision of credible cessation services for SLT users within the existing healthcare delivery system (Ebbert et al. 2007). The many SLT-related challenges described above require us to further our understanding of its role as a temporary behaviour or as a smoking alternative or as a gateway product among new users. This will inform our preventive efforts.

Interstate variations in tobacco trends

Unsurprisingly, the trends of tobacco use were markedly different across the various states of India. Nonetheless, some policy-relevant generalizations could be made. Northeast India recorded the highest prevalence of any form of tobacco use. There is a distinct chewing belt spanning across central India and a smoking belt that engulfs northern and north-western India. Education, income, caste and other socio-economic variables which are recognized as strong predictors of smoking and smokeless tobacco use may explain this interstate variation (Agrawal et al. 2013). Belonging to a particular state has been observed as an independent predictor of tobacco use implying that distal factors such as tradition, politico-legal climate and geography, operating at a state level, also influence tobacco-use behaviour (Subramanian et al. 2004). Some high prevalence states such as Chhattisgarh, Bihar and Sikkim and some states with a higher development index such as Kerala, Andhra Pradesh and Himachal Pradesh have made remarkable progress in tobacco control. As suggested above, there could be many reasons for interstate variations in tobacco control progress but the extent to which each state has been able to implement the anti-tobacco measures probably played a pivotal role.

Measuring progress towards targets

It has been previously projected that India has more than 95% probability of achieving the fifth target specified under the global NCD control framework by 2025 (Bilano et al. 2015). Our observation also confirms that India is on track to achieve this 30% reduction target. In fact, it has achieved this target for female tobacco users. It was evident that although females were late to adopt tobacco, they also quit or avoided the behaviour before the males did. The lower use and steeper decline observed among females was similar to that seen in China (Chen et al. 2015). Although this success is encouraging, the war against tobacco is far from over. Consistent public health efforts are required to respond to the dynamic tobacco scenario and usher into an era where tobacco ceases to be a public health problem. In addition, the target needs to be achieved by most, if not all, of the states for the control to be considered truly successful.

Strengths and limitations

The major strengths of this study lie in its size and representativeness. Drawing from nine rigorous nationally representative surveys, we are looking at one of the largest data pools ever handled to address the question of tobacco trends and patterns in India. Naturally designed, four out of nine data points lie after the implementation of FCTC provisions and the anti-tobacco law allowing us to assess their impact. We have discussed the tobacco epidemic in its entirety (nationwide, state wise, gender wise, product wise and year wise), providing policymakers with the bigger picture rather than piecemeal hunches. Prior to this, there was only a limited examination of SLT trends, but we have provided an in-depth analysis of the SLT trends in tandem with that of smoked tobacco. This is the first study to offer state-wise estimates and insightful interstate comparisons on tobacco trends. We also provided a measure of progress towards the global NCD target for tobacco—at the national and state levels.

There are a few limitations to consider. Not all surveys conducted between 1987 and 2018 were included for reasons already mentioned in the methodology. The heterogeneity across surveys, in terms of their objectives and methodologies, limited our ability to make absolute comparisons, despite our best efforts to make them as homogenous as possible. Firstly, the widely varying upper age limit in the different surveys was a source of major heterogeneity, which was overcome by restricting the analysis to 15–49 years. Secondly, the placement and wording of tobacco-use questions in the different surveys were not the same but we mapped similar questions across

the surveys and made sure that they matched as much as possible, reducing the possibility of errors. Thirdly, although all the surveys employed representative sampling, the varying sample sizes in each survey introduced varying errors of precision, more so at the state level. This imprecision was quantified by 95% confidence intervals, a close examination of which revealed that, except for a few states with very small sample sizes, the uncertainty was agreeable in general. Fourthly, the social desirability bias that often accompanies surveys of unfavourable personal behaviours is likely to have played a role in this analysis. However, it is inconceivable that this bias would have had differential effects across the different surveys and years. Finally, the notion that these different surveys lack absolute comparability because of their primary intentional differences is invalid in so far as the individual estimates for neighbouring time points did not vary to a large extent.

Conclusions

Over the past three decades, the prevalence of smoking has declined steeply and consistently. But, this has been accompanied by an initial rise and lesser magnitude of decline in SLT prevalence. Post-FCTC, the prevalence of all forms of tobacco use declined, setting India on track to achieve the global tobacco target of 30% reduction before 2025. Nevertheless, the achievements among the states have been unequal. The north-eastern states of India, especially Tripura, Mizoram, Manipur, Meghalaya, Assam and Arunachal Pradesh, reported the highest prevalence in smoked, smokeless and any tobacco use. Besides these, Odisha, Jharkhand, Madhya Pradesh, Maharashtra, Bihar and Uttar Pradesh reported high SLT prevalence, while Jammu and Kashmir, Haryana and West Bengal reported high smoking prevalence.

Policy implications for India and the SEA region

Tobacco-control policies with equal emphasis on strategies for SLT and smoked tobacco are recommended. These policies need to take into consideration the unique factors propelling the SLT epidemic and address them accordingly rather than directly transplanting strategies from the experience of anti-smoking efforts. An equitable approach to tobacco control demands specific policies against SLT with a special focus on the economically disadvantaged. Gender-based tobacco policies are required in lieu of the differences observed between male and female tobacco epidemics (Bandyopadhyay and Irfan 2018). Given the diversity in political commitment and socio-economic indicators across subnational units, it would be wise to renounce the 'one-size-fits-all' approach and weave specific strategies to achieve effective tobacco control.

Author contributions RSA conceived the study and managed the overall enterprise; DNS and PCG identified and extracted the data sources; NA carried out data management; RSA, KJ, RSR and DV performed the analysis; DNS, PCG, NA and SK provided critical feedback on the analysis, results and interpretation; KJ, RSR and RSA wrote the first draft. All authors approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. The original surveys included in the analysis had obtained ethical approvals from the participating institution's boards. The data sets of these surveys were publicly available or available on request, and all individual identifying information was removed.

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PLOS ONE

RESEARCH ARTICLE

Attrition and delays before treatment initiation among patients with MDR-TB in China (2006-13): Magnitude and risk factors

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Abstract

Background

China's national tuberculosis programme does not have cohort wise information regarding attrition and delays in the multidrug resistant tuberculosis (MDR-TB) diagnosis and treatment pathway.

Objective

Under the Global Fund programmatic management of drug-resistant TB (2006–13), we assessed the attrition and delay in the pathway and the factors associated.

Methods

Cohort study involving secondary programme data. All patients identified as presumptive MDR-TB (defined as i) previously treated TB patients which included recurrent TB, return after loss to follow up, treatment after failure and ii) new TB patients that were non-converters at three months of treatment or in close contact with a known MDR-TB patient) during October 2006 to June 2013 were eligible for phenotypic drug susceptibility testing (DST). Pre-diagnosis attrition (presumptive MDR-TB not undergoing culture and DST) and pre-treatment attrition (confirmed MDR-TB patients not initiated on treatment) was calculated. Diagnosis delay was the time interval from DST eligibility to DST result, treatment initiation delay was fom DST result to treatment initiation and total delay was from DST eligbility to treatment initiation. Factors associated with attrition and delay were identified using log binomial regression and linear regression, respectively.

Results

Of 78 564 presumptive MDR-TB patients, 2 470 (3.1%) underwent pre-diagnosis attrition. Of 9 283 MDR-TB patients, 3 361 (36.2%) underwent pre-treatment attrition. Median(IQR)

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diagnosis delay was 84 (64, 114) days; treatment initation delay was 23(6,68) days and total delay was 117(77,187) days. Long diagnosis delay was an independent predictor of pre-treatment attrition in a dose response relationship. While pre-treatment attrition was less likely among presumptive criterion 'previously treated' and with increasing time period, it was more likey among elderly and in east and west region. While the diagnosis delay increased with time period, treatment initiation delay and total delay reduced with time period. Short diagnosis delay was associated with west region, smear negative patients and presumptive criterion 'treatment after lost to follow up'. Short treatment initiation delay was associatied with east and west regions while long treatment initiation delay was associated with elderly and presumptive criterion 'recurrent TB'. Total delay predictors were similar to treatment initiation delay. In addition, short total delay was associated with presumptive criterion 'treatment after failure'.

Conclusion

The diagnosis and treatment delay were long and the pre-treatment attrition was considerable high. Long diagnosis delay is likely to predict pre-treatment attrition.

Introduction

Multidrug-resistant TB (MDR-TB), defined as resistance to at least isoniazid and rifampicin, along with rifampicin-resistant tuberculosis (RR-TB), is a major public health concern in many countries and threatens global attempts to end TB [1]. Timely identification and prompt treatment initiation of MDR-TB patients are crucial to prevent the transmission of infection and reduce related morbidity and mortality.

Majority of MDR-TB patients are lost in the diagnosis and treatment pathway. Globally in 2017, there were an estimated 558 000 MDR/RR-TB patients, of whom only 160 684 (29%) were diagnosed and 139 114 (25%) were initiated on treatment [1]. Before universal drug susceptibility testing (DST), TB patients that were at high risk for MDR-TB (presumptive MDR-TB, erstwhile known as MDR-TB suspects) were prioritized for culture and DST. High pre-diagnosis attrition (presumptive MDR-TB not undergoing culture and DST—17%~90%) and pre-treatment attrition (confirmed MDR-TB patients not initiated on treatment—24%) have been reported [2–16]. Delay from eligibility for DST to diagnosis (diagnosis delay) among patients with presumptive MDR-TB who underwent DST and from diagnosis to treatment initiation (treatment initiation delay) among the confirmed MDR-TB patients initiated on treatment are also major challenges. Among studies published during 2000–15, weighted mean time to treatment from specimen collection was 81 days: it was 108 with phenotypic DST and 38 days with genotypic (rapid molecular tests) DST [8]. Though this has significantly improved over the years, this time interval is still considerable.

Globally, the treatment success rates for MDR-TB are between 55–56% [1,17]. Diagnosis and treatment initiation delay could potentially result in such low treatment success rates. However, a systematic review in 2016 revealed a lack of published evidence globally regarding the association between early treatment initiation after diagnosis and high treatment success rates [18].

China contributes to 13% of the global MDR/RR-TB patients. Seven percent of new TB patients and 24% of previously treated patients have MDR-TB. In 2017, there were an

estimated 73 000 MDR/RR-TB patients of whom only 13 069(18%) were diagnosed and 5943 (8%) were initiated on treatment [1]. Treatment success rate is poor (40–50%) [1,19]. Median treatment initiation delay after diagnosis in Shanghai (2011–14) was around six months [20]. There is limited information in China regarding diagnosis delay and factors associated with pre-diagnosis and pre-treatment attrition.

With the support of the Global Fund, China initiated a programme for MDR-TB (the Global Fund programmatic management of drug-resistant TB (GFPMDT)) between October 2006 and June 2014 in a phased manner [21]. In this paper, we assessed the attrition and delay in the MDR-TB diagnosis (through phenotypic DST) and treatment pathway and the factors associated.

Methods

Study design and population

This was a cohort study involving record review of programme data. All presumptive pulmonary MDR-TB patients belonging to the GFPMDT sites and eligible for DST between October 2006 and June 2013 were included. By June 2013, the GFPMDT covered 67 prefectures across 24 provinces in China (Table 1 and S1 Annex).

Presumptive MDR-TB were defined as i) previously treated TB patients which included recurrent TB, return after loss to follow up, treatment after failure and ii) new TB patients that were non-converters at three months of treatment or in close contact with a known MDR-TB patient.

Setting

General setting. China, the world's most populous country, is a unitary sovereign state in East Asia with a population of over 1.4 billion [22]. It has three levels of sub-national administrative divisions: 34 provinces, 334 prefectures and 2851 counties. The prevalence of all pulmonary and bacteriological confirmed pulmonary TB among population over 15 years of age was 442/100 000 and 116/100 000 respectively [23]. The incidence rate of TB/HIV is estimated to 0.82/100 000. For all the hospitalized patients, HIV test was done routinely, but not free of charge.

GFPMDT in China. The launching criteria for PMDT sites included good 'directly observed treatment–short course' foundation, sound local government support and willingness to pilot PMDT. Hence, most of the project sites were located in east and middle region where the economic situation was better than the west region.

Table 1. Implementation of GFPMDT project in a phased manner in China from October 2006 to June 2013.

Phase	Period	Newly launched sites*		No. of cumulative sites*	
		Province	Prefecture	Province	Prefecture
Round 5 phase1	Oct 2006-Sep 2008	2	5	2	5
Round 5 phase2	Oct 2008-Sep 2011	4	26	6	31
Round 7	Oct 2008-Sep 2010	6	10	12	41
SSF	Jul 2010-Jun 2013	12	26	24	67

GFPMDT: Global Fund Programmatic Management of drug resistantTuberculosis;SSF: single stream framework

*newly launched and cumulative sites did not included 10 sites launched in 2013 considering the diagnosis and treatment in these sites was not implemented till the end of that year

Genotypic/rapid DST was not introduced into China during GFPMDT project implementation phase.

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Patients presented at county-level health institutions (basic management units (BMUs)), where sputum testing was conducted and drug-resistance risk assessment was initiated. Sputum specimen from presumptive MDR-TB patients were transported to prefecture or provincial-level laboratory (culture DST facility) for culture. For specimen with a positive culture result, the proportion method was used to determine the susceptibility of isolates against rifampicin and isoniazid. There was one laboratory register at country / BMU level for routine sputum microscopy. There was a culture DST register at prefecture level laboratory or provincial-level reference laboratory. The culture DST facility shared the DST results with the BMU. All the demographic and clinical data including laboratory test results were recorded in the presumptive MDR-TB register at the BMU.

Patients diagnosed with MDR-TB were referred to the designated hospital at prefecture-level. Prior to initiating treatment, patients underwent a thorough medical examination. They received a standardized treatment regimen for 24 months that consisted of 6–8 months of intensive phase (Pyrazinamide, Amikacin, Levofloxacin, Cycloserine, Prothionamide) and 18 months of continuation phase (Pyrazinamide, Levofloxacin, Cycloserine, Prothionamide) [24].

Data variables, sources of data and data collection

During January to April 2014, data were collected from the presumptive MDR-TB register at BMU and MDR-TB register at prefecture level designated hospitals. Data on baseline characteristics like province, prefecture, region, age, gender, GFPMDT phase, sputum smear status at TB diagnosis, presumptive MDR-TB criteria, dates of eligibility for DSTand DST result were collected. Among confirmed MDR-TB, date of DST results, treatment initiation (yes/no) and date of treatment initation were extracted. The procedure of data collection and management included the following steps: 1) the local staff from prefectural level filled the questionnaire which was designed at national level; 2) the provincial-level staff conducted data quality assessment and reconciled discrepancies 3) the national-level staff conducted final data review and assessment to eliminate missing data and errors.

Data management and statistical analysis

Data were single-entered in an MS Excel database in December 2014. The dataset was analyzed using STATA (version 12.1, copyright 1985–2011 StataCorp LP USA).

The following three time intervals were calculated: between eligibility for DST and DST results (diagnosis delay), between diagnosis and treatment initiation (treatment initiation delay), and between eligibility for DST and treatment initiation (total delay). Delays were summarized using median (inter-quartile range-IQR). Pre-diagnosis attrition and pre-treatment attrition were summarized using frequency and proportion.

Predictive modelling using log binomial regression was performed to identify risk factors for attrition. While using the diagnosis delay variable as one of the potential factors associated with pre-treatment attrition, the diagnosis delay was categorized based on quartiles. Linear regression was used to determine the factors associated with delays (one model for each delay: diagnosis delay, treatment initiation delay and total delay). In all the multivariable analyses, variables with unadjusted $p<0.2$ were included.

The associations in the log binominal models were summarized using relative risks (unadjusted and adjusted–RR and aRR) and 0.95 confidence interval (CI). The associations in linear regression models were summarized using Beta (β) coefficients and 0.95 CI. The β coefficient indicated the adjusted mean difference of delay (in days) between the sub-category of interest and the reference sub-category (negative value meant the adjusted mean value in the category

of interest was lower than the reference category; positive value meant the adjusted mean value in the category of interest was higher than the reference category).

As we were dealing with very large numbers of presumptive MDR-TB patients, we assessed the programmatic significance of the β coefficients before assessing the statistical significance. Hence, for the risk factor analysis of diagnosis delay, we considered a β coefficient of at least seven days as programmatically significant association.

Ethics approval

The study was approved by the Ethics Committee of Chinese Center for Disease Control and Prevention. (number 201807 dated 9 April 2018) and Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (The Union), Paris, France (EAG number 23/18 dated 17 April 2018). The study was proposed by National center for TB prevention and control, which was approved by the research department of Chinese Center for Disease Control and Prevention. As this study involved analysis of secondary data, waiver of informed consent was sought and approved by the ethics committees.

Results

A total of 78 564 presumptive MDR-TB patients were included. Their mean (standard deviation) age was 48.1(18.2) years, 58 392 (74.3%) were males and 43 717 (55.6%) were from east region. Most (74 493, 96.1%) patients were sputum smear positive and 51 635 (65.7%) were 'new' TB patients eligible for DST (Table 2).

Pre-diagnosis and pre-treatment attrition

Of 78 564, 77 372 (98.5%) underwent culture. Of those who tested culture positive, 99.2% (64 852/65 353) underwent DST. Therefore, among 78 564, a total of 2470 (3.1%) underwent pre-diagnosis attrition (1192 specimens did not reach DST laboratory, 777 specimens were contaminated and 501 specimen did not undergo DST despite being culture positive).

A total of 9283 were diagnosed as MDR-TB and of them 3361 (36.2%) underwent pre-treatment attrition (Fig 1).

Diagnosis and treatment initiation delays

Median(IQR) diagnosis delay was 84 (64, 114) days; treatment initation delay was 23(6,68) days and total delay was 117(77,187) days (Table 3).

Risk factors for pre-treatment attrition

As the pre-diagnosis attrition was very low and not programmatically significant, we are not presenting the risk factor analysis. Pre-treatment attrition was very high. Pre-treatment attrition was significantly i) lower in round 5-phase 2 and round 7 when compared to round 5-phase 1 of GFPMDT phase, ii) higher among elderly when compared to patients in 15–44 year age group, iii) higher in east and west regions when compared to middle region, iv) higher among patients with recurrent TB, treatment after failure of category I/II regimen and previously treated–others, when comared to new TB patients, and v) high among those with long diagnosis delays (dose response relationship seen) (Table 4).

Factors associated with delays

The diagnosis delay increased in all the phases when compared to round 5 phase 1. It was significantly i) lower among patients with negative sptum smear microscopy status when

Table 2. Clinical and demographic profile of presumptive MDR-TB patients and confirmed MDR-TB patients under GFPMDT project in China, October 2006-June 2013 [N = 78564].

	Presumptive MDR-TB		Confirmed MDR-TB	
Variable	**N**	**(%)**	**N**	**(%)**
Total	78564	(100)	9283	(100)
Age (years)				
• <15	134	(0.2)	10	(0.1)
• 15–44	33704	(42.9)	4569	(49.2)
• 45–64	28153	(35.8)	3651	(39.3)
• ≥ 65	16573	(21.1)	1053	(11.3)
Gender				
• Male	58392	(74.3)	6864	(73.9)
• Female	20172	(25.7)	2419	(26.1)
Region				
• East	43717	(55.6)	4229	(45.6)
• Middle	30131	(38.4)	4327	(46.6)
• West	4716	(6.0)	727	(7.8)
GFPMDT phase				
• Round 5 phase1	22724	(28.9)	2627	(28.3)
• Round 5 phase2	27401	(34.9)	2663	(28.7)
• Round 7	22263	(28.3)	2766	(29.8)
• SSF	6176	(7.9)	1227	(13.2)
Sputum smear microscopy status				
• Positive	75493	(96.1)	9062	(97.6)
• Negative	3071	(3.9)	221	(2.4)
Presumptive MDR-TB criteria				
• New patients	51635	(65.7)	2331	(25.1)
• Previously treated				
○ Recurrent TB	13024	(16.6)	2878	(31.0)
○ Loss to follow-up	600	(0.8)	129	(1.4)
○ Treatment after failure of new regimen*	3703	(4.7)	1755	(18.9)
○ Others	6557	(8.3)	1453	(15.7)
○ Treatment after failure of 'previously treated' regimen#	3045	(3.9)	737	(7.9)

GFPMDT: Global Fund Programmatic Management of drug resistanttuberculosis; MDR-TB:Multi drug-resistant tuberculosis, TB:Tuberculosis; SSF: single stream framework

*Category I regimen–TB treatment regimen under national TB programme for newly diagnosed patients;

#Category II regimen–TB treatment under national TB programme for previously treated patients.

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compared to those with positive smear, and ii) lower among previously treated patients who were lost to follow-up when compared to new TB patients (Table 5).

The treatment initiation delay was significantly i) lower in all phases when compared to round 5 phase 1, ii) lower in east and west region when compared to middle region, iii) higher in elderly when compared to patients in 15–44 year age group, and iv) higher among recurrent TB patients when compared to new TB patients (Table 6).

The total delay was significantly i) lower in round 5 phase 2 and single stream framework when compared to round 5 phase 1, ii) lower in east and west region when compared to middle region, iii) higher in elderly and 45–64 year age group when compared to patients in 15–44

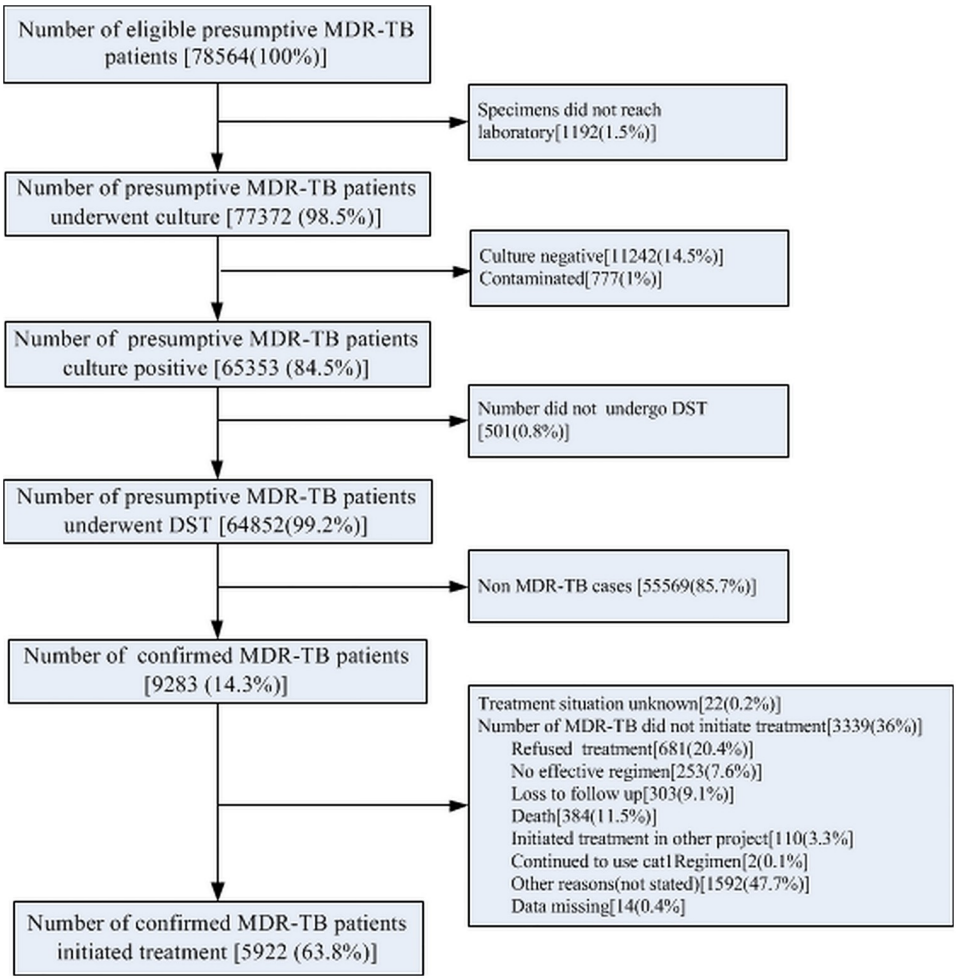

Fig 1. MDR-TB diagnosis and treatment under GFPMDT project in China during October 2006 to December 2013. GFPMDT-Global Fund Programmatic Management of drug resistant tuberculosis;MDR-TB: Multi drug-resistant tuberculosis.

https://doi.org/10.1371/journal.pone.0214943.g001

year age group, and iv) higher among recurrent TB patients and treatment after failure in category I/II patients when compared to new TB patients (Table 7).

Discussion

This is the first study from China to offer many seminal observations on the MDR-TB diagnosis and treatment pathway—a holistic look at flow of a cohort of presumptive MDR-TB patients which includes pretreatment delay calculation from eligibility for DST and the effect of diagnosis delay on pre-treatment attrition. To the best of our knowledge, ours is the first study to fill the 'evidence gap' regarding the association between long diagnosis delay and pre-treatment attrition in the MDR-TB care pathway.

Limitations

We acknowledge some limitations in this study. First, it is likely that not all the presumptive MDR-TB were identified, therefore the selection bias might have led to an underestimation of attrition, especially pre-diagnosis attrition. Second, the treatment outcomes for a significant

Table 3. Time taken (days) for procedures in testing / treatment of presumptive/confirmed MDR-TB patients under GFPMDT project in China, October 2006-June 2013.

Variable	Eligibility–DST [n = 64852]		DST result–treatment initiation [n = 5922]		Eligibility for DST–treatment initiation [n = 5922]	
	Median	(IQR)	Median	(IQR)	Median	(IQR)
Overall median (IQR)	84	(64,114)	23	(6,68)	117	(77,187)
Age (years)						
• <15	83	(68,103)	8	(4,53)	84	(75,114)
• 15–44	81	(62,111)	22	(5,62)	112	(73,180)
• 45–64	86	(65,116)	25	(6,75)	120	(81,191)
• ≥ 65	87	(67,117)	26	(9,73)	127	(85,203)
Gender						
• Male	84	(64,114)	23	(6,68)	117	(78,185)
• Female	83	(63,113)	23	(6,66)	116	(76,190)
Region						
• East	83	(63,115)	22	(6,63)	119	(76,183)
• Middle	87	(66,115)	24	(6,78)	119	(79,196)
• West	75	(56,105)	24	(9,56)	100	(75,154)
GFPMDT phase						
• Round 5 phase1	73	(56,98)	35	(10,86)	123	(82,202)
• Round 5 phase2	88	(68,115)	16	(4,48)	111	(73,171)
• Round 7	93	(70,131)	24	(5,76)	119	(69,205)
• SSF	80	(64,103)	24	(9,59)	116	(87,161)
Sputum smear microscopy status						
• Positive	84	(64,114)	23	(6,67)	117	(77,188)
• Negative	78	(56,105)	32	(12,73)	121	(83,167)
Presumptive MDR-TB criteria						
• New patients	85	(65,113)	23	(8,65)	116	(83,182)
• Previously treated						
○ Recurrent TB	75	(62,104)	30	(8,79)	127	(87,203)
○ Loss to follow-up	80	(59,113)	25	(2,67)	101	(78,174)
○ Treatment after failure of category I regimen*	81	(62,111)	16	(2,53)	103	(60,173)
○ Others	84	(62,118)	27	(7,79)	122	(81,189)
○ Treatment after failure of category II regimen#	85	(65,113)	17	(3,54)	106	(62,165)

TB: Tuberculosis; MDR-TB: Multidrug-resistant tuberculosis; GFPMDT: Global Fund Programmatic Management of drug resistanttuberculosis; IQR–Interquartile range; SSF: single stream framework

*Category I regimen–TB treatment regimen under national TB programme for newly diagnosed patients;

#Category II regimen–TB treatment under national TB programme for previously treated patients.

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cohort of MDR-TB patients was not tracked and collected. Hence, we were not able to study the association between delays and MDR-TB outcomes, evidence for which is limited globally [18]. Third, though this study was unique in calculating the diagnosis delay starting from eligibility for DST, we could not tease out the delay between eligibilty and sputum specimen receipt at DST facility as the date of sputum specimen receipt was not collected. Finally, this being a programme data that is single entered routinely, data entryerrors cannot be ruled out.

Interpretation of key findings

Limitations notwithstanding, there were some key findings. First, the long total delay was majorly contributed by long diagnosis delay (due to the use of phenotypic DST which happens

Table 4. Association of clinical and socio-demographic factors with pre-treatment attrition among patients diagnosed with MDR-TB under GFPMDT project in China, October 2006-June 2013 [N = 9283].

Variable	Total	Attrition					
	[N]	[n]	(%)[@]	RR	(0.95 CI)	aRR[**]	(0.95 CI)
Total	9283	3361	(36.2)				
Age (years)							
• <15	10	3	(30.0)	0.83	(0.21–3.20)	0.56	(0.14–2.26)
• 15–44	4569	1561	(34.2)	1.00	reference	1.00	reference
• 45–64	3651	1255	(34.4)	1.01	(0.92–1.10)	1.05	(0.96–1.16)
• ≥ 65	**1053**	**542**	**(51.5)**	**2.04**	**(1.79–2.34)** ^	**2.21**	**(1.92–2.55)**^
Gender							
• Male	6864	2521	(36.7)	1.09	(0.99–1.20)	1.05	(0.95–1.16)
• Female	2419	840	(34.7)	1.00	reference	1.00	reference
Region							
• East	**4229**	**1598**	**(37.8)**	**1.16**	**(1.07–1.27)** ^	**1.30**	**(1.18–1.42)**^
• Middle	4327	1484	(34.3)	1.00	reference	1.00	reference
• West	**727**	**279**	**(38.4)**	**1.19**	**(1.02–1.40)** ^	**1.34**	**(1.11–1.62)**^
GFPMDT phase							
• Round 5 phase1	2627	1116	(42.5)	1.00	reference	1.00	reference
• Round 5 phase2	**2663**	**766**	**(28.8)**	**0.55**	**(0.49–0.61)** ^	**0.44**	**(0.39–0.50)**^
• Round 7	**2766**	**948**	**(34.3)**	**0.71**	**(0.63–0.79)** ^	**0.64**	**(0.57–0.72)**^
• SSF	1227	531	(43.3)	1.03	(0.90–1.19)	0.88	(0.76–1.03)
Sputum smear microscopy status							
• Positive	9062	3275	(36.1)	1.00	reference	-	-^^
• Negative	221	86	(38.9)	1.13	(0.86–1.48)	-	-
Presumptive MDR-TB criteria							
• New patients	2331	1124	(48.2)	1.00	reference	1.00	reference
• Previously treated							
○ Recurrent TB	**2878**	**984**	**(34.2)**	**0.56**	**(0.50–0.62)** ^	**0.51**	**(0.46–0.58)**^
○ Loss to follow-up	129	65	(50.4)	1.09	(0.77–1.56)	1.13	(0.78–1.63)
○ Treatment after failure of category I regimen[*]	**1755**	**411**	**(23.4)**	**0.33**	**(0.29–0.38)** ^	**0.32**	**(0.28–0.37)** ^
○ Others	**1453**	**547**	**(37.7)**	**0.65**	**(0.57–0.74)** ^	**0.59**	**(0.51–0.68)**^
○ Treatment after failure of category II regimen[#]	**737**	**230**	**(31.2)**	**0.49**	**(0.41–0.58)** ^	**0.48**	**(0.40–0.58)**^
Delay between eligibility for DST to DST result in days							
• 1st quartile (<64)	2272	573	(25.2)	1.00	reference	1.00	reference
• 2nd quartile (64–83)	**2549**	**1003**	**(39.3)**	**1.92**	**(1.70–2.18)** ^	**1.73**	**(1.52–1.96)**^
• 3rd quartile (84–113)	**2396**	**928**	**(38.7)**	**1.87**	**(1.65–2.13)** ^	**1.82**	**(1.60–2.07)**^
• 4th quartile (≥114)	**2066**	**857**	**(41.5)**	**2.10**	**(1.85–2.39)** ^	**2.06**	**(1.80–2.35)**^

GFPMDT-Global Fund Programmatic Management of drug resistant tuberculosis;MDR-TB: Multi drug-resistant tuberculosis, DST: Drug susceptibility testing; RR: reltative risk; aRR: adjusted relative risk, log binomial regression was performed; SSF: single stream framework

[@] row percentage

[*]Category I regimen–TB treatment regimen under national TB programme for newly diagnosed patients;

[#]Category II regimen–TB treatment under national TB programme for previously treated patients

^p<0.05were considered significant difference

[**]log binomial regression, the confounders that were included in the model were age, sex, phase of implementation, region, presumptive MDR-TB criteria and diagnosis delay categorized based on quartiles

^^sputum status not included in the model as unadjusted p value was >0.20

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Table 5. Multivariable linear regression for factors associated with diagnosis delay (between eligibility for DST to DST) among patients with presumptive MDR-TB that underwent DST under GFPMDT project in China, October 2006-June 2013 [N = 68452].

Variables	Beta coefficient**	(95% CI)	p-value
Age (years)			
○ <15	-4.60	(-18.4, 9.21)	0.514
○ 15–44	reference		
○ 45–64	4.79	(3.49, 6.10)	<0.001
○ ≥65	5.62	(4.08, 7.16)	<0.001
Gender			
○ Male	1.57	(0.26, 2.88)	0.019
○ Female	reference		
Region			
○ East	-1.21	(-2.42, -0.01)	0.048
○ Middle	reference		
○ West	**-18.32**	**(-21.17, -15.47)**	**<0.001**^
GFPMDT phase			
○ Round 5 phase1	reference		
○ Round 5 phase2	**14.27**	**(12.8, 15.74)**	**<0.001**^
○ Round 7	**35.73**	**(34.2, 37.26)**	**<0.001**^
○ SSF	**13.24**	**(10.68, 15.79)**	**<0.001**^
Sputum smear microscopy status			
○ Positive	reference		
○ Negative	**-8.91**	**(-12.63, -5.19))**	**<0.001**^
Presumptive MDR-TB criteria			
○ New patients	reference		
○ Previously treated			
○ Recurrent TB	1.28	(-0.29, 2.86)	0.110
○ Loss to follow-up	**-9.38**	**(-15.79, -2.98)**	**<0.001**^
○ Treatment after failure of category I regimen*	-4.13	(-6.86, -1.40)	<0.001
○ Others	5.25	(3.07, 7.43)	<0.001
○ Treatment after failure of category II regimen#	-2.34	(-5.78, 1.11)	0.183

GFPMDT-Global Fund Programmatic Management of drug resistanttuberculosis;MDR-TB:Multi drug-resistant tuberculosis, DST:Drug susceptibility testing; SSF: single stream framework; CI–confidence interval

*Category I regimen–TB treatment regimen under national TB programme for newly diagnosed patients;

#Category II regimen–TB treatment under national TB programme for previously treated patients

**Linear regression, the confounders that were included in the model were age, sex, phase of implementation, region, sputum positivity, and presumptive MDR-TB criteria;

^Due to the large number of patients, adjusted mean difference of at least seven was considered as programmatically significant after whih statistical significance was assessed ($p<0.05$)

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over two rounds: one round for culture of Mycobacterium tuberculosis (6–8 weeks) and another for DST). The total delay was longer than globally reported average figures with phenotypic DST during 2000–15 (117 vs 108 days), probably due to eligbility of DST (not sputum specimen receipt at DST facility) being the starting point for delay calculation [8]. In our study, while the diagnosis delay increased over years, the treatment initiation delay and total delay decreased over the years (2006–13), which might due to the improvement in timely second line drug supply.

Second, the pre-treatment attrition was very high when compared to the global figures of 24% during 2000–15 (reduced to 13% in 2017) [1,8]. Almost half of the patients who

Table 6. Multivariable linear regression for factors associated with treatment initiation delay (between DST and treatment initiation) among bacteriologically-confirmed MDR-TB patients registered for treatment under GFPMDT project in China, October 2006-June 2013 [N = 5922].

Variables	Beta coefficient**	(95% CI)	p-value
Age (years)			
○ <15	-26.71	(-119.88, 66.46)	0.574
○ 15–44	reference		
○ 45–64	5.49	(-1.40, 12.38)	0.120
○ ≥65	**17.26**	**(5.32, 29.19)**	**<0.010^**
Gender			
○ Male	-0.07	(-7.42, 7.28)	0.985
○ Female	reference		
Region			
○ East	**-23.36**	**(-30.16, -16.55)**	**<0.001^**
○ Middle	Reference		
○ West	**-22.36**	**(-36.38, -8.33)**	**<0.001^**
GFPMDT phase			
○ Round 5 phase1	reference		
○ Round 5 phase2	**-35.71**	**(-44.29, -27.14)**	**<0.001^**
○ Round 7	**-14.59**	**(-23.38, -5.79)**	**<0.001^**
○ SSF	**-37.43**	**(-49.65, -25.21)**	**<0.001^**
Sputum smear microscopy status			
○ Positive	reference		
○ Negative	4.92	(-17.04, 26.88)	0.661
Presumptive MDR-TB criteria			
○ New patients	reference		
○ Previously treated			
○ Recurrent TB	**12.90**	**(3.79, 22.01)**	**0.001^**
○ Loss to follow up	7.51	(-24.13, 39.14)	0.642
○ Treatment after failure of category I regimen*	-5.01	(-14.90, 4.87)	0.320
○ Others	5.55	(-5.39, 16.50)	0.320
○ Treatment after failure of category II regimen#	-7.34	(-20.49, 5.81)	0.274

GFPMDT-Global Fund Programmatic Management of drug resistanttuberculosis;MDR-TB:Multi drug-resistant tuberculosis, DST:Drug susceptibility testing; SSF: single stream framework; CI–confidence interval

*Category I regimen–TB treatment regimen under national TB programme for newly diagnosed patients;

#Category II regimen–TB treatment under national TB programme for previously treated patients

**Linear regression, the confounders that were included in the model were age, sex, phase of implementation, region, sputum positivity, and presumptive MDR-TB criteria;

^p<0.05 were considered significant difference

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underwent pre-treatment attrition either refused treatment or were lost to follow-up or died (Fig 1). Patients refused treatment possibly because of a long two month period of hospitalization during treatment at prefecture level. Shortage of second line drugs due to the long turn-around time of international procurement was a possible reason for pre-treatment attrition during the initial phases.

Third, long diagnosis delay contributed to pre-treatment attrition possibly due to patients being very sick at diagnosis to move to a prefecture level facility.

Finally, within the project, around two-thirds of the presumptive MDR-TB patients were new TB patients. In India, majority of the patients were previously treated patients and non-identification of presumptive MDR-TB contributed largely to pre-diagnosis attrition

Table 7. Multivariable linear regression for factors associated with total delay (between eligibility for DST and treatment initiation) among bacteriologically-confirmed MDR-TB patients registered for treatment under GFPMDT project in China, October 2006-June 2013 [N = 5922].

Variables	Beta coefficient**	(95% CI)	p-value
Age (years)			
○ <15	-44.70	(-154.42, 65.03)	0.425
○ 15–44	reference		
○ 45–64	**11.27**	**(3.16, 19.39)**	**0.001**^
○ ≥65	**33.30**	**(19.24, 47.35)**	**<0.001**^
Gender			
○ Male	2.90	(-5.76, 11.55)	0.51 2
○ Female	reference		
Region			
○ East	**-16.45**	**(-24.47, -8.43)**	**<0.001**^
○ Middle	reference		
○ West	**-27.93**	**(-44.44, -11.41)**	**<0.001**^
GFPMDT phase			
○ Round 5 phase1	reference		
○ Round 5 phase2	**-38.78**	**(-48.88, -28.68)**	**<0.001**^
○ Round 7	-6.71	(-17.07, 3.65)	0.200
○ SSF	**-33.32**	**(-47.71, -18.93))**	**<0.001**^
Sputum smear microscopy status			
○ Positive	reference		
○ Negative	8.62	(-17.25, 34.48)	0.51 4
Presumptive MDR-TB criteria			
○ New patients	reference		
○ Previously treated			
○ Recurrent TB	**14.90**	**(4.17, 25.63)**	**0.001**^
○ Lossto follow-up	-6.58	(-44.83, 30.68)	0.729
○ Treatment after failure of category I regimen*	**-16.13**	**(-27.77, -4.49))**	**0.001**^
○ Others	4.59	(-8.30, 17.48)	0.485
○ Treatment after failure of category II regimen#	**-19.22**	**(-34.70, -3.73)**	**0.015**^

GFPMDT-Global Fund Programmatic Management of drug resistanttuberculosis;MDR-TB:Multi drug-resistant tuberculosis, DST:Drug susceptibility testing; SSF: single stream framework; CI–confidence interval

*Category I regimen–TB treatment regimen under national TB programme for newly diagnosed patients;

#Category II regimen–TB treatment under national TB programme for previously treated patients

**Linear regression, the confounders that were included in the model were age, sex, phase of implementation, region, sputum positivity, and presumptive MDR-TB criteria;

^p<0.05 were considered significant difference

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[4–7,15,16]. In our study, though the pre-diagnosis attrition among identified presumptive patients was low, non-identification of large numbers of previously treated patients as presumptive MDR-TB cannot be ruled out. This finding also corroborates with overall low MDR-TB detection rates (8%) as a proportion of estimated MDR-TB in China in 2013 [19].

Implications for policy and practice

There are important implications for China. First, though diagnosis delay was increasing over time period, we expect that by the introduction of rapid molecular DST, we should be able to reduce this delay which will in turn reduce pre-treatment attrition, the total delay and

potentially result in better outcomes. China has recently updated the national TB guidelines (end of 2018). Along with the world health organization guidelines [25], this study provides evidence base to expand the use of rapid molecular tests. China now plans to expand the availability of rapid molecular tests at BMU level in at least 80% counties by 2020. In addition to the high risk group described in this study, the national programme plans to expand its use to all sputum positive pulmonary TB patients.

Second, to reduce the pre-treatment attrition and treatment initiation delays, we recommend decentralized MDR-TB treatment. This may be tried at least for those patients who are not ill at diagnosis [16]. A community-based MDR-TB care model may be tried to improve treatment initiation as reported in Myanmar [26]. There is a need to move towards reducing the mandatory inpatient care for two months which could be a potential barrier to take treatment. Shorter treatment regimen may be further tried in a select group of patients The shorter regimens have shown high treatment success rates in operational settings [27].

Third, low contribution of previously treated patients in the presumptive MDR-TB cohort indicates that there is a critical need to assess this situation nationally in China. The national estimates of presumptive MDR-TB patients based on the presumptive criteria and the number of TB patients that actually undergo culture and phenotypic DST and/or rapid molecular testing (using national TB surveillance data) may be compared.

Finally, a qualitative systematic enquiry is recommended to understand why certain risk groups are more prone to experience attrition and longer delays [28].

Conclusion

In this MDR-TB care pathway from the Global Fund PMDT project in China (2006–13), we holistically documented the attrition, delays and their associated factors. Not only does this study emphasize the importance of early treatment initiation but also brings the focus on early diagnosis by testing patients as soon as they are eligible which can reduce pre-treatment attrition as well as potentially improve treatment outcomes. As China prepares to expand the coverage of rapid molecular DST at the level of counties, similar studies are recommended in future to monitor the reduction of delay and attrition along the MDR-TB care pathway, and the effect of pre-treatment delays (starting from DST eligibility) on treatment outcomes. This is vital if we are to end TB in China and globally by 2035 [29].

Supporting information

S1 Annex. Timeline and strategies under China Global Fund programmatic management of drug-resistant tuberculosis programme scale-up (2006–13).
(TIF)

S2 Annex. Dataset containing data and codebook.
(XLSX)

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Topical Review

Current Treatment of Osteoporosis

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Abstract

Osteoporosis is ever increasing as life expectancy continues to increase across the world. Hypovitaminosis D has been found to be prevalent even in children and adults, and hence, it is imperative to educate the public on the nutrition for bone health. "Love your bones and joints" was the slogan by the World Health Organization to increase the awareness among the public. Bone density is assessed by Dual Energy X-ray Absorptiometry scan and the T score system. Although biomarkers have been studied in research, their clinical utility is still elusive. Regular exercise and adequate intake of Calcium and Vitamin D are important to maintain bone health. Bisphosphonates are the first line drugs in the management of osteoporosis both for primary and secondary prophylaxis. Second-line drugs include denosumab, teriparatide, and newer drugs such as abaloparatide, romosozumab, and calcitonin, which have found more real-life acceptance and efficacious in the long-term management of osteoporosis. Romosozumab, a monoclonal antibody may well become the ideal osteoporosis drug with effects on bone formation and resorption. Surgical treatment choices include – Vertebroplasty and kyphoplasty are being accepted in specific instances and selected centers with variable success.

Key Words: *Abaloparatide, bisphosphonate, denosumab, osteoporosis, teriparatide*

Introduction

Osteoporosis is a disease characterized by low bone mass with microarchitectural deterioration of bone leading to fragile bones and fractures. In India, life expectancy is 67 years and is expected to increase to 71 years by 2025.[1] In 2013, it was estimated that 50 million people in India are either osteoporotic (T-score < −2.5) or have low bone mass (T-score between −1.0 and −2.5).[2] Longitudinal studies of changes in bone mass during growth have confirmed that in girls, the greatest increases in bone mass occur between the ages of 12–15 years, compared with 14–17 years in boys.[3] Hence, children should be targeted first to raise awareness and also increase Vitamin D intake.

The awareness of osteoporosis is low in India with surveys indicating that only 10%–15% is aware of the disease. Urbanization appears to be associated with an increase in the prevalence of osteoporosis due to lifestyle changes, lower physical activity, increase in indoor living, and lower sun exposure. The 1-year mortality after hip fractures is high at 30% in the public hospitals. For both genders, exercise was equally positively associated with Bone Mineral Density (BMD).[4]

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Diagnosis

The standard battery of tests for evaluating osteoporosis includes serum calcium, renal functions, 25 hydroxy Vitamin D3 levels, thyroid function tests, parathyroid hormone (PTH) levels, and alkaline phosphatase. Dual Energy X-ray Absorptiometry scan to estimate the BMD (in the lumbar spine and femoral hip) is the standard investigation for osteoporosis. The World Health Organization (WHO) guidelines on T score and Z score and Fracture risk assessment using the Fracture Risk Assessment Tool (FRAX) tool are recommended to identify high-risk patients. Drug treatment should last for 5 years and thereafter based on the risks. The decision to treat should be taken based on the risks, FRAX tools, patient preference, and benefits. Women with previous major osteoporotic fracture, those who fracture on therapy or others at high risk should generally continue therapy for up to 10 years (oral) or 6 years (intravenous), with periodic risk-benefit evaluation.

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Current practice

All patients taking corticosteroids should be considered for osteoporosis prevention irrespective of the dose and duration of steroids and be given Calcium, and Vitamin D. Patients who have associated risk factors, preexisting bone loss and who are on corticosteroids for longer duration should be considered for antiresorptive therapy.[5]

Although India is a tropical country with abundant sunlight, evidence points to increasing incidence of Vitamin D deficiency, the reasons for which are multifactorial such as traditional clothing (saris, jeans, and salwar kameezes), inadequate dietary intake, poor Vitamin D fortification of food, and highly pigmented skin.[6] Vitamin D deficiency results in ineffective calcium absorption from the gut, which in turn affects the mineralization of bones.[7]

Evidence suggests pharmacologic therapy to be considered based on risk assessment either using FRAX calculator (country specific) or National Osteoporosis Foundation guidelines. The WHO in 1994 and 2004 review, recommends treatment using T scores which is well known. However, the decision to treat rests on the treating doctor and the patients risk and benefits of treatment.[8]

Although bisphosphonates are still the 1st line, newer drugs are increasingly accepted to be the part of the therapeutic armamentarium. Common drugs include alendronate, ibandronate, Risedronate, and parenteral zoledronic acid (ZOL). Rarely osteonecrosis of jaw has been reported in patients with the use of bisphosphonates and denosumab; the incidence is estimated to be 0.001%–0.01%, marginally higher than in the general population.[9]

Teriparatide subcutaneous (sc) injection daily for 18–24 months[10] or denosumab sc injection once in 6 months[11] have proven to be an effective treatment in improving BMD and reducing fracture risk.

Recent advances

In postmenopausal women with osteoporosis previously treated with oral bisphosphonates, denosumab was associated with greater BMD increases at all measured skeletal sites and greater inhibition of bone remodeling compared with ZOL acid.[11]

Tsai *et al*. in the DATA-HRpQCT study (Denosumab and Teriparatide Administration-high resolution QCT) found that 2 years of combined teriparatide and denosumab improves bone microarchitecture more than the individual treatments, particularly in cortical bone and hence beneficial in postmenopausal osteoporosis.[11] The use of abaloparatide sc for 18 months followed by Alendronate for 6 months improved BMD and reduced fracture risk throughout the skeleton and may be an effective treatment option for osteoporosis.[12]

When teriparatide and denosumab are discontinued following treatment, BMD abruptly decreases. The DATA Switch and DATA follow-up studies showed that in the 22 women not receiving follow-up therapy, femoral neck, total hip, and spine BMD decreased by −4.2 ± 4.3%, −4.5 ± 3.6%, and −10.0 ± 5.4%, respectively, while BMD was maintained in those who did receive follow-up antiresorptive drugs (femoral neck, total hip, and spine BMD changes of −0.6 ± 2.7%, −0.8 ± 3.1%, and −1.2 ± 4.7%, respectively, $P < 0.001$ for all between-group comparisons). The benefit with 4 years of intensive therapy was maintained in patients who received prompt antiresorptive therapy but not in those untreated. These results underscore the importance of timely medication transitions.[13]

Romosozumab is a monoclonal antibody that binds to and inhibits sclerostin, increases bone formation, and decreases bone resorption. Studies with romosozumab (originally known as AMG 785/CDP7851) in healthy men and women demonstrated a brisk increase in biochemical indices of bone formation accompanied by a decrease in markers of bone resorption.[14] These divergent effects of romosozumab on bone formation and bone resorption are very distinct from the antiremodeling agents.

In a study by Saag *et al*. the risk of nonvertebral fractures was lower by 19% in the romosozumab-to-alendronate group than in the alendronate-to-alendronate group (178 of 2046 patients [8.7%] vs. 217 of 2047 patients [10.6%]; $P = 0.04$), and the risk of hip fracture was lower by 38% (41 of 2046 patients [2.0%] vs. 66 of 2047 patients [3.2%]. They concluded that romosozumab treatment for 12 months followed by alendronate resulted in a significantly lower risk of fracture than alendronate alone.[15]

In a study by Cosman *et al*., patients were randomly assigned to receive sc injections of romosozumab (at a dose of 210 mg) or placebo monthly for 12 months; thereafter, patients in each group received denosumab (60 mg sc every 6 months) for 12 months. At 12 months, new vertebral fractures had occurred in 16 of 3321 patients (0.5%). In the romosozumab group, as compared with 59 of 3322 (1.8%) in the placebo group (representing a 73% lower risk with romosozumab; $P < 0.001$). At 24 months, the rates of vertebral fractures were significantly lower in the romosozumab group than in the placebo.[16]

The higher rate of serious cardiovascular adverse events in the romosozumab group raises concern that romosozumab may have a negative effect on vascular tissue.[16] Sclerostin is expressed in vascular smooth muscle and upregulated at sites of vascular calcification. Sclerostin inhibits bone formation by inhibiting the osteoblasts and increases bone resorption by increasing the production of receptor activator of nuclear factor kappa-β-ligand by the osteocytes.[17] Further studies would shed more light on this.

Updated Management Recommendations

Life style

Regular exercise and muscle strengthening activities play an important role in keeping the bone health and reducing the risk of falls in the elderly. Smoking cessation will improve the bone health.[18]

Anticatabolic drugs

Adequate calcium and Vitamin D is essential. The use of drugs, such as Bisphosphonates, hormone replacement therapy, estrogen agonists, calcitonin, PTH, and denosumab, are decided as per the affordability and availability of treatment options. Major gaps still remain in the diagnosis and management of osteoporosis, thus highlighting the need for more research.[6]

Most of the drugs licensed for osteoporosis have very good effect on vertebral fracture risk reduction; however, some of them such as calcitonin, teriparatide, and ibandronate have less impact on hip fractures [Table 1] Based on the current evidence following observations can be made;

1. Bisphosphonates remain the first drug of choice and parenteral ZOL acid scores over others as it is once yearly with better compliance and good for patients with gastroesophageal reflux disease[19]
2. Second line would be either denosumab every 6 months or teriparatide for 18 months followed by bisphosphonates to reduce fracture incidence and enable better bone healing
3. Abaloparatide and romosozumab are options for future
4. Romosozumab may well become the ideal osteoporosis drug with effects on both pathways.

Surgery

Vertebroplasty or kyphoplasty is still effective in selected patients. Vertebral augmentation or vertebroplasty is effective for patients with subacute pain, focal tenderness, and edema on MRI concordant with the fracture.[20] During the last 10 years, increasing evidence points to the success of vertebroplasty and NICE (UK) have accepted in selected patients. However, Cochrane review by Buchbinder *et al*. does not support vertebroplasty in routine practice."[21]

Table 1: Currently licensed drugs for osteoporosis and effects on fractures

Drugs	Vertebral fractures	Nonvertebral fractures	Hip fractures	Risk of ONJ
Alendronate	+	+	+	Rare
Ibandronate	+	+	=	Rare
Risedronate	+	+	+	Rare
Zoledronic acid	+	+	+	Few reported
Calcitonin	+	-	-	None
Teriparatide	+	+	-	None
Abaloparatide	+	+	+	Not aware
Denosumab	+	+	+	Very few
Romosozumab	+	+	+	Not aware

ONJ: Osteonecrosis of jaw, +: Beneficial effect, -: No effect, =: Equivocal

Conclusion

Bone health can be maintained by achieving peak bone mass in adolescence, maintaining strong bone with Vitamin D and prevention of bone loss by aerobic and other exercises.

Evidence suggests the emergence of new anabolic drugs and biologics with the sequential approach of drug treatment[22] to prevent fractures and improve long-term health. The development of drugs such as abaloparatide and romosozumab will add further to the therapeutic armamentarium.

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Conflicts of interest

There are no conflicts of interest.

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ORIGINAL ARTICLE

Complications and Management of Paraovarian Cyst: A Retrospective Analysis

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Abstract

Introduction Despite their relative frequency, paraovarian cyst received only scant attention. Clinician should be aware of the complications of paraovarian cyst.

Objective To analyse the clinical profile, complications and management of paraovarian cyst.

Materials and Methods Retrospective analysis of 51 patients with operative diagnosis of paraovarian cyst was carried out at our institution over a 5-year period.

Results Majority (60.78%) of paraovarian cysts were found in the third and fourth decades, and the mean age of the patients was 31.8 years. 62.74% patients with paraovarian cyst presented with abdominal pain, and the rest were an incidental finding. Ultrasound made a correct diagnosis in 47.05% of patients. Mean size of paraovarian cyst was 7.51 cm. Complications of paraovarian cyst noted in our study are cyst enlargement (79.62%), adnexal torsion (18.51%), haemorrhage (7.4%), rupture (1.85%) and benign tumour (12.96%). 84.31% paraovarian cysts were managed by laparoscopy. Fertility-sparing surgery was done in 57.39% of paraovarian cysts.

Conclusion Paraovarian cyst should be considered in the differential diagnosis of adnexal mass. The importance of differentiating it from ovarian cyst cannot be

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overemphasized. Laparoscopic approach and preferably a fertility-sparing surgery should be considered in the management of complications of paraovarian cyst.

Keywords Paraovarian cyst · Complications · Laparoscopy

Introduction

Paraovarian cyst needs to be differentiated from ovarian cyst, as it is not thought to behave in the same way both clinically and biologically. Paraovarian cyst accounts for only 5–20% of all adnexal masses. Paraovarian cyst originates in the broad ligament between the fallopian tube and ovary. The terms paraovarian cyst and paratubal cyst are used interchangeably depending on their proximity to ovary or fallopian tube. They develop either from the mesothelium of broad ligament (68%) or from paramesonephric (30%) or mesonephric remnants (2%). Their exact incidence is not known due to their frequent asymptomatic presence. They are identified in 15.7% of patients undergoing operative laparoscopy. Paraovarian cyst draws clinical attention in the event of complications like cyst enlargement, torsion, rupture, haemorrhage and neoplasm [1, 2].

As there are no clear guidelines for the management of paraovarian cyst and its complications, this study aims to analyse the clinical profile, complications and management of paraovarian cyst.

Materials and Methods

Fifty-one patients with operative diagnosis of paraovarian cyst over a 5-year period from 2012 to 2017 were identified from the medical records of our institution. Age, menarche, marital, menopausal status and the clinical presentation details of the patients were noted from the admission record. Ultrasound and CT/MRI diagnosis and findings in terms of laterality, size, echogenicity, septation and papillary projection were noted.

From the operative records indication for surgery, type of operative procedure, operative findings and complications like adnexal torsion, paraovarian cyst haemorrhage and rupture were obtained. Histopathology reports of these patients were collected. This data was analysed.

Results

Table 1 shows the clinical profile, imaging and complications of paraovarian cyst.

Mean age of the patients in our study was 31.8 years (range 13–72 years), and most (60.78%) of them were in the reproductive age group. About two-third of the patients presented with abdominal pain, and the rest were found incidentally on imaging or surgery done for other reasons. Abdominal pain in paraovarian cyst was due to cyst enlargement in 17 patients, adnexal torsion was noted in 10 patients, haemorrhage was noted in 4 patients and cyst rupture was noted in a patient. Of the cases of incidental paraovarian cyst, 10 cases were identified during evaluation of menstrual irregularity, 4 cases at infertility workup, 1 case at health check-up, 2 cases at Caesarean section and 1 case at puerperal sterilization. Two paraovarian cysts, each found at Caesarean section and puerperal sterilization, were earlier managed by cyst aspiration in first trimester.

Ultrasound made a correct diagnosis in less than half of the patients. Twenty-three patients were misdiagnosed as ovarian cyst and 4 patients as hydrosalpinx. Even MRI made a correct diagnosis only in half of the patients. Paraovarian cyst was more common on the right side, and few were bilateral. Complications of paraovarian cyst noted in our study are cyst enlargement (79.62%), adnexal torsion (18.51%), haemorrhage (7.4%), rupture (1.85%) and benign tumour (12.96%).

Among the 51 patients with 54 paraovarian cysts, 20.34% were $\leq$ 5 cm, 66.66% were 6–10 cm in size, and 12.96% were $>$ 10 cm. The mean cyst diameter in our study was 7.51 cm (range 3–18 cm). As shown in Table 2, out of the 11 paraovarian cysts of $\leq$ 5 cm size, 72.72% were found incidentally. Among the 36 paraovarian cysts of 6–10 cm size, 72.2% presented with abdominal pain. Of the 7 paraovarian cysts of $>$ 10 cm size, 71.4% presented with abdominal pain.

Table 3 shows 10 paraovarian cysts had undergone adnexal torsion. Fifty per cent cases of torsion were noted in reproductive age women. Sixty per cent of torsion occurred in the cyst of 6–10 cm size. Torsion was more common on the right side (3:2). Only 40% of them were managed by detorsion and paraovarian cystectomy.

Among the 54 paraovarian cysts, 47 were simple cyst, 7 were benign paraovarian tumour, and none were malignant paraovarian tumour. As shown in Table 4, among the 6 patients of multiloculated cysts, 5 were simple paraovarian cyst and 1 was a benign tumour. Out of 6 patients of echogenic cysts, 4 were simple haemorrhagic paraovarian cyst and 2 were benign tumour. All the 4 paraovarian cysts with papillary projection turned out to be a benign tumour. Five paraovarian tumours were managed by laparoscopic cystectomy and 2 each by laparoscopic and open adnexectomy. Histological type of the benign tumour was serous cystadenoma in 5 patients and serous cystadenofibroma in 2 patients of paraovarian cyst.

Management (approach and type of surgery) of paraovarian cyst in our study was based on the age of the patient,

Table 1 Clinical profile, imaging and complications of paraovarian cyst

Characteristics of paraovarian cyst	Absolute value	Frequency (%)
Age group		
Adolescent	10	19.6
Reproductive	31	60.78
Perimenopause	6	11.76
Menopause	4	7.84
Clinical presentation		
Abdominal pain	32	62.74
Incidental	19	37.25
Ultrasound diagnosis		
Correct diagnosis	24	47.05
Wrong diagnosis	27	52.94
MRI done in	12	
Correct diagnosis	7	58.33
Wrong diagnosis	5	47.05
Laterality		
Right	29	56.86
Left	19	37.25
Bilateral	3	5.88
Complications		
Cyst enlargement	43	79.62
Torsion	10	18.51
Haemorrhage	4	7.4
Rupture	1	1.85
Benign tumour	7	12.96

Table 2 Analysis of paraovarian cyst of varying size

Characteristic of paraovarian cyst	Cyst size $\leq$ 5 cm (n = 11)	Cyst size 6–10 cm (n = 36)	Cyst size $>$ 10 cm (n = 7)
Age			
Adolescent	–	9	3
Reproductive age	6	22	3
Perimenopause	4	2	1
Menopause	1	3	–
Presentation			
Abdominal pain	3	26	5
Incidental	8	10	2
Complications			
Torsion	2	6	2
Haemorrhage	2	2	–
Rupture	–	1	–
Benign tumour	2	5	–

Table 3 Analysis of torsion of paraovarian cyst

Characteristic of paraovarian cyst	Torsion (n = 10)
Age group	
Adolescent	3
Reproductive	5
Perimenopause	0
Menopause	2
Cyst size (cm)	
$\leq$ 5	2
6–10	6
$>$ 10	2
Laterality	
Right	6
Left	4
Bilateral	–
Operative procedure	
Laparoscopy	
Paraovarian cystectomy	4
Salpingectomy and paraovarian cystectomy	2
Adnexectomy	2
Open	
Adnexectomy	2

Table 4 Analysis of neoplastic paraovarian cyst

Characteristic of Paraovarian cyst	Benign tumour (n = 7)	Simple cyst (n = 47)
Age group		
Adolescent	–	12
Reproductive	6	25
Perimenopause	1	6
Menopausal	–	4
Clinical presentation		
Abdominal pain	3	32
Incidental	4	15
Cyst size (cm)		
$\leq$ 5	2	9
6–10	5	31
$>$ 10	–	7
Content		
Clear	5	43
Echo	2	4
Septation		
Absent	6	42
Present	1	5
Papillae		
Present	4	–
Absent	3	47

Table 5 Analysis of management of paraovarian cyst

Management of paraovarian cyst	Absolute value	Frequency (%)
Surgical approach		
Laparoscopy	43	84.31
Open	7	13.72
Vaginal	1	1.96
Operative procedure		
Laparoscopy		
Paraovarian cystectomy	26	48.14
Salpingectomy and Paraovarian cystectomy	8	14.81
Adnexectomy	11	20.37
Open		
Paraovarian cystectomy	4	7.4
Salpingectomy and paraovarian cystectomy	–	
Adnexectomy	4	7.4
Vaginal		
Paraovarian cystectomy	1	1.85

clinical presentation, size and complications of paraovarian cyst, associated pathology and desire for future fertility. As shown in Table 5, 84.3% of patients were managed by laparoscopy, 13.72% patients by open surgery and 1.96% patients by vaginal route. 57.39% patients of paraovarian cyst were managed by Paraovarian cystectomy, 14.81% patients were managed by salpingectomy and paraovarian cystectomy, and 27.77% patients were managed by adnexectomy.

Discussion

Paraovarian cyst can occur in any age group from neonate to menopause [3]. Risk factors for the development of paraovarian cyst are being studied. Association between obesity and paraovarian cyst has been identified [4, 5]. Paraovarian cyst has been suspected to play a role in infertility and ectopic pregnancy by disturbing tubal motility and by narrowing the tubal lumen.

Enlargement of paraovarian cyst is due to the cystic dilatation of tubal-type lining epithelium. Increase in size of the cyst at the post-pubertal period and during pregnancy suggests hormonal influence on paraovarian cyst growth, but direct link has not been clearly demonstrated. Unlike the ovarian cyst, they are non-physiological and do not respond to hormones. Paraovarian cyst enlargement presents with chronic abdominal pain or as an abdominal or adnexal mass. Clinically, paraovarian cyst is indistinguishable from ovarian cyst. Even on ultrasound, it is difficult to differentiate it from the ovarian cyst.

Adnexal torsion is higher in paraovarian cyst than in ovarian cyst (2.1–16% vs. 2.3%). As the paraovarian cyst has no pedicle on its own, it torts along with ovary or fallopian tube or both. Adnexal torsion is more common on the right side (3:1). So, it is often misdiagnosed as appendicitis and ureteric colic, and the patient may be admitted under surgery department. As it is a surgical emergency, high index of suspicion is essential.

Paraovarian cysts of neoplastic origin are usually underreported. Although malignant paraovarian tumours are very rare and only few cases have been reported in the literature, benign paraovarian tumours are not uncommon. Histological types of the benign paraovarian tumour are serous cystadenoma, papillary serous cystadenoma, serous cystadenofibroma, mucinous cystadenoma and endometroid cystadenoma.

Diagnosis of paraovarian cyst by ultrasound needs greater awareness and accuracy [6]. Findings in ultrasound are well-defined oval or round cyst located close but separate from the ipsilateral ovary, the absence of surrounding follicle and demonstration of split sign. Split sign is the slight opposite oscillatory movement between the cyst and ovary while pushed by the endovaginal probe. Differential diagnosis by ultrasound is ovarian cyst, hydrosalphinx and peritoneal inclusion cyst.

For a more definitive diagnosis, MRI can be done, but the cost is very high and the diagnostic accuracy is still uncertain. In MRI, paraovarian cyst appears as a homogenous mass that lies between the uterus and round ligament but separate from the ipsilateral ovary. Characterization of the cyst by ultrasound helps to differentiate a simple from neoplastic paraovarian cyst. Echogenic paraovarian cyst can either be a haemorrhage or neoplasm. The presence of papillary projection should arouse the suspicion of neoplasm. Apart from the clinical picture, Whirlpool sign in ultrasound helps to diagnose adnexal torsion in paraovarian cyst [7]. Use of Doppler in such situation is not mandatory as the flow can still be normal with partial torsion, leading to delay in management [8].

Management of paraovarian cyst depends on the age, presence and severity of symptoms, cyst size and its complications [9]. Till now, management is just an extrapolation from the ovarian cyst. But unlike the ovarian cyst, they are non-physiological and cannot be expected to resolve in similar fashion; they are more prone for adnexal torsion, and regardless of its size, tumours have been reported [10]. No society has come up with the strict numerical criterion to decide paraovarian cyst of up to which size can be managed expectantly. There is no role for hormonal treatment in paraovarian cyst. Paraovarian cysts found incidentally while surgery needs excision irrespective of its size to avoid possible complications.

Laparoscopic approach is preferred. Intraoperative diagnosis of paraovarian cyst is done by its location and in difficult situations like dense adhesions identification is by the characteristic crossing of vessels over the surface of the cyst. Laparoscopic paraovarian cystectomy is technically easy and less time-consuming than the ovarian cyst and is feasible in almost all cases.

The presence of adnexal torsion in paraovarian cyst should not deter one from doing detorsion and paraovarian cystectomy with adnexal preservation to ensure future fertility and gonadal function [11]. Irrespective of the gross appearance, safety and full functional recovery of the adnexa after detorsion have been well studied.

Age, menopausal status, clinical presentation, cyst size, septation and CA 125 are poor index of neoplasm. Hence, they are not useful in differentiating a simple paraovarian cyst from neoplasm. So, in the presence of papillary projection, caution should be taken at the time of surgery with the use of endobag to prevent intraperitoneal spillage and frozen section analysis [12]. Benign paraovarian tumours are managed by paraovarian cystectomy. Rarity of malignant paraovarian tumour makes its management particularly challenging, and there is no consensus on optimal management. Patients with malignant paraovarian tumour and borderline paraovarian tumour without desire for fertility are managed like malignant ovarian tumour [13]. Borderline paraovarian tumour patients with desire for future fertility can be managed with comprehensive staging and adnexectomy as they usually behave in a benign fashion [14].

Conclusion

Optimal management of adnexal mass requires exact knowledge of nature of the mass. Paraovarian cyst should be considered in the differential diagnosis of adnexal mass both by the clinician and by the radiologist. The importance of differentiating paraovarian cyst from ovarian cyst cannot be overemphasized.

The role of expectant management in paraovarian cyst needs to be studied. Laparoscopic approach is feasible in all cases. Fertility-sparing surgery should be considered in every case. Laparoscopic paraovarian cystectomy is technically easier and not time-consuming.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Statement This study was approved by the institutional ethical committee. Since this study is a retrospective analysis, no ethical issues are involved.

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Original Article

Amlodipine alters hemorheological parameters: Increased efficacy at the cost of edema?

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ABSTRACT

Background: Despite several decades of use of calcium channel blockers, the side effect of edema persists as a class effect, and its mechanism is unresolved. Amlodipine has effects on hemorheology (HR), and its hemodilutory property may partly contribute to its antihypertensive action. This aspect is not well studied, and the literature is sparse in this regard.

Objective: This experiment was planned to determine effect of a single-dose administration of amlodipine on HR parameters in normal human volunteers.

Methods and results: Amlodipine (5 mg) or S (-) amlodipine (2.5 mg) was administered to 27 normal human volunteers. Whole-blood viscosity (WBV) at different shear rates, plasma viscosity (PV), red cell rigidity (RCR), red cell aggregation (RCA), hematocrit (Hct), plasma hemoglobin, along with plasma drug concentration were determined at time intervals, $t = 0$, 4, 8, 12, and 24 h. Statistically significant reductions were observed at $t_{max} = 4$ h in WBV at shear rates of 0.512 s^{-1} ($p < 0.005$), WBV at shear rates of 5.26 s^{-1} ($p < 0.01$), PV ($p < 0.05$), and Hct ($p < 0.01$). At $t = 8$ h, as drug concentration reduced, some of the changes persisted and later slowly decreased with the decreasing drug concentration till $t = 24$ h. Red blood cell–related parameters such as RCA and RCR remained unaltered. WBV values at all shear rates, when corrected for Hct = 0.45, did not show deviation from their original values at any time.

Conclusions: Amlodipine causes a reduction in Hct and blood viscosity, along with hemodilution. These effects persist as long as the drug remains in plasma. Edema resulting from chronic dosing may be explained by the aforementioned effects. It is possible that antihypertensive action of the drug may be due to a combination of vasodilatation and an improvement in the HR properties.

1. Introduction

Clinical hemorheology (HR) studies in the past three decades have proved that hypertension (HT) is accompanied or preceded by an abnormal enhancement in one or more of the HR parameters such as whole-blood viscosity at low rate (WBV_l) or whole-blood viscosity at high shear rate (WBV_h), plasma viscosity (PV), red cell rigidity (RCR), red cell aggregation (RCA), plasma fibrinogen (PFIB), and hematocrit (Hct).[1–3] The nature of association between HT and HR is still debated on, with analogous descriptions stating that the association is like that of a “chick and embryo” or like “two chicks from the same embryo”, or “neither chick nor embryo”.[4,5] Nevertheless, concurrent occurrence of HT and elevated HR parameters is a well-known phenomenon. It is also expected that any therapy for HT would improve the HR profile, along with the lowering of elevated BP.[6] However, these findings are yet to influence the clinical research and management of HT, as outlined in the Joint National Commission (JNC)[7] and the World Health Organization/International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) guidelines on HT.[8]

Amlodipine, owing to its unique pharmacological, pharmacokinetic (PK), and pharmacodynamic profile of high oral bioavailability (BA), half-life between 30 and 50 h enabling once-a-day regimen, minimum peak-to-trough fluctuations, and low cost, is the most common calcium channel blocker (CCB) used in the

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treatment of essential HT.[9] While the effects of CCBs, in general, and amlodipine, in particular, on cardiac muscles and vasculature have been extensively studied, their effects on blood rheology have not received sufficient attention. HR effects of amlodipine have been reported in only one study to date,[10] with no follow-up studies to further evaluate its effects. In this study by Linde et al, amlodipine was found to increase RBC deformability.[10] However, the effects of amlodipine on various HR parameters in normotensives and at various concentrations and its effect on WBV at different shear rates have not been studied. The partitioning of the drug in plasma and erythrocytes and the mechanism underlying reduction in HR parameters are other important aspects, which need to be explored. We, therefore, conducted this study with the objective of evaluating these questions through an interdisciplinary approach involving clinical HR, pharmacological and PK parameters.

2. Methods

The study was designed as an analyst-blind, open-label, balanced, single-dose, PK study of amlodipine under fasting conditions in healthy human volunteers. While the PK study and measurement of plasma drug concentrations were conducted at Drug Monitoring Research Institute (DMRI), Mumbai, the determination of hematological and HR parameters was carried out in the Clinical Hemorheology Laboratory of School of Biosciences and Bioengineering, Indian Institute of Technology (IIT), Bombay. Young male volunteers (age 20.1 ± 2.2 years), free from any cardiovascular, cerebral, or renal diseases were selected. Smoking and consumption of alcohol and medicines other than the drug during the study period was strictly forbidden. The volunteers were made to adhere to the plan of diet, rest, and consumption of medicine under study.

A total of 27 normal volunteers were included in this in vivo study. This was a stand-alone study and not an extension of a BA/bioequivalence (BE) study. The final sample size in our study was determined by the number of volunteers who were available for screening and inclusion within the timeframe stipulated for initiating the study. While no power analysis was performed during the initiation of the study, the number of subjects in our study matches the usual sample size for BA/BE studies which evaluate drug concentration levels with a sample size power at 80% or higher.[11] After an overnight fasting period of at least 10 h, they were administered a single dose of one tablet of amlodipine (5 mg) along with 240-ml drinking water. The plasma drug estimations were carried out by withdrawal of blood samples at $t = 0, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 48, 72$, and 96 h. HR parameters such as WBV at 18 shear rates ranging from 0.512 to 94.5 sec^{-1}, PV, RCR, RCA, and Hct were determined at $t = 0, 4, 8, 12$, and 24 h. A physician carried out a clinical examination of the subjects at the time of check-in and checkout, while vital signs and adverse effect monitoring was performed throughout the study, from the prestudy day to 96 h after administration of dose.

2.1. PK and statistical evaluation

Amlodipine plasma levels were processed using HMS software (E Merck, USA). PK parameters such as AUC $_{0\text{-}t}$, AUC $_{0\text{-}inf}$, Kel, $t½$, C_{max}, and t_{max} were calculated for each volunteer. Amlodipine plasma level data and PK parameters were statistically analyzed using SPSS software. MS Excel was used for calculation of averages, standard deviation, and student's *t*-test.

2.2. Independent ethics committee approval

The independent ethics committee attached to DMRI, Mumbai, approved the protocol and all the amendments. The inclusion of volunteers in the study was subject to their consent, which was documented on the informed consent form for the study.

2.3. Determination of HR and hematological parameters

All the HR measurements were performed at the Clinical Hemorheology Laboratory of School of Biosciences and Bioengineering, IIT, Bombay, in accordance with the norms specified by the International Committee for Standardization in Hemorheology.[12] The procedure and the instruments described below for determination of various HR parameters have been used in our laboratory for the past 15 years and have been reported in several previous publications.[13–15]

About 10–12 ml of blood was withdrawn from the antecubital vein using a plastic disposable syringe with a 21-gauze stainless steel needle applying minimum suction. The blood was immediately transferred to a plastic vial containing a solution of sodium salt of ethylenediaminetetraacetic acid (15 mcg/ml of blood). The determination of HR and hematological parameters was completed within 4 h of withdrawal of the blood sample.

A Low-Shear 30 viscometer (Contraves, Zurich), specially designed for the rheological measurements of small volumes of biological samples, was used for determination of WBV and PV. Blood (mixed with anticoagulant) was sheared in the gap between a cylindrical bob and a coaxial rotating cup. The resistance to the rotating cup is proportional to the shear stress in the fluid; the amount of torque produced by resistance was indicated on the digital display of the instrument. This reading was converted into viscosity in centipoises by multiplication with a factor. Each blood sample was subjected to 18 different shear rates from 0.512 s^{-1} to 94.5 s^{-1}. Whole-blood viscosity determined at 51.2 rpm was designated as WBV_h, while that determined at 0.512 s^{-1} was designated as WBV_l and at 5.26 s^{-1} was designated as WBV_m. After the determination of WBV, the blood sample was centrifuged at 3000 rpm for 15 min and maximum possible volume of plasma was separated from it, taking care not to disturb the cellular layer. PV was determined on the Low-Shear 30 viscometer in a similar manner at shear rates of 20.4, 51.2, and 94.5 s^{-1}.

RCA was determined indirectly by using the following formula[16]:

$$RCA = \frac{(WBV_l)^{45/Hct}}{(PV_{20.4})}$$

where WBV_l is the whole-blood viscosity at 0.512 rpm, $PV_{20.4}$ is the plasma viscosity at 20.4 rpm, and Hct is the hematocrit.

Immediately after the separation of plasma from the centrifuged sample of blood, a suspension of red cells was prepared by pipetting out 500 μl from the middle pack of the red cells and suspending them in 1 ml of Ringer buffer solution. The viscosity of red cell suspension was determined at 94.5 rpm. Its Hct was determined with the help of an autoanalyzer (Sysmex, Japan) by the RBC pulse height detection method.[17,18] The viscosity of the Ringer buffer solution was determined at the same condition and at the same rate of shear. RCR was calculated from the following formula[17]:

$$RCR = \frac{(\text{Viscosity of red cell suspension in ringer buffer solution})^{40/RHct}}{\text{Viscosity of ringer solution at the same shear rate}}$$

where RHct is the hematocrit of the red cell suspension.

The aforementioned equations for RCA and RCR have been used in several clinical studies pertaining to HR and hence have been used here.[16,17]

The magnitude of WBV has been shown by many workers to be a function mainly of Hct. Hence, a corrected value of WBV was calculated using the following formula[19]

$$WBV\_C = (WBV)^{45/Hct}$$

where WBV_C is the corrected whole-blood viscosity at shear rates, WBV is the apparent whole-blood viscosity at shear rates, and Hct is the hematocrit.

2.4. Determination of plasma amlodipine concentration

The blood samples, after phlebotomy, were immediately centrifuged at 10 °C and at 2500–3000 rpm for 10 min and stored at −20° ± 2 °C pending assay. Plasma amlodipine was estimated using a high-pressure liquid chromatography/mass spectroscopy method, developed and used by DMRI for their routine analytical studies. The method was validated in accordance with the principles of good laboratory practices. Sample preparation and analysis for the same has been performed as per Draft SOP/ANA/03/01 of DMRI. The criteria used for validation included specificity and selectivity, sensitivity, accuracy (relative recovery), precision (repeatability and reproducibility), percent extraction yield, and stability including freeze–thaw cycles, long-term stability, and bench-top stability. The data were processed by ANALYTE integrating system software.

3. Results

3.1. Effect of amlodipine administration on HR properties

The results of administration of a single dose of amlodipine on the HR parameters across time points in the subjects in the study are summarized in Table 1. Fig. 1 represents the changes in WBV_h, PV, RCR, WBV_m, WBV_l, RCA, Hct, and plasma drug concentration against time as brought about by amlodipine in systemic circulation. The mean drug concentration increased to a maximum (C_{max}) of 5.938 ng/ml at $t = 4$ h and then steadily declined to about 1.4 ng/ml at $t = 24$ h (Table 1 and Fig. 1). The changes induced by the drug did not totally disappear at $t = 24$ h as the drug was still in circulation.

As the concentration of amlodipine rose to its maximum at $t = 4$, WBV at high, low, or medium shear rates declined; the decline continued even up to $t = 8$ h even as drug concentration started rising. Except for WBV_l at $t = 4$, all changes in WBV values at $t = 4$ or 8 were statistically significant. Later, as drug concentration tended toward zero, WBV values too tended to rise and at $t = 24$ h, showed values lower (WBV_h) or somewhat larger (WBV_l, WBV_m) than their original values at $t = 0$. Changes in Hct and PV were found to be similar to those in WBV_h, although of a less magnitude. Variations in RCR and RCA were statistically insignificant at all time points and did not follow any specific pattern.

Fig. 2 shows the percentage reduction in the magnitude of various HR parameters at $t = 4$. The WBV values at low, high, or medium shear rates, when corrected for Hct = 0.45, showed much less reduction at $t = 4$ when compared with their uncorrected values. However, the WBV values corrected for Hct showed magnitudes between 4.16% and 9.24%, which could not be termed as negligible. Percentage changes at $t = 4$ for most HR parameters were found to be comparable to changes in Hct. However, the changes in RCA and RCR did not correspond well with the changes in Hct.

The average values obtained from regression analysis of plasma drug concentration and each of the HR parameters and the resulting Pearson's correlation coefficient values were also tabulated (Table 2). A strong negative correlation was found between the plasma drug concentration and uncorrected WBV at all shear rates and PV and for Hct and plasma drug concentration. Regression analysis for the plasma drug concentration with RCA and RCR showed poor correlation.

4. Discussion

All the values of HR parameters at $t = 0$ were in conformity with the normal values for the corresponding parameters as reported in previous studies.[20]

The most important findings were the statistically significant reductions in values of WBV_h, WBV_l, WBV_m, PV, and Hct after administration of drug at time = 4 h and/or $t = 8$ h. The drug concentration rises from zero at $t = 0$ to its maximum value (in HR studies) at $t = 4$ and falls slightly at $t = 8$ h. The causative factor for the reduction in these HR parameters is the reduction in Hct. It is interesting to note that the Hct reduces by 10.33% of its original value at $t = 4$ h. This reduction then tapers off to 8.18% at $t = 8$ h, 3.86% at $t = 12$ h, and 1.66% at $t = 24$ h. Thus, it reaches its minimum with the maximum drug concentration, and as the drug concentration reduces steadily, magnitude of Hct slowly comes back to its original value. However, even at $t = 24$ h, as long as the drug level

Table 1
Effect of amlodipine on HR parameters.

Time in hr/parameter	0	4	8	12	24
WBV_h (cp)	5.2 ± 0.78	4.80 ± 0.50***	4.56 ± 0.49***	5.44 ± 0.76	5.38 ± 0.72
WBV_l (cp)	26.42 ± 9.20	21.25 ± 5.72	19.34 ± 6.11*	27.65 ± 9.39	29.53 ± 7.37
WBV_m (cp)	9.32 ± 2.34	7.43 ± 1.44**	7.41 ± 1.46**	9.81 ± 2.02	9.83 ± 1.44
PV (cp)	1.36 ± 0.15	1.27 ± 0.88*	1.29 ± 0.39*	1.39 ± 0.096	1.37 ± 0.083
Hct (%)	44.35 ± 4.55	39.46 ± 3.84**	40.28 ± 4.03*	42.64 ± 3.24	43.62 ± 3.63
RCR	3.80 ± 0.28	3.75 ± 0.12	3.95 ± 0.45	3.69 ± 0.34	4.05 ± 0.40
RCA	19.78 ± 5.43	20.31 ± 5.46	16.99 ± 4.69	20.48 ± 5.94	21.73 ± 3.90
Plasma drug concentration (ng/ml)	0	5.94 ± 0.54	4.14 ± 0.32	2.54 ± 0.45	1.39 ± 0.32

HR, hemorheology; SD, standard deviation; WBV_h, whole-blood viscosity determined at 51.2 rpm; WBV_l, whole-blood viscosity determined at 0.512 rpm; WBV_m, whole-blood viscosity determined at 5.26 rpm; Cp, centipoises; PV, plasma viscosity; Hct, hematocrit; RCR, red cell rigidity; RCA, red cell aggregation.
Values were evaluated by two-tailed Student's 't' test with respect to $t = 0$.
Results were expressed as mean ± SD.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.

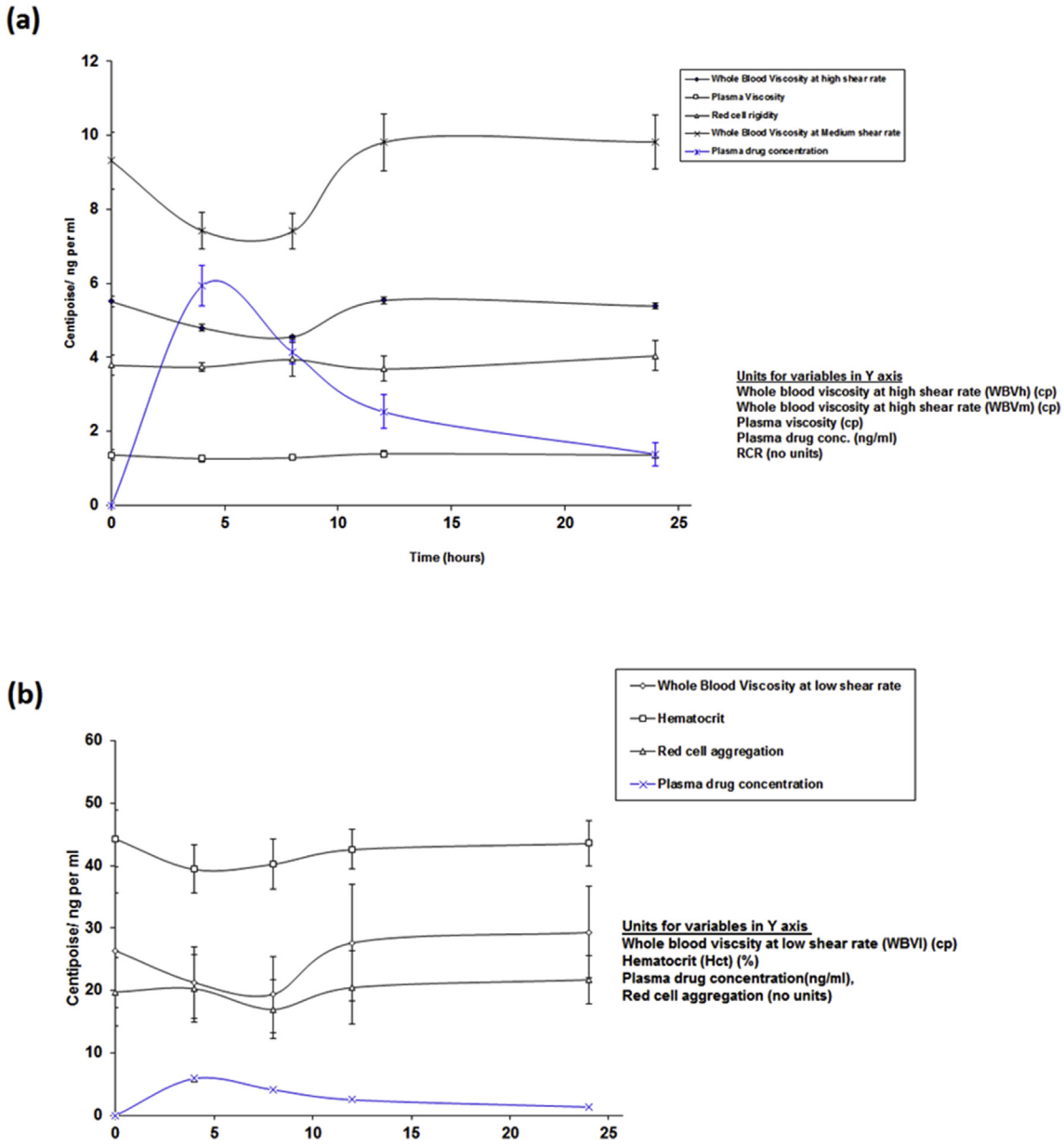

Fig. 1. (a) Effect of time on various HR parameters and drug concentration. (b) Effect of time on various HR parameters and drug concentration. (Units: WBVh (cp), WBVm (cp), PV (cp), Plasma drug conc. (ng/ml), RCR (no units)). HR, hemorheology.

has not reached zero, reduction in Hct too is yet to reach zero. Except for WBV_l, the reduction in HR parameters is higher at $t = 4$ compared with $t = 8$ h.

The centrality of Hct reduction in improving HR parameters in normal controls is corroborated by the contrast in the changes occurring in WBV at various shear rates and their corresponding values corrected for Hct. While WBV_h, WBV_l, and WBV_m show statistically significant reductions at $t = 4$ h and $t = 8$ h, WBV_h_C, WBV_l_C, and WBV_m_C do not show changes of statistical significance. As the average Hct at $t = 0$ is 44.35, WBV_C values (which correspond to Hct = 45) are almost identical to their corresponding WBV values. However, the effect of reduction in Hct at higher t values is totally masked by conversion of WBV values to WBV_C values. Hence, WBV_C values remain relatively unaltered on passage of time. HR parameters related to RBCs, viz. RCR and RCA, do not show much variation on time after administration of amlodipine. In this study, serum erythropoietin levels were not measured in the study subjects.

Because the Hct level at zero hour was measured and it was found to be within the normal range for the study population, the influence of erythropoietin in causing variations in Hct is unlikely. The reduction in Hct and the subsequent reduction in magnitude of selected HR parameters can be attributed to hemodilution. It is postulated that amlodipine, immediately after its administration, absorbs water from surrounding tissues into the blood stream, resulting in hemodilution. This hypothesis is supported by the almost parallel reduction in most HR parameters up to $t = 4$ or 8 h. After $t = 8$ h, all parameters tend to come back to their original values. The relatively low values of WBV_C compared with WBV also support this hypothesis. A strong or very strong negative

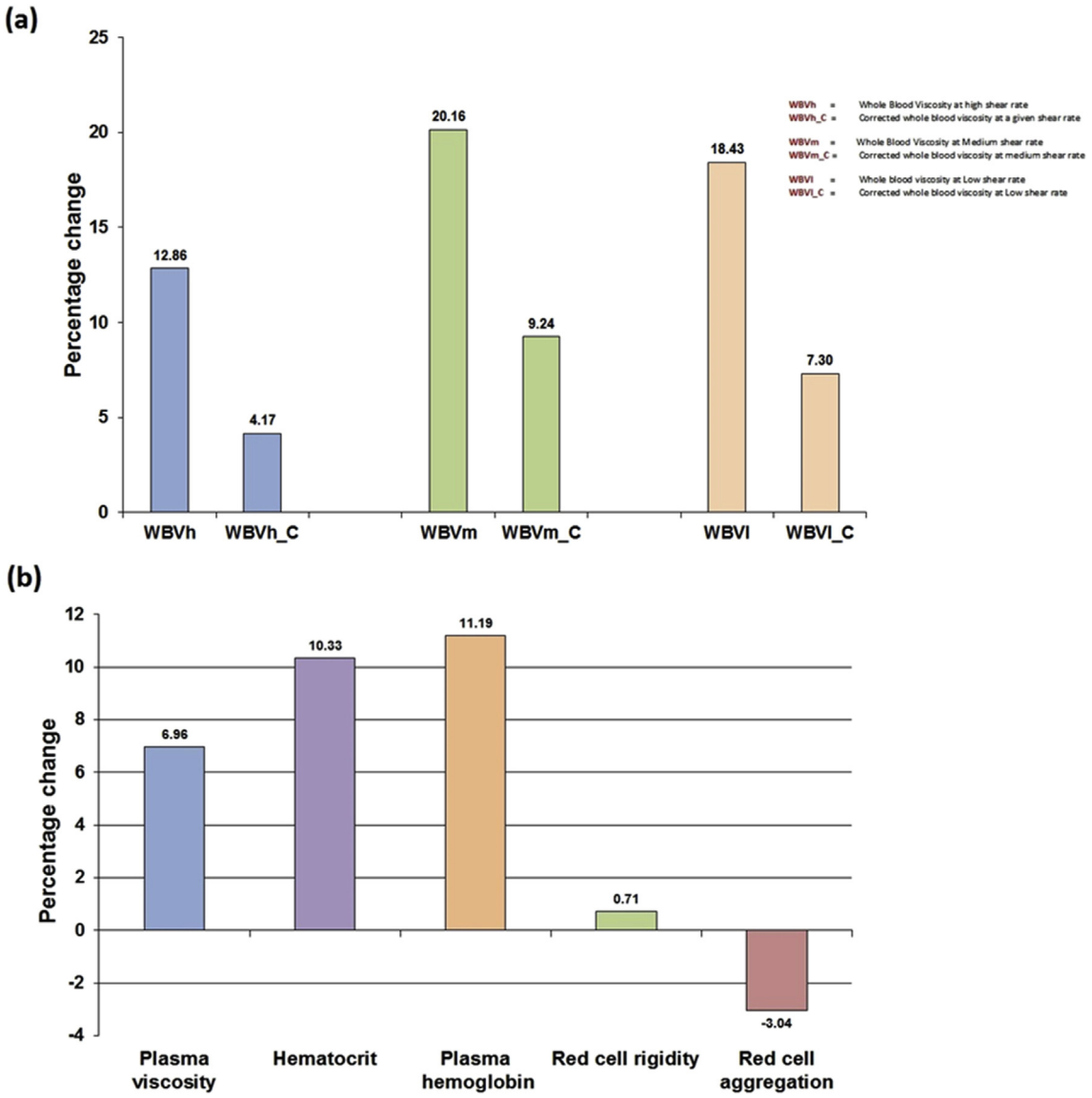

Fig. 2. (a) Percentage change in hemorheologic parameters at C_{max}. (b) Percentage change in hemorheologic parameters at C max (II).

Table 2
Correlation coefficient 'r' between the plasma drug concentration and HR parameters.

HR Parameter	Pearson's correlation coefficient (versus drug concentration)
Whole-blood viscosity at a high shear rate	−0.8
Whole-blood viscosity at a low shear rate	−0.76
Whole-blood viscosity at a medium shear rate	−0.81
Corrected whole-blood viscosity at a high shear rate	−0.41
Corrected whole-blood viscosity at a low shear rate	−0.58
Corrected whole-blood viscosity at medium shear rate	−0.69
Hematocrit	−0.98
Plasma viscosity	−0.83
Red cell aggregation	−0.21
Red cell rigidity	−0.31

HR, hemorheology.

correlation between drug concentration and plasma-related HR parameters (which can be diluted by hemodilution) further strengthens this hypothesis.

An earlier study found that after 4 months of amlodipine treatment, the total peripheral resistance index, WBV, Hct, and serum erythropoietin were found to decrease. The PV decreased, and the erythrocyte deformability increased in most patients, whereas no significant changes were observed in PFIB. The decrease in blood viscosity was attributed by the authors to hemodilution and a decrease in serum erythropoietin.[10] Later studies to substantiate

these findings do not exist. However, studies related to determination of HR effects of other CCBs reported an improvement in blood viscosity, i.e. decrease in various HR parameters. A study carried out in patients with HT using other CCBs for a prolonged period also supports the incidence of hemodilution caused by CCBs.[21] The administration of a single dose of amlodipine on selected hematological parameters has also shown to produce hemodilution.[22]

In an animal study conducted on spontaneously hypertensive rats, administration of intragastric amlodipine at a dose of 10 mg/kg for 6 weeks resulted in a significant decrease in mean blood pressure by 29% but had no effect on PV, PFIB concentration, RBC aggregation, and RBC deformability.[23] This study contradicts our finding, but this study was performed in rats, and dose of amlodipine used was very high (10 mg/kg), which may cause profound hypotension, leading to activation of counter regulatory mechanisms, such as the renin–angiotensin system.

Other antihypertensive drugs have also been shown to exert HR effects. In a study, beta-blocker, angiotensin converting enzyme (ACE) inhibitor, diuretic therapy, and calcium antagonist therapy have shown to alter WBV, PV, fibrinogen, and red blood cell aggregation (RBCA) in a variable way when administered to hypertensive patients with low- and high-shear WBV.[24] Another study demonstrated a positive correlation between intravenous furosemide infusion and RBCA elevation.[25] Adverse HR parameters contribute to HT, but it is also hypothesized that development of microvascular complications such as hypertensive retinopathy may be partly secondary to disordered HR functions.[26] Alpha 1 inhibitor drug therapy has been shown to positively impact HR variables such as WBV, PV, and the fibrinogen level.[27] Venotonic drugs such as ruscus extract and diosmine have been studied to affect leukocyte endothelial interaction and histamine-mediated increase in vascular permeability in the animal model.[27] Probucol has been shown to have positive HR effects on patients with diabetic retinopathy apart from favorable alteration in lipid profile.[28]

A randomized double-blinded study has demonstrated that treatment with enalapril or losartan significantly reduces the mean blood pressure from pretreatment values. However, there was no statistically significant changes in the levels of hemostatic markers such as von Willebrand factor, fibrinogen, soluble P-selectin, and plasminogen activator inhibitor-1.[29] Endocan is a novel marker of endothelial dysfunction in hypertensive subjects. Treatment with amlodipine has been shown to reduce serum endocan and CRP levels, thereby demonstrating anti-inflammatory properties of amlodipine.[30]

An earlier study concluded that a 10.99% increase of Hct produced an increase of one unit relative viscosity, which means approximately a 20% increase in WBV (assuming WBV = 5 cp) for a healthy individual.[31] For the physiologic compensation of 20% increased viscosity, blood pressure increase will be 20% or vasodilation will be 4.66% in radius.[22] Our study shows that 11% reduction in Hct has resulted in 12.94% reduction in WBVh. Thus, this decrease in blood viscosity would result in appreciable reduction in blood pressure. At higher and/or chronic dosing, the contribution of improvement in HR behavior to reduction in BP is expected to be more pronounced.

The association of edema with amlodipine therapy, especially as a dose-related side effect, could also be understood in the context of hemodilution caused by the drug. As shown in this study, the effects of hemodilution are visible even at drug concentration as low as 1.39 ng/ml. Hence, at about 10 times higher drug concentrations (commonly encountered in patients with HT on long-term amlodipine therapy), the accumulation of fluid in the blood would be much higher. The body would attempt to remove this accumulated fluid through renal excretion.

Any imbalance in the kinetics of fluid accumulation and excretion would result in peripheral edema. Another possible mechanism might be hemodilution leading to decrease in intravascular oncotic pressure due to increased compliance of the vascular system compared with the extravascular space, which leads to shifting of fluid to extravascular space, causing edema. Apart from hemodilution, amlodipine was also found to increase the RBC deformability, which can enhance its antihypertensive effect.[10]

The strongly opposite behavior of RCA and RCR compared with other HR parameters with respect to the drug concentration can be understood by classifying the HR parameters as plasma related (WBV, PV, and Hct along with the hematological parameters such as WBC, RBC, and plasma hemoglobin) and RBC related (RCR and RCA). As plasma-related parameters are directly affected by hemodilution, they contribute to the reduction in WBV caused by amlodipine. The difference in the response of plasma-related and RBC-related parameters to amlodipine might result from the preferential partitioning of the drug to plasma rather than to the RBCs, although studies to substantiate this hypothesis have not be reported so far.

Thus, this study shows that administration a single dose of 5 mg of amlodipine could produce statistically significant changes in plasma-related HR parameters such as WBV at different shear rates, PV, and Hct in normal human volunteers, while the RBC-related parameters were found to be unaffected. It is more likely that in hypertensive subjects also, amlodipine would alter HR parameters significantly as shown in other studies.[10] Hence, our study results could be extrapolated to hypertensive subjects.

While hemodilution has been suggested as the main mechanism for this process, the negative correlation between the drug concentration and WBV parameters corrected for Hct suggests that an additional mechanism may also be involved. We also suggest that the antihypertensive action of amlodipine may partly be contributed by improvement in its HR properties. The hypothesis of hemodilution governing the HR behavior and possibly antihypertensive action of amlodipine and of the possible preferential partitioning of amlodipine to plasma rather than to erythrocytes need to be validated through independent studies.

Funding sources

None.

Conflicts of interest

All authors have none to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ihj.2018.10.417.

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Original Article

Coronary artery size in North Indian population – Intravascular ultrasound-based study

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ABSTRACT

Objective: The coronary artery dimensions have important diagnostic and therapeutic implications in management of coronary artery disease (CAD). There is paucity of data on the coronary artery size in the Indian population as measured by intravascular ultrasound (IVUS).

Methods: A total of 303 patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) with intravascular ultrasound underwent analysis along with quantitative coronary angiography (QCA). Of the 492 proximal coronary segments; 221 relating to left main (LM), 164 to left anterior descending artery (LAD), 45 to left circumflex artery (LCX), and 62 to right coronary artery (RCA) were considered.

Results: Patient's mean age was 53.37 ± 3.5 years; men 80%; hypertension 35% and diabetes 24.8%. On IVUS, mean minimal lumen diameter as compared to QCA in LM (4.60 mm versus 4.50 mm, $p < 0.001$), LAD (3.71 mm versus 3.45 mm, $p < 0.001$), LCX (3.55 mm versus 3.16 mm, $p < 0.001$) and RCA (3.85 mm versus 3.27 mm, $p < 0.001$) were significantly larger. Lumen and external elastic membrane (EEM) cross-sectional area (CSA) were larger in males as compared to females with statistical significance for lumen CSA in LM ($p = 0.04$); RCA ($p = 0.02$) and EEM CSA in LM ($p = 0.03$); RCA ($p = 0.006$) but no significance for adjusted body surface area (BSA). In multivariate models, BSA and age were independent predictors of LM and LAD diameters and areas, but age was an independent predictor indexed to BSA.

Conclusion: The coronary artery dimensions by IVUS are significantly larger than QCA. No gender difference in coronary artery size. Age was an independent predictor of coronary artery size in left main and LAD. The coronary artery size may not be a risk factor for acute coronary syndrome.

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality in India. A quarter of all mortality is attributable to CVD, with ischemic heart disease being the predominant cause.[1]The coronary artery size in the general population is variable with multiple factors playing a crucial role such as age, gender, body habitus, genetic, environmental and life style. The outcomes after percutaneous coronary interventions (PCIs) and coronary artery bypass graft surgery (CABG) are mainly determined by the coronary artery size. Intravascular ultrasound (IVUS) is the most widely used intracoronary imaging tool for the quantitative assessment of coronary artery disease, which yields more accurate measurements of vessel geometry and lesion severity than conventional quantitative coronary angiography (QCA).[2] There are no data on the size of normal coronary arteries in the Indian population as measured using IVUS. The primary aim of this study was to determine the coronary artery dimensions by intravascular ultrasound and influence of age, gender, body surface area, diabetes, and hypertension on coronary artery size.

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2. Methods

This is a single-center observational study.

2.1. Objectives

1. To determine the normal dimensions of disease-free coronary artery segments by using intravascular ultrasound.
2. To assess the effect of age, gender, body surface area, diabetes, and hypertension on coronary artery size.

2.2. Study population

This was a single-center observational study carried out in the Department of Cardiology of a tertiary care hospital in North India. From June 2016 to Feb 2019, a total of 303 patients with acute coronary syndrome, who underwent coronary angiography followed by percutaneous coronary intervention with intravascular ultrasound guidance and had proximal disease-free coronary artery segments or minimal atheroma (<20% cross-sectional narrowing to nullify the remodeling effect) were included. Patients with deranged renal function, tortuous coronary vessels precluding IVUS examination, past history of PCI or CABG, and refusal of consent were excluded. All participants provided a written informed consent and study approved by the institutional ethics committee. All procedures were conducted in accordance with principles outlined in the Declaration of Helsinki.

2.3. PCI procedure

All patients were given 325 mg aspirin, clopidogrel 300 mg or prasugrel 60 mg along with intravenous doses of unfractionated heparin titrated to achieve therapeutic range activated clotting time prior to percutaneous coronary intervention procedure. IVUS pull back was taken using a 20-MHz, 2.9 French, Eagle Eye® Platinum RX digital IVUS catheter (Eagle Eye, Philips Volcano, San Diego, CA, USA). All patients were administered 200 mcg of intracoronary nitroglycerine, and IVUS pull back was taken starting 15 mm distal to the lesion till the aorto-ostial junction using an automatic pull back at a speed of 0.5 mm/s before any balloon dilatation. PCI was performed as per standard procedure.

2.4. Angiographic analysis

Coronary angiography was performed in all patients at a frame rate of 15/sec. Standard angiographic views were obtained and all captured angiographic images were analyzed offline. The arteries measured were the proximal left main (LM), left anterior descending (LAD), and left circumflex (LCX) in the right anterior oblique (RAO) 30° projection, and for the right coronary artery (RCA) left anterior oblique (LAO) 60° projection. The proximal coronary artery segments were considered: (1) proximal LAD segment before the first septal, (2) the proximal LCX segment before the obtuse marginal (OM), (3) the proximal RCA segment before the first right ventricular branch. A computer-assisted, automatic contour detection using software Medis Q Angio® XA 7.3 (Medis medical imaging systems, Leiden, the Netherlands), was performed. The outer diameter of the contrast filled catheter served as the calibration standard. Quantitative coronary angiography was carried out in end-diastole when coronary artery segment was contrast filled, uniformly distended and free of tortuosity or overlap.

2.5. Gray-scale IVUS analysis

The IVUS images of all the patients were recorded and stored on a DVD-ROM for offline analysis, which was performed by two independent observers (SK and RK) who were unaware of the patient details or coronary angiograms. A consensus was obtained if there was discordance in the analyses by repeated off line readings. Quantitative and qualitative IVUS analyses were performed in accordance with the American College of Cardiology Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies.[3] All the IVUS analysis was done using a validated and computerized INDEC's Echo plaque 4.3.12J software (INDEC Medical systems, Inc., Santa Clara, CA, USA). After automatic border detection for the lumen and media-adventitia interface by the software, manually correction and confirmation done, to obtain the results calculated and displayed automatically. Lumen cross sections were measured in the disease-free or minimal atheroma segments within 10–15 mm from the ostium before any side branch. After measuring the external elastic membrane (EEM) and lumen cross-sectional areas (CSAs), plaque and media (P&M) CSA was calculated as EEM minus lumen CSA. Plaque burden was estimated as plaque and media CSA divided by EEM CSA multiplied by 100.

2.6. Statistical analysis

All the statistical analysis was done using SPSS version 22.0 (SPSS, Inc., Chicago, Illinois). Categorical data were presented as percentages (%) and frequencies, and Chi-squared test or Fisher's Exact test was used as appropriate. Distribution of the continuous variables was analyzed by Kolmogrov–Smirnov test and presented as mean with standard deviation if normally distributed and median with 25th and 75th percentiles when skewed distribution. Correlations were estimated by Pearson correlation coefficient. Univariate analysis was performed to find association of categorical variables between the two study groups using either Chi-squared test or Fisher's Exact test as appropriate. Continuous variables were compared in the groups by independent t test when normally distributed and Mann Whitney U test with skewed distribution. A stepwise multiple linear regression analysis was done to determine whether age, body surface area, gender, diabetes, or hypertension was independently associated with coronary size. As body size is a confounding variable for coronary size, multiple regression analysis was again performed with the dependent variables in each model, corrected for BSA. A p value < 0.05 was considered statistically significant.

3. Results

A total of 303 patients were examined, out of which 244 were males and 59 were females. Of the 492 proximal coronary segments analyzed include, LMCA (221 sites), proximal LAD (164 sites), proximal LCX (45 sites), and proximal RCA segments (62 sites). The mean age of the patients was 53.37 ± 3.5 years (range 22–90 years). The baseline clinical characteristics of the patients are outlined in Table 1.

The mean diameter of vessels as assessed by IVUS was largest in left main, followed by proximal RCA, proximal LAD, and proximal LCX. There was a strong concordance between IVUS versus quantitative coronary angiography minimal lumen diameter (QCA MLD) in the LM, LAD, LCX, and RCA, separately (Table 2). The IVUS determined coronary artery diameter when indexed to body surface area of left main, proximal LAD, proximal LCX and proximal RCA were 2.64 ± 0.40 mm/m^2, 2.15 ± 0.35 mm/m^2, 2.05 ± 0.30 mm/m^2 and 2.20 ± 0.36 mm/m^2 respectively (Table 2).

Table 1
Baseline characteristics of the patients.

Parameter	Total (n = 303)	Males (n = 244)	Females (n = 59)
Age (years)	53.4 ± 3.5	52.6 ± 11.5	56.36 ± 12.1
Height (cm)	163.5 ± 8.38	165.7 ± 7.2	154.5 ± 6.7
Weight (kg)	68.26 ± 11.60	69.2 ± 11.3	64.3 ± 12.1
BMI (kg/m^2)	25.56 ± 4.15	25.23 ± 4.02	26.9 ± 4.5
BSA (m^2)	1.76 ± 0.17	1.78 ± 0.16	1.66 ± 0.17
Diabetes, n (%)	75 (24.8%)	51 (20.9%)	24 (40.7%)
Hypertension, n (%)	106 (35%)	71 (29%)	35 (59%)
Current Smokers, n (%)	104 (34.3%)	102 (41.8%)	2 (3.4%)
Current Alcoholics, n (%)	83 (27.4%)	83 (34%)	0
Hemoglobin (mg/dL)	13.04 ± 2.07	13.43 ± 1.99	11.34 ± 1.45
Creatinine (mg/dL)	1.05 ± 0.26	1.05 ± 0.24	1.07 ± 0.33
TC (mg/dL)	154.95 ± 51.95	154.47 ± 48.34	157.33 ± 63.84
TG (mg/dL)	137.6 ± 61.05	134.86 ± 59.83	151.02 ± 65.79
LDL (mg/dL)	95.19 ± 48.50	95.30 ± 44.63	94.68 ± 63.68
HDL (mg/dL)	40.17 ± 14.49	39.49 ± 13.34	43.26 ± 18.74

All values are presented as Mean ± SD or number (%).
BMI=Body mass index, BSA=Body surface area, TC = Total cholesterol, TG = Triglycerides, HDL=High density lipoprotein, LDL = Low density lipoprotein.

Table 2
Comparison of MLD by IVUS and QCA.

Dimension	MLD by IVUS	MLD by QCA	R-value	*p*-value
Unadjusted MLD (mm)				
Left Main	4.60 ± 0.69	4.50 ± 0.79	0.332	<0.001
pLAD	3.71 ± 0.60	3.45 ± 0.63	0.479	<0.001
pLCX	3.55 ± 0.56	3.16 ± 0.47	0.302	<0.001
pRCA	3.85 ± 0.62	3.27 ± 0.56	0.649	<0.001
Adjusted to BSA, MLD (mm/m^2)				
Left Main	2.64 ± 0.40	2.53 ± 0.57	0.376	<0.001
pLAD	2.15 ± 0.35	1.94 ± 0.48	0.253	0.016
pLCX	2.05 ± 0.30	1.72 ± 0.22	0.278	0.17
pRCA	2.20 ± 0.36	1.86 ± 0.32	0.749	<0.001

All values are presented as Mean ± SD.
MLD = Minimal lumen diameter, IVUS = intravascular ultrasound, QCA = quantitative coronary angiography, BSA = body surface area.

On IVUS, males had larger coronary artery diameter as compared to females (Fig A1). In females, the diameters were smaller than males in LM by 0.18 mm, in LAD by 0.08 mm, in LCX by 0.27 mm, and in RCA by 0.40 mm. However, it was statistically significant only in RCA. When the coronary artery size was indexed to the BSA, there was no statistically significant difference except in LCX (Fig A2).

Lumen and EEM cross-sectional areas were larger in males in comparison to females and achieved statistically significance in both left main and RCA. However, there was no statistical significance for adjusted BSA (Table 3).

On multiple linear regression analyses, the body surface area was an independent predictor of MLD in all vessels except RCA, and age was significant in both LM and LAD. However, analyses were performed for indexed MLD, as body size was a potential confounding variable, age was an independent predictor for LM ($\beta = 0.269$, 95% confidence interval [CI] 0.004–0.015 and $p < 0.001$), and LAD ($\beta = 0.234$, 95% CI 0.001–0.012 and $p = 0.01$). Similar to findings with diameters, body surface area was also an independent predictor for external elastic membrane cross-sectional area (EEM CSA), except in RCA and age, was statistically significant in both LM and LAD. When analysis performed for indexed EEM CSA, age was an independent predictor for LM ($\beta = 0.255$, 95% CI0.03–0.12 and $p = 0.001$) and LAD ($\beta = 0.30$, 95% CI 0.03–0.10 and $p = 0.001$), and hypertension was an independent predictor in LAD ($\beta = 0.208$, 95% CI 0.13–2.1 and $p = 0.027$) (Table 4). In case of LCX and RCA, no independent predictors were found for luminal

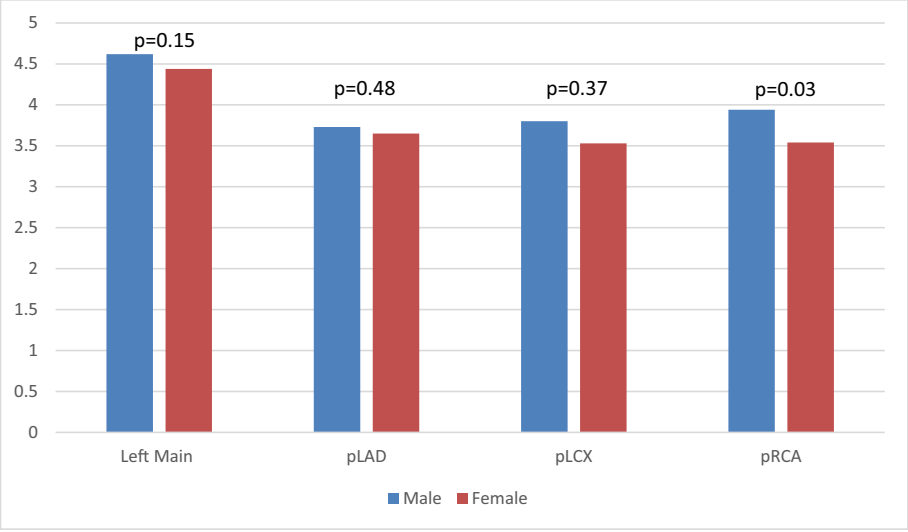

Fig A1. Coronary artery diameter by IVUS (mm), without reference to body surface area.

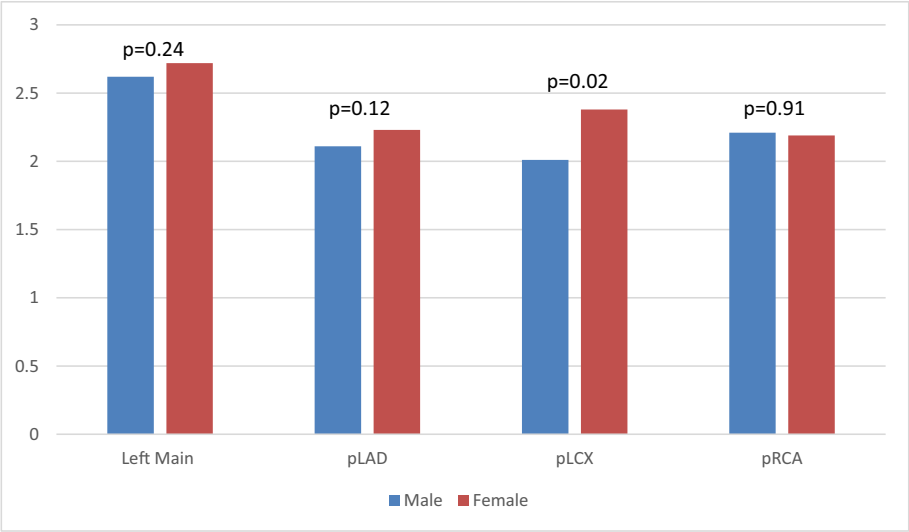

Fig A2. Coronary artery diameter by IVUS (mm) indexed to body surface area.

diameters and EEM CSA. Neither gender nor diabetes independently correlated with any of the measured arterial areas (Table S1-S3, Supplementary data).

4. Discussion

The key findings of this study are as follows: (1) intravascular ultrasound (IVUS)-measured coronary artery dimensions are significantly larger than those measured by quantitative coronary angiography (QCA); (2) minimal luminal diameters and EEM cross sectional areas are larger in males; (3) no gender difference in lumen and EEM cross sectional areas when adjusted to BSA; and (4) age was an independent predictor of coronary artery size and area in LM and LAD.

The absolute size of coronary artery segments does matter during interventional or surgical procedures. Majority of the lesions in acute coronary syndromes involve the proximal segments of the coronary arteries and jeopardize significant amount of myocardium.[4–6] The knowledge of the dimensions helps in choice of devices and stents during the coronary interventions. Smaller arteries tend to decrease the atheroma burden required to develop significant obstructive coronary lesions and further potentiates technical challenges during surgical or interventional procedures.[7]

Coronary angiogram is an established method for assessing the extent and severity of disease but has several limitations.[8] Multiple studies have shown a considerable variability in the visual interpretation of cine-angiograms.[9–11] QCA involves computerized analysis of digital images and automatic edge detection algorithms, but the validity of this angiographic quantification is also questionable.[10–12] Various discrepancies have been observed between coronary angiograms and findings on postmortem examinations.[13] One of the main advantages of IVUS imaging is its ability to precisely define the vessel dimensions and areas.

The coronary artery size is highly variable in the normal population.[14,15] Genetic factors, age, gender, body weight, body surface

Table 3
Comparison of Lumen and EEM Cross sectional area by IVUS.

Dimension	Males (n = 244)	Females (n = 59)	*p*-value
Unadjusted Lumen CSA (mm^2)			
Left Main	20.03 ± 5.93	18.41 ± 3.83	0.04
pLAD	12.79 ± 4.08	12.15 ± 3.84	0.39
pLCX	11.68 ± 3.78	12.74 ± 1.33	0.59
pRCA	13.96 ± 4.29	11.19 ± 3.07	0.02
Adjusted to BSA, Lumen CSA (mm^2/m^2)			
Left Main	11.37 ± 3.14	11.55 ± 2.61	0.80
pLAD	7.36 ± 2.15	7.69 ± 2.32	0.50
pLCX	6.69 ± 2.06	7.97 ± 0.81	0.23
pRCA	7.82 ± 2.60	6.95 ± 1.89	0.27
Unadjusted EEM CSA (mm^2)			
Left Main	25.11 ± 6.43	23.16 ± 4.42	0.03
pLAD	16.98 ± 4.87	15.94 ± 4.76	0.25
pLCX	15.56 ± 5.07	17.01 ± 2.89	0.58
pRCA	18.44 ± 4.79	14.66 ± 3.29	0.006
Adjusted to BSA, EEM CSA (mm^2/m^2)			
Left Main	14.22 ± 3.47	14.26 ± 2.93	0.95
pLAD	9.77 ± 2.55	10.13 ± 2.67	0.53
pLCX	8.91 ± 2.85	10.60 ± 1.29	0.26
pRCA	10.18 ± 2.92	9.14 ± 2.03	0.24

All Values are presented as Mean ± SD.
EEM-external elastic membrane, IVUS = intravascular ultrasound, CSA = cross sectional area, BSA = body surface area.

Table 4
Multiple linear regression models predicting EEM CSA, indexed for BSA.

Characteristic	β	*p*-value
LM model		
Age	0.255	0.001
Gender	−0.047	0.54
Diabetes	−0.029	0.70
Hypertension	0.096	0.21
LAD model		
Age	0.300	0.001
Gender	−0.093	0.33
Diabetes	−0.018	0.84
Hypertension	0.208	0.03
LCX model		
Age	0.136	0.47
Gender	0.122	0.56
Diabetes	0.107	0.60
Hypertension	−0.019	0.92
RCA model		
Age	0.204	0.19
Gender	−0.140	0.38
Diabetes	−0.116	0.45
Hypertension	−0.112	0.48

BSA=Body surface area, LM = Left main coronary artery, LAD = Left anterior descending artery, LCX = Left circumflex artery, RCA = Right coronary artery.

area, weight of the heart, ethnicity, race and environmental factors, have all been correlated with the coronary artery size.[15–21] Smaller coronary artery size has been reported in Indians as compared to the western counterparts.[21,22] This has been attributed to the body habitus and relatively smaller body surface area.[22] Raut et al., observed similar findings, where in coronary artery diameters were larger in Caucasians as compared to Indians, but no difference after correction for BSA.[23] Our QCA findings are comparable and substantiated by a study in similar population contradicting the traditional belief of Indians having smaller coronaries.[24] The vessel diameters in this study by IVUS were significantly larger than dimensions obtained by QCA.

Previous studies from Indian subcontinent by QCA found that males had statistical significant larger coronary artery diameters as compared to females but there was no difference after indexing to BSA.[23,25] However, Elangovan et al found small coronary size in females after correction for BSA.[26] Autopsy series data in humans found smaller coronary artery size in females.[27] In patients undergoing CABG, females had smaller coronary artery diameter and was associated with increased mortality.[28] In the present study by IVUS, males had larger coronary artery dimensions as compared to females but after indexing to BSA there was no statistical significant difference except in left circumflex artery. This finding is probably due small sample size of LCX in females. Our study negates the general belief that women have smaller coronary size.

The size of normal left main artery in present study is comparable to the western population.[29,30] The mean left main minimal luminal diameter was 4.60 ± 0.69 mm in males and 4.50 ± 0.79 mm in females, whereas in study by Kim et al.,[29] it was in males 4.26 ± 0.55 mm and in females 3.92 ± 0.45 mm. In our study, the lower limit of left main coronary diameter as per 2 SD below the mean is 3.22 mm with a 95% CI 4.52–4.68. Multiple factors affect the coronary artery size, and each systemic factor may not affect all coronary arteries in a similar way.[31–33] Kornowski et al. found no gender difference after correcting for body surface area on IVUS but the study was limited by disease vessels at multiple locations.[34] The body surface area and gender were reported to be independent predictors of left main coronary artery size.[29] Sheifer et al. observed gender difference in coronary size in LM and LAD.[35] In contrary, gender was not an independent predictor of coronary artery size in our study. However, body surface area and age were independent predictors of coronary artery size and area in LM and LAD. This could be due to the fact that LM and LAD supply large area of the myocardium. It has been known that ventricular mass strongly predicts the size of coronaries.[29,32,36,37] In the present study, hypertension independently predicted the coronary artery area in LAD. Hypertension causes left ventricular hypertrophy, which increases the myocardial mass (weight). LAD subtends the left ventricular wall, thereby causing larger diameters in this artery.

5. Study strengths and limitations

This is the first of its kind study to assess the coronary artery size by intravascular ultrasound imaging in the Indian population. All coronary artery segments were not analyzed in a single individual. The proportion of females was less.

6. Conclusions

The coronary artery dimensions as assessed by intravascular ultrasound are significantly larger than as measured by quantitative coronary angiography. Body surface area was an independent predictor of coronary artery size. Age was an independent predictor of coronary artery size and area in left main and LAD. Gender and diabetes did not influence the coronary artery size. The coronary artery size *per se* may not be a risk factor for acute coronary syndrome.

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Declaration of competing interest

All authors have none to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ihj.2019.10.005.

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Original Article

Intermediate term outcome after electrogram guided segmental ostial pulmonary vein isolation using an 8 mm tip catheter for paroxysmal atrial fibrillation

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ABSTRACT

Introduction: Pulmonary vein isolation (PVI) is the most widely used procedure for ablation in patients with paroxysmal atrial fibrillation (AF). Not withstanding recent advancements in this field, including sophisticated three-dimensional (3D) based imaging and advanced ablation catheters with contact force technology, many patients and healthcare systems in developing countries will not afford such an expensive therapeutic procedure. There are no data from India analyzing the efficacy of PVI for PAF using conventional mapping and ablation. In this article, we have summarized the intermediate term outcome following PVI in patients with PAF using electrogram-based mapping and a 8 mm tip ablation catheter.
Method: A total of 42 consecutive patients who underwent PVI for symptomatic PAF not controlled with at least one antiarrhythmic drug were studied in a tertiary care institute from March 2011 to June 2018. Patients with rheumatic AF were excluded. The pulmonary vein (PV) anatomy was assessed by pulmonary angiography during the ablation procedure. Using conventional electrophysiologic mapping, a variable curve Lasso catheter placed in the PVs was used to guide the earliest site of breakthrough. The segmental ostial PVI was performed using a 8 mm tip radiofrequency (RF) ablation catheter. Elimination of all PV ostial potentials and complete entrance block into the PV were considered indicative of complete electrical isolation. Follow-up visits were scheduled at one, three, and six months after the procedure, and every six months thereafter. History, symptom review, clinical examination, and 12-lead ECG were performed at each follow-up.
Results: At pre-discharge, 34 patients (81%) were in sinus rhythm, while eight patients (19%) continued to have atrial fibrillation. The age of the study population was 51.5 ± 11.7 yrs. The mean follow-up duration was 44 ± 21 months (range 6–84 months). The number of PVs isolated included one (five patients, 11.9%), two (20 patients, 47.6%), three (12 patients, 28.6%), and four (five patients, 11.9%). In 42 patients, a total of 101 PVs were isolated. The right superior PV (RSPV) was isolated in 37 patients, the left superior PV (LSPV) was isolated in 39 patients, the left inferior PV (LIPV) was isolated in 14 patients, and the right inferior PV (RIPV) was isolated in six patients. The procedure duration was 125 ± 29 min and the fluoroscopy time was 47 ± 13 min. The number of patients who remained in sinus rhythm at 1, 6, 12, and 24 months were 34 (81%), 32 (76%), 30 (71%), and 26 (62%), respectively. Two patients of these underwent repeat PVI, which was successful, and they had freedom from AF episodes. Complications were rare. One patient had a minor pericardial effusion, and one patient had transient sinus pauses, which were conservatively managed.
Conclusion: Conventional RF ablation using PV potential-based mapping and ablation with 8 mm tip catheters is safe for patients with PAF. The intermediate term outcome is satisfactory and cost-effective in our setting with limited resources.

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1. Introduction

Atrial fibrillation (AF) is the most common form of sustained arrhythmia in the world, and it is a major cause of stroke, apart from being symptomatic and sometimes leading to tachycardia-induced cardiomyopathy. When compared with the western population, Indians develop AF a decade earlier,[1,2] and hence, the impact of the disease is very significant.

Haissaguerre et al demonstrated the importance of pulmonary veins (PVs) for the initiation of paroxysmal AF, which started the era of ablation treatment for this arrhythmia.[3] Catheter ablation aims to electrically isolate the foci within the PV, from the atria. It has emerged as a treatment modality for symptomatic patients who have not responded to medication.[4,5]Reports of AF ablation demonstrated that PVI achieved short-term arrhythmic resolution in 80–85% of paroxysmal AF patients[6,7] although some patients required repeat procedures.

Other strategies for PAF include PV antrum isolation, balloon-based radiofrequency (RF)/cryoablation and wide circumferential ablation (WACA) around the PVs. Many of these techniques involve expensive equipment and three-dimensional mapping. The resultant cost is not affordable for most patients in India. Hence, we analyzed our experience using conventional electrophysiology method based on PV mapping using a Lasso catheter. An 8 mm tip catheter was used to ensure better contact, minimize the number of energies necessary, and shorten the procedure time.

2. Method

2.1. Patient selection

A total of 42 consecutive patients admitted with symptomatic PAF for catheter ablation in a tertiary care teaching hospital from March 2011 till June 2018 were prospectively enrolled for this study. All patients gave written and informed consent. All procedures were performed by a single operator.

Inclusion criteria: All patients 18 years or older who had symptomatic non-rheumatic PAF not controlled with at least one antiarrhythmic drug were included for the study.

Exclusion criteria: Patients with rheumatic AF were excluded. Other exclusion criteria were persistent AF (symptoms lasting for more than seven days but less than a year), long-standing persistent AF (symptoms lasting for more than a year), left atrial (LA) thrombus, LA anteroposterior diameter exceeding 50 mm, unstable angina in the previous three months, bleeding disorders, pregnancy, patients with contraindications for anticoagulation, decompensated heart failure, and unwillingness to give informed consent.

2.2. Mapping and ablation technique

PV isolation (PVI) was performed solely by conventional Electrophysiology equipment (EP Tracer, Model V074 version), without using 3D mapping. Before the procedure, PV anatomy was assessed by trans-esophageal echocardiogram (TEE) in all patients: the PVs were visualized and their diameters assessed (mid-esophageal 0° view for right PVs and mid-esophageal 50° view for left PV); the LA was visualized for thrombus, and the LA appendage velocity was assessed (mid-esophageal 90° left and right and 45 ° views). Cardiac CT was used to assess the PV anatomy in selected patients (Fig. 1A and B).

Before the procedure, 25 patients were on oral anticoagulation with vitamin K antagonists or NOAC, while the remaining 17 were taking aspirin. A day prior to the procedure, an oral anticoagulation dose was omitted. All patients underwent TEE prior to the procedure, after confirming that the international normalized ratio (INR) was less than 2. The procedure was performed under local anesthesia. Vascular access was through the femoral veins; two in the right femoral vein (one for ablation catheter and other for SR0 sheath/Lasso catheter) and one access from the left femoral vein was used for placing the decapolar catheter in the coronary sinus. Totally, five patients developed AF during the procedure; two reverted with ibutilide, two with DC version, and one needed both modalities. A left femoral artery sheath was used to monitor blood pressure and for guiding trans-septal puncture. Trans-septal catheterization was performed using RAO 30° and LAO 50° fluoroscopic views, and systemic anticoagulation was achieved with intravenous heparin to maintain an activated clotting time of 250–350 s. Pre-ablation selective angiography of individual PV s was performed in the AP and LAO 40° views **(**Fig. 2A**).**The ostia of the PVs were marked.

In addition, the mapping catheter was used to determine the ostium of the vein as the site immediately before the catheter fell off the venous ridge into the LA during a slow pullback along the trunk of the vein. Through the SR0 sheath, a Lasso catheter was placed in the PV and gradually withdrawn to within 5 mm of the ostium. A Webster 7F thermocouple 8 mm tip catheter was inserted into the LA either through the same trans-septal puncture site or through a second trans-septal puncture and used for ablation (Fig. 2B). Ablation of some of the PVs (especially the right inferior) was not performed in the following situations:

i) The vein was electrically silent
ii) The vein could not be cannulated
iii) The vein was too small in caliber to accommodate the Lasso catheter
iv) There was frequent triggering ectopy that was clearly originating from other (usually superior) PVs.

Focal/segmental PVI was performed during sinus rhythm (right-sided veins) or during distal coronary sinus pacing (left-sided veins) by delivering RF energy at ostial sites that had the earliest breakthrough bipolar potentials (Fig. 2C). RF energy was delivered at the PV ostium with a target temperature of 50–60 °C and a maximum power output of 30–50 W for 20–30 s. Ablation was stopped if the patient had severe pain during ablation.

Patients who had recurrent documented AF paroxysms and consistent PAC morphologies suggestive of a particular vein (usually the upper PVs) underwent isolation of these veins initially. After this, if no further arrhythmias were seen despite isoprenaline, the other veins (usually lower) were left alone. Additionally, 10 PVs showed no electrical activity. Elimination of all PV potentials and complete entrance block into the PV were considered indicative of complete electrical isolation. After ablation, selective angiography of the PVs was performed to look for stenosis. None of the patients had PV stenosis, which usually starts off immediately after ablation. Follow-up cardiac CT scan was not done to assess PV stenosis.

Immediately after PVI, oral anticoagulation was started with warfarin, dabigatran, or apixaban. If warfarin was used, there was a 48 h overlap with enoxaparin or fondaparinux. The anticoagulation was continued for at least three months.

2.3. Follow-up after ablation

Patients were discharged usually within two days after the ablation. Following the procedure, a vitamin K antagonist or direct oral anticoagulant was continued for at least three months. Patients taking an antiarrhythmic drug before the procedure were advised to continue the medication for three months after ablation; this was discontinued thereafter if the patient was free of an AF relapse.

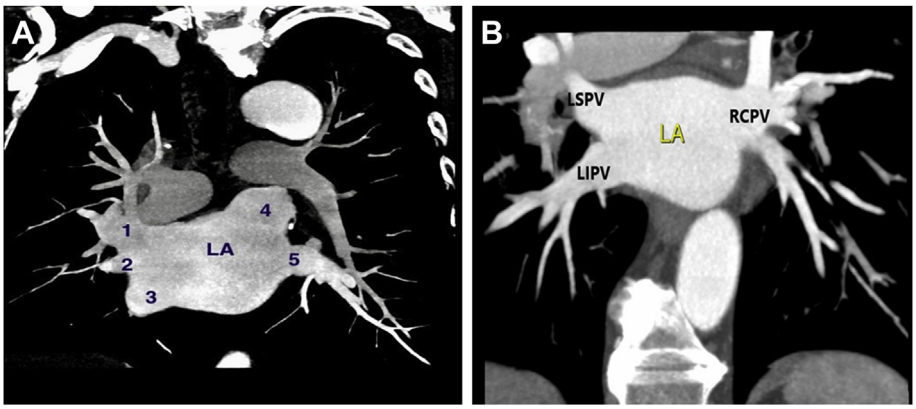

Fig. 1. (A): Posteroanterior projection of cardiac CT showing three left PVs and two right PVs. Labels: LA: Left atrium. 1: Left superior PV. 2: Left middle PV. 3: Left inferior PV. 4: Right superior PV. 5: Right inferior PV. **(B):** Posteroanterior projection of cardiac CT showing two left PVs and a single right common PV. Labels: LA: Left atrium. LSPV: Left superior pulmonary vein. LIPV: Left inferior pulmonary vein. RCPV: Right common pulmonary vein.

Follow-up visits were scheduled at one, three, and six months, and every six months thereafter. An ECG, symptom review, and clinical examination were performed at each follow-up. AF episodes less than 30 min duration are not a significant risk factor for stroke. So, while we may have missed silent AF recurrences in sleep, it is improbable that any significant episodes during the waking hours were missed.

2.4. Freedom from AF

Freedom from AF was defined as no occurrence of even a single episode of documented atrial arrhythmia lasting more than 30 s at any time within the study period after the procedure. No blanking period was used in this study, as there has been conflicting evidence of its validity. Therefore, arrhythmias were classified as recurrent even within the first few weeks of the procedure.

2.5. Statistical analysis

Clinical and laboratory parameters (age, gender, duration of AF, diabetes mellitus, hypertension, hyperthyroidism, coronary artery disease, structural heart disease,(alcoholism, BMI) were assessed for their impact on the outcome of PVI. Discrete variables are described in terms of the frequency and proportion and compared using the $\chi 2$ or Fisher's exact test. Continuous variables are described as the mean ± SD and compared using unpaired *t*-tests for normally distributed data; the median (IQR) was compared using the Mann–Whitney *U* test. Univariable and multivariable predictors of freedom from AF were examined using logistic regression using forward conditional modeling. Kaplan-Meier analysis was used to analyze AF-free survival. Where applicable, two-tailed tests were used in all analyses. A p value ≤ 0.05 was considered significant for all tests. Analyses were performed using IBM SPSS Statistics software V.21.

3. Results

3.1. Baseline characteristics

A total of 42 patients were studied (Table 1**)**. The study population had 30 (71.4%) males and 12 (28.6%) females, aged 51.5 ± 11.7 years (range 24–75 years). The BMI was 22.5 ± 2.55; a BMI > 25 was present in 10 (23.8%) patients. Diabetes was present in 11 (26%) patients, hypertension in 13 (31%), and structural heart disease in two (4.8%) patients. One anti-arrhythmic drug (AAD) had been tried in two (4.8%) of the patients, two AADs in 25 (59.5%) and three AADs in 15 (35.7%) patients. Hence, a mean of 2.31.±.0.AADs had been ineffective in preventing recurrence before the ablation procedure. The commonly used AADs include amiodarone, beta blockers, and flecainide.

3.2. Echocardiographic parameters

The anteroposterior LA diameter was 40.5 ± 4.1 mm and the LVEF was 0.58 ± 0.21. The LA diameter >46 mm was present in six (14.3%) patients, and among them, the failure rate was high: 5/6 (83.3%).

3.3. Procedural details (Table 2)

The procedure duration was 125 ± 29 min, while the fluoroscopy time was 47 ± 13 min. After the procedure, 34 patients were arrhythmia-free, while eight patients continued to have AF episodes. The mean number of PVs isolated was 2.4 ± 0.8. The number of PVs isolated were one (five patients, 11.9%), two (20 patients, 47.6%), three (12 patients, 28.6%), and four (5 patients, 11.9%). The RSPV was isolated in 40, the LSPV in 39, the LIPV in 17, and the RIPV in five patients. The number of RF energy applications was 18 ± 7.

3.4. Complications

Complication occurred in two patients; one patient developed a moderate pericardial effusion and one patient had short-lived sinus pauses, which were conservatively managed.

3.5. Follow-up

The mean follow-up duration was 44 ± 21 months (range 6–84 months). The number of patients who had freedom from AF at 1, 6, 12, and 24 months were 34 (81%), 32 (76%), 30 (71%), and 26 (62%), respectively. The only parameter that showed significant difference between patients who remained in sinus rhythm and AF recurrence was a LA size of ≥45 mm (Table 3**)**. The mean LA size was larger in patients with failed ablation as compared to the success group, 47.8 ± 2.4 mm vs 39.8 ± 2.3 mm ($p < 0.001$).

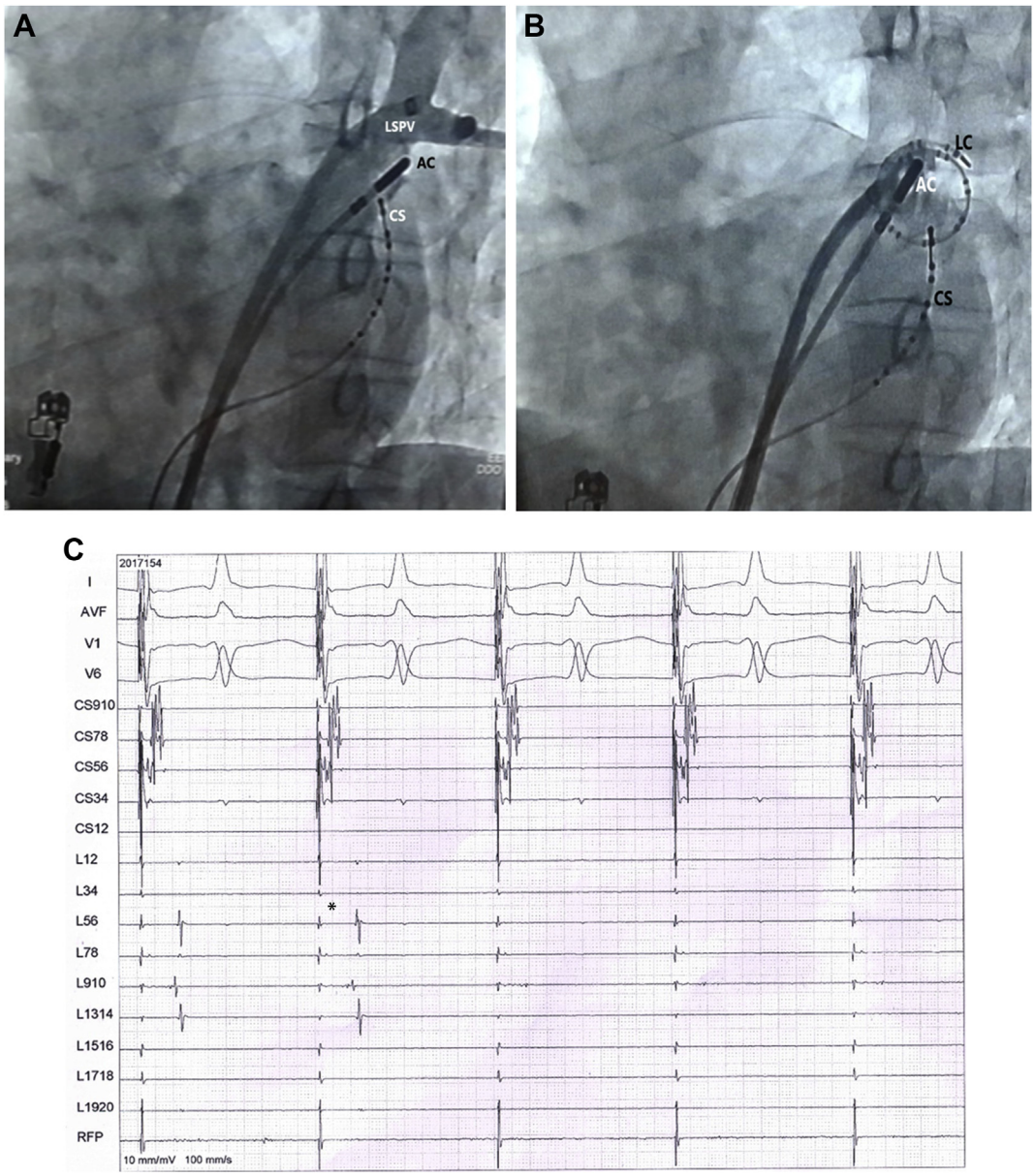

Fig. 2. (A): Preablation selective angiography of the LSPV performed in fluoroscopic LAO view. Labels: LSPV: Left superior pulmonary vein. AC: Ablation catheter. CS: Coronary sinus catheter. (**B):** Left anterior oblique cine-angiographic view demonstrating a Lasso mapping catheter (LC) and 8 mm tip ablation catheter (AC) at the LSPV ostium. CS: Coronary sinus catheter. (**C):** PVI for AF. Note how the PV potential disappears during RF ablation. CS: Coronary sinus. L: Lasso catheter.

3.6. Recurrence of AF and redo PVI

AF recurrence in the first year after initial successful PVI was seen in two patients. In the first patient during the first PVI, the RSPV was electrically silent and other three PVs were isolated, but recurrence was seen after three months. In the redo PVI, he was found to have widespread arrhythmogenic foci in RSPV, and the LIPV showed partial reconnection with good PV potentials. After a successful redo PVI, freedom from AF was noted during two years of follow-up.

In the second patient, the first time she was presented as 'focal' AF arising from the LIPV, which was successfully ablated. After six months, she had multiple hospitalizations for symptomatic PAF episodes. She was taken up for the repeat procedure, which showed good PV potentials in the RSPV, RIPV, and LSPV, which were successfully isolated. During a two-year follow-up period after this, she had freedom from AF episodes.

4. Discussion

The data on epidemiological trends of AF from India are limited.[1,2,8,9] Though non-valvular AF is the commonest cause in the western population, in Indian settings, rheumatic heart disease still contributes to at least for half of the AF cases. In the western population, its prevalence is around 1–2% of the general population, occurring in up to 5% of people aged 75 or more.[10]

Although the aged population is more at risk for developing AF, the mean age of Indian patients with AF is nearly a decade younger than the western cohort.[1,2] In our study, the age was 51.5 ± 11.6 years (range 24–75 years). Hospital data records of patients with chronic AF from Andhra Pradesh revealed a mean age of 45.4 years, with many (51%) aged < 50 years and only 16.3% older than 60 years.[11]In an observational hospital-based study carried among indoor patients of AF in Bihar, Vidya et al reported that the mean age of the patients was 47 years, with 48% of patients aged

Table 1
Baseline characteristics of the study population ($n = 42$).

Parameter		
Age		51.5 ± 11.7 years
Gender		
Male		30 (71%)
Female		12 (29%)
BMI		22.5 ± 2.6
Diabetes mellitus		11 (26%)
Hypertension		13 (31%)
Coronary artery disease		2 (5%)
Structural heart disease		2 (5%)
Duration of symptoms		32 ± 40 months
		1.82
Antiarrhythmics before procedure	1	2 (5%)
	2	25 (59%)
	3	15 (36%)
Antiarrhythmics after procedure	0	7 (17%)
	1	31 (74%)
	2	4 (9%)
LA diameter (AP)		40.5 ± 4.1 mm
LVEF		0.58 ± 0 .21
	1	

Table 2
Procedure-related variables ($n = 42$).

Variable		
Number of veins isolated	1	5 (11.9%)
	2	20 (47.6%)
	3	12 (28.6%)
	4	5 (11.9%)
Procedure time (minutes)		125 ± 29
Fluoroscopy time (minutes)		47 ± 13

Table 3
Factors associated with successful outcome at one year after PVI ($n = 42$).

Variable	Sinus rhythm	AF	p
	Mean ± SD	Mean ± SD	
Age (years)	52.3 ± 12.7	49.8 ± 7	0.655
BMI	22.3 ± 2.6	21.2 ± 1.6	0.307
LA size (mm)	39.8 ± 2.3	47.8 ± 2.4	<0.001

		Sinus rhythm		AF		*p*
		N	%	N	%	
Gender	Male	20	80.0%	5	20.0%	0.418
	Female	10	90.9%	1	9.1%	
Diabetes mellitus	No	22	81.5%	5	18.5%	0.606
	Yes	8	88.9%	1	11.1%	
HTN	No	18	78.3%	5	21.7%	0.277
	Yes	12	92.3%	1	7.7%	

between 51 and 60 years.[12] Data from the IHRS-AF registry[1] and the Indian subset of REALISE AF[13] and RELY-AF[14] study also reaffirmed these findings. The mean age of Indian patients with AF in the REALIZE-AF study[13] was 60 years, while that in the IHRS-AF registry[1] was 54.2 years (range 15–96 years).

Tedrow and colleagues observed a large cohort of women and found that the risk of incident AF was linearly associated with increasing BMI.[15] In our study, out of 42 patients, only eight patients had a BMI ≥25, and the mean BMI of patients in this study was 22.5 ± 2.6.

Atrial enlargement and fibrosis trigger and lead to persistence of AF through changes in the substrate of LA and subsequent electrical remodeling.[16] Moreover, patients with larger LA require more energy and longer lesions to complete the ablation.

Some studies demonstrate that severe LA scarring after ablation predisposes patients to AF recurrences, which seems to result from re-connection between LA and PVs.[17] Zhuang et al showed patients with recurrences had a larger LA size than those without recurrences after a single PVI (95% CI 1.26–2.48, $p < 0.001$).[18] In our study also, the mean LA size was larger in patients with failed ablation than the success group, 47.8 ± 2.4 mm vs 39.8 ± 2.3 mm ($p < 0.001$). The LA diameter was >46 mm in six (14.3%) patients, and among them, PVI was successful in only one patient. Thus, our study finding of larger LA diameter as a predictor of failure of PVI is in concordance with other studies.

In our study, the number of patients who remained in sinus rhythm at 1, 6, 12, and 24 months were 34 (81%), 32 (76%), 30 (71%), and 26 (62%), respectively. Initial studies describing focal ablation within the PVs showed disappointing minimal long-term benefit and a significant risk of PV stenosis.[19] Despite using irrigated tip catheters with contact force technology in PAF patients with mean age of 56 years, the success rate after single PV antral isolation in a multicenter study was 48.6% and the five-year long-term freedom from AF was only 58.3%. About 53% of the PAF patients required second redo PVI, 42% required third redo procedure, and 33% required a fourth redo procedure in that study.[20] Hence, our study results, which used 2D mapping, non-irrigated catheter and few redo ablations, are not inferior.

Marrouche et al[21] clearly demonstrated that distal isolation ablating from within PVs) eliminated AF only in 29% of the patients, whereas ostial isolation eliminated AF in all. In that study, it was also shown that the AF recurrence rate was 21% with 4 mm tip ablation catheter and 15% with cooled tip catheter at a mean follow-up period of 10 ± 3 months and 4 ± 2 months, respectively, whereas no recurrence was documented with use of 8 mm tip ablation catheter at a mean follow-up period of 6 ± 4 months. Though the literature regarding the effectiveness of 4 mm vs 8 mm tip catheter is sparse in catheter ablation for AF, we used 8 mm tip catheter in view of perceived greater effectiveness, especially in cost-constraint situations.

The latest multicentric CABANA trial did not show any significant difference in primary or secondary outcomes between ablation arm and AAD therapy arm in the treatment of AF patients.[22] Regarding cost effectiveness of AF ablation, it had been shown in few decision modeling studies that RF ablation of AF might be cost effective in patients with AAD-resistant AF. The average cost of AF ablation varies between 14,000 and 18,000 US $ in the USA,[23] and such a high cost is primarily secondary to 3D mapping system and special catheters like smart touch catheters/cryoballoon and in Indian settings, such a high cost, is beyond the reach of many deserving symptomatic AF patients. Hence, 2D mapping and 8 mm tip catheter for better contact were used in our study to get better results in a resource-constraint setting.

Complications during AF ablation include vascular access complications, PV stenosis, pericardial tamponade, cerebral embolism, atrioesophageal fistula, cardiac perforation, and death. Currently, the risk of complication during RF ablation for AF is 2–3%, with vascular access complications being the most common, while fatal complications are rare.[24] We had only two complications, none of which was serious.

5. Limitations of the study

In our study, 3D mapping was not used. The current recommendation in AF ablation is to perform PVI of all four PVs, while we have done PVI of only those veins that were considered arrhythmogenic. In patients who had recurrent documented AF paroxysms and consistent PAC morphologies suggestive of a particular vein (usually the upper PVs) underwent isolation of these veins initially.

After this, if no further arrhythmias were seen despite isoprenaline, the other veins (usually lower) were left alone. We did not test with adenosine after ablation. Also, we did not check for an exit block. Our study is a single-center, single experienced operator outcome study. We need to have a multicentric data on AF ablation from developing countries with reduced resources. Our results, though not as good as with current large studies with sophisticated mapping, image merging, and ablation techniques targeting all four veins, are, nonetheless, not much inferior.

We could not analyze whether recurrences correlated with the number of veins isolated. This was because of the small numbers in each subgroup.

Our patients during follow-up did not have routine Holter, external, or internal loop recorder to assess AF recurrence. We now understand that AF episodes less than 30-min duration are not a significant risk factor for stroke. So, while we may have missed silent AF recurrences in sleep, it is improbable that any significant episodes during the waking hours were missed.

Intracardiac echo was not used in our patients due to resource constraints. Intracardiac echo would have possibly helped identify clearly the PV orifice and catheter contact and unmask electrical activity in some of the 'silent' veins.

CT pulmonary venogram was not done routinely in all patients at follow-up to look for PV stenosis due to financial constraints. Immediate postprocedural pulmonary venogram by cine angiogram may not be relied upon to predict the future occurrence of PV stenosis.

6. Summary

To the best of our knowledge, this is the first study from India reporting the intermediate term outcome of PVI for patients with symptomatic PAF. AF was more common in males and in age group >50 years; hypertension was seen in only one-third of the patients. Immediately after the procedure, 34 out of 42 patients (81%) remained free of any symptomatic and/or documented AF episode.

After PVI, the number of patients who remained in sinus rhythm at intermediate term follow-up was 62%. An 8 mm tip catheter ensures adequate contact and energy delivery and was not associated with any major complication. An anteroposterior LA diameter >46 mm was the best predictor of failure of PVI. Though larger LA diameter is associated with higher AF recurrence rates,(larger atria that are associated with healthier myocardium may be easier to ablate.

Hence, this study confirms that PVI using conventional mapping techniques and a 8 mm tip catheter is a safe and economically feasible treatment modality for symptomatic patients with PAF in the Indian setting with reasonable outcomes. Our results, though not as good as with current large studies with sophisticated mapping, image merging, and ablation techniques targeting all four veins, are, nonetheless, not much inferior.

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None.

Declaration of competing interest

All authors have none to declare.

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CASE REPORT

Nanosomal docetaxel lipid suspension based chemotherapy in a pregnant MBC patient – a case report

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Abstract: The current report presents a case of a pregnant woman with breast cancer metastasized to liver and lungs. The standard of care for breast cancer in pregnancy is anthracycline/taxane-based chemotherapy regimens. Docetaxel has shown a favorable toxicity profile during the second and third trimesters of pregnancy. A novel nanosomal docetaxel lipid suspension (NDLS) (DoceAqualip), with a proven efficacy and tolerability profile, has been approved in India for the treatment of advanced solid tumors since 2013. We present here a case of a pregnant woman with metastatic breast cancer managed with NDLS based TAC regimen showing a partial response after six cycles. The patient delivered a healthy male child with normal Apgar score and weight at the 32nd week of gestation.

Keywords: NDLS, PABC, DoceAqualip, pregnancy, breast cancer, metastatic

Introduction

Pregnancy-associated cancer ranges from ~25 to 190/100,000 pregnancies,[1] and has become a leading cause of maternal death.[2] Breast, cervical, lymphoma, ovarian, and melanoma cancers are the most common types reported during pregnancy.[3] Breast cancer is one of the most common malignancy associated with pregnancy, arising in 1/10,000–1/3000 pregnancies.[4] Breast cancer during pregnancy generally occurs in women with advanced age, with only 10% reported under 40 years of age.[5,6] Pregnancy-associated breast cancer (PABC) is defined as breast cancer diagnosed during pregnancy or within one year of delivery.[6,7] The average age of diagnosis of PABC is 33 years with a median gestational age of 21 weeks.[6] The incidence of PABC is ~15–35/100,000 deliveries.[8] During pregnancy, breast cancer is generally diagnosed after it has metastasized due to advanced maternal age and difficulty in diagnosis arising from the pregnancy-related changes in the breast.[9] The average reported delay for diagnosis of breast cancer during pregnancy is 5–15 months from the onset of symptoms.[10]

The optimal treatment of cancer during pregnancy remains elusive because there are limited data from retrospective studies with small samples.[11] The management of PABC is dependent on the trimester of pregnancy, type, and stage of the cancer. Surgery is recommended in all trimesters, chemotherapy in the second and third trimesters, and radiotherapy only in the postpartum period.[6] The National Comprehensive Cancer Network (NCCN) Clinical Practice Guideline suggests chemotherapy followed by endocrine therapy and palliative radiotherapy for the management of breast cancer

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during the second and third trimester of pregnancy.[12,13] In literature, several agents have been evaluated for the treatment of PABC including anthracyclines and taxanes.

We report here a case of a pregnant woman with breast cancer metastasized to liver and lungs. The patient was treated with nanosomal docetaxel lipid suspension (NDLS, DoceAqualip) based TAC (NDLS, doxorubicin, cyclophosphamide) regimen for six cycles.

Case report

A 44-year-old pregnant woman presented with a lump in the left breast and pedal edema. The patient was married from the past 2 years and it was a non-consanguineous marriage. She was a second gravida woman with intracytoplasmic sperm injection conception after 1 year of a previous abortion (conception by in vitro fertilization) a single live uterine pregnancy of 26 weeks (second trimester). She was a known case of hypertension and hypothyroidism, and was receiving nifedipine 10 mg OD and thyroxine 75 µg OD. Medical history showed the patient was anemic with low hemoglobin levels (10.7 gm%). Genotyping for BRCA1, BRCA2, or FANCJ was not performed. Magnetic resonance imaging (MRI) of chest and abdomen (Jun 2018) showed a large soft tissue intensity irregular mass lesion with central necrosis with restricted diffusion measuring 6.1×5.5×5.0 cm in the inner quadrant of the left breast parenchyma with adjusted lobulated prominent ducts suggestive of left breast malignant mass. Multiple enlarged lymphnodes were seen in the left axillary region. Multiple tiny randomly distributed pulmonary nodules with restricted diffusion were observed suggestive of lung metastasis. Multiple mixed hyperintense necrotic, non-necrotic lesions with restricted diffusion of varying size (1.9–8 cm) were seen in both lobes of liver with the largest measuring 8×7 cm. Staging was done as $T_3N_2M_1$ – Stage IV.

Biopsy from the left breast lump showed a tumor occupying 60% of the biopsy specimen. Tumor cells were arranged in a diffuse pattern; few glands and pseudo papillae with surrounding desmoplastic stroma were noted. Tumor cells showed moderate pleomorphic nuclei, coarse chromatin, and moderate cytoplasm. In addition, few benign ductules were noted. Frequent mitotic figures were reported (12/10 hpf). The impression was made as invasive carcinoma, no special type (NOS) (Figure 1).

Immunohistochemical examination showed negative estrogen receptor, weak positive progesterone receptor (10%), and negative HER2 neu status. The final diagnosis was PABC with lung and liver metastases. 2D ECHO with Color Doppler showed normal cardiac functions with an ejection fraction of 63%.

The patient was planned for palliative chemotherapy with 3-weekly NDLS based TAC (NDLS, doxorubicin,

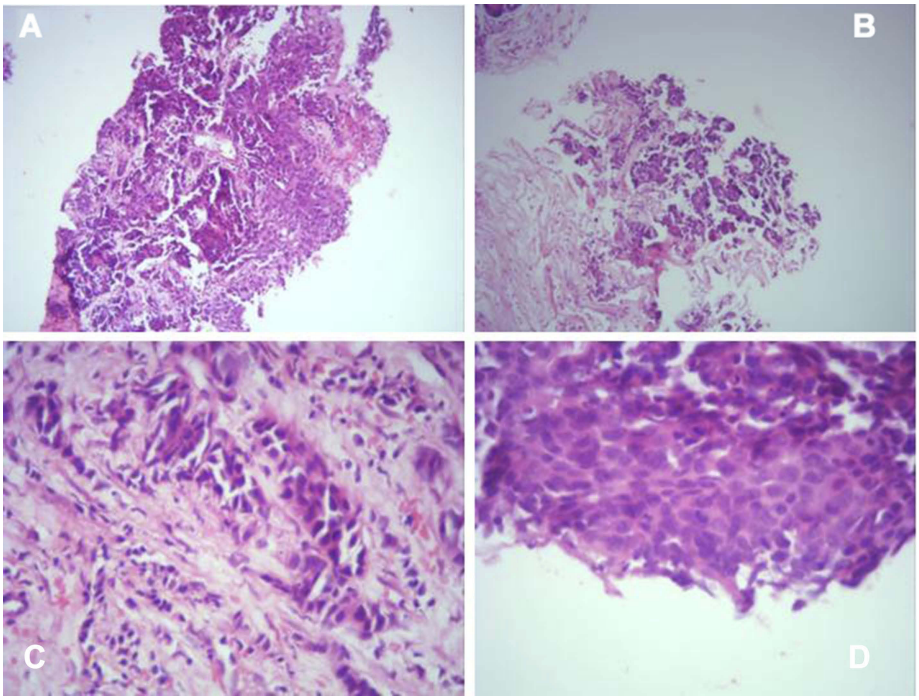

Figure 1 Biopsy of left breast: (**A**) Diffuse pattern (100x); (**B**) Pseudo papilla; (**C**) Occasional tubular pattern with desmoplastic stroma (400x); (**D**) Frequent mitosis (400x).

cyclophosphamide) regimen for six cycles. The first chemotherapy cycle was started in Jul 2018 with intravenous (IV) NDLS 100 mg (75 mg/m^2), IV doxorubicin 80 mg, and IV cyclophosphamide 800 mg on Day 1 of the cycle along with IV ranitidine 50 mg BD, IV dexamethasone 8 mg BD, and IV ondansetron 8 mg BD. Filgrastim 300 µg was given subcutaneously on Days 2–4 of the cycle.

Ultrasound antenatal scan with Doppler done a day before the second chemotherapy cycle demonstrated a single live intrauterine fetus in cephalic presentation with fetal spine to the maternal right side. Active fetal movements and fetal cardiac pulsations were present with fetal heart rate measuring 132/min. Placenta was seen in fundal posterior and left lateral wall with grade II maturity. The average gestational age was 31 weeks and 5 days with normal Doppler parameters. After 3 weeks following the first chemotherapy cycle, a second cycle with NDLS based TAC regimen was administered similar to the first cycle.

At the 32nd week of gestation, the patient presented with the complaints of diminished fetal movements. There was no bleeding or prevaginal draining. Her amniotic fluid index was 7.2 cm. Medical oncologists' opinion was sought for emergency low segment cesarean section (LSCS). She was advised 6 units of fresh frozen plasma and 6 units of platelets transfusion. She was administered 4 doses of IV dexamethasone 6 mg at 6 hourly intervals. Emergency LSCS was performed, and intraoperative findings were normal. The patient delivered an alive male child. The child's birth weight was 1.76 kg which was normal as per the gestational age, and Apgar score was also normal. The child was kept for observation in the neonatal intensive care unit for 2 days as a precautionary measure. Tablet cabergoline 0.25 mg (two tablets) was given to the patient to suppress breast milk secretion. Her Hb level was 7.7 g%, and platelets were 182,000/mm^3; 2 units of packed cell volume was administered, and the patient was asked to check and inform in case of any bleeding. The patient was advised to take plenty of oral fluids, salt-restricted diet, postnatal exercises, to avoid lifting heavy weight, and use of temporary contraception.

Her 3rd, 4th, and 5th chemotherapy cycles with NDLS based TAC regimen were given similar to previous cycles in Aug, Sep, and Oct 2018, respectively. The sixth chemotherapy cycle was given in Nov 2018 similar to the previous cycles except inj. filgrastim 300 µg OD for 3 days was replaced with inj. pegfilgrastim 6 mg OD on Day 2 of the cycle.

After completion of six chemotherapy cycles, MRI done in Nov 2018 showed a small focus of restricted diffusion measuring 8×7 mm noted in the inner quadrant of the left breast. There were no significantly enlarged mediastinal or axillary lymphnodes. Lung parenchyma showed normal densities with no evidence of alveolar densities, intrinsic thickening, fibrosis, or emphysematous changes – suggestive of no lung metastasis. Trachea and main bronchi were within normal limits. A few heterogeneous lesions with restricted diffusion were noted in the liver measuring 2.5×2 cm in segment VIII, suggestive of residual liver metastasis. A cystic lesion measuring 1.2×1.5 cm was noted in segment VIII of the liver. Overall, in comparison to the baseline MRI (Jun 2018; Figure 2) as per RECIST 1.1 criteria a "partial response" was reported with the NDLS based TAC chemotherapy regimen (Figure 3). Table 1 highlights pre- and post-NDLS based chemotherapy MRI findings. The patient is currently stable and receiving hormonal therapy with tamoxifen 20 mg PO. Consent was obtained from the patient for publication of this case report.

Discussion

Pregnancy can delay breast cancer diagnosis, evaluation, and treatment.[7] PABC is not so common and requires thorough workup of breast symptoms to diagnose at early stages so that the treatment can be started as soon as the diagnosis is made.[7] The risk of axillary lymph node metastasis is increased by ~0.9% to 1.8% in case of delayed diagnosis by one month.[14] The incidence of PABC is lower (0.7% in India) in developing countries as the age of the mother at delivery is younger.[15] Pregnancy should not be considered as an obstacle for cancer treatment and majority of PABC woman are candidates for chemotherapy.[16] A multidisciplinary approach is required in the diagnosis and treatment of PABC in order to treat the cancer and at the same time to protect the fetus.[17]

We report here a case of a pregnant woman with advanced stage (Stage IV) breast cancer metastasized to liver and lungs who was successfully managed with NDLS based TAC regimen.

The gold standard for diagnosis of PABC remains the histopathological diagnosis based on core biopsy.[17] This patient underwent MRI and core biopsy to confirm the diagnosis as invasive carcinoma of left breast – NOS. Breast cancer is usually diagnosed with metastasis in pregnant women with advanced age,[9] and similar was the case with this 44-year-old patient in whom the cancer had spread to the lungs and liver. Estrogen and progesterone

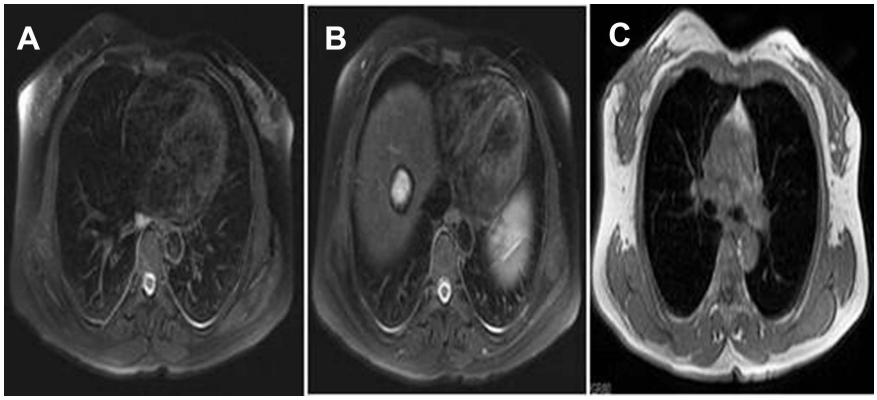

Figure 2 Baseline MRI: (**A**) Malignant mass in the inner quadrant of left breast; (**B**) Multiple mixed hyperintense necrotic, non-necrotic lesions in liver; (**C**) Pulmonary nodules with restricted diffusion in lung.

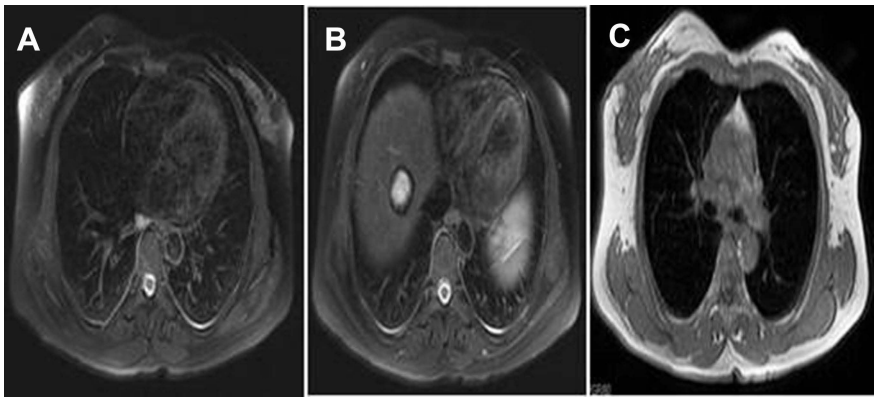

Figure 3 Post treatment MRI: (**A**) Small focus of restricted diffusion in the left breast inner quadrant; (**B**) Heterogeneous lesions with restricted diffusion in segment VIII of liver suggestive of residual liver metastasis; (**C**) Lung parenchyma showed normal densities with no evidence of alveolar densities, intrinsic thickening, fibrosis or emphysematous changes – suggestive of no lung metastasis.

Table 1 Pre- and post-NDLS based chemotherapy MRI findings

Pre-treatment evaluation	Post-treatment evaluation
• Malignant mass (6.1×5.5×5.0 cm) in the inner quadrant of left breast parenchyma • Multiple enlarged lymphnodes in the left axillary region • Multiple mixed hyperintense necrotic, non-necrotic lesions in liver • Pulmonary nodules with restricted diffusion in lung suggestive of lung metastasis	• Small focus of restricted diffusion in the left breast inner quadrant • Heterogeneous lesions with restricted diffusion in segment VIII of liver – suggestive of residual liver metastasis • Lung parenchyma shows normal densities with no evidence of alveolar densities, intrinsic thickening, fibrosis, or emphysematous changes – suggestive of no lung metastasis

Abbrevition: NDLS, nanosomal docetaxel lipid suspension.

receptors are frequently negative in PABC,[18] as evidenced in this patient who was estrogen receptor negative and weak progesterone positive.

PABC is associated with an increased risk of preterm delivery but it does not increase the risk of growth restriction, stillbirths, or congenital malformations.[19] Similarly, this patient also delivered a healthy child at 32nd week, whose birth weight was normal as per the gestational age and had normal psychomotor development.

Anemia is common in pregnant women and in India ~50% of the pregnant women in are anemic as per National Family Health Survey-4.[20] Few studies have correlated the biallelic mutation in BRCA1/BRCA2 with Fanconi's anemia.[21] Bona fide Fanconi anemia proteins, BRCA2 (FANCD1), PALB2 (FANCN), and BRIP1 (FANCJ) genes along with BRCA1 have a role in DNA interstrand crosslink (ICL) repair, and deficiency in BRCA-dependent ICL repair is associated with breast cancer susceptibility.[22] However, genotyping was not performed in our patient.

The management of breast cancer during pregnancy includes surgery during all three trimesters and chemotherapy in the second and third trimesters without increasing

the risk of fetal malformations.[17] However, lower birth weight has been reported in infants exposed to chemotherapy in utero. According to the NCCN guidelines, surgery should not be considered for advanced stage (Stage IV) patients, and the treatment should involve chemotherapy. Several chemotherapeutic agents belonging to pregnancy category D including 5-fluorouracil, doxorubicin, epirubicin, cyclophosphamide, docetaxel, paclitaxel, and trastuzumab have been evaluated for the treatment of breast cancer in pregnancy.[16] During pregnancy, systemic therapy with taxanes and platinum agents can be safely used after careful risk/benefit assessment for mother and child.[23]

The standard of care for PABC is anthracycline/taxane-based chemotherapy regimens.[23,24] As per the published data, doxorubicin in combination with cyclophosphamide and 5-fluorouracil after the first trimester of pregnancy was not associated with antepartum complications,[25] and the anthracycline-based chemotherapy can be used with minimal risk to the fetus in the second and third trimesters.[26] Similarly, taxanes such as docetaxel and paclitaxel when used after the first trimester of gestation showed no increase in the occurrence of fetal malformations and maternal complications.[16,27] A systematic review of 16 studies (50 pregnancies) demonstrated that exposure to docetaxel or paclitaxel was well-tolerated during pregnancy with manageable toxicities and can be considered as an optimal treatment option for patients with PABC.[28]

Preclinical evidence shows low levels of docetaxel/paclitaxel presence in the fetal tissue.[29] Mir et al, in a systematic review mention that an increase (50–100%) in the activity of cytochrome P-450 (CYP) 3A4 during the third trimester of pregnancy may increase the metabolism of docetaxel, a substrate of CYP-450 3A4, that may result in a shorter half-life and a higher clearance.[30,31] Thus, the favorable toxicity profile of taxanes during the second and third trimesters of pregnancy makes it a potential choice toward management of cancers in such cases.

Docetaxel has demonstrated efficacy and tolerability for the treatment of breast cancer as a part of the TAC regimen in several studies in neoadjuvant, adjuvant, and metastatic settings.[32–36] Furthermore, docetaxel has been successfully evaluated for the treatment of PABC. Santis and colleagues published the first case report on the use of docetaxel in PABC in a 34-year-old woman with 15 weeks of gestation and skeletal metastasis. The patient was successfully treated with docetaxel monotherapy for three cycles and the patient delivered a female infant during the 32nd week of pregnancy with normal birth weight and Apgar score without any abnormalities.[37] Potluri et al,[27] reported that adjuvant treatment with docetaxel (four cycles) after doxorubicin and cyclophosphamide-based neoadjuvant treatment (four cycles) resulted in a delivery of a normal child at the 34th week of gestation.[27] Neoadjuvant therapy with doxorubicin/docetaxel at 14 weeks of gestation did not show any fetal malformations after completion of six cycles and resulted in delivery of a normal child at the 35th week of gestation. Potluri et al, concluded that pregnant patients with cancer can be treated with chemotherapy including taxanes during the second and third trimesters without significant risks to the fetus.[27] Several other reports have established the use of docetaxel alone or in combination with other chemotherapeutic agents for the treatment of PABC.[38]

Conventional docetaxel formulation uses polysorbate-80 and ethanol as excipients, which may cause acute hypersensitivity reactions necessitating corticosteroid use as a premedication.[39] Published evidence suggests that solvent-free nanoparticle drug formulations may alter the pharmacokinetic and pharmacodynamics properties of docetaxel resulting in improved efficacy and decreased incidence of adverse effects associated with wide and nonspecific body distribution (eg, neurotoxicity, musculoskeletal toxicity, neutropenia).[40] A novel NDLS formulation of docetaxel was developed based on "Aqualip" technology (patented [WO2008127358] in Europe, Japan, and Canada) with lipids Generally Regarded As Safe (GRAS) by the US Food and Drug Administration. NDLS is devoid of polysorbate-80 and ethanol, thus, not expected to cause adverse effects such as acute hypersensitivity reaction and cumulative fluid retention.[40–45] NDLS has shown efficacy and safety in the treatment of breast cancer without corticosteroid premedication.[46] NDLS is also effective and safe for gastric, ovarian, cervical, penile, hormone refractory prostate, and non-small cell lung cancers.[39,47–50] NDLS has been approved by the Drug Controller General of India. In this patient, NDLS was preferred over conventional docetaxel as it does not contain polysorbate-80 as a carrier, which can potentially trigger acute hypersensitivity reactions and cumulative fluid retention.[41–43]

This report presents the evidence for efficacy and safety of NDLS in the treatment of breast cancer with liver and lung metastasis in a pregnant woman. NDLS, when used in combination with cyclophosphamide and doxorubicin, was effective in controlling the symptoms and have reduced the disease burden in this patient. The

patient delivered a healthy child with normal body weight and Apgar score without any malformations, consistent with published literature of conventional docetaxel.[37] Overall, the patient was clinically asymptomatic with NDLS based chemotherapy and showed a partial response as per RECIST 1.1. Furthermore, the patient completed six cycles of TAC and is on treatment with tamoxifen. On follow-up, both the mother and infant were doing well. The patient tolerated NDLS treatment well without any clinically significant adverse events.

Conclusion

The current report highlights that NDLS with cyclophosphamide and doxorubicin as first-line therapy was effective in a pregnant woman with MBC. The treatment demonstrated a partial response with complete resolution of lung metastasis. The treatment was well-tolerated. Due to its favorable tolerability profile, NDLS can potentially be used in pregnant women.

Ethics approval and consent to participate

The patient provided written informed consent for the publication of this report and the accompanying images. Institutional approval was not required to publish this manuscript.

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Author contributions

RR, NJ, MK, SS contributed to the concept and design, acquisition, analysis, and interpretation of data. RR and SS provided medical care for the patients and collected the data. All authors contributed to data analysis, drafting, or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

Drs. Nisarg Joshi and Mujtaba A Khan are employees of Intas Pharmaceuticals Ltd. The authors report no other conflicts of interest in this work.

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