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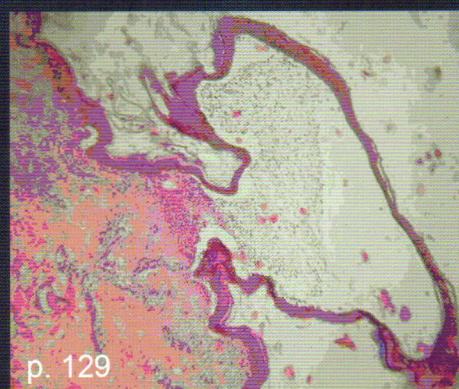
# PUSHPAGIRI MEDICAL JOURNAL

An International Journal



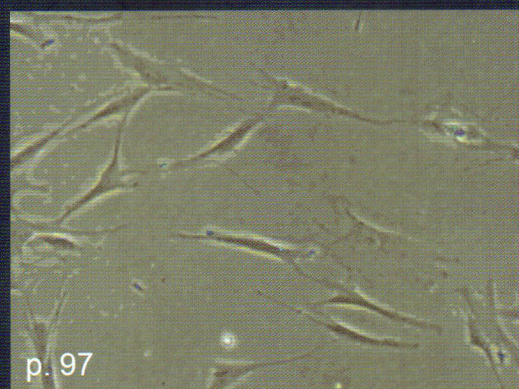
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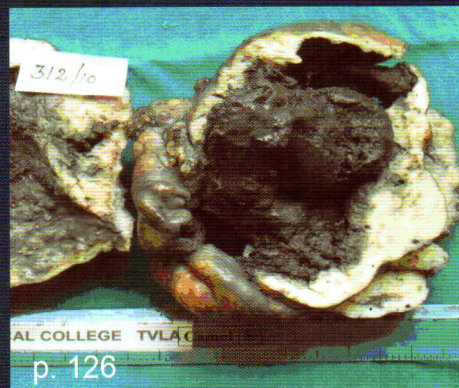
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# Pushpagiri Medical Journal



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To have life for everybody and to have it in abundance through Science and Technology for a Knowledge Society and for improving the Health of our immediate community, the State, the Country and the World at large.

### OUR VISION

We care God cures

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'Pushpagiri Medical Journal', an International Journal, is the official publication of Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla. It is a peer reviewed multi-disciplinary journal providing health professionals with a forum to discuss current challenges in healthcare, sharing innovative evaluation and treatment techniques, learning about and assimilating advanced methodologies being developed in various disciplines in modern medicine as well as related professions, and communicating information regarding newer developments and research programmes. The journal serves as a valuable tool for helping therapists deal effectively with the emerging problems, stumbling blocks and challenges in the field, and emphasizing articles and reports that are directly relevant to medical practice and public health. It will publish original research articles, concise evidence based review articles, unusual and interesting case reports and technical reports. We offer an online submission facility, and a fast reviewing process and editorial decisions so as to avoid delay and inconvenience to the authors. The journal is being included and indexed with many international databases, and it will be published half-yearly, in March (January-June) and September (July-December).

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## EDITORIAL

### Research methodology training for Medical students

**Research**: the term itself is cloudy in the minds of most medical students and many of the members of faculty. This could be because they are not aptly trained on research, as a part of their curriculum. Many Universities round the world insist that post graduate medical students undergo systematic training in research methodology, biostatistics and publication of research articles. This is primarily targeted at ensuring good quality theses and dissertations to be brought out by postgraduates in all specialties of medical science. Most often the PGs are expected to know many details on research methodology by the time the thesis protocol is prepared, and they are totally in the dark. This scenario recurs year after year, because there is no exposure to any type of formal training.

#### **Defects currently encountered**

- ❖ There are few innovative ideas from the medical students. They should know that medical education is much more than just reading books and treating patients accordingly.
- ❖ Most of the studies done by PG candidates involve only data analysis (data collected from medical records), which don't necessitate application of their own skills and ideas.
- ❖ Plagiarism is a serious issue in post graduate and PhD theses, and articles for publication. Since most studies are available online, they are simply copy - pasted without even acknowledging the author or source.
- ❖ Most of the clinical research is in the form of clinical trials by pharma companies, which is a lucrative business, and often gives biased results.
- ❖ Biostatistics is a difficult field for most of the medical students. Not having much knowledge about statistical analysis, they get the work done by statisticians, without any active involvement of their own.
- ❖ Poor computer and communicative skills of many medical students form a big hindrance to acquiring updated knowledge, and presenting it in the appropriate manner, at suitable venues.

#### **Objectives of research methodology training to medical students**

- The training should not be aimed at the preparation and presentation of PG theses.
- Every student should have done one research project (at least a retrospective observational study) before being awarded the MBBS degree certificate. During the MBBS course itself not less than 30% of the students should be able to get funded research programmes like ICMR/ STS projects.
- A proper awareness of clinical trials should be imparted to the students. As doctors, they should know the importance of conducting clinical trials, ethical issues involved in them and biasing in results.
- Knowledge about the current studies and previous clinical trials done by others will help to bring out further advances.
- Create awareness on the lacunae in the present lines of clinical management and the wide scope for improvement through research.
- All medical professionals should be able to assess the study designs and statistical details of clinical studies so as not to get misguided by the drug promotional literature poured into their hands.

Medical Council of India could address the issue and make 'Research Methodology Training' a practical component in the Undergraduate curriculum, similar to the autopsy training. And, without waiting for the MCI to make a move, the institutional authorities can take initiatives in this direction.



## ✪ MEDICAL ETHICS

### High suicidal rates in Medical profession..... When shall we start helping ourselves?

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As is known to us all, suicidal tendency is complex with many psychological, social, biological, cultural and environmental factors involved. Let us observe certain real-life incidents:

#### Scenario 1

For the fresh batch of MBBS students it was the day of their first gross anatomy spotting. The faculty members were busy arranging specimens and setting questions. Two boys came running to the dissection hall screaming, Mr X has hanged himself ..... senior students in the hostel had tried to revive him.... ..... he was rushed to the casualty ..... but to what avail?

#### Scenario 2

A final year MBBS student, quite religious, belonging to a middle class family, not able to 'concentrate well' in the hostel, went home a week prior to the university examination. Two days prior to the examination, he spent a very pleasant time during dinner with parents, and went to bed late, but kept sending SMS to close friends seeking their prayers. Early next morning the mother found her offspring hanging himself, leaving a note of apology to the dear and near ones .....

#### Scenario 3

An Anaesthesiologist on night duty was found in the morning, lying down lifeless in the duty room, with a slow intravenous drip still running ..... As a student, a brilliant candidate, loved by all who interacted with this doctor at least once in their lifetime ..... what had gone wrong?

#### Scenario 4

A senior medical faculty member, deeply attached to the family members, fully aware that the children were very much dependent on her,

sustained 70% burns at home, and passed away the very next day, despite all efforts, in a known centre of excellence.....

Any medical professional will have such memories as these. But as usual, we end up attending the funeral ceremonies, with a few tears falling in fond remembrance, convening a condolence meeting, sharing words of helplessness and possibly regret, and at last, forgetting in a few weeks time, with everyone returning to their daily chores and heavy responsibilities. Medical Professionals and students are all any way trained to encounter accidents, tragedies, deaths and dead bodies and postmortems, very frequently, without developing any empathy. They know to forget ..... *they have to learn to.....!!!*

#### Magnitude of the problem at the International level

In 1997, the National Institute of Occupational Safety and Health (NIOSH) and other government agencies in USA analyzed death certificates of the individuals from all occupations and found statistically significant higher rates of suicide in white male physicians. A leading voice for physicians, *KevinMD.com*, says that every year about 300 to 400 doctors take their own lives, making up to roughly one daily. *Another interesting observation is that even though male suicides outnumber female suicides by about four times in the general population, the suicide rate among male and female doctors is almost equal.*

#### Published data: Aggravated suicide rates in Kerala

The *New Indian Express* (last updated: 05 May 2011) observes that on several social indices like literacy, public health and life expectancy at birth, Kerala is ranking highest, with the rates comparable to developed

countries; at the same time, Kerala has the highest rates of suicide and divorce in India. As per the data available from National Crime Records Bureau (NCRB), New Delhi, the suicide rate is consistently high in Kerala, and is almost three times the National average (roughly 10 in India, and 26 in Kerala).

The *Business line* (Aug 09, 2004), noted that the scale and extent of this dangerous affliction can be gauged from the fact that the State accounts for 25 per cent of sales of psychiatric drugs in the country, making it a favourite destination for the drug manufacturers according to the *State Mental Health Authority (SMHA)*. Mental illness is cited as the cause of suicide in only five per cent of the total cases in the country as a whole, while it is 14.3 per cent in Kerala.

Sad to admit, no data is available regarding the suicide rates among medical professionals and students in Kerala, in the current literature.

### Probable contributory factors:

#### ➤ Depressive tendencies

The recent documentary, "*Struggling in Silence*", exposes one among the features on the darker side of the profession - the barely known and very rarely discussed problem of depression and suicide among doctors and medical students. "Undiagnosed and untreated depression is the culprit here," says Dr. Charles Reynolds, Professor of Psychiatry at the University Of Pittsburgh School Of Medicine, who appears in the film. He was one among the authors of an article (one in the few) on physician suicide, published in *The Journal of the American Medical Association* in 2003. He observed that the incidence of depression during the entire period of life is basically the same for male physicians and the general population of men (about 12 percent). At the same time the male doctors' suicide rate is 1.4 times higher than that in general male population. Female doctors, on the other hand have double the rate of depression and 2.3 times higher rate of suicide, compared to general women population.

It is very popularly known that when depression prevails if one gets the proper therapy the distorted thinking process usually gets cleared in a short span of time. Once settled, the same person can find pleasure, energy, and hope in life again. But while someone is seriously depressed, suicidal thinking is a real concern, which needs to be addressed without doubt or delay. Sometimes people who feel suicidal tendencies may not even realize they are depressed.

Could it be true that medical professionals are not able to seek treatment for a common illness that millions of people all over the world have subdued with therapy? Why are we not able to make proper use of the readily available medications? Is it because we are unable to admit to have a mental-health problem, since we could lose respect in the society, referrals, income and even our practicing licenses?

#### ➤ Need to be projected strong

It is a strong notion that doctors are supposed to be the physically and mentally agile persons who care for the sick with pride and confidence, and are not fragile and disabled ones who need to be cared for. From the very young age itself these individuals are generally brilliant, exceptionally hard working, and enthusiastic in all academic as well as co-curricular activities. By adolescence their mental makeup is such that they are not easily able to accept failures and setbacks.

#### ➤ Insurmountable stress

Medical curriculum is entirely different from other professional courses in that the subjects are exceptionally vast and difficult to be reproduced with precision. The methodology of medical studies is entirely different from that followed during the schooling period, and some students find it terribly hard, having sleepless nights and restless days. The stress of frequent examinations, rigorous professional training, staying away from home without parental support, lack of religious activities in which they were trained from early childhood, and at least in some cases, the regret of being a financial burden to the family could make life miserable for many medicos.



Once the medical course is completed, the empathy some doctors could feel for their patients, unavoidable setbacks and misfortunes encountered in patient management, the stressful duty timings, professional rivalry, inability to secure higher education in the choice specialty, etc. could make life seem totally insecure. So also, a persistent, emotional victimization for various reasons increases the stress.

At the same time many of those interviewed after failing in a suicide attempt confess that they were trying to escape from a situation that seemed impossible to deal with, or to get relief from unbearably disturbing thoughts or feelings. Their wanting to die was actually a surfacing of their wanting to escape from what seemed beyond their capabilities. And at that specific moment death appeared to be the best way open.



### ➤ Substance abuse

Frequent consumption of alcohol and regular intake of some drugs, even for a short duration, have depressive effects on the brain. Misuse of these substances, readily available to medical personnel can bring about serious depression episodes. That's especially true for some individuals who already have a tendency to depression because of their family history, biological peculiarities or other stressful environmental factors. The chances of their depression getting worse rise because many people who are depressed are known to turn to alcohol or narcotic drugs as an escape. This happens without them realizing that the depressive effects alcohol and drugs have on brain, can actually aggravate their depression.

In addition to depressive effects, alcohol and drugs could, on their own, alter a person's judgment. It may interfere with the ability of an individual to assess risk factors, make appropriate choices, and evaluate the available solutions to problems, clinical or otherwise. Not infrequently, suicide attempts occur while the person is under the influence of alcohol or drugs, and this may also be due to over dosage, deliberate or accidental.

### ➤ Why High rates in female doctors?

Several explanations have been proposed to explain the high suicidal rate among female doctors. While being a doctor can be stressful for anyone, it may conflict with the life goals of women more than men. Given that women, on average, tend to place more value on spending time with family and friends, and engaging in activities in their households, neighbourhood, etc. The long hours of work take them away from all these things. The regular, lengthy clinical duties make it more difficult for females to maintain stable marital relationships, to have children in time, and to be a good mother who caters to the physical, emotional, academic, healthcare and spiritual needs of children. So also women may be more negatively affected by any setbacks in lives of children, parents, in laws, etc., than men. An unintended pregnancy, the severe moral pressure in being forced to do abortions sometimes, resistance from workplaces to get maternity leave (if at all with salary), all put severe stress on female doctors.

Most male doctors are able to engage in social activities and entertainments, despite their tight schedules; but such is not the case of females, who still bear most of the family burden. Men are generally able to spend more time at leisure/ academic activities, with very little time spared for household responsibilities. Generally, by the time females reach workplace, they are already tired, but do the same volume of work that males do, and go back home to pursue work there.

Many female doctors who have a brilliant academic record during their undergraduate period, fail to stand high in their higher academic pursuits

later, owing to family commitments after marriage. This leads to disappointment in the career, and is a factor that leads to family discordance and depression.

### ➤ Is suicide always planned?

A depressed person probably plans a suicide well in advance, but many a time suicide attempts are impulsive, in a moment of feeling desperate loneliness and complete loss of hope over anything in life. A situation like a breakup in relations, even a frail disagreement with relatives or at work place, getting ousted from preferences or being victimized in any way can cause someone to feel desperately miserable. Often, a frustration like this, added to an existing depression, acts like the final straw. Some people who attempt suicide really mean to end their lives, but many aren't completely sure they want to die. Many suicide attempters actually do not desire to be dead; they just want escape from the pain that is torturing them. So also his impulse to end life, however overpowering, usually does not last forever.

Sadly, many people who really didn't mean to kill themselves end up dead or critically ill or crippled for their lifetime.

### Do religions, faith and morals help?

The way societies view suicide varies widely according to culture and religion. Most religions do not justify intentional taking away life, whatever be the limits of joy and pain, success and failure, pleasure and suffering, happiness and sorrow that comes in this life.

The *Catechism of the Catholic Church* (#2280) asserts, "Everyone is responsible for his life before God who has given it to him. It is God who remains the sovereign Master of Life. We are obliged to accept life gratefully and preserve it for His honour and the salvation of our souls. We are stewards, not owners, of the life God has entrusted to us. It is not ours to dispose of". Therefore, we must be mindful that the preservation of our life is not something discretionary, but obligatory.

In Hinduism, there are various views with regard to suicide. Generally, committing suicide is considered a violation of the code of *ahimsa* (non-violence) and therefore equally sinful as murdering another. Some scriptures state that to die by suicide (and any type of violent death) results in becoming a spirit wandering earth until the time one would have otherwise died. Islam, like other Abrahamic religions views suicide as one of the greatest sins and utterly detrimental to one's spiritual journey. *Quran* instructs; "do not kill yourselves, surely God is most merciful to you." (4:29).

At the same time, all religions teach that we should only pity, that some troubled persons faced something so seemingly unbearable, insurmountable or agonizing, that force them to withdraw from the love of God and others, and kill themselves.

However, poor observation of religious principles frequently leads to breaking down of moral value systems and weakened social support, ultimately paving the way to depressive tendencies. Gay, lesbian, and other misguided youth are more at risk in thinking about and attempting suicide.

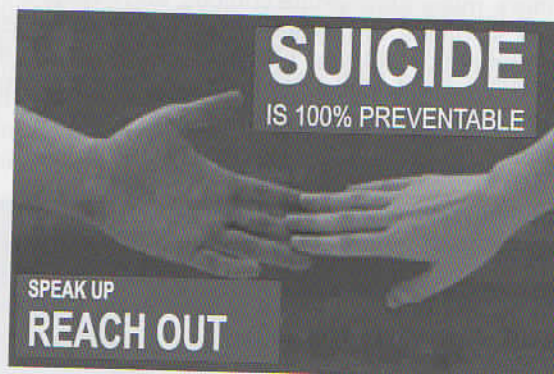
### How do we plan suicide prevention?

The WHO as part of its suicide prevention drive, has issued *three* directives to its member nations. The *first* one requires the nations to regard suicidal tendency as a mental condition for arriving at a suitable course of treatment. The *second* one demands that all suicide prevention programmes should be dovetailed with the prevailing health policies of the individual country. The *third* directive called for effective coordination at all levels to ensure that all these efforts yield a workable policy format.

### Specific strategies for doctors and medicos

- Promote awareness among doctors and medical students that suicidal tendencies and depression are not-very-uncommon health problems, which are quite preventable. Steps are to be taken to reduce the stigma associated with being a person on medication for mental health problems.
- Implement training for recognition of individuals with at-risk behaviour, and warning signs (eg:- talking about suicide, seeking out lethal means, preoccupation with matters related to death, expressing hopelessness about future, self-loathing, self-hatred, abrupt attitude change putting affairs in order, saying goodbye to even distant relatives and friends, sudden withdrawal from others, self-destructive behaviour, sudden sense of calm in a generally agitated type of personality).
- Give better training for health professionals on counseling, and employing crisis counseling organizations, with round the clock services.
- Reduce access to lethal means.
- Improve reporting and portrayals of suicidal behaviour, mental illness and substance abuse in the entertainment and news media. Highlight obviously negative factors of suicide such as pain of suicide attempts, and bitter experiences of the families and friends. Lessons from suicide survivors, conveying message to choose methods other than suicide to solve our innumerable problems, can also be projected.
- Awareness has to be created about the dangers of bullying in those who may be contemplating suicide.
- Expand surveillance systems for suicidal tendencies. There are various screening tools in the form of self-report questionnaires to help identify those at risk.

- Promote and support research on suicide and its prevention by initiating and participating in research projects to develop, implement and evaluate innovative approaches. The primary goal of each project should be to disseminate information about what has been concluded, through scientific articles and seminars that reach both professionals and lay public.
- Reducing substance abuse and consequent domestic discordance, as long-term strategies.
- Promoting mental resilience through assured optimism and a feeling of attachment to those in close circles. The deficiency of open communication, especially among professionals has to be rectified, allowing a person to ventilate oneself, and give a feeling of being listened to, with empathy and concern. Through understanding, reassurance, and support, we can help our loved ones overcome thoughts of suicide.



- A practical way to address doctors' suicide is to focus on medical students and residents, since depression often starts in young adulthood. Academic setbacks/failures, examination fever etc. need to be addressed by authorities and parents with caution, taking care to give sufficient emotional support, in addition to appropriate disciplinary measures.
- A guidance cell has to be constituted in medical schools including psychiatrist, clinical psychologist, interested faculty members and appropriate people from the administration, to give the necessary treatment and counseling.
- The academic system needs a wholesome revamp, in order that ethical, moral and spiritual values come to be embedded at a young and impressionable age.

"If we teach doctors to recognize depression in themselves," says Dr. Paula Clayton, the medical director of American Foundation for Suicide Prevention, "they will recognize it in their patients." And then everybody will feel better.



## ✪ ORIGINAL ARTICLE

# Epidemiological factors affecting anaemia prevalence in rural adolescents in South India

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## Abstract

**Objectives:** To find out the prevalence of anaemia in adolescents of rural area and correlate it with anthropometric, social and physiological variables like gender, BMI, height for age, stage of adolescence, attainment of menarche in girls and social class. **Subjects:** Adolescents (age 10-19 years) from the selected area, who do not have any chronic illness or recent hospital stay, and who consent for participation in the study. **Method:** Two camps were conducted in April 2010 in a rural area (Panchayat) 10 km from our Medical College, and the adolescents were motivated to attend. The attendance was cross checked with the previously prepared list. Socio economic status as well as growth parameters were recorded in a pretested proforma. Capillary blood was collected from each adolescent and haemoglobin was estimated by cyanmethaemoglobin method on the same day. Anaemia was defined according to WHO definition (Hb <11.5g/dl upto 12 years; Hb <12g/dl for girls 12 to 19 years and boys 12 to 14 years; Hb <13g/dl for boys 14 to 19 years). The results were entered in master chart along with other social and growth parameters. Chi square test was used to find out significant correlations with variables like sex, stage of adolescence, low BMI, low height for age, attainment of menarche. **Results:** 269 adolescents participated in the study, of which 172 were girls and 97 were boys. Total 88 were found to be anaemic (32.71%). 72 girls out of the total 172 (41.86%), and 16 boys out of 97 (16.49%) were anaemic. The increased prevalence of anaemia in adolescent girls compared to boys was found significant ( $p < 0.001$ ). Though there was an apparently increased prevalence of anaemia in early adolescence (53 out of 143, 37.06%), compared to mid (24 out of 84, 28.57%) and late adolescence (11 out of 42, 26.19%), this was not found to be statistically significant ( $p > 0.05$ ). There were 49 girls below the 3<sup>rd</sup> centile for BMI (thinness), of which 32 had anaemia (65.31%), and among the 123 girls with normal BMI, 40 had anaemia (32.52%). The increased prevalence in adolescent girls with lower BMI was found to be statistically significant ( $p = 0.001$ ). In girls with stunting also, there was statistically significant increase in the prevalence of anaemia ( $p < 0.0001$ ). The present study could not find out any statistically significant increase in the prevalence of anaemia in boys with either thinness or stunting. Out of 62 girls who had attained menarche, 27 were anaemic (43.55%) whereas 45 were anaemic among 110 girls who had not attained it (40.91%), the difference was not statistically significant. On socioeconomic categorization of the participants, 15 out of 27 in Modified Kuppuswamy's class V were anaemic (55.56%), whereas only three out of 19 in class II were anaemic (15.79%). On comparing prevalence of anaemia in class II and class V, the low socioeconomic status was found to significantly correlate with the prevalence of anaemia ( $p = 0.005$ ). The prevalence was comparable in class III and IV (36.14% in class III and 37.85% in class IV). **Conclusions:** Anaemia is prevalent in the adolescent population of rural Kerala. Girls are significantly more affected than boys. Low socioeconomic status is a risk factor for anaemia. Anaemia prevalence is significantly more in girls, whose BMI is < 3<sup>rd</sup> centile and height for age is < 3<sup>rd</sup> centile. Attainment of menarche has no direct correlation with prevalence of anaemia.

**Key words:** Adolescence, Anaemia, Prevalence

## Introduction

The adolescent growth period is called 'the second chance' for completion of growth of an individual, during which we get a chance to correct the nutritional inadequacies of childhood. The likelihood of anaemia at this age is very high, both for boys and girls because of rapid somatic growth as well as expansion of blood volume. Anaemia at this period is riskier in girls compared to boys because anaemia contributes to increased maternal mortality, increased foetal growth retardation, perinatal mortality and low birth weight. In general, iron deficiency anaemia is a major contributing factor to lowered resistance to infection, poor cognitive development, fatigue, lowered physical activity, poor mental concentration, and productivity.

National programme to tackle iron deficiency anaemia has been established long back. Still we need to collect frequent updates on the prevalence, to get objective evidence whether the programme is reaching the intended beneficiaries.

There are several Indian studies on anaemia prevalence done on adolescent girls in different parts of the country, which show different prevalence ranging from 22.0 to 96.5% (Vasanthi and Pawashe, 1994<sup>1</sup>; Chaturvedi and Kapil, 1996<sup>2</sup>; Seshadri, 1997<sup>3</sup>; Aggarwal, 1998<sup>4</sup>; Rajaratham *et al.*, 2000<sup>5</sup>; Sivakumar *et al.*, 2001<sup>6</sup>; Gawarikar *et al.*, 2002<sup>7</sup>), but there are few studies which include adolescent boys.

From Kerala, there are some studies on anaemia prevalence on preschool children<sup>8</sup>, but only very few on adolescents. Hence this study was conducted to know the status of anaemia prevalence among rural adolescents in this state.

## Subjects and methods

It was a cross sectional study conducted in a Panchayat 10 km away from Pushpagiri Medical College, Tiruvalla. The approximate number of adolescents residing in the chosen area was obtained from the local RCH co-ordinator. Two medical camps were conducted there, in the month of April 2010. Information regarding medical camps being organized in the community halls of the selected areas was circulated through *Kudumbasree units*, printed leaflets, and through the local administrators and RCH coordinator. All the adolescents were motivated to attend. The attendance was cross checked with the available list of adolescents residing in the area. Informed consent was taken for enrolment into the study.

A pretested proforma was used to record the information on socio-demographic characteristics like age, educational status, monthly family income, and medical history like age at menarche given by the subjects, as well as to record the growth parameters and physical examination findings. Weight and height were measured using standard methods, and BMI was calculated using the formula weight in kg/ height in m<sup>2</sup>. BMI less than third percentile was taken as thinness, and height for age less than 3<sup>rd</sup> percentile as stunting

according to WHO growth charts 2007. The socioeconomic categorization was done using Modified Kuppaswamy's scale. Haemoglobin estimation was done using cyanmethaemoglobin method on the same day of blood collection.

Inclusion criteria were any adolescent (defined as aged between 10 and 19 years) residing in the ear marked areas, who consent for enrolment and blood collection. Exclusion criteria were those outside the age range and outside the locality, having chronic illnesses or recent hospitalization and those who do not consent. The criteria for detecting anaemia were set as per WHO guidelines, (values < 11.5 g/dl for children up to 12 years, < 12 g/dL for girls from 12 to 19 years and boys < 14 years, and less than 13 g/dL for boys from 15 to 19 years of age<sup>10</sup>). All the data were entered in a master chart.

Table 1: Distribution of boys and girls according to their stage of adolescence and the proportion of people with anaemia

Stage of adolescence	Girls (total 172)			Boys (total 97)		
	No. of girls	Anaemic girls		No. of boys	Anaemic boys	
		No.	Percentage		No.	Percentage
Early (10-13 years)	91	42	46.15%	52	11	21.15%
Middle (14-16 years)	49	20	40.82%	35	4	11.43%
Late (17-19 years)	32	10	31.25%	10	1	10%
p value	p>0.05, not significant			p>0.05, not significant		

## Results

The total number of adolescents was 760, of which 352 attended the camp. Seventy five were not willing for enrolment to the study and blood collection and eight were excluded because of various reasons. A total of 269 participants were enrolled for the study, which included 172 girls and 97 boys. Among the total, 88 were found to have anaemia (32.71%). Only 16 boys (16/97- 16.49%) were anaemic whereas 72 (72/172- 41.86%) girls were anaemic. This difference between boys and girls was statistically significant (p<0.001). Taking the split up of the total into early, mid and late adolescents, 53 out of 143 early adolescents were anaemic (37.06%). Of this, 11 out of 52 (21.15%) were early adolescent boys and 42 out of 91 (46.15%) were early adolescent girls.

In mid adolescence, prevalence of anaemia was 28.57% (24 out of 84) and in late adolescents, 11 out of 42 (26.19%) were anaemic. In mid adolescence four out of 35 boys (11.43%) were anaemic and 20 out of 49 girls were anaemic (40.81%) and in late adolescence 10% (one out of ten) of boys were anaemic while for girls of similar age group, 31.25% (10 out of 32) were anaemic. The study thus revealed that in all age groups anaemia was prevalent in girls and even though there is an apparently increased prevalence of anaemia in early adolescence for both boys and girls, this difference was not statistically significant (p>0.05).

This observation is shown in Table 1. Total 62 adolescent girls had attained menarche, of whom, 27 were anaemic (43.54%) whereas out of 110 who had not attained menarche, 45 were found anaemic (40.90%). So, attainment of menarche was not significantly related to prevalence of anaemia in adolescent girls (p> 0.05).

Among a total of 49 girls with BMI less than 3<sup>rd</sup> percentile according to WHO, 32 were anaemic (65.30%) whereas among the group of girls with normal BMI, which was 123 in number, only 40 were anaemic (32.52%). This increased prevalence of anaemia in thin girls was found to be statistically significant ( $p < 0.001$ ) (Table 2).

The same trend was seen in girls with stunting also. Among 22 girls with stunting, 16 (72.73%) were anaemic whereas among 150 normal height girls, only 22 (14.67%) were anaemic ( $p < 0.001$ , significant) (Table 2).

Table 2: Correlation of thinness & stunting with anaemia prevalence

Total 269	Girls (172)			Boys (97)		
		Anaemia - 72 (41.86%)			Anaemia - 16 (16.49%)	
	$p < 0.001$ , significant					
Kuppuswamy's social class	Class II (19)			Class V (27)		
	Anaemia - 3 (15.79%)			Anaemia - 15 (55.55%)		
	$p < 0.005$ , significant					
WHO growth parameter	Girls (172)			Boys (97)		
	Total	Anaemic	%	Total	Anaemic	%
BMI <3rd centile	49	32	65.30	34	8	23.53
Normal BMI	123	40	32.52	63	8	12.70
	$p < 0.001$ , significant			$p < 0.005$ , significant		
Height: <3rd centile	22	16	72.73	6	1	16.67
No stunting	150	22	14.67	91	28	30.77
	$p < 0.001$ , significant			$p < 0.005$ , significant		

Among boys, prevalence of thinness and stunting was less, and also statistically significant increase in incidence of anaemia was not observed in them in the present study.

There were no representatives from Modified Kuppuswamy's class I in the present study, probably because of the peculiarity of the area we chose for study, which is a rural area where most adults were manual labourers or skilled or semiskilled workers. Among adolescents from Modified Kuppuswamy's class II, three were having anaemia out of 19 (15.79%), in class III 30 out of 83 were anaemic (36.14%), in class IV 53 out of 140 were anaemic (37.86%) and in class V 15 out of 27 were anaemic (55.55%). Though the prevalence of anaemia was comparable in class III and IV, there was statistically significant increase in the prevalence of anaemia in class V compared to class II ( $p = 0.005$ ).

### Statistical tools

Chi Square test was calculated to find out the statistical significance of different proportions.

### Discussion

The prevalence of anaemia in 630 adolescent girls (13-16 years) of rural Wardha<sup>9</sup> was found to be 59.8 per cent. A study conducted by Kalpana *et al.*, (2007)<sup>11</sup> on 500 adolescent girls (13 to 15 years) of low income families in Coimbatore revealed that 76.4% of adolescent girls were anaemic and only 23.6% were

non-anaemic.

At the international level, a study conducted by Adgeppa *et al.*, (1997)<sup>12</sup> in Indonesia including 805 adolescent girls showed that 21.1% of the girls (170) were anaemic, having haemoglobin level less than 12 g/dl.

In a Sri Lankan study<sup>13</sup> on 690 adolescent girls the prevalence of anaemia (Jayatissa and Piyasena, 1999) was 21.1%.

Shahabuddin *et al.*, (2000)<sup>14</sup> conducted a study on nutritional status of adolescents in a rural community of Bangladesh and reported 98 per cent (1453 out of 1483) prevalence of anaemia in adolescent girls.

In our study the prevalence of anaemia in adolescent girls was 41.86% which was not too high as in the study by Shahabuddin *et al.*, or as low as in Srilankan and Indonesian studies. The difference could be due to the differences in socio-demographic variables between the study populations.

A study from Chandigarh conducted in 2002<sup>15</sup>, on 1180 school students including both boys and girls of age 12 to 18 years, showed a higher prevalence of anaemia in girls compared to boys (23.9% v/s 7.7%). This increased prevalence in girls compared to boys was similar to our study, though we got prevalence higher than the former study, both for boys and girls, probably due to the reason that, the former study included both rural and urban children, whereas we did study on rural children alone. Also in the same study, the prevalence of anaemia in girls whose BMI was low was higher than those who had normal BMI ( $P < 0.001$ ). Same observation was repeated in the present study, but our study could not find such a correlation among adolescent boys probably because of the small number of adolescent boys participated in our study.

As observed here, the study conducted by Kaur S *et al.*,<sup>9</sup> in rural Wardha also showed increased likelihood of anaemia in adolescents of low socio economic status. Their study also did not show any correlation between prevalence of anaemia and status of menarche. This also was keeping with the present study.

According to Gowarikar *et al.*, (2002)<sup>7</sup>, adolescent girls belonging to weaker section of society has very high prevalence of anaemia (97%). This observation is similar to the present study where we could find a statistically significant increase in the prevalence of anaemia among adolescents belonging to modified Kuppuswamy's class V.

### Conclusion

Even though we have programmes to improve adolescent nutrition and prevention of anaemia, a significant number of adolescents are anaemic, probably because of lack of awareness and reluctance on the part of adolescents to seek medical and nutritional advice, or that the programme fails to reach many of the intended beneficiaries. We need to strengthen our national programme by more activities

and awareness programmes at the community level and by house visits to reach the beneficiaries, for the successful implementation of the programme.

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## ✦ ORIGINAL ARTICLE

# Correlation of Heart rate variability analysis to Blood pressure indices in young healthy subjects

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## Abstract

**Background and objectives:** Heart rate variability (HRV) is related to lifestyle in adults. Regular physical exercise has beneficial effect on autonomic control of heart, with decreased resting HR often accompanied with increase in HRV. The purpose of the present study was to determine whether readily measured BP indices and responses to autonomic reflex tests could be used as surrogates of short term HRV, which is an established marker of autonomic regulation of SA node. Therefore, we examined the correlation between short-term HRV and heart rate (HR), BP indices viz. systolic BP, diastolic BP, pulse pressure (PP), and rate-pressure product (RPP), during supine position and standing erect.

**Methodology:** The study evaluated 75 male subjects for their resting cardiovascular parameters. HR, and systolic and diastolic BP were recorded, and RPP calculated using HR and systolic BP, along with autonomic function test parameters. Valsalva ratio was calculated after Valsalva maneuver, and change in diastolic BP and PP after isometric hand grip test. They were also subjected to short term heart rate variability recording in supine and standing erect positions.

**Results:** Non-parametric Wilcoxon signed rank test was applied to analyze the changes in HRV parameters because of the peculiar distribution of data. Pearson correlation coefficient was used for correlation matrix and  $p$  value less than 0.05 was considered significant. Mean R-R interval and SDNN (standard deviation of normal-to-normal RR intervals) of time domain analysis, and LF and HF power, total power and LFnu (low frequency in normalized units) of frequency domain analysis were highly significant on comparing supine to standing positions. We looked for correlation between short term HRV and resting cardiovascular parameters, but no significant correlation was observed. **Conclusion:** Our data indicated a decrease in HRV that seems to be an expression of reduction in autonomic modulation in postural change from supine to erect. The findings were suggestive of a shift in cardiac autonomic regulation towards sympathetic activation in response to real life stressors, which includes the decrease in parasympathetic modulation in sinus node. Results suggested no significant correlation between readily measured BP indices to HRV in the study group. Hence we suggest that mean HR, SBP, DBP and RPP cannot be used as surrogates of HRV.

**Key words:** Autonomic function, Isometric handgrip, Heart rate variability, Rate-pressure product.

## Introduction

The term heart rate variability conventionally describes the beat-to-beat fluctuations in the heart rate or the variations in consecutive RR intervals. HRV is mainly caused by efferent modulations of sinus node, the pacemaker of heart. Casting a retrospective glance, existence of physiological rhythms in the beat-to-beat heart rate signal was noted about twenty years prior to the first clinical application of HRV, and was first

appreciated in 1965 by Hon and Lee<sup>1</sup>. Twelve years later, Wolf *et al.*,<sup>2</sup> found an association between a higher risk of post infarction mortality and reduced HRV. For many years, the HRV has only been expressed in mean values and standard deviations as a representation of the time domain analysis, until Akselrod *and his team* described the relation between quantitative evaluations of the beat-to-beat cardiovascular control by a power spectral analysis of HR fluctuations.

Nowadays, the frequency domain analysis obtained by mathematical processing of the RR intervals is well accepted to assess the neural mechanisms controlling HR. Thereby two main spectral components which are considered as markers of the sympathetic and parasympathetic control of the heart have been discriminated: a high frequency component (HF) which ranges from 0.4-0.15 Hz, and a low frequency component (LF) ranging from 0.15-0.04 Hz. Furthermore, the HRV measure is apparently easy to derivate because of the availability of new, digital, high-frequency, long- and short term multi channel ECG recordings. Due to this, HRV is considered a useful method for both clinical and research studies, to examine the autonomic nervous modulation of heart. Especially specific experimental conditions as awake and sleeping situations, different body positions, and physical training, as well as pathological conditions provide a good insight into the vegetative control of heart. Nevertheless the susceptibility of ANS to factors such as respiration, and internal and external influences have to be considered. To avoid that affecting HRV results, standardized measurement conditions in accordance with the guidelines of the Task Force<sup>4</sup> have been established.

## Objectives

- To study the cardiovascular reactivity to postural changes, and performance challenges
- To test whether readily measured BP indices and responses to classic autonomic reflex tests could be used as surrogates of short term HRV.

## Methodology

The present study was conducted in the Department of Physiology, Navodaya Medical College, Raichur. Seventy five healthy young males of age 18-21 years, drawn from various socio-economic groups were randomly selected. The study was approved by the Institutional Ethics Committee of Navodaya Medical College.

The protocol was explained to the healthy volunteers, and informed consent was obtained. Those selected, according to the laid down inclusion and exclusion criteria (subjects with cardiovascular abnormalities and systemic illnesses excluded), were given specific dates to visit Department of Physiology. All tests were conducted between 10.30 and 13.30 hours. During the first visit complete general physical examination was performed along with anthropometric parameters, body mass index (BMI) and ECG. Each subject was given specific dates to visit autonomic function laboratory to evaluate ANS functions. They were also instructed not to have coffee/ tea/ cola 12 hours prior to the tests, and to have light breakfast two hours before the tests.

Subjects had to relax in supine position for 30 minutes in the laboratory, and then various autonomic function tests were performed on them. A chest lead

ECG was recorded throughout supine rest and standing position, using the ECG V: 52 system (Niviqure Meditech Pvt. Ltd.). Beat to-beat variations in instantaneous HR were derived offline using a rate-detector algorithm. For computing HRV indices during supine rest and tilt, recommendations of the Task Force on HRV were followed<sup>4</sup>. Briefly, a five-minute ECG was acquired at a sampling rate of 1000 Hz, during supine rest and standing erect, with the subjects breathing normally at 12-18/minute. RR intervals were plotted using ECG V: 52 software. RR series was extracted using a rate-detector algorithm after exclusion of artifacts and ectopics. A stationary 256 second RR series was chosen for analysis.

In the time domain, SDNN was taken as an index of overall HRV. The RR series was re-sampled at 4 Hz, the mean and trend were removed, Hann window was applied, and the 1024 data point series was transformed by fast Fourier transformation. Low frequency (LF) and high frequency (HF) spectral powers were determined by integrating the power spectrum between 0.04 and 0.15 Hz, and 0.15 and 0.4 Hz respectively. Total power was calculated by integrating the spectrum between 0.004 and 0.4 Hz, and included very low frequency, LF and HF components. Spectral powers were expressed in absolute units of milliseconds squared. LF and HF powers were also expressed in normalized units LFnu & HFnu as described.

Later the subjects were tested for autonomic function tests with resting HR, SBP, DBP and BP for isometric hand grip performance as indices of sympathetic system, and HR changes to deep breathing and Valsalva ratio as indices for parasympathetic system. All data were expressed as mean  $\pm$  SD; Spectral powers were expressed as median (interquartile range); Wilcoxon-matched pairs test was used for skewed data. Correlation between HRV indices and cardiovascular parameters as determined by autonomic function test were analyzed using the Pearson correlation coefficient, and a two tailed *p* value less than 0.05 was considered significant.

## Results and analysis

The data (n=75) age and anthropometric measurements are expressed as mean  $\pm$  SD along with the range of the variables in Table 1.

Table 1: Baseline characteristics of subjects

Characteristics	Mean + SD	[Range]
Age ( Years)	19.69+0.93	[18 - 22]
Height (m)	1.65+0.042	[1.58 - 1.74]
Weight (Kg)	62.10+4.96	[50 - 76]
BMI (Kg/m <sup>2</sup> )	22.58+1.74	[19.04 - 26.29]

The cardiovascular parameters, systolic BP, diastolic BP, PP, HR and RPP are presented in Table 2.



Table 2: Resting cardiovascular parameters of subjects

Parameter	Mean $\pm$ SD	Range
Systolic BP (mm Hg)	118.18 $\pm$ 6.56	106 - 136
Diastolic BP (mm Hg)	76.98 $\pm$ 3.64	70 - 84
Pulse pressure (mm Hg)	41.18 $\pm$ 5.89	27 - 56
Heart rate (beats/min)	73.53 $\pm$ 3.88	64 - 82
RPP[SBP X 10 <sup>-2</sup> ]	86.96 $\pm$ 7.44	68 - 107

Table 3: Autonomic function test parameters of subjects

Parameter	Mean $\pm$ SD	Range
Valsalva Ratio	1.67 $\pm$ 0.36	1.14 - 2.66
I - E HR difference	25 $\pm$ 3	17 - 35
Increase in DBP during IHG (mm Hg)	35 $\pm$ 2.73	30 - 41
Change in PP during IHG (mm Hg)	9.96 $\pm$ 0.85	8 - 12

The autonomic function parameters i.e., parasympathetic component represented by heart rate changes during Valsalva maneuver and during deep breathing test, and sympathetic component by change in diastolic BP to sustained isometric hand grip test, along with change in pulse pressure are tabulated in Table 3.

Table 4: Comparison of HRV test parameters in different posture of subjects

Parameter	Supine	Standing	P value
Mean R - R (ms)	888.52 $\pm$ 102.8	770.19 $\pm$ 89.10	P<0.0001
SDNN (ms <sup>2</sup> )	45.57 $\pm$ 5.63	43.8 $\pm$ 5.41	P<0.0001
LF power (ms <sup>2</sup> )	114.13 $\pm$ 24.53	240.62 $\pm$ 51.72	P<0.0001
HF power (ms <sup>2</sup> )	173.61 $\pm$ 13.42	81.06 $\pm$ 6.26	P<0.0001
Total power (ms <sup>2</sup> )	966.62 $\pm$ 75.25	718.74 $\pm$ 55.96	P<0.0001
LF nu	39.31 $\pm$ 5.11	74.21 $\pm$ 4.13	P<0.0001

Table 4 shows a comparison of HRV parameters to different positions i.e., supine and standing. Both the time domain and frequency domain analysis were used as parameters of HRV and the statistical test applied was Wilcoxon-matched pairs test, as the data was skewed. Mean R-R interval and SDNN of time domain analysis were highly significant on comparing supine to standing (P<0.001).

Similarly LF, HF power, Total power and LFnu of frequency domain analysis were highly significant on comparing supine to standing (P<0.001).

The correlation between short term HRV indices to BP indices, on application of Pearson correlation coefficient test and Spearman's rank correlation test suggested no statistically significant (P>0.05) correlation between the two sets of variables, as displayed in Table 5.

Table 5: Comparison of HRV test parameters in different posture of subjects

	Mean HR	SBR	DBR	PP	RPP	DBP(IHG)
SDNN	0.3	-0.1	0.02	-0.1	0.1	0.133
LF power	0.2	0.08	-0.05	0.1	0.2	0.12
HF power	0.2	-0.04	-0.03	-0.02	0.07	0.99
Total power	0.2	-0.03	-0.03	-0.02	0.07	0.99
LF nu	0.1	0.09	-0.05	0.2	0.2	-0.2
Valsalva ratio	0.2	0.08	-0.06	0.1	0.2	0.12
I-E HR difference	0.06	-0.2	-0.009	-0.2	-0.09	0.5

## Discussion

The focus of this study is on non-invasive, quantitative tests of autonomic function that are currently used in autonomic laboratories. Two points are worth considering:

1. There is a concept that evaluation is not only an extension of clinical examination; the result of many tests is best interpreted together. It is too simplistic to ascribe a single function to a single test. A particular clinical question may require the selection of a specific battery of tests tailored to answer it.
2. Majority of tests of autonomic function evaluate end-organ responsiveness so that end-organ failures can affect test results.

The analysis of HRV has long been applied as a research tool for evaluation of ANS. Power spectral analysis is a mode of classification of spectral changes in the neural regulation of heart. Therefore sympathetic activity is classed with low (LF 0.15-0.04 Hz), and vagal activity with high frequency (HF 0.4-0.15 Hz) fluctuations. The HF consists of pure vagal, and the LF of sympathetic and vagal activity, which may not be numerically quantized. So the ratio between the sympathetic and parasympathetic is expressed by the normalized LF and HF units, and LF/ HF ratio is designated as a marker of sympatho-vagal balance.

In time domain, analysis of mean R-R interval and SDNN indicates overall heart rate variability. To test the significance of HRV parameters and their correlations, tests are conducted in different positions, altering the nervous control of heart, to obtain modulated HR analysis<sup>5,6,7,8</sup>.

### Relationship of HRV parameters to position

An HRV index to change in position, SDNN encompasses all components responsible for RR variability, and is a simple domain measure of overall HRV. High frequency spectral power reflects parasympathetic modulation of RR interval at controlled respiratory frequency. LF power in absolute units of power quantifies baroreflex-mediated modulation of RR intervals in the 0.04-0.15 Hz range. Changes in sympathetic as well as vagal nerve traffic to the heart are thought to contribute to LF power. Total power is also an index of overall HRV. At least in physiologic states

characterized by sympathetic excitation, low frequency spectral power expressed in normalized units of power (LFnu) has been shown to be a useful non-invasive index of sympathovagal balance<sup>9,10</sup>.

Our results in study, showing a decrease in mean RR, increase in LFnu and decrease in HF power during standing erect, are well known concomitants of sympathetic excitation, which is associated with an increase in mean HR and reduces the magnitude of respiratory sinus arrhythmia. The experimental maneuver from supine to standing induces a shift of the sympathovagal balance towards sympathetic activation.

As expected our study confirmed that mean RR is associated with progressive sympathetic activation and parasympathetic withdrawal. We also noted that LFnu can track the gradual increase in the cardiac sympathetic modulation, and HFnu exhibits a reciprocal trend as a result of the relationship between LFnu and HFnu, as shown in total spectral power.

Our results were similar to those obtained by Malliani, A *et al.*,<sup>11</sup> and some other studies<sup>12,13,14</sup> also. In the supine position healthy subjects always present LF and HF components, the latter being greater in adolescence and smaller in adulthood. In the active upright position (or during passive tilt), in addition to increase in HR and small adjustments in BP, marked change occurs, as a rule, in the spectral profile - LF component is increased, and HF component is reduced. Variance usually decreases in the upright position, causing a reduction in the absolute value of both spectral components. Hence, in the upright position, LF tends to be decreased in its absolute values, by the reduction of variance, but also tends to be increased, in nu, by the greater concentration of power in this part of the spectrum.

Numerous data, collected in various human and animal studies, have been summarized previously<sup>15,16,17,18</sup> to support the following assumptions:

1. The respiratory rhythm of heart period variability (HF) is a marker of vagal modulation (a view widely accepted).
2. The rhythm corresponding to vasomotor waves and present in heart period and arterial pressure variability (LF) is a marker of sympathetic modulation of, respectively, heart period and vasomotion.
3. The reciprocal relation existing in R-R variability spectrum between LFnu and HFnu is a marker of the state of sympatho-vagal balance modulating sinus node pacemaker activity (also deducible from LF/HF, which, like any ratio, can emphasize the opposite changes).

This hypothesis does not imply that LF and HF components should be confined to sympathetic and vagal activities, respectively. Actually, the opposite is true, because they are simultaneously present in the

discharge of both autonomic outflows. However, rhythm, being a flexible and dynamic property of neural networks, should not necessarily be restricted to one specific neural pathway to carry a functional significance<sup>15,16,17,18</sup> (as in the case of different EEG patterns).

*Correlation matrix, to study correlations between resting mean HR, BP indices, and autonomic function test parameters to short term HRV indices*

In our study on comparing the normally distributed data of cardiovascular variables (mean resting HR, systolic BP, diastolic BP, PP and RPP) to non-normally distributed data of HRV indices (SDNN, LF, HF, TP, LFnu) no correlation was seen between them.

Our results, in agreement with the study of Raine Virtanen *et al.*,<sup>19</sup> and others<sup>20,21,22</sup> stated that although young subjects revealed an association between PP and BRS, PP was not independently associated with any measure of HRV. This suggests that PP does not have any significant effect on tonic autonomic cardiovascular control, and that it affects baroreflex-mediated heart rate fluctuations.

In contrast to our study report, Madan Mohan *et al.*,<sup>23</sup> reported a positive correlation between PP and LF power during supine rest. The explanation given for this was, as PP value is higher, loading of high-pressure baroreceptor afferents is greater, and consequently, the reflex modulation of RR interval is also higher.

The significant positive correlation between RPP and the change in LFnu (i.e., LFnu during tilt - LFnu during supine rest) is possibly because of sympathetic excitation during head-up tilt. Among other parameters, SDNN, mean HR, SBP and DBP on comparing to HRV parameters did not show any significant correlation.

Studies by Grossman and Kollai<sup>24</sup>, Convertino, Victor A<sup>25</sup>, and de Boer RW *et al.*,<sup>26</sup> compared RR-interval fluctuations to ripples on a sea, of varying depths. Their study suggested no arguments in the sympathovagal literature to support the view that fluctuations of nerve traffic (ripples on the surface of sea) are important, rather than the absolute levels (depth of the sea).

Such justification is necessary. Evidence indicates that during moderate changes of arterial pressure, the physiologically relevant variable is the nearly constant fluctuations of vagus nerve traffic, rather than the varying absolute levels of vagus nerve traffic that are proportional to arterial pressure.

## Conclusion

Our data indicate a decrease in HRV that seems to be the expression of a reduction in autonomic modulation in postural change from supine to standing. The findings are suggestive of a shift in cardiac autonomic regulation towards sympathetic activation in response to real life stressors,

which also includes a decrease in parasympathetic modulation in sinus node. At the same time results indicated no significant correlation between readily measured BP indices to HRV in both male and female groups. So we suggest that mean heart rate, SBP, DBP and RPP cannot be used as surrogates of HRV.

However, further studies are to be done in healthy subjects belonging to various age groups, and in patients having autonomic dysregulation.

### Limitations of our study

Despite the large number of experimental and clinical studies published, the measurement of HRV is still a research technique and not a routine clinical tool. There are several potential reasons that can explain this situation.

1. Clinical applications of HRV assessment is limited by lack of standardized methodology due to variability of the parameters according to gender, age, drug interferences and concomitant diseases.
2. Despite the relative evidence of the robust character of parameters such as SDNN and HRV index, there is still no consensus about the most accurate HRV parameter for clinical use.
3. The sensitivity, specificity and positive predictive accuracy of HRV are limited. Particularly, its positive predictive accuracy is modest, ranging from 14 to 40%. It has, however, a higher negative predictive value ranging from 77 to 98%.

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## ✪ ORIGINAL ARTICLE

# Experimental evaluation of anticonvulsant activity of hydrocotyle asiatica linn (*Centella asiatica*) in albino mice

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### Abstract

**Objective:** 1.To evaluate the antiepileptic activity of Hydrocotyle asiatica linn (aqueous extract) in preventing maximal electroshock (MES) induced convulsions. 2. To compare its efficacy with the standard anticonvulsant drug, phenytoin. **Method:** Twenty four male albino mice weighing 18-30 gm are selected and divided into four groups - Group C mice were administered distilled water (0.25ml), Group S phenytoin (50 mg/kg), Group T1(100 mg/kg) of aqueous extract of Hydrocotyle asiatica linn and Group T2 (300 mg/kg) of aqueous extract of Hydrocotyle asiatica linn. Seizures were induced via., ear clip electrodes with a current of 50 mA for 0.2 seconds. Each mouse was pretreated with drugs (p.o.) one hour before MES test. Statistical analysis was done by student 't' test. **Results:** The aqueous extract of Hydrocotyle asiatica at a dose of 300mg/kg showed statistically significant anticonvulsant activity against MES convulsions; its anticonvulsant activity is similar to that of the standard drug phenytoin. **Conclusion:** This study demonstrated the anticonvulsant activity of aqueous extract of Hydrocotyle asiatica in albino mice. This plant can be a therapeutic potential to treat epilepsy in addition to the conventional antiepileptic drugs.

**Key words:** Hydrocotyle asiatica, Maximal electroshock seizures, Phenytoin.

### Introduction

Epilepsy has been recognized since antiquity. The word epilepsy is derived from the Greek word meaning "to seize" or "take hold of", indicating that the person having a seizure is "possessed" or at least out of control<sup>1</sup>. Epilepsy is a relatively common neurological disorder. Approximately 5-10% of the population will have at least one seizure, with the highest incidence occurring in early childhood and late adulthood<sup>2</sup>. Antiepileptic therapy has many drawbacks such as long duration of therapy, adverse effects of drugs, need for therapeutic drug monitoring, etc.<sup>3,4,5</sup> There is clearly a need for more specific and effective drugs<sup>4</sup>. Medicinal plants have been an important source of new drugs. Hydrocotyle asiatica linn has been extensively used in Ayurvedic medicinal practice for the treatment of epilepsy, various skin diseases, leprosy and malaria<sup>6,7</sup>. Experimentally, drugs with a potential antiepileptic activity are assessed by injecting medullary stimulants or by applying a maximal electrical shock. The drugs which antagonize the chemically induced

seizures are effective in petit mal epilepsy, and those which protect against electrically induced seizures are useful in grand mal epilepsy.<sup>8</sup>

### Materials and methods

#### Preparation of aqueous extract of Hydrocotyle asiatica linn

The dried powder of whole plant was obtained from Head of the Department of *Rasayanashastra, Ayurvedic maha vidyalaya*, Hubli. The aqueous extract was obtained by cold maceration method. Thirty grams of the powder was soaked in 200 ml of cold water for approximately 18 hours at room temperature. The extract was first filtered through Whatman no. 1 filter paper and then through a 0.45 µm membrane filter. The filtrate was evaporated to dryness at room temperature in a steady air current and the yield recorded as a percentage of the quantity of initial plant material used<sup>9</sup>. The test solution of Hydrocotyle asiatica linn was prepared by dissolving two grams of aqueous extract of Hydrocotyle asiatica linn in 100 ml of distilled water at room

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temperature. This solution had a concentration of 20 mg/ml.

**Preparation of solutions of standard drug**

*Phenytoin sodium*: The standard solution of phenytoin sodium was prepared by dissolving 400 mg of phenytoin sodium powder (Ciron pharmaceuticals) in 100 ml of distilled water at room temperature. It was protected from light. This solution had a concentration of four mg/ml.

**Animals**

Twenty four male albino mice weighing 18 to 30 gm were obtained from Central animal house, KIMS, Hubli. They were divided into four groups, each group consisting of six animals.

**Experimental methods**

Ethical clearance was obtained from the Institutional Animal Ethical Committee (IAEC) of KIMS, Hubli. All test animals were allowed food and water *ad libitum*, both being withdrawn just prior to experimentation<sup>10</sup>. All the test animals which were tested for standard convulsive responses with MES stimuli were subjected to further experiments of this study after 24 hours.

**Maximal electroshock (MES) method**

The mice were subjected to maximal electroshock convulsions with a current of 50 mA for 0.2 second via ear clip electrodes. The electrodes were moistened with saline solution before application. The resultant seizure passes through various phases: phase of tonic limb flexion, tonic limb extension, clonus and post-ictal depression followed by recovery or death<sup>11</sup>. Only those animals showing the above phases of convulsion were selected for this study. The mouse was considered as protected if the drug prevented the appearance of hind limb tonic extensor component of the seizure<sup>10</sup>.

The mice were divided into four groups, each consisting of six of them.

(a) *Control group (C)*: were administered 0.25 ml of distilled water orally. After one hour<sup>12</sup>, they were subjected to maximal electroshock with an alternating current of intensity 50 mA for 0.2 second through ear clip electrodes. The duration of various parameters like tonic hind limb flexion, tonic hind limb extension, clonus, post-ictal depression and the incidence of recovery or death were noted.

(b) *Standard group (S)*: received 50 mg/kg of phenytoin orally<sup>12</sup>. After one hour, they were subjected to maximal electroshock with an alternating current of intensity 50 mA for 0.2 second through ear clip electrodes. The results obtained were recorded in a similar way as for group C.

(c) *Test group-1 (T1)*: received 100 mg/kg of aqueous extract of *Hydrocotyle asiatica* linn orally<sup>13</sup>. After an hour, they were subjected to maximal electroshock with

through ear clip electrodes. The results obtained were recorded in a similar way as for group C.

(d) *Test group-2 (T2)*: mice received 300 mg/kg of aqueous extract of *Hydrocotyle asiatica* linn orally.<sup>13</sup> After an hour, they were subjected to maximal electroshock with alternating current of intensity 50 mA for 0.2 second through ear clip electrodes. The results obtained were recorded in a similar way as for group C.

The observations are presented in tables 1-6.

**Statistical analysis**

The results of this study were expressed as mean± standard error of mean (mean± SE), and were analyzed by student 't' test. Significance was established when probability value (p value) is less than 0.05. [P values are denoted as \* P < 0.05 as significant, \*\* P < 0.01 as highly significant and \*\*\* P < 0.001 as very highly significant].

**Results**

The parameters observed were the duration of tonic hind limb flexion, tonic hind limb extension, clonus, post-ictal depression and incidence of recovery and death. The mouse was considered protected if the drug abolished the tonic hind limb extension<sup>10</sup>. The mean duration of various parameters of different groups are shown in tables 1-4.

Table 1: Mean duration of various parameters of group C (control, 0.25 ml distilled water)

Parameters (in seconds)	Serial no. of animals						Mean
	1	2	3	4	5	6	
Tonic hind limb flexion	1	4	3	3	4	2	2.83
Tonic hind limb extension	13	16	15	10	11	12	12.83
Clonus	16	14	20	13	16	14	15.5
Post-ictal depression	178	180	212	170	120	182	173.66
Recovery(R)/ Death (D)	R	R	R	R	R	R	

Table 2: Mean duration of various parameters of group S (standard, 50 mg/kg phenytoin sodium)

Parameters (in seconds)	Serial no. of animals						Mean
	1	2	3	4	5	6	
Tonic hind limb flexion	-	-	-	-	-	-	
Tonic hind limb extension	-	-	-	-	-	-	
Clonus	16	14	18	14	13	14	14.83
Post-ictal depression	-	-	-	-	-	-	
Recovery(R)/ Death (D)	R	R	R	R	R	R	

Table 3: Mean duration of various parameters of group T1 (test, 100 mg/kg of aqueous extract of *Hydrocotyle asiatica* linn)

Parameters (in seconds)	Serial no. of animals						Mean
	1	2	3	4	5	6	
Tonic hind limb flexion	2	2	-	2	-	-	1
Tonic hind limb extension	10	12	-	11	-	-	5.5
Clonus	-	18	19	20	17	16	15
Post-ictal depression	-	90	-	63	-	-	25.5
Recovery(R)/ Death (D)	R	R	R	R	R	R	

Table 4: Mean duration of various parameters of group T2 (test, 300 mg/kg of aqueous extract of *Hydrocotyle asiatica* linn)

Parameters (in seconds)	Serial no. of animals						Mean
	1	2	3	4	5	6	
Tonic hind limb flexion	-	-	2	-	-	-	0.33
Tonic hind limb extension	-	-	10	-	-	-	1.66
Clonus	14	16	18	15	16	15	15.66
post-ictal depression	-	-	90	-	-	-	15
Recovery®/ Death (D)	R	R	R	R	R	R	

**Comparison of various parameters of different groups with control**

- **Tonic hind limb flexion:** A comparison of mean duration of tonic hind limb flexion of control group with other groups (Table 5, Fig. 1) indicates that there is a decrease in mean time of tonic hind limb flexion in group T1, T2 and S. The test compound has shown statistically significant protection ( $p < 0.01$ ) in both groups T1 and T2. In group S, there is complete abolition of flexor phase, which is statistically significant ( $p < 0.001$ ).
- **Tonic hind limb extension:** A comparison of mean duration of tonic hind limb extension of control group with test groups (Table 5, Fig. 2) indicate that there is a decrease in mean duration of tonic hind limb extension in both groups T1 and T2 and it is statistically significant ( $p < 0.001$ ). In group S, there is complete abolition of tonic hind limb extension which is statistically significant ( $p < 0.001$ ). The abolition of tonic hind limb extension has occurred in three out of six mice in T1 and five out of six mice in T2.
- **Clonus:** Analysis of results when compared with control (Table 5, Fig. 3) suggest that there is a decrease in mean duration of clonus in groups S and T1 while a slight increase in group T2. However these values are not statistically significant
- **Post-ictal depression:** A comparison of mean duration of post-ictal depression with control (Table 5, Fig. 4) indicates that there is a decrease in the mean duration in groups T1 and T2, which is statistically significant ( $p < 0.001$ ). Group S has not shown statistically significant ( $p < 0.001$ ) post-ictal depression at all.

Table 5: Comparison of mean duration (in seconds) of different parameters in MES method (with control)

Parameters (in seconds)	Group-C1	Group-S1	Group-T1	Group-T2
Tonic hind limb flexion	2.83 ± 0.5	0***	1**	0.33***
Tonic hind limb extension	12.83 ± 0.95	0***	5.5 ± 0.4***	1.66***
Clonus	15.5 ± 1.05	14.83 ± 0.75 <sup>NS</sup>	15 ± 0.66 <sup>NS</sup>	15.66 ± 0.57 <sup>NS</sup>
Post-ictal depression	173.66 ± 12.45	0***	25.5 ± 7.95	15***

Data expressed as mean±SE  
 n = 6; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (compared with control), NS - not significant

**Comparison of various parameters of different groups with standard**

- **Tonic hind limb flexion:** A comparison of mean duration of tonic hind limb flexion of test groups (T1 and T2) with standard (S) [Table 6, Fig. 1] indicates that there is no significant difference between S, T1 and T2.
- **Tonic hind limb extension:** A comparison of test groups (T1 and T2) with group S (Table 6, Fig. 2) indicate that there is significant difference between S and T1 ( $p < 0.001$ ) while no significant difference between S and T2.
- **Clonus:** Analysis of results when compared with standard (Table 6, Fig. 3) suggests that there is no significant difference between groups S and test groups T1 and T2.
- **Post-ictal depression:** A comparison of mean duration of post-ictal depression with standard (Table 6, Fig. 4) indicates that there is no significant difference between the groups S, T1 and T2.

Table. 6 Comparison of mean duration (in seconds) of different parameters in MES method (with standard)

Parameters (in seconds)	Group-C1	Group-S1	Group-T1	Group-T2
Tonic hind limb flexion	2.83 ± 0.5	0	1 <sup>NS</sup>	0.33 <sup>NS</sup>
Tonic hind limb extension	12.83 ± 0.95	0	5.5 ± 0.4***	1.66 <sup>NS</sup>
Clonus	15.5 ± 1.05	14.83 ± 0.75	15 ± 0.66 <sup>NS</sup>	15.66 ± 0.57 <sup>NS</sup>
Post-ictal depression	173.66 ± 12.45	0	25.5 ± 7.95 <sup>NS</sup>	15 <sup>NS</sup>

Data expressed as mean±SE  
 n = 6; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (compared with control), NS - not significant

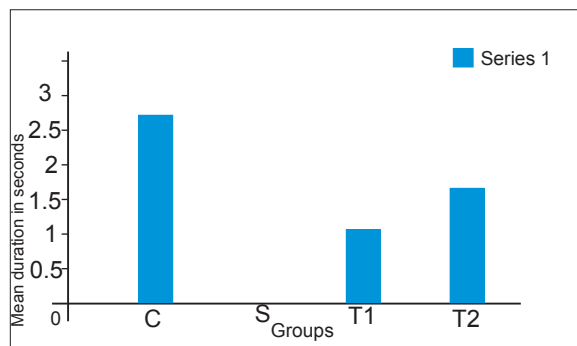


Fig. 1: Mean duration of tonic hind limb flexion

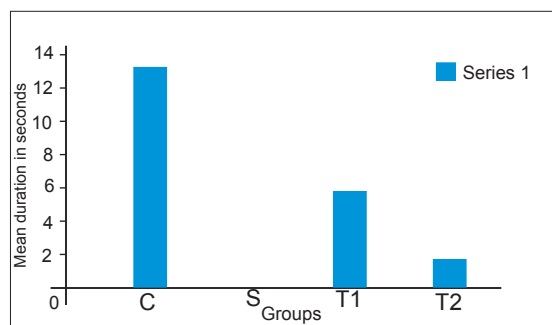


Fig. 2: Mean duration of tonic hind limb extension

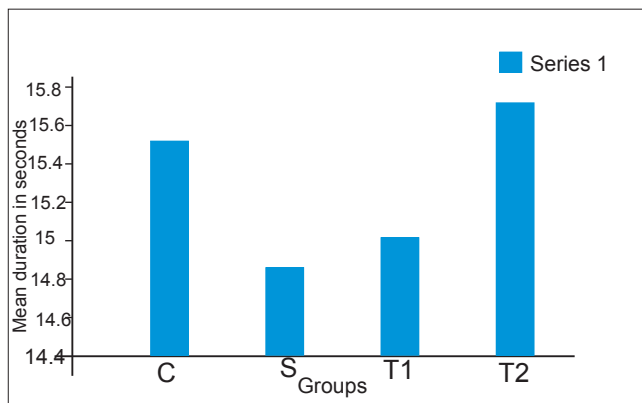


Fig. 3: Mean duration of clonus

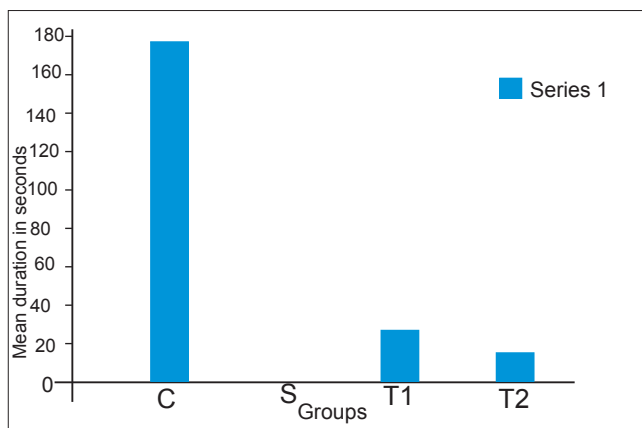


Fig. 4: Mean duration of post-ictal depression

## Discussion

In the present study, anticonvulsant activity of aqueous extract of *Hydrocotyle asiatica* was screened by maximal electroshock (MES) method. MES method has a high degree of predictivity for drugs useful in managing tonic-clonic seizures in man<sup>8</sup>.

The comparison of mean duration of various parameters of control group and test groups has indicated that there is a decrease in the mean duration of all the parameters in test groups T1 (100 mg/kg of aqueous extract of *Hydrocotyle asiatica*) and T2 (300 mg/kg of aqueous extract of *Hydrocotyle asiatica*). The values are statistically significant for all parameters except for clonus.

The abolition of tonic hind limb extension has occurred in three out of six mice in T1 and five out of six mice in T2. Since this abolition is considered suggestive of protection against MES convulsions<sup>11</sup>, the aqueous extract of *Hydrocotyle asiatica* has anticonvulsant effect against MES convulsions at a dose of 300 mg/kg.

A comparison of test group T1 with group S (Table 6, Fig. 2) indicates that there is no significant difference between S and T1 for all parameters except clonus. A comparison of test group T2 with group S indicates no significant difference between S and T2. This effect is comparable to that of phenytoin in this study.

It is apparent from the results that the test compound, aqueous extract of *Hydrocotyle asiatica* at a

dose of 300 mg/kg, has shown similarity to phenytoin in this experimental study. Like phenytoin, it has protected the experimental animals against both MES convulsions. It has abolished the tonic hind limb flexion and extension in all but one mouse subjected to MES. In view of its ability to abolish tonic component of MES, it is likely to find a place in the management of generalized tonic-clonic seizures.

At therapeutically relevant concentrations, phenytoin limits the repetitive firing of action potentials evoked by a sustained depolarization of neurons. This effect is mediated by a slowing of the rate of recovery of voltage-activated Na<sup>+</sup> channels from inactivation, an action that is both voltage- and use-dependent. At these concentrations, the effects on Na<sup>+</sup> channels are selective, and no changes of spontaneous activity or responses to GABA or glutamate are detected<sup>3</sup>. Hence it may be proposed that the mode of action of aqueous extract of *Hydrocotyle asiatica* may involve blockade of voltage gated sodium channel.

*Hydrocotyle asiatica* has recently been shown to have an anti-lipid-peroxidative and antiepileptic activity in the lithium pilocarpine model of status epilepticus<sup>14</sup>. In a recent study it has been found that *hydrocotyle asiatica* causes perceptible changes in the cholinergic system as one of the facets of its anticonvulsant activity<sup>15</sup>. *Centella asiatica* significantly prevented the cognitive impairment and attenuated the oxidative stress induced by PTZ kindling<sup>13</sup>.

## Conclusion

The test compound at a dose of 100 mg/kg has abolished tonic hind limb extension in three out of six animals in MES convulsions. Hence this dose is not adequate to prevent experimentally induced MES convulsions. At a dose of 300 mg/kg, the aqueous extract of *Hydrocotyle asiatica* has shown statistically significant anticonvulsant effect against MES convulsions.

Thus the aqueous extract of *Hydrocotyle asiatica* has shown efficacy in MES convulsions in mice in the present study. Since the clinical correlates of MES seizures are tonic clonic convulsions, the aqueous extract of *Hydrocotyle asiatica* is likely to be useful in the treatment of tonic clonic seizures. Further detailed studies of the active principle/s of this plant are worth pursuing in this regard.

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## ✪ ORIGINAL ARTICLE

# Early detection of autism: Comparison of two screening tools

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## Abstract

**Background:** The incidence of autism is on the rise in India. There are not many well conducted studies in this subject to identify the various risk factors based on the geographic, cultural and genetic differences or similarities of Indian population with the rest of the world. **Objectives:** To study the comparative efficacy of two screening tools for early detection of Autism namely Checklist for Autism in Toddlers (CHAT) and Trivandrum Autism Behaviour Chart (TABC) and to study the prevalence and various factors influencing the incidence of Autism. **Setting:** Pushpagiri Institute of Medical Sciences & Research Centre, Tiruvalla, a tertiary care Medical College in central Kerala. **Period:** Six months from September 2008 to February 2009. **Method:** Two hundred children between 18 and 36 months attending the child development centre of our medical college were screened for autism using the two tools, CHAT and TABC in a single setting after carefully taking the history and recording the information in a proforma. The observations were evaluated and results drawn. Statistical analyses of the results were done using *kappa*, chi-square, and Fisher exact tests, wherever appropriate. **Results and Conclusions:** Ten children were screened positive for autism by CHAT and 16 children by TABC. Kappa analysis showed that both these tools are in good agreement ( $K=0.75$ ) with each other. No statistically significant association could be drawn between sex, birth order, area of residence, parental age, mother's education or gestational age. Statistically significant association was obtained between low paternal education status ( $P<0.037$ ), developmental delay like no social smile at two months, no talking by twelve months, no protodeclarative pointing, and no wave bye-bye ( $P<0.00001$ ), and autism.

**Key words:** Autism, Checklist for Autism in Toddlers (CHAT), Trivandrum Autism Behaviour Checklist (TABC).

## Introduction

Autism is a neuro development disorder characterized by impaired social interaction, communication, and restricted and repetitive behaviour. These signs all begin before a child is three years old<sup>1</sup>. Autism affects many parts of the brain; how this occurs is not understood<sup>2</sup>. Autism is a highly variable brain development disorder<sup>3</sup> and symptoms tend to continue through adulthood, although often in more muted form<sup>4</sup>. Autism's individual symptoms occur in the general population and appear not to associate highly, without a sharp line separating pathologically severe from common traits<sup>1</sup>. Recent studies from the United States report prevalence as high as three to 6.7/1000 children<sup>5</sup>. It is interesting to note that the prevalence

is increasing in India as well. Autistic has disorder, the most severe of the PDDs, is four times more common in boys than in girls. Many children with autism also have mental retardation. The specific diagnosis of Pervasive Development disorders are made according to the definition set forth in the DSM-IV (The American Psychiatric Association, 2000)

The awareness of Autism spectrum disorders has started to increase in our country with the establishment of various child development centers and associations for children with special needs. However there are not much well conducted systematic studies in this subject to identify the various risk factors based on the geographic, cultural and genetic differences or similarities of Indian population with the

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rest of the world. This study is hence conducted with the intention to find the efficacy of two different screening tools, one which is well established and widely used in the western population (CHAT) and the other devised by the Child development centre in Trivandrum (TABC) in the early detection of autism.

The present study also aims at looking into the various risk factors for the development of autism in the Indian scenario and the comparison of the data with the available literature. The study hopes to result in the establishment of a uniformly organized and comprehensive health care system which allows access to health care personnel and psychologists enabling extensive evaluation, diagnosis and management of autism in our country.

## Methods

Total 200 children between 18 and 36 months attending the child development clinic of our medical college mostly urban, suburban (80%) and around 20% rural population were included in the study. It was done over a period of six months from September 2008 to February 2009. The children diagnosed to have developmental delay, mental retardation, chromosomal anomalies, diagnosed cases of neurometabolic or neurodegenerative disorders or structural anomalies of the brain were excluded from the study.

All children were evaluated by taking a detailed history, and the information was recorded in the standard proforma. They were then screened for features of Autism using the tools Checklist for Autism in Toddlers (CHAT)<sup>6</sup> and Trivandrum Autism Behaviour Checklist (TABC) by the principal investigator and a development therapist at a single sitting. The screening was conducted one after the other on the same day with the two investigators not knowing about the result of the other screening test.

The Checklist for Autism in Toddlers is a screening instrument that was devised to test the prediction that those children not exhibiting joint attention and pretend play by the age of eighteen months might be at risk for receiving a later diagnosis of autism. It takes 5-10 minutes to administer and is simple to score. The order of the questions avoids a yes/no bias. Those children who fail all key items are predicted to be at the greatest risk for autism. Children who fail both items measuring proto-declarative pointing, but who are not in the high risk for autism group, are predicted to be at medium risk. Children who do not fit either of these profiles are predicted to be at low risk. TABC is developed by child development centre, Trivandrum, with each item carefully selected from Childhood Autism Rating Scale (CARS)<sup>7</sup>. The screening tools used are given in Annexure 1 and 2.

The observations were evaluated and results drawn. Statistical analyses of the results were done using *kappa*, *chi-square* and *Fisher exact tests* wherever appropriate.

## Results

**TABC screening:** A total of 184 children had a score between 20 and 35 and were screened negative for Autism. Twelve children had score between 36 and 43, and four had score more than 44. These 16 children were screened positive for Autism. Kappa analysis proved that both these tools are in good agreement with each other ( $K=0.75$ ). The proportion of agreement was 97%. See table 1.

Table 1. Comparison between CHAT and TABC

CHAT	TABC		
	Autism	No Autism	Total
Autism	10	0	10
No Autism	6	184	190
	16	184	200

The 16 children screened positive for Autism by TABC were studied in detail and their parameters compared with non-Autistic children are given below.

- \* *Prevalence of autism:* Present study the prevalence was five percent according to CHAT and eight percent according to TABC.
- \* *Sex distribution:* There are 12 boys and four girls who are screened positive for Autism, showing a ratio of 3:1. Even though 75% of total positive cases were boys, a statistical significance could not be obtained ( $P<0.15$ )
- \* *Birth order:* Of the 16 children, ten (62.5%) were first born, five (31%) were second born, and one (6.37%) were third born. There was no statistically significant correlation with any of the birth order of the child ( $P<0.57$ ) and incidence of autism.
- \* *Area of residence:* Eleven children are from urban area and five from rural area. The percentage of urban children having autism was 69% but it was not statistically significant ( $P<0.24$ )
- \* *Father's age:* two children had father's less than 30 yrs and the rest 14 ie, 87.5% had father's more than 30 years. This association between increasing father's age and autism was not statistically significant ( $P<0.37$ ) using Fisher exact test.
- \* *Mother's age:* Ten children had mothers less than 30 yrs and six children more than 30 years. However this was also not statistically significant.
- \* *Father's education:* Ten children had fathers without secondary school graduation and six had graduated fathers. 62.5% fathers were having poor educational status and the correlation with autism was statistically significant ( $P<0.037$ ).
- \* *Maternal education:* Nine children had mothers without secondary school graduation and seven had graduated mothers. There was no statistically significant correlation between mother's education and autism.
- \* *Gestational age:* Two children were preterm, 14 were term and there was no statistically significant correlation ( $P<0.54$ ) (Fisher exact).

- \* **Developmental history:** Of the 16 autistic children, 9 (56%) did not develop social smile at 2 months which was found to be statistically significant ( $P < 0.00001$ ). Twelve children (75%) did not talk by 12 months ( $P < 0.00001$ ), nine children (56%) did not have proto declarative pointing ( $P < 0.0001$ ), and nine (56%) children who did not wave bye-bye during examination or according to the mother's report were later found to be positive for autism ( $P < 0.0001$ )

## Discussion

Two hundred children were screened for autism using the two tools, CHAT and TABC. CHAT is used worldwide as a baseline, simple screening tool for identifying children with features suggestive of Autism<sup>6</sup>. TABC is developed by child development centre, Trivandrum, with each item carefully selected from CARS<sup>7,8</sup>.

**Prevalence of Autism:** A total of 16 were screened to be positive (8%) using TABC. The prevalence in United States is 3 to 6.7% in one study and 4 to 6% in another study, which is comparable with the present study<sup>5,8</sup>. No Indian study with reliable observation could be obtained. An apparent rise in prevalence in our study may be due to the fact that the study was conducted in a tertiary referral centre.

**Comparison between CHAT and TABC:** These two screening tools were compared with each other for efficacy. While CHAT picked up 10 children with Autism, TABC picked up 16. Those 4 children not picked up by CHAT were having scores between 36 and 39, which indicate that they had mild features suggestive of Autism. Kappa statistics revealed that these tools are in good agreement ( $k=0.75$ ) with each other.

**Relationship between various risk factors and the incidence of Autism:** Present study did not have any statistical correlation between a particular sex and increased incidence of autism. However, many studies have proved that male sex has got increased predisposition for boys than girls<sup>5</sup>. No correlation was obtained between a birth order and autism. Research has suggested that birth order especially first and last born is a risk factor for autism<sup>9</sup>. No correlation was obtained between the area of residence and autism. But studies have shown that urban population is more predisposed to have autistic children<sup>10</sup>. No correlation was seen between parental age and autism. Some studies suggest that increased parental age had predisposes to having autistic offspring and some other studies indicate that parental age has no relation with autism<sup>11,12</sup>.

Present study showed a significant statistical correlation between poor paternal education and incidence of autism. Western studies have shown a contradiction finding that autism is seen more in parents with high educational qualifications<sup>9</sup>. This may be because parents with low education are not aware of the

opportunities of early screening and diagnosis of autism.

The observation in the present study may be a bias because of the geographical area where the study is conducted. Here many of the fathers are working in gulf countries and their educational qualifications are skill based and as stated by the mothers. Another probable reason is autism being known to have a genetic basis, these fathers with poor education could have had autistic features as boys themselves, which ended up in poor scholastic performance. However there was no association between mother's education and autistic offspring in present study.

The present study did not have any statistical correlation between gestational age and autism. Some studies have shown that autism is more seen among preterm babies<sup>9</sup>. Of the 200 children screened, only 21% were preterm and that might have been the reason for not obtaining a more accurate data.

**Developmental Delay and Autism:** Children with significant developmental delay were initially excluded from the study. However we could obtain history of developmental delays which showed a very significant association with autism. The specific domains which had more prognostic importance on the future diagnosis of autism were absence of social smile by two months, no talking by 12 months, no protodeclarative pointing, and no wave bye-bye. This observation was well supported by many studies in the literature<sup>13,14</sup>, thus showing that routine, regular developmental surveillance is still the best form of monitoring to prevent developmental disabilities and deviations.

## Conclusions

A total of 200 children were screened for early detection of autism using two independent tools CHAT and TABC in a single setting. Ten children were screened positive for autism by CHAT and 16 children by TABC. This showed that TABC detected more children with autism and may be considered as a useful tool. Kappa analysis showed that both these tools are in good agreement ( $K=0.75$ ) with each other.

No statistically significant association could be drawn between sex, birth order, area of residence, parental age, mother's education or gestational age and autism.

Statistically significant association was obtained between low paternal education and autism in the offspring ( $P < 0.037$ ) however this could not be supported by other studies or data available from literature.

A very highly significant association ( $P < 0.00001$ ) was obtained between history of developmental delay like no social smile at two months, no talking by 12 months, no protodeclarative pointing, and no wave bye-bye.

### Trivandrum Autism behavioural checklist (TABC)

NEVER	SOMETIMES	OFTEN	ALWAYS
1	2	3	4

#### I. Social Interaction

- Inability to establish and/or maintain eye contact
- Child does not respond when called, sometimes appears to be deaf
- Difficulty in mixing and playing with other children of same age
- Lack of appropriate emotional responses
- Can do certain tasks well, but not the tasks involving social understanding

#### II. Communication

- Difficulty in Comprehension/communication
- May/may not indicate needs by gestures or leading adults by the hand
- Echolalia/ Using nonsensical words and muttering to self
- Lack of pretend play

#### III. Behavioural characteristics

- Like sameness in everyday routine
- Inappropriate attachment to objects
- Unusual body movements such as flapping hands, or rocking and jumping and muttering to self
- Extreme restlessness Hyperactivity/Over passivity or prefers to be alone all the time
- Not responsive to normal teaching methods

#### IV. Sensory Integration

- Doesn't like to be hugged or touch/Apparent insensitivity to pain
- Intolerance/Addiction to certain sounds, taste, odours, visuals
- No understanding or fear of real dangers/ Excessive fear for heights, change in position
- Enjoys spinning or rotating objects. Inappropriate laughing and giggling/ crying spells with extreme distress for no apparent reasons
- Difficulty in fine motor skills/ a tendency to fall/ clumsiness/ resistance to new motor movement activities

Responses:

Never-1; Sometimes-2; Often-3; Always-4

Scoring:

20-35: Non-autistic; 36-43: Mildly-moderately autistic; 44 and above: Severely autistic.

(With permission : Child Development Centre, Trivandrum)

#### Recommendations

Screening for autism should be carried out to all children between 18 and 24 months for early detection and intervention strategies for better outcome. A large community basis screening in both rural and urban population may be done to know the incidence and prevalence of autism, so that early intervention strategies could be developed for the large scale benefit of the community and the nation as a whole.

#### What is already known

- Autism is a neurodevelopmental disorder which is on the rising trend in India.
- It is possible to diagnose Autism before 3 years.
- CHAT is a well known screening tool for autism.

#### What this study adds

- The prevalence of autism is between 5 and 8% according to the present study done in a tertiary level teaching hospital in central Kerala.
- A screening test at an age as early as 18-24 months can identify features of Autism.
- TABC is equally good in screening children for Autism especially for early detection of mild to moderate categories of autism.

#### Acknowledgements

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## ✦ ORIGINAL ARTICLE

# Intussusception: A short term retrospective analysis

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## Abstract

**Background:** Intussusception is the most common abdominal emergency in infants and small children. There has been great progress in diagnostic and therapeutic management of this condition. The incidence of intussusception is very high among the paediatric population in Central Travancore. **Aim:** The purpose of our review was to analyse the clinical presentation, atypical manifestations in particular, diagnostic modalities, treatment options and also to analyse the non-idiopathic variety of intussusception admitted to our hospital. **Materials and Methods:** We performed a retrospective analysis of all intussusception cases seen at the Department of Paediatric Surgery from June 2011 to January 2012. Twenty nine patients with radiological evidence of intussusception were included in the study. The data obtained included age, sex, clinical presentation, diagnostic procedures, mode of treatment, and results. Patients included children from one month to 12 years of age. Of all, 64 percent of children were under one year of age, and 66% were boys. The duration of symptoms before treatment was less than 24 hours in 38%, 24-48 hours in 28%, and more than 48 hours in 34% of patients. **Results:** A significant number of babies presented with atypical features. There were two children with no pain at all, and three with urticaria as the presenting symptom. One baby was brought with features mimicking epilepsy. The success rate of hydrostatic reduction was 95.4%. Four patients underwent surgery and, among these, abnormalities in the intestinal wall necessitated resection in two cases. An association between the duration of symptoms and the outcome of the non-surgical treatment was not very evident in our study, as compared to earlier literature. **Conclusion:** Identifying atypical presentations and early diagnosis is very important to avoid unnecessary morbidity and mortality. Hydrostatic enema is a safe and effective approach for uncomplicated intussusception reduction with a high success rate, but the older age groups need surgical exploration, especially when in doubt regarding a specific diagnosis.

**Key words:** Intussusception, Red current jelly stool, Hydrostatic reduction, Jejunio-jejunal intussusception.

## Introduction

Intussusception is a process in which a segment of intestine invaginates into the adjoining intestinal lumen, causing bowel obstruction. Eighty percent of all cases of intussusception are observed in children or infants, and the condition is observed more often in boys<sup>1</sup>. Adult intussusception is extremely rare. Intussusception can be divided into ileo-caecal (more than 90%), ileo-ileal, colo-colic and rarely jejuno-jejunal. Almost 75% of intussusceptions occur below the age of two years<sup>2</sup>. Idiopathic variety i.e., without a lead point, is

almost always the ileo-caecal type and is associated with lymphoid hyperplasia of intestine<sup>3</sup>. Pathologic lead point is seen in up to 12% patients, mainly in children above two years of age, and the commonest cause is Meckel's diverticulum<sup>4</sup>. Intussusception develops suddenly; typically a completely healthy child begins to cry periodically. Usually the child calms for 10-15 minutes, until the next attack of pain. He may vomit repeatedly. The typical triad of the findings of intussusception is: periodic abdominal pain, passage of blood and mucus per rectum (red current jelly) and palpable

abdominal mass in upper right quadrant of abdomen<sup>5</sup>. The condition is likely to be fatal if not corrected promptly. The diagnosis can be confirmed by ultrasonography supplemented with colour Doppler imaging,<sup>6,7</sup> or radiographically, with the help of air insufflation or barium enema<sup>8</sup>.

Early hospitalization and the appropriate conservative or surgical treatment usually provide favourable outcomes. Conservative reduction is globally employed and, as a rule, is effective; but in different institutions different agents are used. Liquid i.e., hydrostatic reduction under ultrasound guidance is very effective<sup>9,10</sup>. In most of the series, the success rate of the reduction is about 85 - 95%<sup>11</sup>. In cases of delayed diagnosis of intussusceptions, presence of peritonitis, or when there is a suspicion of a lead point, surgical intervention is required<sup>12</sup>. The mortality rate has steadily declined in the past century to less than one percent in most countries.

**Materials and methods**

A retrospective analysis of all cases of intussusceptions in children admitted to the Department of Paediatric Surgery at Pushpagiri Institute of Medical Sciences, Tiruvalla, Kerala from June 2011 to January 2012 were undertaken. A total number of 29 children in the age group of three months to twelve years were included in the study.

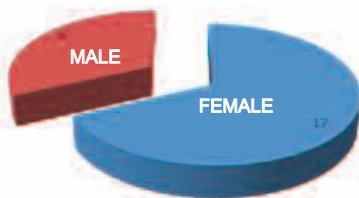


Fig. 1: Incidence of intussusception in the two sexes

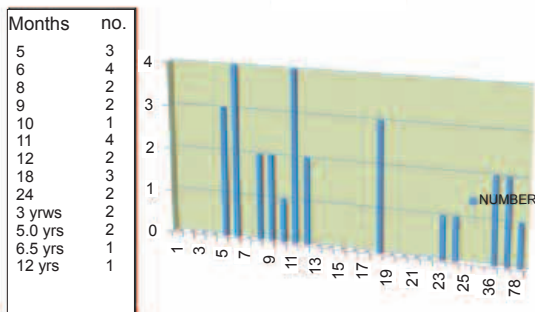


Fig. 2: Age incidence in intussusception

**Results**

The purpose of this review was to analyse the clinical presentation, atypical manifestations in particular, diagnostic modalities, treatment options and also to analyse the non-idiopathic variety of intussusception admitted to our hospital. Study period was nine months from June 2011 to January 2012. Twenty nine patients with Radiological evidence of intussusception were included in the study. Of these twenty two patients were referred from peripheral hospitals. Almost two-thirds of the children (Fig. 1) were male (M-19, F-10). Around 79% (no=23) of them were below the age of two years, which represents the commonest age group for idiopathic intussusceptions (Fig. 2). All children above the age of four years (no=4) had to undergo laparotomy for identifying the lead point. Out of 29 children, four, i.e., 13.7% had spontaneous reduction of the intussusception, with improvement in or disappearance of symptoms after admission, and repeat scans showed disappearance of the mass.

Those who presented within the age of three years or below, and having ultrasound confirmed ileo-colic intussusception, were treated by hydrostatic reduction irrespective of the duration of symptoms. Only in one child hydrostatic reduction failed, and it was required to proceed with laparotomy (Fig. 3). The earlier concept of not doing non-operative reduction after 48 hours was not practised. Absolute contraindications such as features of peritonitis, and old children with intussusceptions other than ileo-colic, were excluded from operative reduction.



Fig. 3: Ileo-colic intussusception

There was a definitive seasonal variation in the incidence of Intussusception. In the idiopathic group, 64% (n=14) of patients came during the months of August, September and October.

Clinical presentations were varied (Fig. 4), and many of the kids presented with atypical symptoms. Classical triad of symptoms were seen, as follows: colicky pain in 23 patients (80%), bleeding per rectum in nine babies (31%), and mass in six children (20%), thus

the combination triad of symptoms all together were seen only in 20% (n=6) of patients.

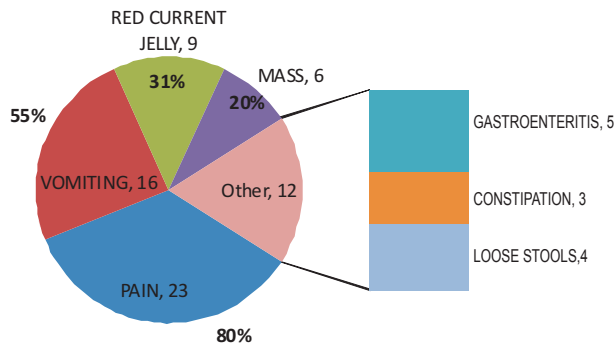


Fig. 4: Variegated clinical presentations in intussusception

The duration of symptoms varied from few hours to five days (Fig. 5), and a trial of non-operative reduction was attempted in all patients with clinically suspected idiopathic intussusception.

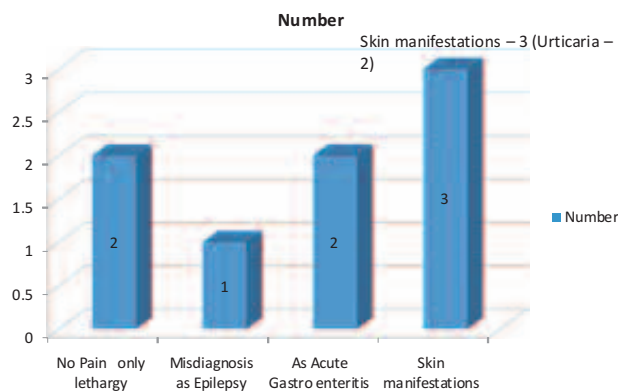
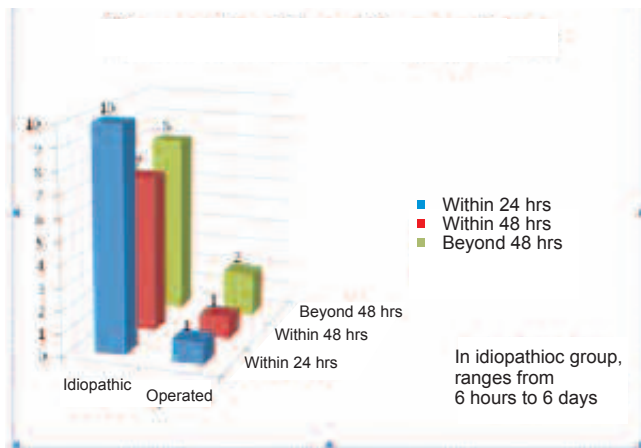


Fig. 6: Cases with atypical presentations

Among those with atypical symptoms and signs (Fig. 6), absence of definite colicky pain was noticed in two patients; they had only lethargy. One baby presented with features similar to epilepsy, and three had generalized urticarial skin lesions and for which the possibility of Henoch Scholein purpura was considered.

The success rate of hydrostatic enema reduction method in our series was 95% (23 out of 25 babies in whom reduction was attempted), and the complications were negligible.

### Recurrent intussusception

Two children came back with recurrence. One child, who had an initial reduction two months before the second attack, was one year old; and was managed successfully with hydrostatic reduction. Another child, a four year old male, had a third attack (initial two reductions about three years back, in an interval of two months), which was reduced spontaneously.

The most unusual case was a six year old boy, who presented with features of upper small intestinal obstruction and found to have Jejuno-jejunal intussusceptions. Emergency laparotomy showed multiple lesions on jejunum and stomach with an obstructing intussusception in jejunum (Fig. 7).

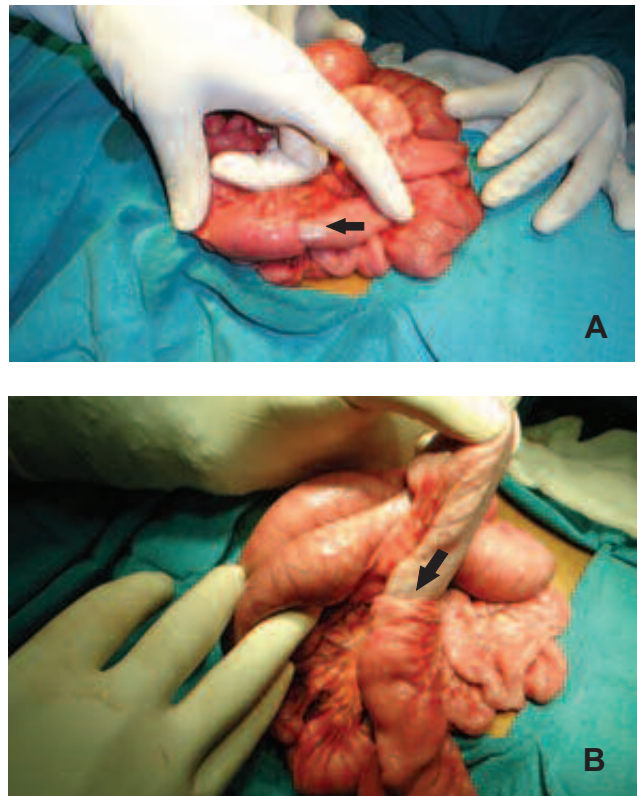


Fig. 7: A,B Multiple intussusceptions in jejunum (Arrows)

Resection of the lesion was done and the biopsy revealed non-Hodgkin's lymphoma. Even though lymphoma is the most common malignant lesion of small bowel in children, it is very rare to manifest as intussusception in jejunum (Fig. 8).



Fig. 8: Jejunal lesion - Lymphoma

## Discussion

Although the classical picture is typical, failure to recognize it may result in delayed diagnosis. A good number of babies present with atypical manifestations. Viral infections followed by intestinal lymphoid hyperplasia can be implicated in the aetiology of intussusception and that could be the reason for seasonal increase in incidence<sup>3</sup>. But the presence of an infectious disease does not preclude the presence of a surgical concern.

A typical presentations form an important reason for delay in diagnosis. Few of them can present without pain and a very high index of suspicion is essential for the proper diagnosis<sup>13</sup>. Any children belong to the vulnerable age group even with atypical presentation should get a screening abdominal ultrasound done, particularly so, as the test is very cheap and highly specific and sensitive in diagnosing intussusceptions.

In general, most children with intussusception can be treated conservatively. There are many types of contrast agents used for the purpose.

The success rate of hydrostatic enema reduction method in our series was 95%, and the complications were extremely rare, and usually avoidable. Morbidity depends on the time interval between the onset of symptoms and the diagnosis. Proper and timely resuscitation and correction of electrolyte imbalance, if any, must be done without any delay.

Correlation between the duration of symptoms and effectiveness of non-operative reduction was not very evident in our study, as babies with symptoms of more than four days also had successful reduction. Older children presenting with intussusception must be investigated thoroughly, and may end up in laparotomy in most of the cases. Any lead points must be resected for biopsies as lymphomas and other malignancies are common. The incidence of NHL acting as a lead point in intussusception is reported to be as high as 17%, in children over 4-6 yrs of age usually in ileum or colon<sup>14</sup>.

The mortality rate has steadily declined in the past century to less than 1% in most countries<sup>3</sup> with the advent of early diagnostic modalities and effective non-surgical management.

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## ✦ ORIGINAL ARTICLE

# Pulmonary function in Tyre factory workers in Central Kerala, South India

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### Abstract

**Introduction:** The workers involved in tyre manufacturing are exposed to a mixture of chemicals and dusts which could potentially lead to respiratory obstruction and/ or restriction. **Objective:** To estimate the prevalence of respiratory dysfunction due to occupational exposure in a tyre manufacturing factory. **Methods:** A cross sectional study was conducted in a major tyre manufacturing factory in Kerala. Two hundred and ninety six workers were interviewed from mixing plant, tyre plant, tube plant and pre-cured tread rubber (PCTR) plant, and administrative staff of a tyre factory. Pulmonary function test was done using a portable spirometer. Regression equations were calculated using SPSS for windows using normal population data from 51 healthy people. Fifth percentile of these parameter values were taken as lower limit of normal (LLN) adjusted for age and height. Respiratory dysfunction of either restrictive or obstructive nature was estimated based on these values in the tyre workers. **Results:** The duration of work exposure ranged from 1.6 to 37.4 years. A significant 53.7% of the workers complained of breathlessness. 35.5% of the workers were smokers. The proportion of individuals with obstructive disorder was 4.4% and that of restrictive disorder was 54.1%. Tube plant had higher proportion (60.3%) of workers with respiratory dysfunction. However, the tyre plant, where the duration of employment was the shortest among the various sections, had 50% of the workers having respiratory dysfunction. Smoking habits and family history did not significantly explain the presence of abnormality of pulmonary function. LLN method was found better correlated with respiratory problems than conventional method of fixed percentage of predicted values. **Conclusion:** Rubber tyre making is associated with high prevalence of respiratory dysfunction which in majority of cases, are subclinical. Regular spirometry assessment can detect individuals at risk of problems and they could be better educated about regular protective mask usage which is currently not enforced.

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**Key words:** Pulmonary function, Rubber tyre manufacture, Lowest limit of normal, Respiratory dysfunction.

### Introduction

It is a fact that the industrial revolution brought many tremendous improvements in the lives of common man and the consumers enjoy the benefits of these luxuries without any heed to the sufferings of the very providers of these various comforts. Occupational diseases in the work force form a major health issue that has come about due to modernization. If one looks at the data of the estimates of occupational disease and occupational injury in India, the figures (1.83 and 18.3 million respectively) indicate that, as a nation, India is contributing nearly

20% of the global burden in this respect<sup>1</sup>.

Industries using chemicals in the manufacturing process expose the workers to great health hazards. For example, it is a well known fact that workers involved in tyre manufacturing are exposed to heterogeneous mixture of chemicals and dust through inhalation and absorption via skin. Inhalation is probably the most important route of exposure in the workplace and it is an inescapable route of exposure. The relationships between occupational exposures and respiratory symptoms in industrial workers have been studied since early

1970s when protective measures were not much popular. Respiratory problems like reduction in pulmonary function, chest tightness, shortness of breath etc. were some of principal adverse effects reported.<sup>2-5</sup>

Respiratory system involvement among these workers could be either due to airway obstruction or any restrictive pathology in lung tissue. As toxicity data are not available on most of the chemicals to which rubber factory workers are exposed, it is difficult to predict the type of pathology that might have caused a reportedly higher prevalence of breathlessness among them. This study was designed to estimate the prevalence of respiratory dysfunction due to current occupational exposure in the rubber products manufacturing industry, taking flow volume curve patterns as the main diagnostic criterion.

## Subjects and Methods

This study was designed as a cross sectional study in a major tyre manufacturing factory in Kerala, South India. Ethics committee approval was obtained from the Institutional Ethics Committee, and absolute confidentiality of data was maintained throughout the study. All male employees present on day shift who volunteered for a pulmonary function test were included in the study irrespective of their clinical complaints. Total 296 workers were interviewed with regard to demographic data, smoking habit, type of work and respiratory symptoms. Details of nature and duration of employment were collected from the Employees Welfare Department of the tyre manufacturing factory selected for study. Pulmonary function tests were conducted by a technician on the same portable Pulmonary Function Testing (PFT) machine. Four main production processes/ personnel groups were distinguished viz. mixing plant, tyre plant, tube plant and pre-cured tread rubber (PCTR) plant in addition to the administrative section staff.

### Study setting

The study site consisted of a cluster of four major plants such as mixing plant, tyre plant, tube plant and PCTR plant. The raw materials were mixed in mixing plant, and further processing was done in the rest of the plants. The employees were categorized as engineering, material handling, plant technical, production quality assurance and administrative staff.

### Pulmonary function tests

Forced expiratory flow-volume curves were recorded using a computerized portable pulmonary function test machine. Three forced vital capacity maneuvers were obtained for each subject. The largest of three Forced Vital Capacity (FVC) and Forced Expiratory Volume in one minute (FEV1) values were accepted even if the two volumes did not come from the same curve. The ratio of FEV1 to FVC was expressed as a percentage.

## Statistical analysis

To generate regression equations for Lowest Limit of Normal (LLN or 5<sup>th</sup> percentile) values of pulmonary function parameters as recommended by the latest American Thoracic Society (ATS) guideline, pulmonary function test was conducted among 51 healthy people from the general population of central Kerala (including some occasional smokers) with no occupational or domestic exposure to dust or chemicals. Regression function of SPSS software was used to generate these best fitting cross sectional regression equations as given in table 1. The square of age (since its relationship with pulmonary function was found to be non-linear) and height were taken as 'X' (independent) variables and the specific pulmonary function parameter was taken as 'Y' (dependent) variable in the prediction model.

Table 1: Regression equations for 5<sup>th</sup> percentile of parameters from control population

Dependent variable	Regression equation
FVC (l)	$-0.000221a^2 + 0.02957h - 1.487$
FEV1 (l)	$-0.00022a^2 + 0.02279h - 1.771$
FEV1/ FVC (s/l)	$-0.00143a^2 - 0.0546h + 89.873$

a - Age in years; h - Height in centimeters

Workers with FVC less than 5<sup>th</sup> percentile of the expected volume were diagnosed to have restrictive disorder and those with FEV1 / FVC less than fifth percentile of the expected ratio were diagnosed to have obstructive disorder. Respiratory dysfunction was assumed in any person if any one of the disorder was diagnosed using the fifth percentile method and this was correlated with the complaint of breathlessness. Respiratory dysfunction was also estimated from the standard method of fixed percentages of expected values and was compared with our estimation using fifth percentile as LLN.

The data collected from the factory were entered in Microsoft Excel and was analyzed using SPSS for Windows (SPSS Inc. Chicago, Illinois, USA). Frequency of workers in different plants and their mean ages with standard deviation were calculated. They were grouped according the duration of employment and work site for analysis. All continuous variables were summarized as means and standard deviation. All categorical variables were summarized as frequency tables. Student's t test and Chi-square were used for testing significance, wherever necessary. A p-value of less than 0.05 was considered statistically significant.

## Results

The range of age of 296 male workers in the study was 23 to 57 years (mean 39.65, SD 6.92). The pattern of age distribution was in such a way that the tyre plant employed younger workers while the older ones were working in the tube plant (P = 0.0021). The duration of employment of subjects varied from 1.6 years to 37.4 years (mean 14.6 years, SD=6.7).

Workers of the tyre plant had complaints of shortest duration of employment of  $11.2 \pm 3.5$  years ( $P < 0.0001$ ). Out of 296 workers, 159 (53.7%) complained of breathlessness as shown in table 2. Specifically, 93 (31.4%) of them also gave history of allergic rhinitis, 22 employees (7.4%) had complaints of asthma and 38 (12.8%) of the workers reported family history of respiratory diseases indicating familial predisposition. One hundred and five (35.5%) workers were smokers. Only 54 (18.2%) of the workers reported using masks while at work.

Table 2: Frequency of workers with respiratory problems, family history and smoking

History of respiratory problems	Present %	Absent %	Association	
			Smoking	Family history
Breathing difficulty	159 (53.7)	137 (46.3)	0.242	0.58
Allergic rhinitis	93 (31.4)	203 (68.6)	0.562	0.003+

\* *p*-value for chi-square for bivariate analysis † statistically significant

From the analysis of the respiratory function parameters it was found that obstructive disorder was present in 13 (4.4%) and restrictive disorder in 160 (54.1%). Restrictive disorder was more or less equally common among the people above and below 40 years of age (55.9% vs. 52.7%,  $P = 0.58$ ). Those who were in occupation for more than 15 years (58.7% vs. 51.6%,  $P = 0.243$ ) had considerably more restrictive disorder, though the association was not statistically significant. A total of 169 workers (57.1%) were in one or other category of respiratory disorders which was consistent with the reported rates of symptoms.

When workers of tyre plant were compared against the rest of the factory workers, it was observed that respiratory dysfunction was more common among other departments (50% vs. 58.8%,  $P=0.223$ ). On closer look among other departments, more people working in the tube plant were found to be having respiratory dysfunction (60.3%), which was partly because of the older cohort working in this area.

Respiratory dysfunction as determined by the method of 5<sup>th</sup> percentile as LLN correlated better with complaints of breathlessness as reported by the employees as shown in table 3. Respiratory dysfunction (both obstructive and restrictive disorder) was correlated with smoking habits, family history of respiratory problems, and regular mask usage. These had no significant effect on prevailing respiratory dysfunction as shown in table 4. Allergic rhinitis, on the other hand, had significant relationship with family disposition.

Table 3: Symptom correlation of two methods of estimation of respiratory dysfunction

Respiratory dysfunction according to		Breathing difficulty as complained by worker		P value
		Yes	No	
Fixed % of predicted as LLN	Present	63	47	0.345
	Absent	96	90	
5 <sup>th</sup> percentile method	Present	95	65	* 0.034
	Absent	64	72	

\* Statistically significant

Table 4: Comparison of respiratory dysfunction with smoking, family history and use of mask

Other influencing factors		Respiratory dysfunction		P value
		Yes (%)	No (%)	
Smoking	Present	64 (39.7)	41 (32.3)	0.321
	Absent	105 (62.1)	86 (67.7)	
Family history	Present	17 (10.1)	21 (16.5)	0.099
	Absent	152 (89.9)	106 (83.5)	
Mask usage	Present	27 (15.9)	27 (21.2)	0.244
	Absent	142 (84.1)	100 (78.8)	

## Discussion

This study has revealed a high prevalence of both restrictive disorder (54.1%) and obstructive disorder (4.4%) among tyre manufacturing factory workers of South India. This accounted for the high prevalence of complaints of breathing difficulty (53.7%) among them. National Institute for Occupational Safety and Health (NIOSH) in USA conducted a health hazard evaluation at the Kelly-Springfield Tyre Company in Freeport, Illinois, in 1992 and reported that one-third of the workers complained of cough, and one-fifth reported chest tightness, wheezing, or shortness of breath<sup>6</sup>. Compared to this, the present study revealed a higher prevalence of breathing difficulty.

Data analysis was done by stratifying employees as young and old according to their duration of employment and age. Though statistically not significant, restrictive disorder was higher in those with longer duration of employment which was independent of age effect.

Tyre plant workers showed lower prevalence of respiratory dysfunction and this can be attributed to their younger age. Short duration of employment in the tyre section is worrisome if these workers are migrating to other sections after a period of strenuous work and persistent damage to lung. Thus it can be considered as indirect evidence of a healthy worker effect indicating association of respiratory dysfunction and duration of employment in the tyre section.

Gupta *et al.*, studied 667 rubber factory workers during 1990-91 and found that respirable fraction of the particulate size ( $< 0.5$  micron) showed high mean concentration of suspended particulate matter, benzo(a) pyrene, benzo(e) pyrene and benzo(a) anthracene in the compounding section when compared with vulcanising and packing loading units<sup>7</sup>. While comparing the lung functions amongst these groups, the higher results of residual volume, residual volume/total lung capacity ratio, forced respiratory capacity and lower values of total lung capacity were observed in group III workers as compared with other two groups. And also these results seem to be correlated with the high pollutant concentrations to which group III workers were exposed, and reflect a clear combination of obstructive and restrictive pattern of lung functions in them.

There was no statistically significant association with smoking habits of the workers and presence of respiratory dysfunction, same with family history of respiratory problems and regular mask usage. This might be due to confounding effect of different variables beyond the scope of this study i.e., workers with family history of respiratory problems might tend to use protective masks regularly but some of them will nevertheless land up in respiratory dysfunction because of high familial predisposition. Pattern of respiratory dysfunction also did not show any significant association with smoking habits in contrast to previous studies which showed significantly higher prevalence in smokers<sup>8</sup>.

## Conclusion

In this study an attempt was made to verify the significance and authenticity of most recent recommendation for diagnosis of restrictive and obstructive disorder given by American College of Occupational and Environmental Medicine (ACOEM) i.e., taking 5<sup>th</sup> percentile values of lung volumes of normal population as lower limit of normal (LLN). Diagnosis with new recommendation correlates well with their reported complaints of breathing difficulty whereas no association is found with diagnosis using conventional fixed percentage of predicted lung volume. Moreover conventional method can cause under-diagnosis of respiratory disorders, especially in this type of study set up, where young and apparently healthy subjects are studied.

## Acknowledgements

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## ✪ CASE REPORT

# Perforation of Gastrointestinal stromal tumour of small bowel - a rare case

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### Abstract

Gastrointestinal stromal tumours are by themselves relatively rare tumours and their presentation as acute abdomen due to intestinal perforation is extremely rare. A case of perforation of a Gastrointestinal Stromal Tumour (GIST) of the small intestine is reported here. The patient was being evaluated for a mass in the abdomen, and developed signs of perforation while admitted in the hospital. An emergency laparotomy was done, which revealed rupture of the tumour mass arising from the jejunum, and RO resection was required. Histopathology and immunohistochemistry revealed the tumour mass to be GIST and regular follow up was advised, along with Imatinib therapy.

**Key words:** GIST, Perforation, KIT antigen, Laparotomy, Imatinib.

### Introduction

Gastrointestinal stromal tumours are rare malignancies. Although they form the most common sarcoma of the gastrointestinal tract, they represent only 0.2% of all GI tumours<sup>1</sup>. The term GIST was first coined in 1983 by Mazur and Clark to describe non-epithelial tumours of the GI tract that lacked the ultrastructural features of smooth muscle cells as well as the immune-histochemical characteristics of Schwann cells<sup>2</sup>.

Many GIST are asymptomatic, discovered usually during imaging or laparotomy for other reasons. Patients with advanced disease may present with a mass lesion or vague abdominal pain. GIST can be highly vascular, and bleeding is one of the common presenting symptoms<sup>3</sup>. Some cases present with small bowel obstruction. Here we present an unusual case presentation of GIST, who was admitted with vague general and abdominal complaints, and ultimately went into small bowel perforation.

### Clinical presentation

A 62 year old male patient was referred from a nearby local hospital with history of loss of appetite and weight loss during the past one month, malena of 20 days duration, and fever with chills and rigours for 15 days,

associated with burning micturition. He had no significant past history, apart from being a known hypertensive, on regular treatment. On examination, there was pallor, but vital signs were normal. Local examination of the abdomen revealed a palpable mass in the hypogastric region, extending into the right and left iliac fossae. The mass was firm, smooth surfaced with all its borders palpable, except the lower border. Mobility of the mass was restricted. The rest of the abdomen appeared clinically normal.

The investigations showed a haemoglobin level of 7.5gm/ dl and PCV of 22.5. Ultrasound evaluation of abdomen showed a mass in the hypogastric region, but the organ involved could not be identified conclusively. After admission he was being prepared for a contrast enhancement CT abdomen, when he had a sudden episode of passing large amounts of black tarry stools, followed by severe abdominal pain, distension and breathlessness.

On examination, all the vital signs were visibly deteriorating, with a heart rate of 120/ min, respiratory rate 36/min and BP 90/ 60mmHg. His abdomen was distended and showed a generalized tenderness. Abdominal wall exhibited guarding and rigidity. In addition, clinically there was

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obliteration of liver dullness. He was resuscitated with IV fluids and a chest X-ray was taken which showed free gas under right dome of diaphragm, thus confirming the presence of hollow viscus perforation. An USG abdomen was also done which showed moderate amount of free fluid in the peritoneal cavity.

On laparotomy around 1.5 litres of foul smelling sero-sanguinous fluid was present in the abdominal cavity. A mass of about 15 x 12 cm size was found on the anti-mesenteric border of the jejunum about 15 cm distal to the duodeno-jejunal flexure (Fig. 1), with a perforation on it. Adjacent jejunum was found in a pre-ischemic state due to rotation of the mass around the mesentery. The inferior surface of the mass was adherent to the fundus of the urinary bladder. All the adhesions were separated and the mass was excised *in toto* along with five centimeters of the jejunal segment on its either side. End to end anastomosis of the cut ends of the jejunum was done, followed by thorough peritoneal lavage.

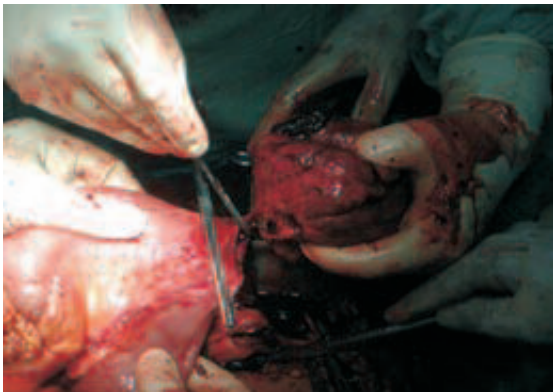


Fig. 1: Perforated GIST, with segment of jejunum (intra-operative)

Histopathology specimen was an irregular nodular mass weighing 450 gms, and 14 x 12 x 3 cm in size (Fig. 2). One side of the mass showed a rent. The cut section showed areas of haemorrhagic necrosis and cystic degeneration. Microscopic examination of the mass showed spindle shaped cells arranged in sheets, and interlacing fibres, with 1-2 mitotic figures/ 50 HPF. The HP diagnosis reported was *small bowel gastrointestinal stromal tumour - high risk type*. Immunohistochemistry was also done but CD117/C-KIT, CD34 and S-100P were negative; only smooth muscle actin was positive.



Fig. 2: Cut section of the excised GIST

Later the patient developed surgical site infection in the lower part of the incision, which improved with antibiotics and daily dressings. He was discharged after secondary suturing of the wound, and was referred to a cancer center for further medical management of the condition GIST.

## Discussion

Primary GIST can arise throughout the gastrointestinal tract, but are most common in the stomach (40-70%), small bowel (20-40%), colo-rectum (5-15%) and oesophagus (<5%)<sup>4</sup>. GIST usually presents in patients from 40 to 60 years of age. Incidence is almost equal in males and females, and occurs in all racial and ethnic groups.

The tumour is believed to originate from gastrointestinal pacemaker cells known as interstitial cells of Cajal. In the vast majority of GIST cases, there is mutation of the *c-kit proto-oncogene*<sup>5</sup>, which codes for the protooncogene protein, c-kit (CD 117). Normally c-kit antigen serves as a receptor for the enzyme tyrosine kinase, which is involved in many of the steps of haematopoiesis and gametogenesis and functioning of gastrointestinal pacemaker cells<sup>6</sup>.

GIST is nowadays diagnosed by immunohistochemistry from the expression of CD117 (positive in 95% cases). A variety of other immune-markers have also been demonstrated, such as BCL-2 (80%), muscle-specific actin (50%), smooth muscle actin (35%), S-100 (10%) and desmin (5%)<sup>7</sup>.

Many of the cases with GIST are asymptomatic, discovered upon imaging or at laparotomy for other reasons. Patients may present with bleeding, a mass lesion or vague abdominal pain. Obstruction of the GI tract is a rare presenting condition and perforation of GIST extremely rare.

For imaging studies, USG may be helpful. CT scans are critical to determine the anatomic extent of GIST and to assist with planning the operative procedure. PET (Positron Emission Tomography) is also useful. Specific diagnosis is often delayed until complications develop. Even with the newer diagnostic modalities, and even if complications occur, exact diagnosis is difficult preoperatively<sup>8</sup>.

Surgery with complete resection of the tumour remains the standard therapy for most of the GIST cases. Because lymph node metastasis is extremely uncommon, regional lymphadenectomy is not recommended. Following surgery, all patients should receive adjuvant treatment with Imatinib mesylate. Imatinib (Gleevec), a small molecule oral drug is a selective inhibitor of type 3 tyrosine kinase, and has shown remarkable efficacy against the KIT oncoprotein. The prognostic factors of GIST are mitotic rate, tumour size and its anatomic site<sup>9</sup>. The five year overall survival rate following complete resection ranges from 40-55%<sup>10</sup>, but with perforation it goes down further a bit.

## Conclusion

GIST represents a rare group of multiform tumours with varying biological behaviour. Their presentation as acute abdomen due to perforation is extremely rare. But even then the aim of management remains the same with complete resection of the tumour followed by adjuvant therapy with molecular inhibitors like Imatinib.

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## ✦ CASE REPORT

# Non-specific bullous lesions in Systemic Lupus Erythematosus

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### Abstract

**Bullous lesions in Systemic lupus erythematosus (SLE) patients are uncommon but a well recognized condition. A patient with SLE who developed vesiculo-bullous lesions is being reported. Diagnosis was confirmed by histopathological examination and Immuno-fluorescence (IF). Dapsone has produced a dramatic improvement of her skin lesions. This case is reported to highlight an uncommon manifestation of SLE and to discuss the pathological features of the condition.**

**Key words:** Systemic Lupus Erythematosus, Non-specific bullous lesion, Dapsone

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### Introduction

Lupus erythematosus (LE) is a group of heterogeneous auto-immune diseases with widely varied clinical manifestations. In this condition, auto-antibodies are directed against cell nucleus. Skin lesions in this condition may be specific or non-specific. Rarely (SLE) presents with bullous lesions, and this could even it could be an initial manifestation of SLE<sup>1</sup>.

Simultaneously, the patient had also noticed blisters on her body which were associated with reddish raised lesions. When the blisters had appeared, acyclovir was started on suspicion of chicken pox. With steroid therapy, all her symptoms had improved except the blisters. The blisters had persisted even after more than a week's therapy with acyclovir. Three months after acyclovir therapy, she had reported to this hospital.

### Clinical presentation

A pale and afebrile 35-year-old lady reported to the Department of Dermatology with multiple tense vesicles, small bullae and crusted lesions distributed in a generalized manner, but predominantly on sun exposed areas. Oral cavity showed erosions of the palate and buccal mucosa. Hypopigmented macules were seen on sites of healed lesions. Cutaneous examination showed multiple erythematous plaques seen on the exposed areas (Fig. 1). She gave a history of evening rise of temperature, joint pains and lymph node enlargement of the neck three months back and had been on anti-tuberculosis treatment (ATT) elsewhere. While on ATT, she had developed severe ulceration of the mouth. She had then been re-evaluated and a diagnosis of SLE was made based on investigation findings. ATT was then stopped and she was started on oral prednisolone.



Fig.1: Vesiculo-bullous lesions and resolved lesions over the extremities

### Investigations and Management

The relevant investigations done in some outside centre at the time of diagnosis of SLE had revealed haemoglobin levels of 3.8 gm/dl, erythrocyte sedimentation rate (ESR) of 50 mm in the first hour, platelet count of 49000/cumm and peripheral smear showing bicytopenia. Bone marrow examination had showed dyshematopoietic changes without any abnormal cells, positive anti-nuclear antibody, hypo-complementemia,

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and dsDNA. Patient was already on systemic steroids and symptoms were alleviated when she reported to the authors.

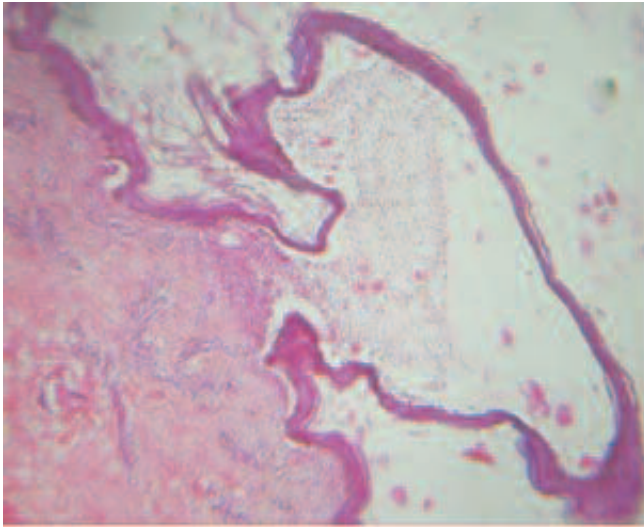


Fig.2: Histopathology of the skin lesion (H&E - 50x)

On re-evaluation, her Hb level was 8.9 gm/dl; total WBC count was 5600/cumm; ESR was 90 mm in the first hour and ANA was positive. Histopathology of a lesion showed a sub-epidermal bulla containing numerous neutrophils and papillary microabscesses resembling dermatitis herpetiformis (DH), typical of bullous SLE (Fig. 2). Direct immuno-fluorescence showed moderately strong granular IgG in basement membrane zone (BMZ) band going to the floor of the split. Moderately strong bands of IgM and IgA were also seen with weak C3 band in BMZ and in blood vessel walls. The diagnosis of LE nonspecific bullae (DH like) was confirmed and she was put on dapsone 100 mg in addition to prednisolone 40 mg daily. The addition of dapsone resulted in rapid and marked improvement of her skin lesions.

## Discussion

Bullous SLE is defined as a transient autoimmune blistering condition that occurs in the setting of SLE. This skin change is an uncommon but well recognized entity. Bullae in SLE may occur due to various causes. They may be an expression of the disease process itself, an associated state such as porphyria cutanea tarda, a coincidental finding such as bullous pemphigoid or dermatitis herpetiformis or the result of a drug reaction<sup>1</sup>. However a histo-pathological study helped to differentiate between these conditions. Histopathologic differentiation is important as the therapy and expected course of disease differ. Vesiculobullous lesions in SLE can be divided on the basis of histopathology into LE- specific and LE- non specific<sup>2</sup>.

Histopathology of LE- specific skin disease is characterized by vacuolar basal cell degeneration, basement membrane thickening, dermal mucin

deposition and mononuclear cell infiltration of dermoepidermal junction where as LE-non-specific bullous lesions are characterized by marked neutrophilic infiltration with papillary microabscess formation resembling DH and the inflammatory variant of epidermolysis bullosa acquisita. However the direct immunofluorescence findings are typical of those of LE<sup>2</sup>. Linear bands of IgG, IgA, IgM and C3 are seen in BMZ. Immuno-electron microscopy study has demonstrated that circulating IgG antibodies bind below lamina densa. Circulating auto antibodies reactive with type VII collagen is present in some cases. Other dermal and epidermal antigen can also be involved, and in some cases no circulating antigen can be detected<sup>3</sup>.

Clinically bullous SLE is characterized by widespread non-pruritic vesiculobullous eruption which is unresponsive to high dose steroids. However these lesions show a dramatic response to dapsone<sup>1</sup>. It is important to recognize LE-nonspecific lesions because they may be a marker of underlying systemic disease activity.

Zecevic RD *et al.*<sup>4</sup>, have conducted a study which showed LE-nonspecific disease had significantly increased disease activity when compared to those with only LE-specific lesions and those with both kinds of lesions. Thus the type and number of cutaneous diagnoses are important in assigning risk and in following the activity of SLE.

This case was initially misdiagnosed as a manifestation of TB and was started on anti-tuberculosis treatment. Later on, even while she was being treated for SLE, when the bullae had appeared, she received treatment with acyclovir with a diagnosis of chicken pox. Upon referral, a lesional biopsy of this patient in the present centre showed a DH like picture which was consistent with bullous SLE. Direct immunofluorescence findings were also confirmatory. Patient had not shown any improvement of the bullae with systemic steroids, even though other systemic features improved. On starting dapsone she showed a dramatic improvement, once again, confirming the diagnosis of LE non-specific bullae (DH like).

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## ✦ CASE REPORT

# Bacteremia caused by *Acinetobacter ursingii* in a tertiary care hospital of South India

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### Abstract

**Acinetobacter** species are known to cause bacteremia. We report a case of bacteremia by a rare species of *Acinetobacter*, namely *Acinetobacter ursingii*. The organism was isolated from the blood of a forty year old man with pulmonary tuberculosis admitted in our ICU. To our knowledge this is the first case report of bacteremia caused by *Acinetobacter ursingii* from India.

**Key words:** *Acinetobacter ursingii*, Bacteremia, VITEK 2C

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### Introduction

The genus *Acinetobacter* is an important causative pathogen of nosocomial infections in the healthcare setting. The genus has at least 32 genomic species<sup>1</sup> and the one most frequently isolated from humans is *Acinetobacter baumannii*<sup>2</sup>.

Due to their inherent capacity of for long term survival on inanimate surfaces, they are commonly isolated from the hospital environment<sup>3</sup> and are associated with skin colonization of inpatients and hospital personnel<sup>4</sup>. Despite their low pathogenic potential, the *Acinetobacter* species have increasingly been recognized as opportunistic pathogens mainly in immune-compromised patients and patients hospitalized in intensive care units (ICUs)<sup>5</sup>. We report a case of bacteremia due to a rare species of *Acinetobacter* from a patient admitted in the ICU.

### Clinical presentation

A forty year old male, a known case of pulmonary tuberculosis on antitubercular drugs, but defaulted after two months of therapy, was admitted at our hospital in the ICU with symptoms of cough with haemoptysis, evening rise of temperature, weight loss and loss of appetite of one week duration. He was a known diabetic, which was poorly controlled.

On examination, the patient was febrile, had hypotension and his O<sub>2</sub> saturation was 95%. Examination of respiratory system showed bilateral

crepitations, and chest X- ray revealed upper lobe fibrosis with cavitation. There was no focal neurological deficit.

Investigations revealed a haemoglobin level of 10.7gm/l. Total leucocyte count was  $10.6 \times 10^3/\text{l}$ , with a differential count of 90% neutrophils, 3.6% lymphocytes, 5.5% monocytes, 0.1 % basophils and 0.8% eosinophils. Serum sodium level was 129 mEq/l, potassium 3.6 mEq/l, bicarbonate 28.3 mEq/l, urea 19 mg/dl, random blood sugar 392 mg/l and glycosylated haemoglobin 10.57%. The blood cultures sent grew *Acinetobacter ursingii* (*A. ursingii*).

The patient was again started on antitubercular drugs, specifically treated with intravenous imipenem, and was also put on inotropic support. He was in the ICU for six days and because of the intensive therapy, improved and was shifted to ward, and subsequently discharged.

### Phenotypic identification

Blood culture was processed using the BacT/ALERT (bioMérieux) automated system, and A grew *Acinetobacter ursingii* after twenty four hours of incubation. *Acinetobacter* isolate derived from blood specimen had typical properties of the genus *Acinetobacter*, being non-motile, strictly aerobic, oxidase-negative and glucose non-fermenting gram-negative cocco-bacilli. Biochemical characterization was achieved by performing with an ID-GNB card, VITEK 2C (bioMérieux) in accordance with the manufacturers' instructions.

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The positive biochemical reactions by VITEK 2C for this organism include D-glucose, lipase, urease, citrate, L-lactate assimilation, sucrose and L-lactate alkalization.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility of the clinical isolate was determined by the disk diffusion method on Mueller-Hinton agar according to the recommendations by CLSI standards. The disks were supplied by Bio-Rad Laboratories, Mumbai, and the following antibiotics were tested: ampicillin/ sulbactam, ticarcillin, piperacillin, piperacillin-tazobactam, ceftazidime, ceftriaxone, cefepime, imipenem, tigecycline, tobramycin, gentamicin, amikacin, levofloxacin, ciprofloxacin, and trimethoprim/ sulfamethoxazole, and the isolate was found to be sensitive to all the above antibiotics tested. MICs were determined for the same antibiotics (see Table 1), using VITEK 2C, AST card - N090 (bioMérieux) according to the manufacturers' recommendations.

Table: 1 Antimicrobial susceptibility profile of *A. ursingii*

Antimicrobial	MIC ( $\mu\text{g/ml}$ )	Interpretation
Ampicillin/ Sulbactam	4	S*
Ticarcillin	$\leq 8$	S
Piperacillin	$\leq 4$	S
Piperacillin/ tazobactam	$\leq 4$	S
Ceftazidime	$\leq 1$	S
ceftriaxone	$\leq 1$	S
Cefoperazone/Sulbactam	$\leq 8$	S
Cefepime	$\leq 1$	S
Imipenem	$\leq 1$	S
Amikacin	$\leq 2$	S
Gentamicin	$\leq 1$	S
Tobramycin	$\leq 1$	S
Ciprofloxacin	$\leq 0.25$	S
Levofloxacin	$\leq 0.12$	S
Tetracycline	$\leq 1$	S
Tigecycline	$\leq 0.5$	S
Trimethoprim/ Sulfamethoxazole	$\leq 20$	S

\* S - susceptible

### Discussion

Although *A. ursingii* has been isolated solely from humans, its natural habitat is not known. We presume that this isolate colonized the patient's skin and that the intravascular catheterization could have facilitated its spread to the bloodstream. The accurate identification of the species of *Acinetobacter* is important for epidemiological and therapeutic reasons. *A. ursingii* has not been reported in infectious processes apart from its recent description as a new species<sup>6</sup>. Furthermore, *A. ursingii* strains may have the potential to spread to other patients, as demonstrated by molecular typing<sup>7</sup>.

Bacteremia caused by this organism has been reported from few countries like France<sup>8</sup> and from United Kingdom<sup>9</sup>. Studies show that *A. ursingii* can inhabit the hospital environment<sup>10</sup>. For this reason, clinical microbiologists must be aware of the

opportunistic pathogenicity of this newly described species, which deserves further studies to determine its prevalence in humans. Although identification of these rare isolates by the routine phenotypic methods used in the clinical setting can be difficult, precise assessment of the genus *Acinetobacter* at the strain level is necessary to evaluate the clinical significance of each isolate, and to determine the source and route of transmission, for the purpose of preventing nosocomial infections.

Although the possible source and route of transmission of the infection in our patient was not clear, previous reports and our identification of this organism support the view that *A. ursingii* is a clinically important causative pathogen of blood stream infections and we should be aware of its potential resistance to antimicrobials.

### Conclusion

The reliability of phenotypic tests for the identification of *Acinetobacter* species is inadequate, especially for species other than *Acinetobacter baumannii* complex. Newly described species, such as *Acinetobacter ursingii* is frequently misidentified due to inadequacies of conventional biochemical testing.

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## ✪ CASE REPORT

# Management of a Hemi-Mandibulectomy defect with a definitive Guiding Flange Prosthesis

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### Abstract

Acquired defects of the orofacial structures must be analyzed as to the specific cause and the consequent objectives of rehabilitation. Resection of oral tumours of the tongue, floor of the mouth and mandible results in functional disability and cosmetic disfigurement which presents a major challenge to the rehabilitation team and to the maxillofacial prosthodontist. A guiding flange guides the resected mandible into correct position. Guiding Flange made of acrylic polymers which lacks the principles of Removable Partial Denture design may affect the longevity of the remaining teeth. A guiding flange attached to a cast partial denture was fabricated to be used as a long term prosthesis restoring reasonable function and appearance.

**Key words:** Hemi-mandibulectomy, Guiding flange, Elastomeric impression

### Introduction

The treatment of malignant tumours of the oral cavity often requires the resection of structures essential to the functions of mastication, deglutition and speech. Mandibular discontinuity defects present a major challenge to the rehabilitation team and to maxillofacial prosthodontist. Patients have functioned with resected mandible for many years using their proprioceptive feedback on the non-resected side to compensate for deviation towards resected side<sup>1</sup>. However absence of the muscles of mastication on the surgical side results in a significant rotation of the mandible upon closure.

When viewed from the frontal plane, the mandibular teeth on the nonsurgical side move away from the maxillary teeth towards the surgical side. (Fig. 1) Thereby occlusal contact between maxillary and mandibular teeth is prevented, compromising mastication, speech and appearance. The amount of deviation depends on the extent of resection, method of closure, nerve innervations and presence of remaining teeth<sup>2</sup>. A guiding flange may be employed to guide the mandible into its correct position with the maxilla. Rehabilitation would be made much easier and effective if a guiding flange was constructed immediately due to the proprioceptive receptors present on remaining teeth<sup>1</sup>.

### Clinical presentation

A 66 year old male patient was referred to Dept. of Prosthodontics, Pushpagiri College of Dental Sciences, Tiruvalla for prosthetic rehabilitation of a hemimandibulectomy defect. His chief complaints were difficulty in sleeping, eating and speech due to deviation of mandible to left side. Intraoral examination showed partial glossectomy and mandibular defect extending from midline to left condyle, which is classified as Class 3 according to Cantor and Curtis<sup>2</sup>, with a plastic surgical reconstruction of floor of the mouth and defect. All remaining teeth were present on maxillary and mandibular arches with generalized periodontitis and associated severe deviation towards surgical side (Fig. 1).



Fig.1: Deviation of mandible to the surgical site

### History

Patient had an ulcer of the tongue of four months duration opposing mandibular left second premolar and first molar teeth with sharp lingual cusps. The ulcer (4x4 cm)

was an ulcero-infiltrative lesion about one centimetre from the tip of the tongue, with minimal extension to the floor of the mouth. Further investigations suggested well differentiated squamous cell carcinoma of tongue. The surgery involved a wide excision of the tongue along with left segmental mandibulectomy and vein preserving neck dissection. Reconstruction of the floor of the mouth was done by Pectoralis Major Myocutaneous Flap (PMMC).

Physiotherapy like maximal opening and closing of the mandible (patient to guide the mandible while closing) was suggested to loosen scar contracture and reduce trismus. The patient was recalled after one month during which the amount of mandibular deviation to the surgical side had reduced mildly, and the main concern remained difficulty in sleeping due to deviation of the mandible to the surgical side.

An acrylic based guiding flange on the mandibular arch was planned as an *interim prosthesis*. Following a full oral prophylaxis, alginate impression was made and a bite registration made with wax by manually guiding the mandible to centric occlusion. Maxillary and mandibular casts were articulated with the bite registration record. Prosthesis was fabricated on the non-surgical side with a lingual supporting plate, and two modified Adams clasps used as retentive elements attached to the lingual plate. Each Adams clasp had a projection extending from the buccal side going in a superior direction to buccal sulcus of maxilla, which was then reinforced with acrylic resin (Fig. 2). Teeth were not replaced in the interim prosthesis. The prosthesis was finished and inserted in the patient's mouth. He was asked to wear it during day and night (Fig. 3) and was evaluated every week.



Fig.2: Modified Adams clasps with projection extending to buccal sulcus



Fig.3: Interim acrylic guiding flange guiding mandible into centric occlusion. (Arrow - direction of guiding)

Since this design lacked the principles of stress distribution, the remaining mandibular teeth started showing periodontal problems. A definitive type guiding flange on a cast partial denture with acrylic teeth on the surgical side was planned. Maxillary and mandibular Impressions were made of alginate, and diagnostic casts made, surveyed using dental surveyor, and articulated as during acrylic guiding flange fabrication. All components of the cast partial denture were designed, mouth preparation done, and rest seats prepared on the abutment teeth. Final impression was made with elastomeric impression material and master cast poured.

A lingual plate major connector, with maximum number of direct retainers for retention, was fabricated in wax. Extending from the shoulder portion of the direct retainers, the guiding flange projection unto the maxillary sulcus was waxed (Fig. 4). The length was planned according to the level of mouth opening and was done in three steps.

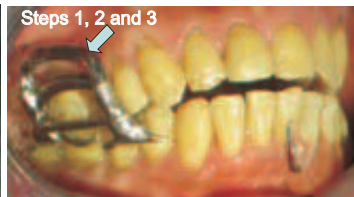


Fig. 4: Metal framework with teeth set in wax for trial. Teeth not set to posterior-most extent.

Fig. 5: Finished Guiding Flange prosthesis inside mouth (Three steps of the guiding flange are shown)



Fig. 6: Guiding Flange in action. (Arrow shows the direction of guiding)



Fig. 7: Occlusal contact on the resected side while Guiding Flange is in place

Once the wax up was finished it was cast in metal. Then the metal frame work try in was done in the patient's mouth (Fig. 5). Jaw relation was recorded again in the manually guided centric occlusion. Final try in was completed with the teeth on surgical side till second premolar. The finished prosthesis was delivered to the patient and instructions given (Fig. 6 and Fig. 7).

After six months of continuous wear the patient was able to bring his mandible to a sliding centric occlusion without the guiding flange, as shown in Fig. 8.

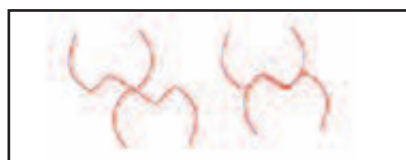


Fig. 8: Sliding Centric Occlusion: Mandibular cusp slides from initial point of contact to centric occlusion.

## Discussion

Aramany and Myers found that the use of an immediate intermaxillary fixation after segmental resection of the mandible during the first six post operative weeks reduces the degree of mandibular deviation<sup>3</sup>. The mandibular guidance therapy begins when the immediate post surgical sequelae have subsided usually at about two weeks after surgery. An exercise program, with maximum opening of the mouth, and during closure manipulating the mandible away from the surgical side by grasping the chin, will loosen scar contracture, reduce trismus and improve maxilla-mandibular relationship<sup>2</sup>.

Robinson *et al.*, stated that the fabrication of a provisional guide plane facilitates the fabrication of a definite restoration and a cast restoration is appropriate such that the mandible can be manipulated into an acceptable maxilla-mandibular relationship<sup>4</sup>. Sahu S K stated that acrylic guide flange prosthesis was a simple and cost effective method of managing the mandibular deviation<sup>5</sup>. But it may not be used for a longer duration. It is prudent not to replace teeth in the transitional acrylic guiding flange to reduce undesirable forces on the abutment teeth used for the prosthesis retention (Fig. 3).

A common feature among all removable resection prosthesis is that all framework designs should be dictated by basic prosthodontic principles of design. These include broad stress distribution, cross-arch stabilization using a rigid major connector, stabilization and retention of components at locations within the arch to best minimize dislodging functional forces, and replacement tooth positions that optimize prosthesis stability and functional needs<sup>6</sup>. Modifications to these principles are determined on an individual basis and are greatly influenced by unique residual tissue characteristics and mandibular movement dynamics<sup>6</sup>. Mandibular guiding flange attached by two precision attachments was used quite effectively both for physiotherapy and eating by Prencipe MA *et al.*<sup>7</sup> Teeth were not set to the posterior most extent, in this case, to decrease the cantilever effect on the remaining natural teeth.

Difficulty in sleeping due to the mandibular deviation was this patient's chief complaint. He was asked to wear the prosthesis during day and night as the sleep was affected due to deviation of mandible. After six months of continuous wear the patient was able to bring the mandible to a sliding centric occlusion without the guiding flange (Fig. 8). Frequent reviews are mandatory and it needs to be seen whether further continuous wear will help the patient improve further in achieving proper centric occlusion without guiding flange and also whether he will be able to retain that ability once the guiding flange is discontinued. If so, guiding flange may be considered as a definitive treatment modality in correcting mandibular deviation subsequent to mandibular resection. The guiding flange extension was done in three steps and as the patient coordination in bringing his mandible to centric

occlusion increases, the length of the flange will be reduced by cutting out each step and once all the steps are removed, the end to mandibular guiding therapy has reached.

The success of hemimandibulectomy rehabilitation depends on the nature of surgical defect, patient's cooperation and prosthetic management with early physiotherapy program. The presence of teeth in both the arches creates a better proprioceptive sense and the prosthesis which re-educates the mandibular muscles to re-establish an acceptable occlusal relationship will control the opening and closing of the mandibular movements adequately and repeatedly<sup>1</sup>. This clinical report illustrates that earlier the treatment begins with exercise program and with an interim guiding flange, a more definitive restoration on a cast partial denture can be fabricated.

## Conclusion

Complete rehabilitation of a hemimandibulectomy case is a challenging task, especially due to the lack of bony foundation on the surgical side and difficulty in speech due to partial glossectomy. Application of the 'Principles of Designing' in guiding flange construction will achieve long term successful results.

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## ✦ CASE REPORT

# Isolated splenic lesion in a case of *Herpes simplex* encephalitis

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## Introduction

Herpes simplex encephalitis (HSE) is a serious consequence of Herpes simplex virus infection of the central nervous system<sup>1</sup>. In children older than three months and adults, HSE is usually localized to the temporal lobe and is commonly caused by type1 HSV<sup>2</sup>. In the neonates there is generalized brain involvement and the common etiological agent, HSV type 2 is commonly acquired at the time of delivery. Early diagnosis and effective antiviral treatment significantly improve outcomes. We present a case of acute HSE with multiorgan dysfunction in a patient, admitted to a tertiary care centre in Central Travancore.

## Clinical presentation

A 37 year old woman was admitted to the Medical Intensive Care Unit of Pushpagiri Medical College and Hospital, Tiruvalla on 3/11/2011, after she had presented with a history of lethargy, generalized weakness, intermittent fever with chills, loss of appetite, cough with expectoration accompanied with state of confusion (intermittent for 2-3 days), and irritability. The patient also gave a history of irrelevant speech, unsteady gait, hallucinations and an episode of urinary incontinence.

Virology biochemical and haematological investigations were done. Peripheral smear showed toxic granules in neutrophil series. The level of ALT was elevated (SGPT-126 IU/L) and the raised bilirubin (total - 0.6mg/dl, direct - 0.23mg/dl) was an indication of compromised hepatic functions. CSF analysis showed raised RBC count, elevated protein level (166gm/dl), and normal glucose (68mg/dl), which suggested a HSV encephalitis. Magnetic Resonance Imaging (MRI) showed focal solitary lesion in splenium of corpus callosum; which exhibits T2W/FLAIR hyperintensity

with diffusion restriction. The MRI features in conjunction with clinical findings suggested acute febrile encephalopathy (AFE).

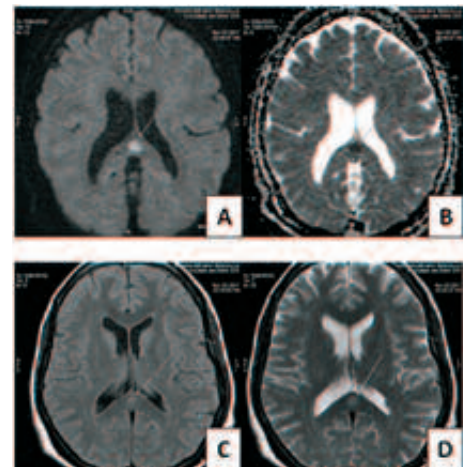


Fig. 1: A) Diffusion weighted B) ADC (apparent diffusion coefficient map C) FLAIR and D) T2W Focal solitary area of diffusion restriction in splenium of corpus callosum with low ADC values. It exhibits T2W and FLAIR hyperintensity.

Cerebrospinal fluid (CSF) sample was received at the Pushpagiri Center for Virology for anti-HSV IgM antibodies. An immunocapture (Diasia Diagnostica Senese Italy) enzyme linked immunosorbent assay (ELISA) was done, having diagnostic sensitivity 95.6% and specificity 97.8%. The test is based on the capture of anti-HSV antibodies by monoclonal antibodies (anti-human antibodies) bound to the solid phase (microtiter wells) and subsequent binding to an antigen conjugated to peroxidase substrate. The HSV IgM titre was high, suggesting a case of HSV encephalitis. The patient was treated with methyl prednisolone (1 g/day x 3 days) and acyclovir (1 injection 800 gm/ 8 hr). On the third day, 05/11/11, her condition improved and was shifted to the ward with recovering neurological status and blood picture. The final diagnosis was acute meningoencephalitis, acute disseminate encephalomyelitis with acute renal failure and anicteric hepatitis. A follow

up of MRI was done on 08/11/2011 Fig.2: (A, B) which showed a complete resolution of isolated splenial hyperintensity in TRW/FLAIR images as well as restriction in diffusion weighted images. The patient was discharged on 08/11/2011, and asked to follow up after ten days.

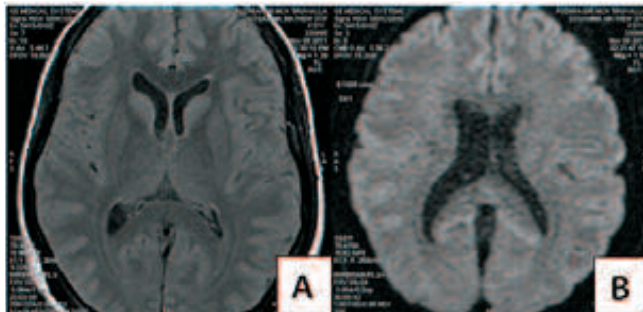


Fig. 2: A) FLAIR B) Diffusion weighted  
Near-total resolution of splenial hyperintensity on FLAIR sequence and diffusion restriction not seen.

## Discussion

HSE, a treatable condition has an estimated incidence of about a case per million per year worldwide. Encephalitis is a common sporadic viral disease of the brain and if left untreated can result in death<sup>3</sup>. Mortality among untreated patients exceeds 70%. Although antiviral treatment has reduced mortality due to herpetic encephalitis, majority of survivors present with residual neurological deficits or neuropsychiatric symptoms.

Polymerase chain reaction (PCR) for the detection of herpes DNA is the gold standard for laboratory diagnosis of HSE. It has a sensitivity of 95% and a specificity of 98% However, due to financial feasibility most laboratories in India use ELISA systems<sup>4</sup>. Usually HSV IgM antibody production begins several days after a primary (initial) HSV infection and lasts for several weeks.

## Conclusion

Early diagnosis is the key to improve HSE outcomes. The incidence of HSE in Kerala remains unknown due to lack of appropriate diagnostic facilities. Neurodiagnostic evaluation is also an important tool in the determination of herpetic encephalitis, since other treatable disease mimic HSV encephalitis. Early diagnosis based on clinical and biological data are essential to distinguish them from the other CNS diseases thereby facilitating early and appropriate therapy<sup>5,6,7</sup>. This case report is presented to increase awareness among clinicians to this potentially fatal but treatable condition.

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## ✦ TECHNICAL REPORT

### Nanomedicine in Radiation protection

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#### Nanomedicine

Nanomedicine denotes the application of nanotechnology in medicine. There are basically two concepts defining nanomedicine. One which defines it as a technology that uses molecular tools and knowledge of the human body for medical diagnosis and treatment<sup>1</sup> and the other explains it as the use of physical effects occurring in nanoscale objects that exist at the interface between the molecular and macroscopic world in which quantum mechanics is considered supreme<sup>2</sup>. The first scientist to voice his outlook about the many applications of nanotechnology in medicine was the late Nobel physicist Richard P. Feynman, who envisaged the use of nanoscale robots to perform surgery<sup>3</sup>. Since most of the natural processes take place in the nanometre scale regime, a confluence of both nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine<sup>4</sup>. Nanosized organic and inorganic particles are amenable to biological functionalization and are currently employed as a tool to explore certain avenues of medicine in several ways including imaging<sup>5</sup>, sensing<sup>6</sup>, targeted drug delivery<sup>7</sup>, gene delivery systems<sup>8</sup>, artificial implants<sup>9</sup>, etc.

#### Radiation and the need for Radioprotectors

Ionizing radiation or particle radiation produce ions during passage through the matter and while interacting with living cells, it causes a variety of changes depending on exposed and absorbed dose, duration of exposure and the interval after exposure, and susceptibility of tissues<sup>10</sup>. Ionizing radiations cause immediate chemical alterations in biological tissues, damaging DNA and membranes of cells.

Ionizing radiations are

encountered in different spheres of human life. Human beings are constantly getting exposed to natural background radiation which accounts for approximately 80 percent of human exposure, mostly from indoor radon, followed by radiation from space and Earth's crust while man-made sources of radiation account for the rest 20 percent exposure<sup>11</sup>.

There are several beneficial purposes for radiation or radioactive materials, such as radiation being used in medicine as diagnostic X-rays, radiopharmaceuticals for diagnosis and treatment, radiation therapy for cancers, for sterilization of medical equipment and food products, etc. in industry, radioactive elements used in locating oil and minerals in the earth, processing semiconductor chips for computers, in nuclear power reactors for energy generation, etc., and also in determining the age of materials through radiocarbon dating. This widespread use of radiation in diagnosis, therapy, industry and energy sector, and inadvertent exposure during air and space travel, nuclear accidents and nuclear terror attacks, etc., has increased the exposure of living beings to radiation and radiation induced damages<sup>12</sup>. Recent reports indicate the possibility of cancer induction due to exposure of humans to radiation during therapeutic and diagnostic X rays<sup>13</sup> and CT scans<sup>14</sup>. For beneficial use of radiation, the risks of radiation exposures are to be carefully restricted. Thus, the role of radioprotection is very important in all clinical situations of radiation exposure<sup>12</sup>.

Radioprotectors can be defined as *any medicinal agent or device applied prior to, or during radiation exposure that actively prevents or limits injury, whether that injury be at the molecular, cellular, tissue or organ system level*<sup>15</sup>. Radioprotectors have the ability to

reduce the biological effects of ionizing radiation on normal tissues, including lethality, mutagenicity and carcinogenicity<sup>16,17</sup>, and have applications in clinical oncology, space travel, radiation site clean-up, radiological terrorism and military scenarios<sup>18</sup>.

Among the many radioprotective compounds that have been developed over the years, a majority was designed to reduce the levels of radiation-induced free radicals within the cell. Thiol compounds like Amifostine (WR-2721), which are efficient free radical scavengers, have been studied extensively. Amifostine is the only Food and Drug Administration (FDA) approved radioprotector in use, and is currently employed in the clinic for reducing the incidence and severity of xerostomia in head and neck cancer patients undergoing radiation therapy. Unfortunately, application of this drug has so far been less than hoped for, owing to toxicity often being evidenced at optimal radioprotective doses<sup>19,20</sup>.

In view of these scenarios, a radioprotector for therapeutic (protects tissues when administered after radiation exposure) or preventive (protects tissues when administered prior to radiation exposure) application, capable of attenuating the deleterious effects of radiation on human normal tissue needs to be developed for use in various planned or unplanned radiation exposure situations, especially for cancer patients undergoing radiotherapy. Thus, the search to identify or develop less toxic or non-toxic agents to counter the effects of ionizing radiation remains an area of intense focus. Recently nanoparticles are gaining interest in the field of radioprotection as cerium oxide nanoparticles, yttrium oxide nanoparticles, carbon nanoparticles, etc were found to possess antioxidant properties and several works have shown the ability of these nanoparticles to offer protection against radiation damages.

### Nanoparticles in radiation protection

Nanoparticles constitute a new generation of free radical scavengers. Carbon nanoparticles, CeO<sub>2</sub> nanoparticles, Yttrium oxide nanoparticles, silver nanoparticles, gold nanoparticles, platinum nanoparticles, poly (lactic-co-glycolic) acid (PLGA) nanoparticles, etc., function as potential biological free-radical scavengers or antioxidants and these nanoparticles may be used to scavenge ROS responsible for radiation-induced cell damage.

An allotropic variation of carbon known as *Fullerenes*<sup>21</sup> consisting of 60 carbon atoms connected by sp<sup>2</sup>-bonds, which has a uncharacteristic delocalization of  $\delta$ -electrons enables it to react with oxygen free radicals and can be used as tools for regulation of free radical processes and for reducing the severity of oxidative stress in biological systems<sup>22,23</sup>. *Fullerenes* and its derivatives have attracted considerable attention in biologic applications due to their high reactivity toward radicals<sup>24</sup>, especially reactive oxygen species (ROS) such as superoxide<sup>25</sup>, hydroxyl radical<sup>26,27,28</sup>, peroxy radicals<sup>29,30</sup>, and nitric oxide<sup>30,31</sup>.

Water soluble *fullerenes* have shown promising results in mitigating neuro-degenerative diseases related to oxidative stress<sup>32,33,34,35</sup> in addition to its promising cardioprotective<sup>36</sup>, hepatoprotective<sup>37</sup>, nephroprotective and radioprotective<sup>38</sup> ability, because of its virtue as an antioxidant<sup>39</sup>.

Various studies have revealed the prospective biological application of the CeO<sub>2</sub> nanoparticles as an antioxidant and radioprotector<sup>40</sup>. The CeO<sub>2</sub> nanoparticles provide selective protection to normal cells during radiotherapy<sup>41</sup>. These nanoparticles have oxygen vacancies due to the dual oxidation state (Ce<sup>4+</sup> to Ce<sup>3+</sup>) which is responsible for the interesting redox chemistry exhibited by the CeO<sub>2</sub> nanoparticles and makes them attractive for the radical scavenging properties<sup>42</sup>.

Yttrium oxide nanoparticles are also able to rescue cells from oxidative stress-induced cell death. There are three alternative explanations for the observation that the cerium oxide and yttrium oxide particles protect from oxidative stress. They may act as direct antioxidants, they may block ROS production in cells by inhibiting a step in the programmed cell death pathway, or they may directly cause a low level of ROS production that rapidly induces a ROS defense system. Nanoparticles of aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) also behave as potential free radical scavenger<sup>43</sup>.

Another interesting candidate which shows radioprotecting property because of its excellent free radical scavenging, antimicrobial and anti-inflammatory activities is silver nanoparticles<sup>44,45</sup>.

The results of several studies suggest that nanocrystalline silver play a role in altering or compressing the inflammatory events in wounds and facilitating the early phases of wound healing<sup>46</sup>. The flexibility of silver nanoparticle have made it possible to bind antioxidant molecules its surface<sup>47,48</sup>, thereby making the conjugate much more radioprotective than its individual components<sup>49,50</sup>. Silver nanoparticle complexes of PASAG, Glyceryrrhic acid, sesamol and lipoic acid have been found to be effective in protecting biological systems under *in vitro*, *ex vivo* and *in vivo* conditions<sup>47-50</sup>.

It has been shown that Gold nanoparticles could act as an anti-oxidative agent, by inhibiting the formation of ROS, scavenging free radicals; thus increasing the anti-oxidant defense enzymes<sup>51</sup>. Also it has been shown that functionalization of the vitamin E-derived antioxidant with gold nanoparticles could efficiently enhance the antioxidant activity<sup>52</sup>. Preliminary investigation on gold nanoparticles conjugated with antioxidant compounds has presented promising results as a worthy radioprotector<sup>53</sup>.

Platinum nanoparticle has been shown to scavenge of superoxide anion and hydrogen peroxide thereby inhibiting lipid peroxidation under *in vitro* conditions<sup>54,55</sup> and enhancement of life span of *Caenorhabditis elegans*<sup>56</sup>.

Functional surfactants with antioxidant properties can be used to form nanostructures of inherent antioxidant activity<sup>57</sup>. Poly (lactic-co-glycolic) acid (PLGA) nanoparticles with entrapped alpha-tocopherol and ascorbic acid showed a promising design for the effective delivery of antioxidants necessary to combat oxidative stress<sup>58</sup>. Recently it has been shown that Melanin coated silica nanoparticles can be used for protection of bone marrow during radiation therapy of cancer<sup>59</sup>.

The role of reactive oxygen species in ionizing radiation injury and the potential of antioxidants to reduce these deleterious effects are well established. As mentioned, ionizing radiation generates free radicals that in turn lead to DNA damage. Most of the radiation induced biological damage arises from the interaction of the radiation-induced free radicals with the biomolecules. The chemicals that can scavenge free radicals may also reduce the occurrence of the DNA strand breaks. Thus agents that can prevent the formation of free radicals or destroy free radicals by reacting with them, thereby inhibiting their reaction with biomolecules, can function as radio-protectors. Nanoparticles of carbon, silver, gold, cerium oxide, etc are reported to possess radiation protection and since the other nanoparticles discussed above are shown to possess free radical scavenging activities, they must also be screened for their possible radiation protection efficiency.

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## ✦ TECHNICAL REPORT

# Edible Vaccines: Strides in Immunization

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### Abstract

Vaccination is one of the greatest achievements of medical science and it has revolutionized the management and eradication of infectious diseases in humans and animals. Conventional vaccines bear several disadvantages from the viewpoint of production, preservation and delivery. The turn of the twenty second century has witnessed an exponential phase in the evolution of trailblazing approaches in vaccine development, including successful establishment of edible vaccines that utilize transgenic plants. This has inspired biotechnologist to explore the chances of food crops as prophylactic agents serving as vehicles for both production and delivery of vaccines.

**Key words:** Edible vaccines, Transgenic plants, Prophylactic agents

### Introduction

Edible vaccines are emerging innovations in medical science and plant biology for the efficacious and affordable pharmaceuticals<sup>1,2</sup>. Here genes encoding immunogenic proteins of pathogens are transferred into a plant and these transgenic plants then produce the desired immunogenic protein subunit vaccines.

Plants have provided humans with useful molecules for many centuries, but only in the recent past it has become possible to use plants for the production of specific heterologous proteins<sup>3</sup>. The first plant made protein was human growth hormone, in transgenic tobacco in 1986<sup>4</sup>. The structural authenticity of plant-derived recombinant proteins was confirmed in 1992, when plants were used for the first time to produce an experimental vaccine: the hepatitis B virus (HBV) surface antigen<sup>5</sup>.

### Production of edible vaccine

Plant molecular farming deals with the large-scale production of recombinant proteins by using crop plants as expression hosts. Plants can be transformed via., two different multistep sophisticated strategies: 1) stable transformation, 2) transient transformation<sup>6</sup>.

*Stable transformation* refers to the insertion of foreign DNA into the

genome of the plant nucleus or plastids (eg. chloroplasts), resulting in the transfer of the genetic information to the subsequent generations of transformed plants. Two approaches are applied here a) the mostly used *Agrobacterium tumefaciens* - mediated gene transfer into the nuclear genome and b) biolistic process (particle bombardment<sup>6,7</sup>). The gene containing DNA coated metal (gold) particles are fired at the plant cells using gene gun. Those plant cells that take up the DNA are then allowed to grow in new plants, and are cloned to produce large number of genetically identical crop. This method is quite attractive because DNA can be delivered into cells of the plant which makes gene transfer independent of regeneration ability of the species. But the chief limitation is the need for costly device particle gun. The advantage of using transgenic plastids is the high-level expression of recombinant protein, compared with stable transformation of the nucleus. Unlike nuclear-encoded recombinant proteins, the recombinant proteins emanating from the chloroplast genome will not be glycosylated<sup>8</sup>. However stable transformation is time consuming takes several months until positive transformants are obtained<sup>6</sup>.

*Transient transformation*, in which foreign DNA never enters the genome of the plant cell, hence cannot

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be inherited by the offspring. Transient transformation is mainly based on genetically modified plant viral vectors (e.g. TMV)/ agroinfiltration (Agrobacterium-mediated delivery). During agroinfiltration, leaf discs are inoculated with bacterial culture which results in transient expression of non-integrated transfer DNA (T-DNA) within the plant cells. Transient transformation only requires a few days until recombinant protein can be detected within the plant tissue<sup>6,7,9</sup>, however, this procedure needs to be frequently repeated. The scheme for the construction of an edible vaccine is pre-arranged in Fig. 1<sup>10</sup>.

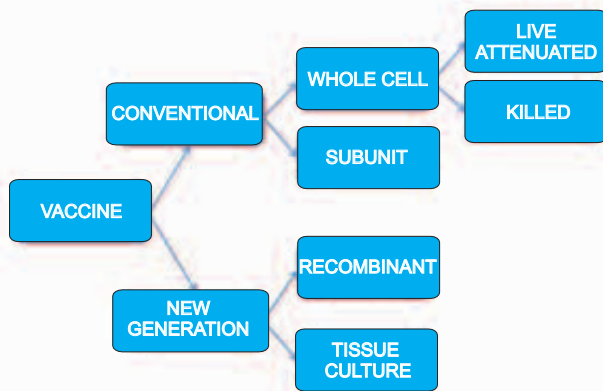


Fig. 1: Schematic representation of the evolution of edible vaccines

### Transgenic plants as vaccine production units

The possibility of producing recombinant proteins in plants has opened a new field for designing innovative vaccine models. Mainly two approaches are utilized in the development of plant-based vaccines: i) plants as factories (biofactories) for potential vaccine antigens, or ii) plants as both production and delivery systems (edible vaccines).

In the first approach the recombinant protein is purified from the host plant and subsequently used for injective immunization. This has benefits compared with expression of recombinant protein in bacterial/ yeast cells. The bacterial expression system, like *Escherichia coli*, brings a pathogenic risk factor when using protein purified from such cells for use in humans while this is absent in biofactories. Moreover, the bacterial expression system lacks the ability to perform post-translational modifications of the recombinant protein. Furthermore, glycosylation of proteins produced in yeast cells differ greatly from that of proteins expressed in mammalian cells. However, the glycosylation of recombinant proteins produced in biofactories gives patterns similar to those performed by a mammalian cell<sup>8</sup>. When the production of edible vaccines is considered, it is desirable to select a plant whose products are consumed raw to avoid degradation during cooking like tomato, banana, cucumbers etc. A list of edible vaccines and their expression hosts are showed in Table 1<sup>11,12,13,14,15,16</sup>.

Table 1. List of edible vaccines

Sl. No.	Name of the vaccine	Vector plant	Disease
1	HIV	Tobacco, tomato	AIDS
2	Rabies	Tobacco, tomato, potato	Rabies
3	Hepatitis B	Potato, tobacco	Hepatitis B
4	Vibrio cholera	Potato	Cholera
5	Enterotoxigenic E.coli	Tobacco, potato, maize	Traveller's Diarrhoea

There are several reports on development of transgenic plants that express antigenic proteins of human and animal pathogens<sup>17,18</sup>. The edible vaccine approach is of interest when constructing a vaccine model against pathogens that have the mucosal tissues as their primary site of infection (e.g. HIV-1). Via an oral administration route, the mucosal surface will be exposed to the vaccine antigen and the immune cells in these tissues can be primed. The mucosal immune system is known as the first line of defense against many pathogens<sup>19</sup>. Owing to the possibility of using plants as both production and delivery system for vaccine antigens, the added advantages are i) low production costs, ii) no maintained cold chain is necessary, iii) needle-free vaccination, iv) bioencapsulation of the antigen, v) proper glycosylation of proteins, vi) local production in resource-poor settings, vii) ease for frequent boosts and viii) induction of mucosal immune responses<sup>2</sup>.

Different plants have been tried as gene expression systems. Hepatitis B surface antigen (HBsAg) expressed in tobacco showed buoyant density and antigenicity similar to human- and yeast-derived HBsAg, suggesting a typical characteristics plant protein folding. Mice immunization with recombinant HBsAg (rHBsAg) demonstrated stimulation of T-cell proliferation. B- and T-cell epitopes of HBsAg were preserved when the antigen was expressed in transgenic tobacco<sup>20</sup>. Constructs carrying the gene encoding *Escherichia coli* heat-labile enterotoxin (LT-B) were introduced into tobacco and potato plants<sup>21</sup>.

LT-B is produced by enterotoxigenic *E. coli* (ETEC) and also immunogenically interacts with the cholera toxin of *Vibrio cholerae*. Thus, LT-B is a candidate vaccine against both ETEC and cholera. The toxin protein was expressed in potato microtubers and at much higher levels in plants containing the fusion protein. The mice immunized by feeding with LT-B expressing potato tubers developed serum immunoglobulin G (IgG) and mucosal immunoglobulin A (IgA). The cholera toxin B subunit (CTB) against *Vibrio cholerae* expressed in potato tubers showed similar responses in mice. The potato was also used for the production and delivery of the human insulin antigen<sup>17</sup> and human experiments showed enhanced serum IgG- and mucosal IgA-specific antibodies<sup>22</sup>. Norwalk virus capsid protein was expressed in potato tubers and found to be immunogenic in mice. Feeding this potato showed a modest serum level antibody increase in 19/20 healthy human volunteers<sup>15,16</sup>. The first multi-component vaccine in potato against the three enteric pathogens – *V.cholerae*, rotavirus and ETEC was tried In another study and mice fed these tubers

produced both serum and mucosal antibodies against these pathogens<sup>23</sup>. Soyabean was used for production of the glycoprotein B antibody of the herpes simplex virus 2 (HSV-2)<sup>24</sup> and corn was used for production of an LT-B subunit vaccine. Stoger *et al.*<sup>25</sup> expressed the single-chain Fv (ScFv) antibody of the human carcino embryonic antigen (CEA), a marker to diagnose tumor onset, in both rice and wheat grains.

### Edible vaccines: a safer alternative

Plants provide a very attractive alternative that can be scaled up to a high production system for recombinant proteins<sup>18,19</sup>. Recent advances in the understanding of transgene expression in plants allowed the development of using edible plants for delivery of antigens for immunization<sup>20,21,22</sup>. Mostly edible vaccines stimulate of mucosal antibody production than injectable vaccines. Eating fruit or drinking juice of the transgenic plant should induce the mucosal immune system. Influence of the mucosal system is expected to lead serum system to produce pathogens specific immunoglobulins. Also producing and delivering vaccine in juice can curtail the costs of hypodermic needles, thereby preventing hazards from reuse of needles and, impoverished countries. There is also concern that plant material that contains recombinant proteins could inadvertently enter the food chain. Although it is apparent that pharmaceutical crops do not suffer the same acceptability problems as genetically modified food crops, the risk assessment and environmental impact studies must be carried out to the same level, to ensure the highest standards of responsibility and regulatory compliance.

### Conclusion

Conventional vaccines are associated with a number of disadvantages forcing the medical community to search for alternative strategies. The use of foods as vehicles for production and delivery of vaccines is an intriguing field of Biotechnology and should provide incentives for both human health and the agricultural sector in the near future. Plant-based vaccines will provide a new use for food crops as these crops can then be grown for the sole purpose of producing and/or delivering biopharmaceutical or medicinal products. This lead to a future of safer and more effective immunization. They would overcome some of the difficulties associated with traditional vaccines, like production, distribution and delivery and they can be incorporated into the immunisation plans. But still there is lack of production and investment in this new technology but it will be likely conquered to make plant derived vaccine more efficient and dependable.

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## ✦ TECHNICAL REPORT

# Using financial equations in Microsoft Excel<sup>®</sup> for demographic projections

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The population of India as per the provisional figures of Census 2011 staggering 1210.19 million. The next question in the mind of most of us is “when will we touch the two billion mark?” Since the mathematical calculations are an anathema to a majority of the gentry, it seems very pertinent to call for the use of computers and general purpose software for answering such a million rupee question. As we are all aware, population grows exponentially like most other indices governing our lives such as inflation, interest and income. General purpose softwares incorporate financial features into them which we can tweak to give us the results for the growth of population of the country. But, who among us are using a programme such as Microsoft Excel<sup>®</sup> spreadsheet, even for money matters? The majority of excel users hardly ever encounter terms such as present value (PV), future value (FV), rate of growth (RATE) and such. However, the good news is that demographic calculations are quite similar to financial ones such that tweaking some of those built-in functions could easily solve the problems such as “what rate of growth would get us to two billion population in 20 years?”, as easy as they would those on the lines of, “how many years of systematic investment would make my deposit to a million rupees at the current interest rate?”

### Growth rate of population

From apocalyptic exponential models of calculation to more digestible arithmetic progression models of population growth, several methods have been proposed for planning and allocation purposes on the governance side. But just like financial projections, the warning note at the bottom would say that the past trends are not highly dependable as a number of variables determining growth can change and take the human civilization to

new highs or plateaus. Natural increase in the population happens because of net additions in the form of births subtracted by number of deaths just as interest money will increase a bank deposit and inflation would reduce its worth (Table 6). A zero population growth would mean equal number of deaths and births. However natural increase is not the only phenomenon which adds to the growth of population. Population movements in the form of immigration and emigration would also result in changes in population size even if the birth and death rates are balanced. It is interesting to note that even a ‘replacement’ level fertility (wherein a set of parents produce exactly one girl and a boy) would still cause the population to increase because of overlapping life-periods between generations.

### Method of natural increase

Let us implement the method of natural increase in an excel spreadsheet before moving on to more complex functions in the programme. The following figure illustrates the use of simple calculations possible in the spreadsheet for additions and subtractions etc. The example cited in Table 1 is that of a town which had 134,000 (mid-year) population in 1990. The vital processes governing growth are also shown in the figure. The spreadsheet can be used to add and subtract based on relative cell locations (such as A2 which refers to the second row in the first column). An addition between two cells would be achieved by a simple equation such as “=A2+B2” in another cell. The net increase in population in a year is thus calculated by the inclusion of the notation “=B2-C2+D2-E2” which means that the births (B2) and immigrants (D2) are added whereas deaths (C2) and emigrants (E2) are subtracted. The row numbers change as the years progress down the columns.



Table 1: Natural Increase method as implemented by MS Excel spreadsheet

	A	B	C	D	E	F	G
1	Year	Births	Deaths	Immigrants	Emigrants	Growth	Comment
2	1990	3705	1164	8220	8433	=B2-C2+D2-E2	Ans = 2328
3	1991	3813	1114	9135	9895	=B3-C3+D3-E3	Ans = 1939
4	1992	3856	1108	11688	11606	=B4-C4+D4-E4	Ans = 2830
5	1993	4037	1127	8695	9542	=B5-C5+D5-E5	Ans = 2063

**Projected population**

Projection of population is realistically achieved from the table given above by finding the cumulative net increase from 1990 onwards till 1993. One may compute the yearly additions to the population in the above example as 2328, 1939, 2830 and 2063 respectively. Any carry forward of the estimate has to be done by other methods such as progression, in the absence of speculation. The future projection without real numbers of births, deaths and migration has to be achieved based on such mathematical models. The simplest would be arithmetic progression wherein average yearly increase is calculated and applied uniformly till the next census data becomes available. Typically, in India, we have decennial censuses which get us the count of population at intervals of ten years. The difference between two consecutive censuses will give us the decennial population growth. The average yearly growth is then given by  $1/10^{th}$  of decadal increase in population.

	A	B	C	D	E
	Year	Pop (m)	Growth	Annual Growth	Comment
1	1901	238.40			
2	1911	252.10	=B3-B2	=C3/10	1.37
3	1921	251.30	=B4-B3	=C4/10	-0.08
4	1931	279.00	=B5-B4	=C5/10	2.77
5	1941	318.70	=B6-B5	=C6/10	3.97
6	1951	361.10	=B7-B6	=C7/10	4.24
7	1961	439.80	=B8-B7	=C8/10	7.87
8	1971	548.20	=B9-B8	=C9/10	10.84
9	1981	683.30	=B10-B9	=C10/10	13.51
10	1991	843.90	=B11-B10	=C11/10	16.06
11	2001	1027.00	=B12-B11	=C12/10	18.31
12	2011	1210.20	=B13-B12	=C13/10	18.32

The expressions needed to get yearly growth between the censuses of India are given in Table 2. This increase can be added in yearly increments to the latest census data to get population estimates of subsequent years e.g. population in the year 2012 would be  $1210.2 + 18.32$  i.e., 1228.52. Accordingly, it would take another 43 years to make India two billion strong at this constant rate. This, we know to be not true because the population does not grow at a constant rate. Just like other facts in life such as interest rates and inflation rates, population also tends to grow at an exponential rate. We would, thus, stick to the commonest model of growth, which is a compounded interest rate which is

applied to our cumulative fixed deposit in banks. This is easily implementable using excel spreadsheets using built-in functions. This mode simulates the common geometric progression of population growth which is found to be a reasonable approximation from available examples in India and elsewhere.

**Arithmetic growth versus geometric progression**

A model which adds a fixed quantity to the starting value repeatedly would generate a linear growth pattern of the order,  $P_t = P_0(1+rt)$  shown as the straight line in Fig. 1. Population of India from 1901 AD, however, did not follow the arithmetic progression. Rather, the growth was exponential in nature with the velocity of growth increasing as the years progressed.

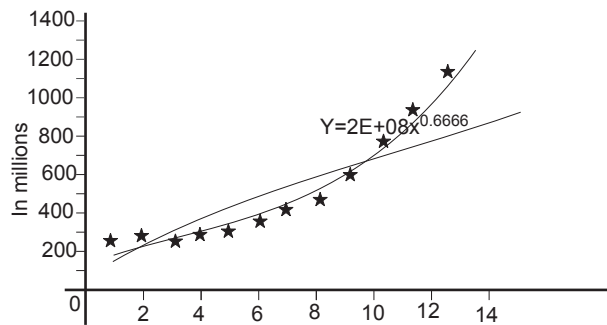


Fig. 1: Growth of Indian population: the arithmetic and geometric fit lines

This meant that simple arithmetic would not be useful in predicting the points or the future growth by simple and repetitive additions. The method to use would be geometric progression where the growth rate remains constant resulting in faster growth as time passes.

The equation here would be  $P_t = P_0(1+r)^t$ , which is the same equation used to compute compound interest rate in a bank deposit or to find inflationary increase of commodity prices. This calculation is easily possible in Microsoft Excel® and would be used here to find the growth in population of India at a given rate.

The application of function,  $FV(rate, nper, pv)$ , is demonstrated in table 3 below to project the population of census 2011 to a future census of 2021 which is 10 years away which will give a value of 1,425,381,042.28. The caveat is that there is no surety that the growth is going to be 1.65% per annum as in the last decade and could vary in the years to come.

Table 3: Use of FV function in MS Excel Spreadsheet

	A	B	C	D
1	Future	Census	Annual Rate of Growth	Comment
2	Year 2021	Census 2011	(in percentage)	Projection
3	=FV(C3,10,-B3)	1,210,200,000.00	1.65%	1,425,381,042.28

Finding the rate for growth is also possible from excel spreadsheets as shown in table 4. The growth of Indian population in a geometric fashion in the last 110 years was at a rate of 1.49% on an average.

Table 4: Calculating rate using MS Excel spreadsheet

	A	B
1	Year	Population
2	1901	238,400,000.00
3	1911	252,100,000.00
4	1921	251,300,000.00
5	1931	279,000,000.00
6	1941	318,700,000.00
7	1951	361,100,000.00
8	1961	439,800,000.00
9	1971	548,200,000.00
10	1981	683,300,000.00
11	1991	843,900,000.00
12	2001	1,027,000,000.00
13	2011	1,210,200,000.00
14	Rate	=Rate(110,-B2,B13)

**Doubling time**

Let us use the combination of these functions to find the doubling time required in various growth scenarios just as we would project our financial position adjusting with rates of interest and inflation. At the current rate of 1.65%, India would touch 2 billion mark by the year 2042 and would double by the year 2054 AD!

This projection is based on past performance and does not take into account the carrying capacity of the nation. The calculation is demonstrated as a function with the growth rates of the last decade and of the century (1.65% and 1.49%) in table 5. A general approximation of doubling time is possible by dividing 69.3 with the rate of growth in percentage. 'Goal-seek' feature of excel is useful for arriving at further complicated scenarios.

**Logistic Progression**

Growth of population in India appears scary at current levels. However, 2001-2011 was the first decade (with the exception of 1911-1921) which actually added lesser population compared to the previous decade. Hence, going by the slowing down of the population juggernaut of India, it appears the exponential growth may be on its final legs. If the growth flattens at this rate, the usual models of population growth would be out of place. This is akin to a situation where growth of our funds based on interest rates (as similar to birth rates) is cancelled out by higher and higher inflation rates (as similar to death rates).

Table 5: Projecting the population growth by two different rates

	A	B	C	D
1	Year	@1.65%	@1.49%	Comment
2	2011 AD	1,210,200,000.00	1,210,200,000.00	1,210,200,000.00
3	2012 AD	1,230,168,300.00	=FV(1.49%,1,-,C2)	1,228,231,980.00
4	2013 AD	1,250,466,076.95	=FV(1.49%,1,-,C2)	1,246,532,636.50
5	2014 AD	1,271,098,767.22	=FV(1.49%,1,-,C2)	1,265,105,972.79
6	2015 AD	1,292,071,896.88	=FV(1.49%,1,-,C2)	1,283,956,051.78
7	2016 AD	1,313,391,083.18	=FV(1.49%,1,-,C2)	1,303,086,996.95
8	2017 AD	1,335,062,036.05	=FV(1.49%,1,-,C2)	1,322,502,993.21
9	2018 AD	1,357,090,559.64	=FV(1.49%,1,-,C2)	1,342,208,287.81
10	2019 AD	1,379,482,553.88	=FV(1.49%,1,-,C2)	1,362,207,191.29
11	2020 AD	1,402,244,016.02	=FV(1.49%,1,-,C2)	1,382,504,078.44

Table 6 shows how a yearly deposit growing according to interest rate will be deflated by an equal inflationary depreciation. Please note the empty parameter in the function inside the notation for present value, **PV(8%,A2,-,C2)**.

Table 6: Growth & depreciation due to inflation demonstrated in MS Excel spreadsheet

	A	B	C	D	E
1	Year	Yearly deposit	Interest@8%	Inflation@8%	Comment
2	1	1,00,000.00	=FV(8%,A2,-B3)	=PV(8%,A2,-,C2)	Depreciation decreases the future value in spite of the growth
3	2	1,00,000.00	208,000.00	178,326.47	
4	3	1,00,000.00	324,640.00	257,709.70	
5	4	1,00,000.00	450,611.20	331,212.68	
6	5	1,00,000.00	586,660.10	399,271.00	
7	6	1,00,000.00	733,592.90	462,287.97	

The result is a population which grow into a logistic model because of successful population control programmes and a change in the attitude of people towards large families as shown in Fig. 2. Cost of living definitely aids in the curtailing of family sizes. Against all odds, the planning wizards of India are expecting the stabilization of the size of Indian population to about 1.5 billion by 2050 AD and about two billion by 2100 AD.

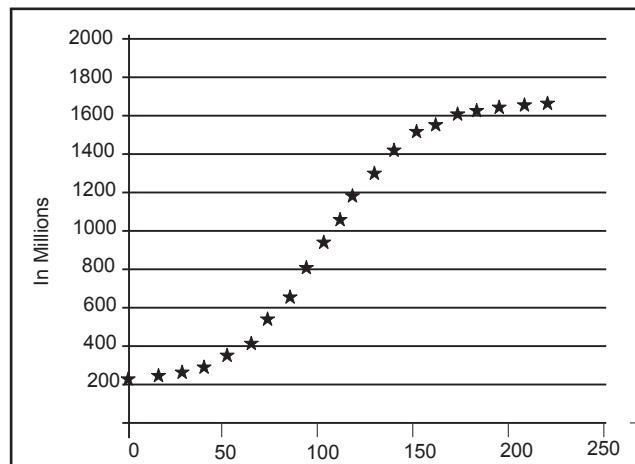


Fig. 2: Logistic model of growth of Indian population

## Conclusion

Only very few of us use software such as Microsoft Excel® even for financial calculations. However it is striking to note that the financial functions available in excel spreadsheet are useful even for population projections in demography. The model used for such calculations is '*exponential*' and is useful in generating geometric series of census estimates. Useful functions include FV, RATE and PV among others. Empirical calculation to estimate doubling time of population is also provided for non-users of computers.

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