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Can Medical Research Help Modulate The Rate of Aging?

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Abstract

Research to understand the mechanisms behind attaining old age and senescence has received great impetus during the past 2 decades. While the inevitability of the process and the end is obvious, many interesting features of this evolutionarily opted phenomenon are being surfaced. Aging and life span of species seem to be influenced both by genetic component as well as environmental forces. It is also apparent that life span of species has a relation to the time taken to reach reproductive maturity. Work with model organisms and some human syndromes characterized by accelerated aging has indicated that there are about 250-300 genes that are closely linked to the process of aging. Any subtle changes in the expression of these genes seem to significantly alter the rate of aging and life span. Most of these genes are found to code for pathways related to energy and stress handling metabolism and maintenance and repair mechanisms. Among the maintenance and repair pathways, DNA repair mechanisms, in particular those pathways like base excision repair pathway vested with the function of repairing 'in house' damage to DNA are emerging as important modulators for the aging phenomenon and associated debilities like neurodegenerative disorders. These advances in aging research are

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also becoming initiators for a hope to achieve a considerably lengthy health span in human populations.

Key Words: Aging, DNA repair mechanisms, genetic component, environmental forces

Introduction

History has never witnessed so much information and knowledge on human body and the molecular mechanisms that control its miraculous functioning, as it is now. The two fundamental and awe inspiring natural phenomena, the birth and death had so much influence on human civilization and attracted the capabilities of the best minds to understand these phenomena. Medical science has gained some significant ground in the case of former. Today the detailed knowledge about how a baby is conceived, the kinetics of its growth in mother's womb, the delivery of the baby and the requirements for the growth of the delivered infant, is available. As a result of this entire advancement one is able to control the whole process and decide when to have a baby. Even the sex of the baby to be born can be ascertained through appropriate scanning procedures.

However, it has not become possible for scientists to display the same confidence as far as the second phenomenon mentioned above, the death, is concerned. Even then, the progress in the medical sciences in the areas of nutrition and healthcare has achieved improved life span of people in general although there seems to considerable difference between advanced countries as compared to the developing nations. In India too the average life span has increased from around 42 at the time of independence to 63 as of today. It is generally believed that this is due to significant decrease in infant mortality coupled with improved health care which has become affordable for more number of people. People are living longer and with the percentage of people above 60 in India reaching the double digit, this section of population started drawing the attention of the government, the planners and more importantly the researchers. Little is known about why we become old and then die. It is a challenge that greets this millennium. It is a challenge whether the rapid advances in science and technology would enable maintenance of good health and the postponement or reversal of that type of old age marked by debilitating diseases, frailty and senility. Would it ever be possible to

preserve youthfulness for the later years in life?

It is the purpose of this article to present an update on the status of Science research seeking to explain the molecular mechanisms behind the aging process. Further, to point out the leads that this research has provided to offer hopes to modulate, if not gain complete control, the process of aging. Finally, the very modest contribution made from the laboratory of this author towards a slightly better understanding of the DNA Repair mechanisms in brain and its tight relationship to aging and age associated neurodegenerative disorders will be mentioned.

Molecular biology of Aging

Up until 50 years ago, research emphasis on the biology of aging was scanty. However with the advent of molecular biology, research in medical sciences and healthcare improvement coupled with good nutrition resulted in extended longevity of populations particularly in developed countries. With this, certain debilitating disorders like Alzheimer's and Parkinson have started making their appearance more frequently in these aging populations. Living longer is fine but it became clear that it brings in some associated problems to the society and to the government committed to the welfare measures like providing medicare to senior citizens. Intensive research began to understand the mechanism behind getting old and associated debilities; both out of curiosity and necessity.

There were theories galore to explain why living organisms age and die. As the research went along, several questions emerged that need to be addressed. For example, primitive unicellular organisms that multiply by binary fission have no death. Each cell keeps on dividing as long as nutrition is available and practically no part of the parental cell seems to have been lost during vegetative division. However, death of the parental cell/organism has come into operation when the evolution opted for sexual reproduction (1). It appears that while sexual reproduction offers the hybrid vigor to the offspring, the price for this is parental death at different points of time after the sexual act depending upon the species and their potential longevity. Another striking observation of the researchers is the average life span of different species which must be a product of genetic as well as environmental contributions. The average life span of some species is given in Table 1 to emphasize this point. What is interesting is the apparent positive correlation between the times taken to reach adult hood (reproductive maturity) in any given species, to the length of the life span of that species. This gave an important clue about the

network relationship between the attainment of reproductive maturity, the life span and the aging process which supposedly begins from the onset of reproductive maturity.

Table 1 : Longevity and Time taken for Reproductive Maturity

Species	Longevity (Years)	Age of Puberty (Years)
Humans	100	12-14
Elephant	70	12-14
Chimpanzee	40	10
Dog	30	1
Rhesus Monkey	25	3
Cat	25	1.5
Rat	3	0.25
Mice	3	0.20

In spite of the fact that some evolutionary biologists along with their experimental scientist friends vehemently argue that there are no special genes coding for aging process and that human longevity is made up by genes only to an extent of 25 % (2, 3) there is overwhelming evidence accumulating over the years that genetic component exerts a major influence in determining the longevity potential. For example microsomal transfer protein gene located on chromosome 4 has been identified as a longevity contributor in a group of centenarians(4). Further the genetic component in centenarians is increasingly becoming apparent from studies on human populations(5,6). To cap it all, as of today, there are about 280 genes that have been identified in various organisms that influence the life span one way or the other(7). The most extensively studied organisms include *C.elegans*, Drosophila, yeast, and mice. For obvious reasons information from human studies is scanty or indirect. However, knowledge from the homologous genes, cell culture studies and last but not the least from various progeroid syndromes characterized by accelerated aging in humans, *viz.*, Werner's syndrome(WS), Hutchinson Gilford Progeroid syndrome(HGPS), Down syndrome (DS), Bloom's syndrome (BS), Cockayne's syndrome (CS) and Ataxia telangiectasia (AT) is helping to understand the genetics of aging process.

In Table 2, the numbers of genes in different organisms that are known to exert influence on rate of aging process and therefore on the life span of those species, are listed. The numbers indicated are approximate as the information is constantly undergoing revision with newer findings. The present data are obtained largely from the data base available at University of Washington, Seattle web site (7). Some genes, when mutated, are

known to extend the life span while the other shorten the life span. These numbers are also indicated in the table to highlight the evolutionary trend where in the number of genes that could extend the life span decrease in more advanced species. For example in humans, as on today, there appears to be no genetic disorder that would result in extended life span!

Table 2 : The number of genes,when mutated, known to either extend or shorten the life span in different species.

Organism	Total Number of genes Known to modulate life span	No of genes that shorten life span	No. of genes that extend life span
C.elegans	116	12	104
S.cerevisiae	94	48	46
Drosophila	35	5	30
Mice	24	13	11
Humans	8	8	NIL

Information gathered largely from University of Washington data base (7)

The extensive genetic data mentioned in Table 2 have been carefully analyzed by many scientists to examine whether or not all those genes that have been found to have some relation to life span would fit into any discrete metabolic pathways. An excellent review has appeared most recently on this subject(8) and it turns out that most of the life span modulating genes would fall into 3 or 4 metabolic/signaling pathways. In general the accumulated information, indicated in Table 2 already reveals that while moving up the evolutionary ladder, the genomic complexity increases and the number of genes with a clear impact on life span decreases. Nevertheless, there seem to be a general trend emerging in that life span determining genes are generally involved in certain metabolic and signaling pathways while the interactive network may be more complex in higher species like humans. The following pathways seem to be very intimately involved with the process of aging and therefore the life span.

1. Insulin/Insulin like growth factor-1(IGF-1) signaling :

Originally, the genetic regulation of life span has become evident from the studies on the nematode, *C.elegans*. Mutations in genes like daf-2 and age - 1 resulted in extended life span in these worms (9). There was homology between these genes and mammalian genes encoding Insulin receptor and IGF-1 receptor and phosphatidy-linositol-3-OH kinase (10, 11). It is now certain that even in higher animals like mice and humans the insulin signaling pathway coupled with growth hormone releasing hormone and growth hormone are closely linked to the life span. Inhibition of this pathway results in increased life span (12, 13). It is interesting that mutations in IGF-1 receptor with low plasma IGF-1 levels were found in Italian centenarians (14). Thus glucose utilization pathways seem to have profound effect on the rate of aging and life span.

2. Stress related pathways:

One of the major stresses that all the oxygen dependent organisms have to live with is the oxidative stress. The very energy metabolism in mito-chondria would produce reactive oxygen species (ROS) and therefore oxidative stress on the cell but usually this is handled by two important enzymes, catalase and superoxide dismutase (SOD). In mammals three SOD genes have been identified for handling the oxygen derived stress. Catalase and SOD2 (Mn-SOD) variant seem to be more important and related to aging and life span (15). There is also a claim that over expression of SOD2 in mice leads to increased life span. Reactive oxygen species and such other chemicals can, if not removed, cause damage to macromolecules including DNA and this can have adverse effects. Apart from the oxidative stress, other forms of stress are common in all the higher organisms. Cells suffer from high temperature stress and also endoplasmic reticulum stress due to improper folding of proteins. Heat shock proteins and such other factors are considered to take care of these stressful situations. Further cardio-vascular stress particularly in individuals with advancing age plays a major role in the aging process and longevity determination. Apo lipoprotein E variants are known to be important players in human ailments involving cardiovascular stress and neurocognitive deterioration (16). The ability to handle stress is tightly linked to aging process (17).

3. Macromolecular repair and maintenance mechanisms:

During evolution, organisms have developed various strategies to repair any damage that may have occurred to

any of the cellular components including the macromolecules like DNA, RNA, proteins and membrane structures. The most important of all these strategies is perhaps, the ability to repair damaged DNA, so essential to maintain the structural integrity of DNA so that faithful transfer of genetic information from generation to generation is assured. A number of DNA repair pathways have been identified in both prokaryotes and higher organisms. In mammalian cells four major DNA repair pathways are identified: (1) A simple reversal of the damage, (2) Nucleotide excision repair (NER) including mismatch repair and transcription coupled repair, (3) Base excision repair (BER) and (4) Recombinational repair (RR) including non homologous end joining repair (NHEJ). It is beyond the scope of this article to discuss these pathways here. However, informative and critical reviews have appeared in recent times and these may be referred to for more information (18-22). There are a couple of reviews specially dealing with DNA repair pathways in brain (23, 24). Suffice it to say here that all these pathways and in particular the NER and BER seem to have a tight relation to the phenomena of aging and neurodegenerative diseases (25-27). Furthermore, BER is the pathway that appears to be eminently equipped to handle the simple damage that occurs to the bases in DNA as a result of very cellular metabolism itself that is known to generate reactive oxygen species. Spontaneous deamination and loss of DNA bases could also occur eventually resulting in mismatched base pairs and baseless sites. In view of this BER is being considered to have a major role in maintaining the structural integrity of genomic DNA and therefore to the processes like aging and neurological disorders (24, 28-32).

In view of the special interest of this author in brain aging and associated neurological disorders and their relation to BER pathway, this pathway is briefly outlined in Fig 1. Essentially there are four steps in this pathway and two sub pathways could be visualized, one concerned with 'short patch or single nucleotide replacing pathway' and the other 'long patch pathway' involving filling up of a patch of up to 13 nucleotides. Some details about the steps are also presented in the legend for Fig 1. In recent years, BER is drawing the main attention as one of the most crucial DNA repair pathways for the following reasons: (1) This is a pathway which is conserved as well as evolved through the evolution to take care of the DNA damage concerned with loss or modification of the bases that are likely to happen in the normal course of cell's life and

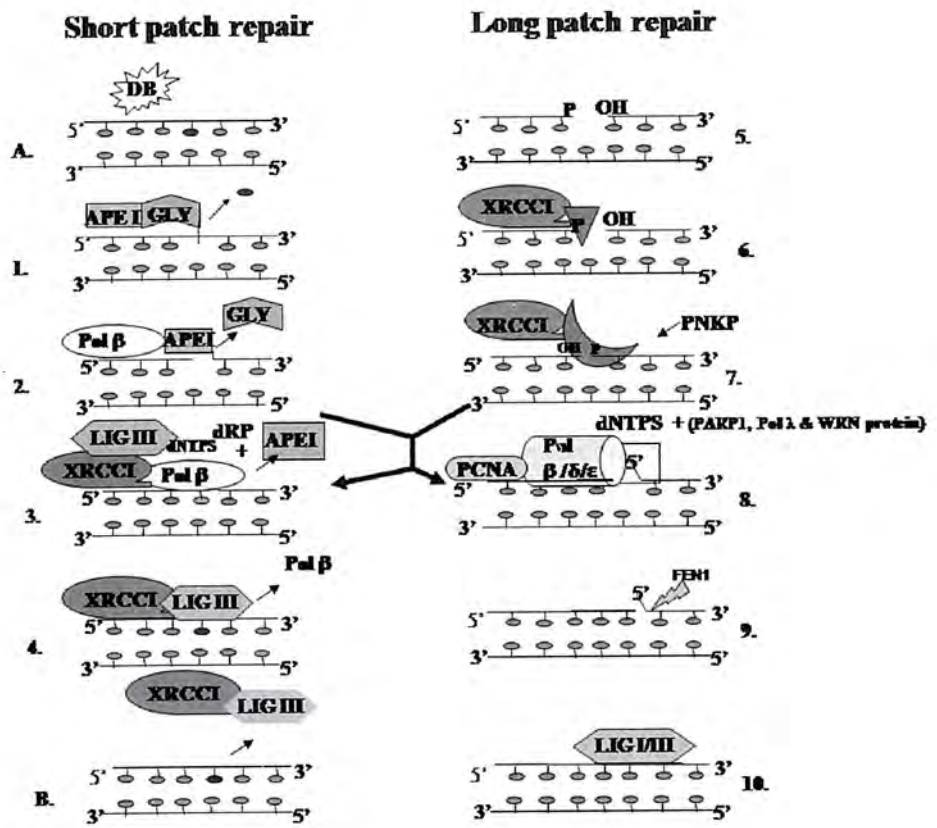

Fig 1: Base Excision Repair (BER) Pathway. On the left and right side are the 'Short patch' or single nucleotide pathway and the 'long patch' pathways respectively. Crossing over of the pathways can occur at points 2 and 7.

In step one of short patch pathway (left panel) the altered base (A) is recognized and cleaved from the deoxyribose phosphate moiety by an appropriate DNA-glycosylase. At the same time, the AP endonuclease (APE1) reaches the site. The second step consists of breaking the chain at 5'-side of the abasic site. The major endonuclease specific for abasic site in DNA in humans is APE1. In step 3, the pol β fills up the one nucleotide gap and also releases the 5'-deoxyribose phosphate (dRp). At the same time DNA-ligase III - XRCC1 complex arrives at the site. Step 4 consists of DNA-ligase III sealing the nick and pol β dissociating from the site. Subsequently the XRCC1 and ligase III come off from the site leaving behind repaired DNA (left panel, B).

The predominant route for BER is the 'short patch or single nucleotide pathway' discussed until now and shown on the left panel. In cases where the terminal sugar phosphate after the AP endonuclease incision (Step 2) develops a complex structure that cannot be acted

upon by the dRpase activity of pol β (for example, oxidized abasic site) the repair synthesis would nevertheless continue but in a strand displacement manner (right panel) This long patch synthesis is catalyzed either by pol β itself or a bigger polymerase like pol δ/ε with associated proof reading activity. Also, this pathway is stimulated by PCNA and requires a 'flap' structure specific endonuclease-1 (FEN1) activity to cut the flap like structure produced by the strand displacement type of synthesis by pol β. PCNA seems to stimulate the FEN1 activity and the repair patch size is about 7 nucleotides. The ligation can be achieved; it appears, either by DNA-ligase I or III. At this time, it is a matter of speculation as to what determines the type of DNA polymerase to be recruited for long patch BER pathway. The involvement of four other proteins in BER is reported in literature. These are PARP-1, PNKP, DNA-polymerase lambda (polλ) and Werner syndrome protein.

Abbreviations: DB-damaged base; APE1-human apurinic/apyrimidinic endonuclease 1; GLY-DNA-Glycosylase; Pol β/δ/ε- DNA-Polymerase β/δ/ε respectively; dRP-deoxyribose-5'-phosphate; XRCC1-X-ray repair cross-complementing, gene1; LIGI/III- DNA-ligase I / III; PARP1-Poly (ADP-ribose) Polymerase1; PNKP- Polynucleotide Kinase 3-prime phosphatases; FEN1- 'Flap' structure specific endonuclease 1; dNTPs- deoxynucleoside triphosphates. There is some recent suggestion that one of the several DNA-polymerases being discovered newly, the DNA-polymerase lambda (λ) has similar properties as that of pol β and may participate in BER. Figure taken from reference 28.

metabolism, (2) The relatively high importance of this pathway in post mitotic organs like brain where high oxygen dependent metabolic activity is seen in spite of lack of cell replication and (3) Being recognized as main pathway for repairing the oxidative damage to DNA bases.

In our laboratory, we have measured the activity of DNA polymerase β, a crucial enzyme in BER pathway, in rat neurons at different ages of the animal. The findings are summarized in Table 3. As can be seen, the activity of this important enzyme markedly decreases by adult hood itself with further decrease at the old age. In the subsequent studies we have examined the most crucial step in BER pathway, the gap filling activity after the damaged base is removed (a combination of steps 2 and 3 in the left panel of Fig 1). For this, synthetic oligo duplexes (32-mers) with one or four nucleotide gap in one of the strands are used as substrates and the gap filling activity in young, adult and old neuronal extracts is measured (Fig 2). Gap repair involves two steps: the creation and filling of the gap by the addition of the required number of nucleotides followed by the ligation

Table 3 : DNA Polymerase activity in rat neuronal and astroglial cells of different ages with 'Activated Calf Thymus DNA'

SUBSTRATE		AGE	
	YOUNG (5 days postnatal)	ADULT (6 months)	OLD (28 months)
'Activated DNA'			
Neuron	2023±1076	719 ± 541*	568 ± 412*
Astroglia	1471± 550	822 ± 512*	694 ± 652*

Values are averages ± S.D. and expressed as picomoles of the radioactive deoxynucleotide incorporated into the acid insoluble fraction in 1hr/mg protein.

*These values are significantly different ($p<0.001$ for neurons and 0.02 for astroglia) from the corresponding value at "young". Data taken from ref.24 and 33

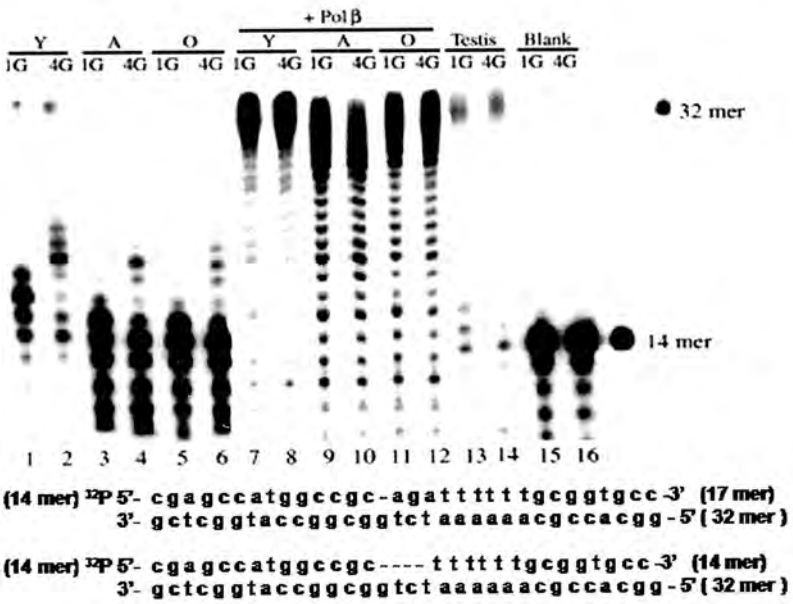

Fig 2: Gap repair activity in 'Young', 'Adult' and 'Old' neuronal extracts supplemented with recombinant pure rat liver pol β .

A typical autoradiogram from three different experiments is shown. Lanes 1-6 neuronal extracts from young brain (Y, 5 days postnatal), adult brain (A, 6 months) old brain (O, 2 years) Lanes 7-12 neuronal extracts supplemented with 1 unit of pol β. Lanes 13 and 14 are

with testis extracts alone as positive control. Lanes 15 and 16 are without any neuronal extracts (Enzyme blanks). The mobility of labeled standard 14-mer and 32-mer are also shown.Lanes 1,3,5,7,9,11,13,15 are with 1-gap substrate(1G) while lanes 2,4,6,8,10,12,14,16 are with 4-gap substrate(4G). The oligoduplexes with 1 and 4 nucleotide gap used in the study are also shown. Figure taken from ref 34.

with the 5'-phosphorylated downstream primer. This repair process, if completed properly should give a radioactive spot on the sequencing gel corresponding to the 32-mer since one of the deoxynucleotide triphosphates used in the reaction mixture is 32P labeled. However no addition of nucleotides to upstream primer (14-mer) was seen in adult and old extracts (Fig 2). In the young, some addition of nucleotides was seen and ligation to downstream primer also occurred although quite feebly. On the other hand, when the extracts were supplemented with pol β, addition of nucleotides occurred all the way to extend the upstream primer to a 32-mer apparently in a distributive strand displacement manner. It was found that excessive amounts of pol β would result in strand displacement type of addition of nucleotides while low amounts of pol β would add just the required number of nucleotides. Even then ligation was achieved only in young extracts and no ligation could be visualized in adult and old (Fig 3). Finally efficient gap filling followed by ligation, that means complete gap repair, was achieved and for this to happen, conditions required are the presence of 5'-PO_4 on the downstream primer, and supplementation of aging neuronal extracts with both pol β and DNA ligase (Fig 4). These studies thus demonstrated that aging neurons are unable to affect BER due to deficiency of pol β and DNA-ligase and fortifying the neuronal extracts from aged animals with these two factors can restore the lost BER activity.

Some of our most recent experiments confirmed the above results with gap filling activity with respect to overall BER activity as well. The overall BER activity is drastically reduced in neurons of aged animals and the activity could be restored back to a significant extent by the supplementation of aging neuronal extracts with pol β and DNA ligase, an observation similar to that with gap filling activity. Further, our very recent experiments also showed that limited (40%) dietary calorie restriction initiated in adult rats and continued upto old age (2 years) resulted in improved pol β activity in neurons (recent unpublished observations). In view of the importance of BER activity in a tissue like brain

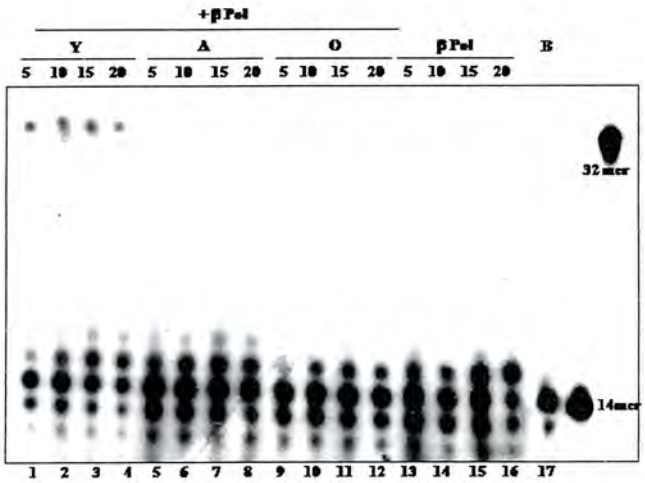

Fig 3: Time course of Gap repair activity with 'Young', 'Adult' and 'old' neuronal extracts supplemented with *low amount* of recombinant pure rat liver pol β (0.2 units)

A typical autoradiogram is shown. Lanes 1-12, neuronal extracts from young brain(Y), adult (A) and old (O) rat brain with 0.2 units of added pol β. The incubation times in minutes are also indicated above each lane. Lanes 13-16 are with pure pol β only. Lanes 17 is without any neuronal extracts (Enzyme blanks). The mobility of labeled standard 14-mer and 32-mer are also shown. Other details and notations are similar to the Figure 2. Only 1 gap duplex was used as substrate. Figure taken from ref 34.

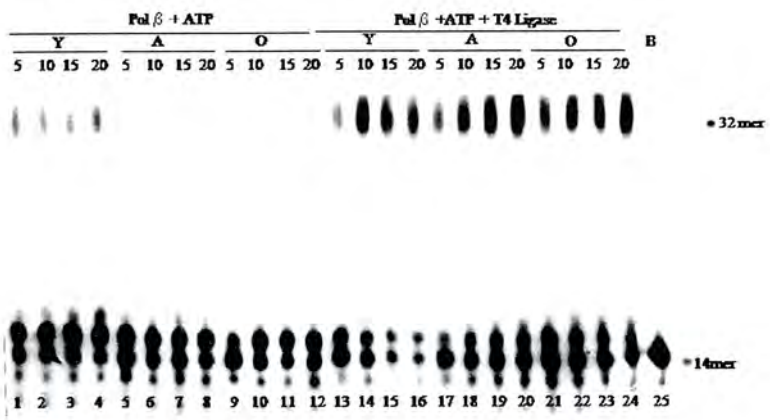

Fig 4 : Restoration of the gap repair activity in adult and old rat neuronal extracts when supplemented with limited amounts of pol β (0.2 units) and 20 units of T4 DNA ligase.

All the experimental details and notations are as in figures 2 and 3.

and the role of DNA repair in maintaining genomic integrity and therefore sustaining the health span of the individuals, these observations are refreshing in the sense that attempts can be made to extend these *in vitro* observations to an *in vivo* situation.

Epilogue

Aging and death are the products of biological evolution influenced by both genetics and environment. At this point of evolution, both these phenomena are inevitable. Yet when science started looking at these processes and the molecular mechanisms behind them, it has become one of the most fascinating journeys of scientific research. Today, even though so much information has accumulated about the process of becoming old, yet it is not clear why there exists so much diversity in life spans in different species. The precise molecular relationship between the reproductive system and the life span is yet to be understood. While many individual genes have been identified in model organisms like *C.elegans*, drosophila, yeast and mice and sometimes a mutation in a single gene is known to extend or decrease the life span of the model organism, the situation in humans seem to be much more complicated and extrapolation of observations from lower organisms to higher organisms must be done with caution. It is indeed interesting that if one looks up the evolutionary ladder, the dramatic influence of single genes on life span seem to be distinctly disappearing(Table 2). It is becoming clear that in highly evolved species like humans one may have to look for system analysis of discrete pathways rather than single genes.

Be that as it may, research on aging has reached a critical stage where it began to identify certain metabolic pathways that are likely to influence the rate of aging and the extent of life span. Two or three pathways are emerging as crucial in this context: (1) The efficiency of glucose utilization for various purposes including energy, (2) Pathways related to handling stress of various kinds including oxidative stress and (3) Maintenance and repair pathways like DNA repair and protein folding. Among these, the glucose utilization and DNA repair pathways have been receiving high attention and perhaps for valid reasons. Glucose metabolism is related to both energy and production of reactive oxygen species. DNA repair is related to maintenance of genomic stability for proper information transfer. If DNA damage accumulates and DNA repair potential diminishes with age then it is to be expected that a chaotic situation

would develop in cellular events leading to cell death and aging among other things. Whether this is nature's design to trigger senescence and death is a matter for pondering. What is more important is the fact that increased understanding of these pathways would lead to capacity to modulate these processes and therefore to achieve, perhaps, a lengthy health-span in human life.

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Non-Alcoholic Fatty Liver Disease : The Global Epidemic

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Abstract

Non- alcoholic fatty liver disease (NAFLD) comprises a spectrum of liver disease which encompasses steatosis, steatohepatitis and cirrhosis. Over the past 2 decades, NAFLD is being increasingly recognized as a manifestation of insulin resistance and metabolic syndrome.

The prevalence of NAFLD in community based studies varies from 2.8% (based on unexplained raised serum transaminases in USA) to 23% (based on USG evaluation of "fatty liver" in Italy). Contrary to earlier notion that NAFLD is a disease of affluent countries, data from developing countries, including India show similar high prevalence of 18 % (India) to 20% (China).

Natural history of NAFLD, as elucidated from serial liver biopsy studies, suggest slow progression in upto one third. While patients with steatosis have <1% risk of liver related mortality, 9-25% of those with NASH die due to end stage liver disease within 10 years.

Since NAFLD is considered as hepatic manifestation of metabolic syndrome, treatment of NAFLD entails correcting components of the same (Obesity, hypertension, diabetes mellitus, hypertriglyceridemia).

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Lifestyle modification, including restricted dietary intake of high energy foods, and aerobic exercises to achieve ideal body weight are the key components of treatment of NAFLD. Results from controlled trials have shown improvement in liver enzymes, insulin resistance and quality of life with lifestyle modification. Long-term sustainability of weight reduction by lifestyle modification needs constant patient motivation. Promising pharmacological options include insulin sensitizers and anti-obesity drugs.

With rising prevalence of obesity, diabetes, hypertension, NAFLD is emerging as a global epidemic with far reaching implications. "Prevention is better than cure". Promoting healthy lifestyle holds the key to check this growing global epidemic.

Keywords: non-alcoholic fatty liver disease, steatosis, steatohepatitis, cirrhosis

Introduction

Nonalcholic fatty liver disease (NAFLD) is a condition in which excess fat accumulates in the liver in subjects who do not consume significants amounts of alcolhol. This condition was described about 5 decades ago in obese individuls, but remained largely ignored till recent times, when it is being recognized as a emerging global epidemic. It is also becoming apparent that this condition is not always innocuous, and that the disease may progress in a small but significant proportion of subjects to liver cirrhosis and liver failure (1,2).

NAFLD is best described as the hepatic manifestation of the metabolic syndrome; most subjects have or develop one or more of the following features: central obesity, type 2 diabetes mellitus, hypertension and hyperlipidemia, and have insulin resistance as their core pathogenetic mechanism. The liver morphology comprises a spectrum ranging from mere accumulation of fat within hepatocytes (steatosis) to associated inflammation and fibrosis (steato-hepatitis); some cases progress to cirrhosis, liver failure or liver cancer.

How common is NAFLD?

How are community surveys conducted?

With the prerequisite of low (less than 20 g/day) or no consumption of

alcohol, two approaches have been used to identify subjects with NAFLD in the community; those with elevated levels of aminotransferase enzymes in the serum (3) or bright echoes on a routine ultrasound examination of the liver (4). Although puritans insist that a liver biopsy is the most definitive method to diagnose the condition, especially its progressive variety called non-alcoholic steatohepatitis (NASH), this invasive test is not practicable in community surveys. Nevertheless, the non-invasive tests of assessing liver functions or detecting excess fat in the liver, although surrogate, serve to provide useful data on the prevalence of NAFLD in the community.

What is the prevalence of NAFLD in the community?

Around 2.8 % of the American population was found to have abnormally elevated ALT values (>43IU/L) which could be ascribed to NAFLD (4). Recently, however, studies have suggested that the cut-off values for ALT should be brought down to 30 IU/L for men and 19 IU/L for women (5). With these lower cut-offs, 12.4% of men and 13.9 % of women are suspected to have NAFLD in the community.

Using ultrasound examination, frequency of NAFLD has been found to be 15-30% in various countries from across the globe. Some of the important epidemiologic population surveys (6-14) are listed in Table 1. The high prevalence of NAFLD is not limited to developed countries alone. Fan *et al* evaluated prevalence of fatty liver based on USG abdomen among adults in Shanghai, China. Of 3175 patients examined, 20.8% had fatty liver (7).

What are its common associations?

The increasing prevalence of NAFLD in the population reflects the growing pandemic of obesity and type 2 Diabetes Mellitus (15-17). The risk of NAFLD has been estimated to be 6 times higher in obese individuals compared with those with normal body weight. In a study from Japan, the prevalence of NAFLD was 3.5 % in non-obese and 20% in those who were obese (18).

Patients with type 2 DM frequently have NAFLD. Its prevalence in this group has been reported to be as high as 50 %, and is around 2-3 times higher than in non-diabetics. Insulin resistance is central to both these disorders. The common pathogenetic mechanism of these two disorders explains the high frequency of their co-existence in the same individual.

Does it affect children?

NAFLD can affect children as well. With the current world wide epidemic of pediatric obesity, pediatric NAFLD

Table 1: Epidemiology of NAFLD

Author (year)	Study	Diagnostic method	Country	No. of individuals screened	Prevalence of NAFLD (%)	Prevalence of NASH (%)
Bedogni (2005)	Population-based	Ultrasonography	Italy	598	23	ND
Fan (2005)	Population-based	Ultrasonography	China	3175	15	ND
Nomura (1988)	Population-based	Ultrasonography	Japan	2571	11	ND
Ruhl (2003)	Population-based	Aminotransferases	USA	5724	2.8	ND
El-Hassan (1992)	Outpatient	Ultrasonography, CT	Saudi Arabia	1425	10	ND
Araujo (1998)	Outpatient	Ultrasonography	Brazil	217	33.5	ND
Lee (1989)	Hospital series	Liver biopsy	USA	543	ND	9
Nonomura (1992)	Hospital series	Liver biopsy	Japan	561	ND	1
Byron (1996)	Hospital series	Liver biopsy	USA	1226	ND	11
Daniel (1999)	Hospital series	Liver biopsy	USA	81	51	32

is increasingly being diagnosed. This increase is also associated with increasing prevalence of type 2 DM and hypertension in children, reflecting an increase of insulin resistance and metabolic syndrome. A recent survey found 31 % of American school children to be overweight and 16 % to be obese; these figures are three times the prevalence noted in a survey conducted in 1996 (19).

Does it occur in developing nations too?

The earlier notion that obesity, metabolic syndrome and NAFLD are

diseases of the developed "Western" world is being challenged by recent reports demonstrating similar trends in developing countries.(Fig.1) A survey of the Indonesian population (9) reported a 30 % prevalence of NAFLD, a figure higher than even that from USA (24%) or Japan (15%). Another recent study

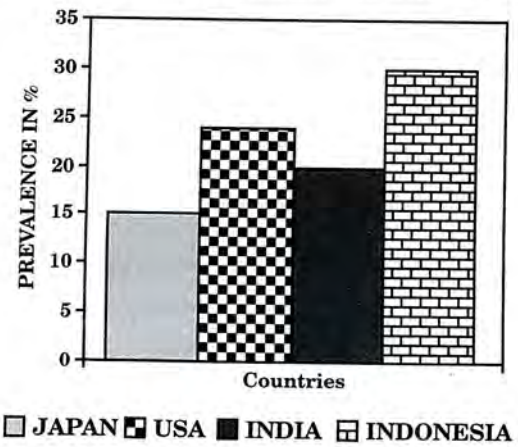

Figure 1 : Prevalence of NAFLD in general population

from Shanghai found 24% of the Chinese subjects to have the disorder (7). The increasing and widespread availability of high calorie "junk" food and sedentary lifestyles cutting across cities all over the world, is probably contributing to NAFLD becoming so ubiquitous.

How common is this entity in India?

Several reports from India suggest that NAFLD is quite common in this country as well (20-24). Epidemiological data on the prevalence of NAFLD in the general population in India though scarce, report figures of 16.6 and 24.5%. The survey of residents of railway colonies in Mumbai by Deepak Amarapurkar found 18.9% of those above 20 years to have bright echoes on ultrasound examination, suggestive of NAFLD. The prevalence was more in males (24.6%) than in females (13.6%) and was more often seen in those with central obesity or diabetes (22). In another ultrasonographic survey from the coastal areas of eastern India, 24.5% of "healthy" relatives of patients visiting the hospital were found to have NAFLD. This study also confirmed a higher frequency in males, and in those who were overweight (21).

NAFLD is commonly seen in type 2 diabetics. Around half of diabetics screened by ultrasound were found to have fatty liver (20). In a hospital based study from Chandigarh aimed at describing the clinicopathological spectrum of NAFLD, 100 NAFLD patients with increased liver enzymes were prospectively evaluated for clinical presentation and components of metabolic syndrome. Risk factors for the grade and stage of the disease on histology were studied in 38 biopsy-proven patients. Twenty percent of

patients were overweight, 68% had obesity, and 78% had central obesity. Abnormal cholesterol, HDL, and triglycerides were present in 36%, 66%, and 53% of patients, respectively. Twelve percent of patients had diabetes mellitus and 16% patients had various associated diseases. All 22 (100%) patients studied by ITT and all but one (98%) studied by HOMA-IR were found to have reduced insulin sensitivity and 50% were found to have metabolic syndrome by the modified ATP III criteria. Twenty patients of 38 (53%) had histological evidence of NASH (class 3=6, class 4=14). The other 18 (47%) qualified for class I (n=1) or class II (n=17) NAFLD. Four (10.5%) patients had bridging fibrosis and none had evidence of cirrhosis liver.(23)

In a similar hospital based study from Delhi, the clinical and biochemical profile at initial presentation of patients with histologically proven NASH was evaluated. Fifty-one patients with NAFLD formed the study population. Their median age and BMI were 34(17-58) years and 26.7(21.3-32.5) kg/m(2) respectively and 90.1% were males. The majority of the patients had mild inflammation, either grade 1 (63%) or grade 2 (31%) and only (6%) patients had severe (grade 3) inflammation. Twenty-three (45%), 19 (37%), 8(16%) and 1(2%) patient had stage 0, 1, 2 and 3 fibrosis respectively on index biopsy and none had cirrhosis. On multivariate logistic regression analysis, hypertriglyceridemia >150 mg% was the only factor independently associated with presence of high grade of inflammation (OR = 1.6; 95% CI: 1.3-22.7, P = 0.02), while none was associated with advanced fibrosis. Triglyceride levels correlated positively with inflammatory grade (r = 0.412; P = 0.003). A closer look at this study however shows that the mean age of subjects was (34 y) around 3 years less than the study from Chandigarh (37 y), thereby explaining the milder histological changes and lower association with other components of the metabolic syndrome (24).

In 65 patients of NASH (mean age 38 years) studied in Lucknow, 72.8% were found to have high BMI; however 98.3% had increased waist-hip ratios indicating that central obesity was a more sensitive indicator of NAFLD in Indian subject. This study also highlighted the limitations of applying the NCEP ATP III criteria in Indian patients for the diagnosis of metabolic syndrome; the Indian criteria with lower anthropometric cut-offs for diagnosing central obesity showed a better correlation with NAFLD (25).

The recent rapid increase in prevalence of obesity amongst Indian children is causing concern. In a survey of Indian urban school children, Marwaha (26) found 19% to be overweight and 5-6% to be obese. Hypertension and early onset of type 2 DM are being increasingly seen in Indian children. Fast food, sedentary habits, lack of sports and outdoor activities are common in Indian cities where these trends are being observed. With India already acquiring the dubious label of becoming the "Diabetic Capital" of the world, these trends in unhealthy life style are likely to result in a burgeoning epidemic of NAFLD and metabolic syndrome in the population

Is NAFLD a cause of cryptogenic hepatitis?

On the basis of liver biopsies, features of NASH could be identified in 65-9% of cases of 'cryptogenic' hepatitis. All large surveys (27-29) of cirrhotics from across the globe have found a proportion of 15-30% in whom no etiologic cause for their chronic liver damage could be identified; many of these patients are diabetics or provide a history of having been obese. It is conjectured that these patients have liver cirrhosis due to long standing and progressive NASH.

What is the natural history of NAFLD?

The natural history of NAFLD can be gauged by longitudinal follow up of a large number of subjects with this disorder. As the rate at which this disease progresses is slow, requiring a very long period of follow up to assess outcome, studies on the natural history of NAFLD are difficult to perform. They are based on two approaches

1. serial biopsy studies
2. cohort studies with clinical end points

There are significant limitations in each of these. While serial biopsies are limited by selection bias in patients undergoing repeat liver biopsies, cohort studies have limited follow up. Despite these limitations, the studies have provided data on which some inferences can be drawn. There have been no studies from India on the natural history of NAFLD; hence the few studies available from other countries are reviewed here.

NAFLD is a slowly progressive disease. Serial liver biopsy studies (30,38,40) have shown that around half of the subjects with NAFLD seem to remain stable at the same stage for 5 - 13 years, 15-20% in fact show some improvement while only 30-40 % progress from one stage to another as shown in (Table 2).

Table 2 : Fibrosis progression in liver biopsy based follow up in NAFLD.

Author (year)	No. of patients	Average time interval (years) between biopsies (range)	Progressed *n* (%)	Stable *n* (%)	Improved *n* (%)
Harrison (2003)	22	5.7 (1.4-15.7)	7 (32)	11 (50)	4 (18)
Adams (2005)	103	3.2 (0.7-21.3)	38 (37)	35 (34)	30 (29)
Ekstedt (2006)	70	13.8 (10.3-16.3)	29 (41)	30 (43)	11 (16)

NAFLD comprises a histological spectrum; those with steatosis alone (without necro-inflammation or fibrosis) seem to run a very benign course. Less than 5% of these subjects showed progression to cirrhosis over more than15 years. This contrasts sharply with those who had NASH at initial evaluation; progression to cirrhosis was seen in twice the number (10%) and over half the time (8 years) in this group. Higher BMI, greater insulin resistance or the presence of type 2 diabetes constitute risk factors for a higher rate of fibrosis progression (31).

With the development of fibrosis and morphological features of cirrhosis over time, many of the classical changes of excess fat accumulation in the liver such as steatosis, or features of inflammation such as presence of inflammatory cells or ballooning of hepatocytes, disappear. In a grossly scarred cirrhotic liver, it is therefore difficult to establish what caused the damage. As a corollary, liver biopsy features other than fibrosis severity, may not be useful to predict the long-term prognosis in an individual patient with NAFLD.

Long term prognosis of patients with NAFLD studied by following cohorts till clinical end points occur, have shown slow disease progression over time; the prognosis however varies with the stage of NAFLD (Table 3). Patients with bland steatosis have <1% chance of dying due to their liver disease. On the other hand, those with aggressive steatohepatitis or cirrhotic stage NASH have a worse prognosis, as demonstrated in three recent studies; 9-26% of patients died within 4-10 years

Table 3 : Long term prognosis of NAFLD

Author (year)	Diagnosis	*n*	Cirrhosis prevalence (%)	No. of liver-related deaths (%)	No. of deaths overall (%)	Average follow-up (years)
Dam-Larsen (2004)	Bland steatosis	109	1	1 (0.9)	27 (24.8)	16.7
Matteoni (1999)	NAFLD	98	20	9 (9)	48 (49)	8.3
Adams (2005)	NAFLD	420	5	7 (1.7)	53 (12.6)	7.6
Ekstedt (2006)	NAFLD	129	7.8	2 (1.6)	26 (20.2)	13.7
Lee (1989)	NASH	39	16.3	1 (3)	10 (26)	3.8
Powell (1990)	NASH	42	7	1 (2)	2 (5)	4.5
Hui (2004)	Cirrhotic-stage NASH	23	100	5 (21)	6 (26)	5.0
Hashimoto (2005)	NASH with septal fibrosis or cirrhosis	89	48	6 (6.7)	8 (9)	3.7
Sanyal (2006)	Cirrhotic-stage NASH	152	100	22 (14.5)	29 (19.1)	10

of follow-up, with most causes of death being related to end-stage liver disease (34-37). Risk of progression to liver failure is increased five fold among obese NASH patients as compared to non obese patients.[5% Vs 1%] (39). When compared with other etiologies of chronic liver disease, NASH-cirrhosis has outcomes comparable with HCV cirrhosis (34).

Overall, a diagnosis of NAFLD is associated with a shorter survival than expected (Fig. 2). In a community based study at Minnesota, USA, NAFLD patients had significantly lower 10 year life expectancy than the healthy general population [77 Vs 88%, $p<0.05$] (38) Liver failure, variceal hemorrhage and HCC were important causes of mortality in this study population.

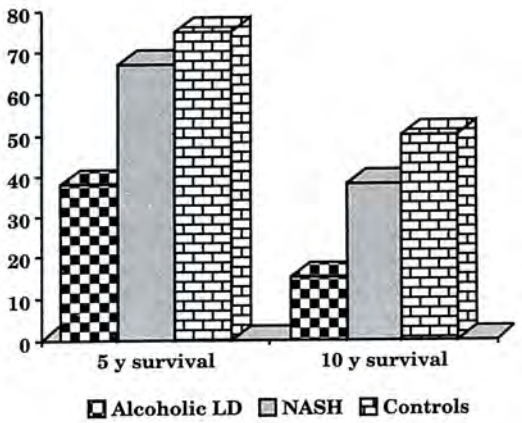

Fig 2: NAFLD: Life expectancy

Conclusion

Non-alcoholic fatty liver disease is a recently recognized entity characterized by accumulation of fat in the liver in non-alcoholic subjects. It is associated with insulin resistance and metabolic syndrome. A significant proportion of Indians are being affected by this disorder. A subgroup of patients runs a progressive course with damage to liver cells and development of cirrhosis.

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Economic Implications of Preventive Cardiology : Indian Perspective

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Abstract

India, which had been fighting the problems of infectious and nutritional diseases in the past is now facing the challenge of cardiovascular diseases (CVDs) such as hypertension (HTN), coronary artery disease (CAD), type 2 diabetes mellitus (T2DM), stroke, obesity and metabolic syndrome. It is well known that south Asians are more prone to vascular diseases mainly HTN, CAD, T2DM and stroke. These diseases have now slowly but steadily crept into the poorer segments of the society and that too amongst the most productive and working force of the country. Number of people affected with HTN, CAD, T2DM, stroke, and rheumatic heart disease (RHD) works out to be around 268.7 million based on current prevalence and 2001 census. RHD with its attendant complications continues to be our problem. Unfortunately, the number of studies that report health costs of these diseases is quite few in India. This article tries to address this issue with regard to the economic burden of CVDs in India and provides an overview of the resultant economic loss if they are not timely prevented.

According to the most conservative estimate the approximate cost of treating CVDs diseases particularly HTN, CAD, T2DM , stroke, and RHD, in India would be around Rs 3178.1 billion whereas if simple and practical life style measures are adopted to prevent these diseases the burden would be reduced to a mere Rs 615.7 billion. This huge

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saving of Rs. 2562.4 billion will provide an answer to the big question: what is best: Prevention or Cure? Lest we adopt preventive measures right now at community and individual levels the direct and indirect health economic toll due to man days lost and social support will be devastating for our economy. Further, the economical implications of obesity, metabolic syndrome and stroke in the population < 40 years mandate in depth research.

Key words: Economics of prevention, coronary artery disease, hypertension, type 2 diabetes mellitus, stroke, metabolic syndrome, rheumatic heart disease.

Introduction

Cardiovascular Diseases (CVDs) particularly hypertension (HTN), type 2 diabetes mellitus (T2DM), coronary artery diseases (CAD), rheumatic heart diseases (RHD), obesity and metabolic syndrome have emerged as a major cause of morbidity and mortality in developed as well as developing countries. India with its population crossing the one billion mark (1) is facing major brunt of these diseases on account of several reasons like (a) changes in life style consequent to economic improvement, migration to cities and urbanization (b) ageing (c) ethnicity (d) increasing population (2). If the current rising trend of these CVDs is not contained well in time it is feared that the bare minimum cost of managing and treating these diseases will be beyond the economical means of our resources.

It is also to be appreciated that due to unprecedented technological advances in medicine and cardiology a mad race for providing tertiary care treatment for CVDs has taken a front seat, while preventive strategies remain more or less ignored. Corporate-sponsored medical emporia have flooded the entire developing world and the glamour for interventional cardiology is further compromising the basics of preventive measures which may be more useful for preventing its further spread in an individual as well as society at large (2).

The economic implication of treating the CVDs particularly CAD and T2DM when fully developed is enormous. The situation turns more serious when we realise that, this epidemic is also affecting the most productive age group of the country i.e. the incidence of CVDs among

population less than 40 years is increasing (3).

This paper aims to illustrate the current CVD burden and its impact on Indian economy and an urgent need to adopt the simple and practical measures to prevent this epidemic and its effect on overall national economy.

Burden of Cardiovascular Diseases (CVDs)

During the last four decades cardiovascular diseases have been steadily increasing and over the time they have affected approximately 1.5-26.5 % of the Indian population albeit more in urban areas compared to rural population. Most important aspect of this scenario is that most of these illnesses are preventable. (Table 1)

It is estimated that India presently has 35.8 million of CAD, 200 million of HTN, 1.2 million of CVA (stroke), 31.0 million of T2 DM and 0.7 million of RHD (Table 2). These figures have been derived from the current prevalence rate and assuming total population as derived from 2001 census to be 1027 million. Though a tentative prevalence rate of obesity and metabolic syndrome have been calculated on the basis of recent studies done in urban cities, however hard figures regarding their prevalence in rural and urban areas both need to be worked out.

Table 1: Prevalence Trend of Cardiovascular Disorders (CVDs) in India

Disease	1950-1970	1970-1980	1980-1990	1990-2000	2000 onwards
CAD (4)	5.5%	6.5%	9.7%	10.9%	11%
HTN (5,6)	1.2%		*		20%
DM (7.8.9)	2.1%	2.3.%	12.1%	13.5%	14.3%
CVA (10)	*				0.9-2.2 /1000 persons
Obesity (11)	*				26.5%
Metabolic syndrome (12)	*				25.8%
RHD (13,14)	1.8-11	1.8-11	1-3.9	1-3.9	1.5-4

* The exact prevalence needs to be worked out.

Table 2: Burden of Various Cardiovascular Disorders in India as of 2005

Disease	Total Population affected	Age Group <40 years affected (Young Burden)
CAD (1)	35.8 million	13.4 million
HTN (6)	200 million	*
DM(1)	31.0 million	6.4 million
CVA (15)	1.2 million	0.1 million
Obesity	*	
Metabolic syndrome		
RHD (15)	0.7 million	Mostly involves young people
Total	268.7 million	*

* The exact prevalence needs to be worked out.

Rural and Urban Distribution of Various Cardiovascular Disorders in India

Various studies have revealed variable distribution of CVD in urban and rural areas.

Table 3: Urban and Rural Distribution of Cardiovascular Disorders in India

Disease	Urban %	Rural %
CAD (16)	9.7.	3.5
HTN(17)	24	17
DM (8)	15.5	3.77
CVA	*	*
RHD(15)	2.56	7.42

* The exact prevalence in rural and urban areas needs to be worked out.

Coronary Heart Disease

1) **Prevalence of Coronary Heart Disease (CHD) in Indians**

As evident from Table 4, within a short span of 5 years the number of patients having CHD has increased by almost 10 million. This rising trend is observed both in urban as well as rural population. Total number of people suffering from CHD work out to be 35886789 . (Table 4 and Fig 1)

Table 4: Prevalence of CHD in India (1)

Age	2000			2005		
(years)	Rural	Urban	Total	Rural	Urban	Total
20-29	1799691	2711501	4511192	2012363	413805	6150408
30-39	2854247	2635019	5489266	3383816	3869904	7253720
40-49	3342472	2776974	6119446	4127201	4116830	8334032
50-59	3590885	2288412	5879296	4544974	3171320	7716294
60-69	3153512	1888199	5041711	3849544	2582790	6432334

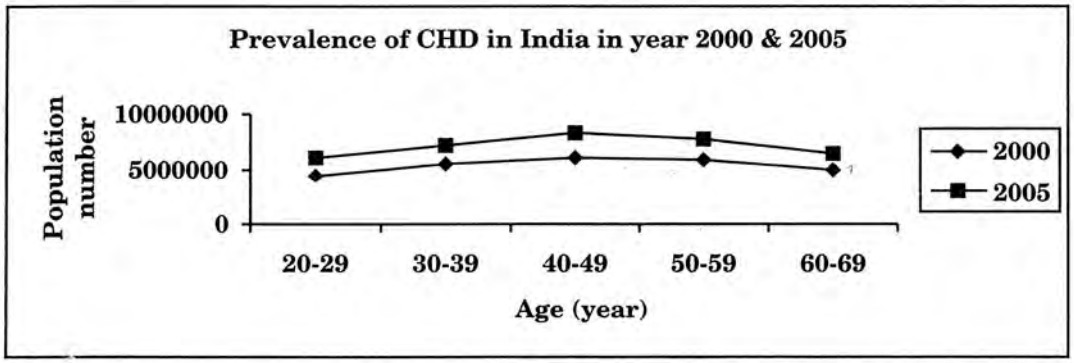

Fig 1: Graph based on National Health Profile 2006 (1)

As evident from Fig 1, the prevalence of CHD has increased among all the age groups in a span of 5 years.

2) Rural and Urban Distribution of Coronary Heart Disease (CHD)

The number of patients of CHD in rural population in India has increased from 1,47,40,808 to 1,80,07,899 whereas it has increased from 1,23,00,104 in to 1,78,78,889 in urban population within a period of 5 years (2000- 2005). (Table 4 and Fig 2). The increase in the number of subjects with coronary heart disease in the age group 35 -45 in rural population is a matter of concern from public health point of view.

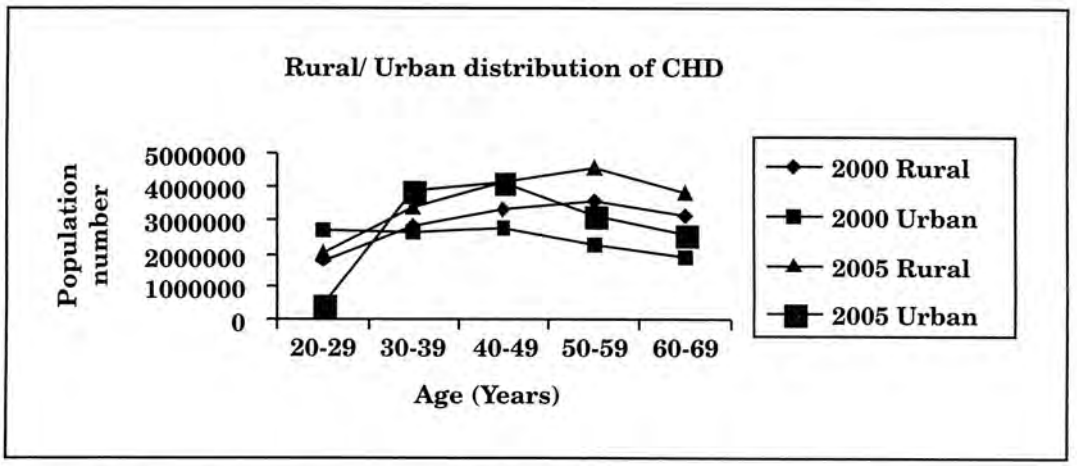

Fig 2: Graph showing rural and urban variation of CHD in India based on National Health Profile 2006 (1)

3) CAD in Young Adults

The problem of CAD in India is becoming alarming as the disease has started affecting the most productive age group people i.e. younger generation. The definition of young CAD has been varying from 35 to 45 years in different series. For the sake of working out economic impact of young CAD on society we have taken a cut off limit of = 40 years to label young CAD (3). The prevalence of premature CAD in Indians is up to 3 times higher when compared with people of similar age group in the western world (22).

In West young CAD is about 5% and in India it is 12-16% in = 45 years. But, unlike in Whites, CAD in young Asian Indians is known to be severe, extensive, and malignant (23, 24). This is attributed to an accelerated atherosclerotic process that begins

early in life due to heredofamilial reasons (25).

4) **CAD in Women**

Despite the fact that the lifetime risk of death from CAD is more than 10 times greater than that from breast cancer, yet most of the women consider their risk of CAD to be 1% only (26). This lack of concern for CAD by women itself explains the vulnerability of this subgroup of population to the modern epidemic of CVD. The incidence of CAD increases with age in women, although the clinical presentation of the diseases lags 10 years behind that in men (26). Lifetime risk of developing CAD after the age of 40 years is 49% for men and only 32% for women, but women are more likely to experience significant morbidity and mortality associated with acute coronary syndrome (27). Typical angina has less predictive value in females pretest probability is 50-60%, whereas it has 80-99% value in males (28). The young women are increasingly presenting with acute coronary syndromes. Though they enjoy certain degree of cardioprotection because of their hormonal effects, but this effect vanishes as they start smoking. And in case if early menopause superimposes on the deadly effect of smoking, the risk of premature CAD (Age =40 years) increases manifold. DM and or hypertension are considered to be the major culprit for premature CAD in women in absence of smoking (29, 30).

Type 2 Diabetes Mellitus

The global epidemic of T2 DM is progressively increasing in India and the toll has risen from 25 million cases in year 2000 to 31 million by the year 2005. (Table 5 and Fig. 3)

Table 5: Prevalence of Diabetes in India (1)

Age	2000			2005		
(years)	Rural	Urban	Total	Rural	Urban	Total
20-29	549102	1003310	1552412	609128	1209725	1818853
30-39	1425108	2567970	3993077	1518041	3126311	4644352
40-49	2232090	3882005	6114095	2488845	4968478	7457323
50-59	2628455	4188171	6816626	2966586	5394860	8361446
60-69	2053095	2783100	4836195	2237319	3445602	5682920
70+	1100412	1401300	2501712	1267086	1807951	3075038
Total	**9988262**	**15825855**	**25814117**	**11087005**	**19952927**	**31039932**

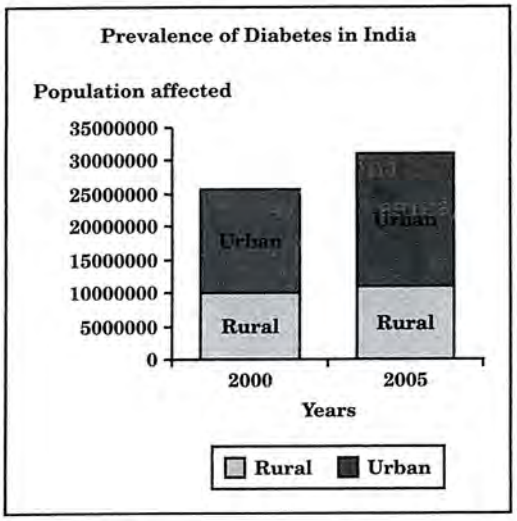

Fig 3: Graph based on National Health Profile 2006 (1)

Rheumatic Heart Disease

With improvement in overall socioeconomic status of society the prevalence of RHD is gradually declining over the years. So much so that the number of RHD cases in the last five years has remained almost same as evident from reports of National Commission on Macro-economics and Health (15).

Estimated cases of RHD in 2000: 7, 64,556
Estimated cases of RHD in 2005: 7, 44,510

Stroke

Although we have pretty good information about the prevalence of CAD, T2 DM, HTN in the society; same is not true with stroke. It is only recently that some data is available regarding stroke. The number of cases of stroke has marginally increased in the last five years from 1,081,480 to 1,247,812. (Table 6)

Table 6: Prevalence of Stroke in India (15)

Age Group	Estimated cases in Year 2000	Estimated cases in Year 2005
20-39	92746	104693
40-59	457365	534032
60-79	531369	609086
Total	**1081480**	**1247812**

Reasons for Increasing Prevalence of CVD

Most important causes of this phenomenal rise in CVDs are smoking, obesity, metabolic syndrome, increasing longevity and increase in total population itself. However, most worrying aspect is its progressively increasing premature occurrence in younger segment of the society in the last two decades.

Apparently there has been radical change in our lifestyle, dietary pattern and physical nature of job. Each of these conditions is linked to life style. Parallel to increase in obesity is the concurrent spurt of metabolic syndrome.

1) **Smoking**

Tobacco is the major cause of cardiovascular related deaths in the world today. Unfortunately India has the privilege of being the second largest producer of this killer. Nearly 5 million people die due to tobacco use every year and this figure will increase to 10 million tobacco attributable deaths per year by 2020 (34). In India alone it has been estimated that the number of people dying due to tobacco use every year are 8, 00,000 -9, 00,000 (35).

The consumption of smoking has been increasing at an alarming rate as a result of sophisticated global promotion strategies developed by multinational cigarette companies and local bidi industry.

Table 7: Prevalence of Smoking in India (15)

Age group (years)	Males %	Females %
15–19	4.4	0.2
20–24	13.7	0.6
25–29	25.1	1.1
30–39	37.6	2.2
40–49	45.0	4.0
50–59	45.3	5.7
60+	38.2	5.3
Average	29.4	2.5

Bidi being the cheaper option than cigarette is the most popular way of smoking among low socioeconomic group of people and in villages. Despite containing less tobacco than a cigarette, an unfiltered bidi releases two to three times more tar and nicotine, making them potentially more dangerous health hazard (36). And among their primary targets are the most vulnerable group namely woman and adolescent population who are ill equipped to cope with the slick marketing techniques and the dirty tricks perfected by the tobacco industry. It needs no over emphasis that chronic severe smoking ultimately leads to premature and accelerated atherosclerosis (37).

2) **Obesity**

Estimated prevalence of obesity in India is 26.5% based on body mass index criterion. This modern disease is rising unexpectedly at a rapid pace and is engulfing the vulnerable young population in developing countries like India (Table 8).

Currently more emphasis is being laid on measuring waist circumference which is a marker for central obesity. According to IDF standards the cut off points for waist circumference are 90 and 80 cms. for males and females respectively (12). One important factor for this is the rising life expectancy and

Table 8 : Prevalence of Obesity in India

Prevalence of obesity (11, 31, 32)

Prevalence of obesity as per western cut off	BMI=30kg/m^2	4%
	BMI=25.5kg/m^2	9.9%
Prevalence of obesity as per Asia Pacific obesity criterion	BMI=25kg/m^2	26.5%
Prevalence of obesity as per CURES study,2007	BMI=23kg/m^2 *	45.9%

* Time has come to consider this as a cut off as we are ethnically and phenotypically more susceptible for CAD and T2 DM.

the others are related to the rapid and chaotic urbanization with accompanying life style changes and to the powerful economic and cultural influences of globalisation. Junk food is replacing dietary fiber and the complex carbohydrates of fruits and vegetables. Junk foods are the foods that contain little or no proteins, vitamins or minerals but are rich in salt, sugar, fats and are high in energy (calories) (33). The Burgers of Mc Donald and the Pizzas are replacing healthy food in cities particularly in the metropolis. Calorie intake is multiplying whereas the physical activity is reducing. This mismatch between energy intake and energy output has manifested itself in a pandemic of obesity (2).

3) **Metabolic Syndrome**

The prevalence of central obesity, glucose intolerance, hypertension, high triglyceride (TG) levels, and low levels of high-density lipoprotein cholesterol (HDL-c) - the five 'axes of evil' of metabolic syndrome is highest among the Indian community in south Asians. It continues to increase at a rapid pace (38, 39). In India the prevalence of metabolic syndrome has been estimated to be 23.2% by WHO criteria, 18.3% by ATPIII criteria and 25.8% by IDF criteria (12).

Economic Burden of CVDs

Already reeling under the burden of poverty and illiteracy, the new burden of rising CVD in India has strained Indian economy to a great level. The big question is what the best option is: prevention or cure? An important warning sign about the road not to travel is now provided by the USA, the chief advocate of a technological approach to health. The American people are now grappling with a health care crisis without early solution in sight (2). Based on most conservative estimate the current cost of managing CAD cases would be as below (Table 9)

Table 9: Economic Burden of CAD #

	Conservative Rx Polypill Approach for 1 year (18)	Coronary Angiography CAG (18)	Coronary Angioplasty PTCA (18)	Coronary Artery Bypass Graft CABG (18)	Ancillary Costs (Staff, Clinics, Hospitalisation) (18)	Life Style for 1 year
Cost Per patient	Rs 5500/- year (*)	Rs 5000/- event @	Rs 1,00,000/- event	Rs 1,00,000/- event	—	Rs 7/- person/day **
Cost for Total population	For 8 million patients *Direct Cost:* 44 billion *For Ancillary medical services# #:* 44 billion	For 1.27 million events/year 6.5 billion	For 30,000 events/year 3 billion	For 20,000 events/year 2 billion	— 100 billion	For 8 million patients/year 20.4 billion ***
Grand Total Cost per year			**Rs 200 billion**			**Rs 20.4 billion**

\# We have used traditional costing method for calculating cost of medicines / polypill/ angiography / PTCA/ CABG.

@ Based on the assumption that each procedure costs Rs.5000

* Considering that total population affected from CAD in 2002 was 32 million out of which at any given time only 1/4th will be aware of their disease and will be on a minimum basic prescription following 'polypill approach' (Aspirin, beta blocker, ACE inhibitor and statin) (18, 40, 41). The average cost of generic form of these drugs in India is about Rs 15/day, that amounts to Rs 5500 per year.

** Based on the assumption that if a person stops smoking, the amount saved will be Rs 3/day/bundle of bidi and if the person consumes healthy food i.e. two fruits (Orange, Jamun,) and two vegetables (White-Gourd/Lockey, Ginger,), then the expenditure will be Rs 10 per day/per person; So that the net expenditure per day on adopting Life style measures will be Rs 10- Rs 3 =Rs 7/Day/person. (Table 10)

\# Ancillary medical services include cost of investigations and hospital visits.

*** Calculations are done on those 8 million patients who would have been protected from CHD if life style measures have been adopted timely but instead now require either conservative or invasive management.

The cost of treating CHD most conservatively in India is huge and amounts to the tune of Rs 200 billion whereas if early preventive measures are taken the cost will be reduced to a minimal of Rs 20.4 billion. The net saving of Rs 180 billion is enormous for our country. (Table 9)

Healthy life style measures include eating vegetables, fruits, increasing physical activity and stop smoking/ tobacco use. The approximate cost per person is calculated in Table 10.

Table 10: Estimated Expenditure on Healthy Life Style

Life Style Measure	Expenditure
Eating Two Vegetables/day (Lockey, Karela)	Rs 5/- day
Eating Two Fresh fruits (Orange, Jamun)	Rs 5/- day
Walking	Nil
Yoga	Nil
Stop Smoking	-Rs 3/- day * (Saving)
Total/person/day	Rs 7/- day
Total/person/year	**Rs. 2555/- person/year**

* Presuming one bundle of bidi costs Rs 3.

The total cost for adopting healthy life style by a person will be Rs. 2555/ person/year.

Table 11 provides a comparison between the cost differences if various CVS disorders are treated instead of adopting preventive strategy. The estimated cost for treating various cardiovascular disorders is Rs 3178.1 billion whereas if simple lifestyle measures are adopted the cost shrinks to a mere Rs 615.7 billion. The net saving of Rs 2562.4 billion to the country is probably the evidence based answer for adopting preventive strategies at an early age.

Prevention

There is enough convincing data that simple life style measures lead to prevention of CAD in first stance and/ or reversal of the atherosclerotic lesion (38, 44). Thus adopting prevention at an early age is an economical option than therapeutic interventions. As mentioned earlier the cost of treating various cardiovascular disorders would be Rs.3178.1 billion; India obviously cannot afford the mammoth expenditure of providing treatment to such a large number of diseases which are easily preventable. Moreover cardiovascular death strikes not as an unexpected bolt of lightning but as the culmination of a slowly evolving process marked by readily recognizable signposts. Pictorial signposts for individuals requiring preventive measures for CVS diseases are depicted below and graded as follows (Fig 4):

Table 11: Approximate Financial Burden of Various CVDs in India

Disease	Total population affected	Population <40 years affected (Young Burden)	Total Economical burden/year for **treatment of entire affected** population (Rs billion)	Total Economical burden/year for **treatment of <40 years age (Young Economical Burden)** (Rs billion)	Total Economical burden/year if **Life Style measures are adopted** by entire affected population (Rs billion)	**Net Saving** if Life Style measures are adopted for prevention rather than treatment of CVDs (Rs billion)
CHD *	35,886,789	13,404,128	200	74.7	20.4	179.6
DM #	31,039,932	6,463,205	618.12	128.5	79.3	538.9
CVA **	1,247,812	104,693 (20-39yrs)	1292	107.2	3.1	1288.9
RHD ***	744,510	Not Applicable	192 (tertiary prevention)	192 (tertiary prevention)	1.9	190.1
HTN @	200 million		876	****	511	365
			Rs 3178.1 billion	****	**Rs 615.7 billion**	**Rs 2562.4 billion**

* Table 9 above

\# based on the assumption that the mean total annual cost direct (ambulatory plus hospitalization) as well as indirect is Rs. 19,914/person (7).

** Based on the assumption that cost of treating CVA in US is $103 576/ person and in India the cost may be 1/4th of this cost i.e. $25894/person i.e. Rs 1,035,760/ person (42).

*** Based on the costs of tertiary prevention of RHD in Pondicherry (43).

@ based on the assumption that a combination of diuretic and ACE inhibitor will cost approximately Rs 12/day to a patient.

**** The exact burden needs to be worked up.

Fig 4: Pictorial signposts requiring preventive measures

Country shall be saving an estimated amount of Rs 2562.4 billion apart from uncalculated morbidity and mortality by adopting three simple steps namely (a) cessation of smoking, (b) start walking and (c) eating healthy food individually as well as at the community level.

Types of Prevention

Three types of prevention have been advocated by WHO:

Primordial Prevention

Primordial prevention was first suggested by Strasser in 1980, and it begins before the emergence of risk factors in a population. Inclination of healthy life style (No tobacco, Physical activity) has to be implemented at a very young age preferably in the entire population at primordial level to prevent 8 million potential cases. It begins in early life, childhood and/ or adolescence when the health risk begins. Primordial prevention of CAD rests on the following core principles:

1. Prevention of maternal malnutrition.
2. Smoking cessation by mother during fetal life and subsequently by adolescent child.
3. Changes in dietary habits, healthy nutrition.
4. Regular non-occupational physical activity and increased occupational physical activity.
5. Control of mental stress – by yoga and stress management techniques.

As cardiovascular diseases are multifactorial there is a small possibility that even after adopting

healthy life style and polypill approach, some patients may still develop CAD, HTN, and or DM requiring secondary and tertiary care.

Primary Prevention

The preventive strategies aim at reversing the risk factors that have already established themselves in a population. It includes mass screening, health education and control of various risk factors. Prevention may be population based or targeting high-risk groups.

Primary Prevention in High Risk Individuals

1. Cessation of smoking.
2. 150 minutes of moderate physical activity such as brisk walking per week. It reduces the risk of developing T2 DM (8).
3. Dietary interventions: With the rapid urbanization and busy working schedules, the fast food culture has steadily crept in India. Young people get hardly any time for planning healthier meals and the burgers and pizzas provide an easily accessible alternative for their food. Such fast food frenzy is adding to the rapid increase in burden of obesity. The best preventive step to curtail this is either to avoid eating at such outlets or at least eating healthy food at these outlets. Consuming a diet with less than 30% of energy as fat and less than 10% energy as saturated fat. It is also advisable to take wide range of carbohydrate foods rich in dietary fiber and of low glycemic index (cereals, vegetables, legumes and fruits).
4. Maintaining tight glycemic control has been shown to reduce risk of CHD in diabetics (8).
5. Systolic blood pressure reduction by 5 to 10 mm Hg results in 20-30% risk reduction in CHD events in diabetics (8).

Population Based Strategy for Prevention

This approach is based on the community participation and the government's will power to lower the risk factors in the whole population. And in developing countries like India with a huge population, a small change is likely to result in large benefits to the society as compared to large changes in a small number of high risk patients.

STEP	Role of community	Role of government
1	People should eat healthy foods with adequate starch and fiber.	Make healthy foods like vegetables vegetables, fruits cheaper and easily available.
2	People should restrict eating junk food with high saturated fat.	Ban or restrict advertising of unhealthy food or allow them to advertise them with a word of caution.
3	Stop smoking	Smoking should be banned. Alternative to tobacco farming should be given financial support.
4	People should walk and exercise daily.	Government should ensure that there are open and safe spaces for sports and outdoor activities.
5	People should adopt lifestyle intervention programmes like yoga.	Yoga should be a part of regular training at schools and colleges.

Secondary Prevention

Not withstanding the role of antiplatelets, beta-blockers, ACE inhibitors and statins, the role of smoking cessation, healthy diet and physical activity maintains its utility in secondary program also.

1) Antiplatelet agents:

Aspirin: The antiplatelet trialists collaboration showed a 15% reduction in mortality due to vascular reasons and a 30% reduction in non-fatal events. Treatment with aspirin results in significant reduction in restenosis following percutaneous coronary events (45).

2) Beta- blockers:

Beta blockers help by BP control as well as by other mechanisms. Beta-blockers offers benefits beyond blood pressure control especially by their anti ischemic, anti fibrillatory, anti thrombotic and anti atherosclerotic effects, regression of LVH, reduction in heart rate leading to prolongation of coronary diastolic filling.

3) ACE inhibitors:

The principal ACE-inhibitors are captopril, enalapril, ramipril, quinapril. Studies have shown that there is a dose dependent but blood pressure independent reduction in cardio-vascular events with the use of ACE-inhibitors (45).

4) Statins:

They are now considered to be an integral part in the management of CVDs and have surpassed all the other classes of medicine in reducing the incidence of major adverse outcomes of death, heart attack and stroke (46). Amongst various statins atorvastatin is considered to be the goal standard for prophylaxis of cardiac ischemia and stroke (46).

Conclusion

Reviewing the whole scenario of CVD and its health economics it can be safely concluded that a small investment in promoting healthy life style and dietary habits at primordial and primary level will reap larger economic and health benefits to the society run rather than spending colossal amount on curative medicine alone.

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Immunohistochemical Study of Aging Rat Brain – Effect of High Aluminium and Restricted Calcium in Diets

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Abstract

Certain environmental factors which accumulate slowly with age could be associated with the pathogenesis and/or progression of neurodegenerative disorders with associated cytoskeletal abnormalities in neuronal cells. In aluminum (Al) induced encephalopathy, abnormally high phosphorylation states of the neurofilament proteins affect crucial interactions between the cytoskeletal components leading to disruption of integrity of architecture. Dietary factors such as deficiency of micronutrients viz iron, magnesium and calcium have been implicated to favor Al absorption and accumulation in the body and contribute towards the etiopathology of Al associated neurological disorders. Therefore, this study was conducted to examine any alteration in the neuronal cytoskeletal components which could arise due to chronic feeding of moderately high levels of Al to experimental rats maintained on low dietary calcium in their diets. This situation simulates the one found in India where Al vessels are commonly used for cooking and storing food and intake of calcium is well below the optimum. The high number of neurofibrillary tangles found mainly in the substantia nigra region in the brains of rats fed moderately high levels of Al merits attention. It also throws light on the contribution of dietary aluminium in the causation of neurotoxicity at the cellular level.

Key words: Aluminium, Cytoskeletal proteins, Low calcium diet, Neurodegeneration, Neuropathology

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Introduction

Most of the neurological disorders like Senile Dementia of the Alzheimer's type (SDAT) and Parkinson's Dementia (PD) where Aluminum (Al) is implicated as one of the risk factors are associated with aging (1,2). This could probably suggest that certain environmental factors which accumulate slowly with age, (like Al) could be associated with the pathogenesis and/or progression of these neurodegenerative disorders which have been shown to reveal defects in cytoskeletal architecture of the neuronal cells (3).

The neuronal cytoskeletal components viz. NeuroFibrillary Proteins (NFPs) and Microtubules Associated Proteins (MAPs) esp. tubulin associated unit (tau) have been shown to be the main targets of Al toxicity (4, 5). Microtubules are involved in various cellular processes viz. chromosomal movement, axonal transport and stabilization of cell shapes. Tau is more abundant in axons and appears to promote tubulin assembly. Neurofilaments (NFs) are the major structural components of the axons, dendrites and neuronal cell bodies. Apart from maintaining the structural integrity of neuronal cells, NFs are also suggested to be involved in axonal transport of lysosomes, enzymes, neurotransmitter molecules and other metabolites. Normal nerve cells and dendrites contain NF in its nonphosphorylated form.

In Al induced neuropathy, selective impairment in the axonal transport of neurofilaments leads to the accumulation of neurofibrillary material in the perikaryon and in the proximal parts of the axons and dendrites (6).This has been viewed as a consequence of abnormal phosphorylation of the neurofilament proteins which might alter certain crucial interactions between the cytoskeletal proteins (7,8). It has also been proposed that specific interactions of Al with the phosphate groups of the cytoskeletal proteins may lead to the aggregation of highly phosphorylated NFs and MAPs in experimental toxic states (9-11). As an alternative hypothesis for the Al induced neurofibrillary degeneration, inhibition of activated proteolytic degradation of neurofilaments by Al has been proposed (10, 12).

Aluminum has been shown to be an important link between tau protein and Alzheimer's disease (AD) (13), where it is suspected to be a cofactor in the formation of "NeuroFibrillary Tangles" (NFTs) by interacting with paired helical filaments - tau (PHF-tau) (4). Al has been shown to cross the blood-brain barrier as L-glutamate

complex and cause neurotoxicity especially in the hippocampus region (14). Aluminum maltolate treated rabbits also have been reported to show neuronal cell loss and neuronal pathology similar to Alzheimer's disease viz. neurofibrillary pathology (15). A study on explant cultures of cortical neurons, established from rat embryos, showed that aluminum enters neurons and induces possible conformational changes in tau as detected by the Alz-50 antibodies (16). Environmental factors have also been implicated in the pathophysiology of most Al associated neurological disorders (17)

Dietary factors such as citrate, lactate, ascorbate etc. and essential nutrients like iron and calcium have been shown to play an important role in modifying the gastrointestinal absorption of aluminium (18). While citrate promotes formation of soluble Al-complexes and thereby increases its absorption, reduced intake of iron or calcium also increases Al absorption and retention in experimental animals (18, 19). The citrate content of the most vegetarian diets is high and calcium requirements are also not generally met in Indians (20). Based on the available evidence, and the potential risk of Al toxicity in the Indian sub-continent a study was conducted to investigate the effects of chronic toxicity of dietary Al coupled with Ca restriction on the neuronal cytoskeletal proteins in the brain.

Materials and Methods

Experimental Design

A total of 24 weanling, male, Wistar (WNIN) rats were randomly divided into four groups of six animals each. The animals were housed in individual plastic cages with stainless steel wire mesh bottom and lid under recommended conditions of 12 hr light/ dark cycle, 25±1°C room temperature and relative humidity of 55 to 60%. Stainless steel diet cups and plastic water bottles with stainless steel nozzles were used. Rats were given diet daily along with double distilled water and the concentration of Al in the drinking water was negligible. The actual food intake was recorded every day and body weights were taken on a weekly basis. Serum Ca levels were also estimated. The animal's dietary regimens were:

(a) Normal Ca diet - Low Al (0.5% Ca, 20-30 ppm Al).

(b) Normal Ca diet - High Al (0.5% Ca, 220-230 ppm Al).

(c) Low Ca diet - Low Al (0.125% Ca, 20- 30 ppm Al).

(d) Low Ca diet - High Al (0.125% Ca, 220 - 230 ppm Al).

The rats were fed on their respective diets for 18 months and then euthanized. The brain samples were removed and subjected to histopathology study as well as tissue Al levels estimation.

Composition of diet used

Diet component	g/kg diet
Starch	350
Sucrose	350
Casein	200
Groundnut oil	50
Salt mixture	40
Vitamin mixture	10

Choline chloride (1g/kg diet) was first added to the vitamin mixture and then mixed with the diet.

One kg of control diet contained 10g $CaCO_3$ (5g Ca/kg diet). Casein provided 20.4 mg Zn/kg diet (the recommended amount for Zn being 12.0mg/kg diet). Therefore, additional Zn was not added in the salt mix. The amount of CaCO3 was adjusted in the experimental diets so as to obtain 1.25g of Ca per Kg diet (75% restriction of Ca). These diets were found to contain 20 to 30 ppm of Al. Additional $Al_2 (SO_4)_3$ $16H_2O$ was added to the diets to achieve 220 to 250 ppm of Al for the high Al diet groups. Ca: P was maintained at 1.25:1 by manipulating the concentration of KH_2PO_4 in diet. All diets had 0.1% citric acid.

Methodology

Brain Al level estimation was done as per the method of D'Haese *et al* (21) and serum Ca level estimation was done as per the method of Zettner *et al* (22)

For histopathological examination of brain, the tissues were fixed in 10% neutral buffered formalin and paraffin embedded 5 μm thick sections were cut uniformly from 3000μm posterior to bregma so as to obtain the hippocampus and substantia nigra regions of the brain in the same section. The sections were stained with Mayer's Hematoxylin-Eosin (H & E) stain as per the conventional staining protocol. Histological alterations if any (granulation / vacuolation), in these regions were observed under a light microscope. Immunohistochemistry was performed on paraffin embedded sections as per the method of Takeda M. *et al* (23). Briefly, antigen specific primary antibody was applied to deparaffinized hydrated tissue sections. Following a brief wash, the section was incubated with a biotinylated secondary antibody. With the addition of Avidin-Peroxidase reagent, a stable avidin-biotin complex is formed with the bound biotinylated secondary antibody. Sites of antibody deposition are visualized by addition of freshly prepared substrate containing hydrogen peroxide and the electron donor chromogen 3-amino-9-ethyl

carbazole (AEC). Bound peroxidase catalyses the oxidation of AEC to form a rose red to reddish brown insoluble precipitate at antigen sites which is observed under a light microscope.

Mouse derived monoclonal MAP anti-tau (clone Tau-2) antibody and anti-neurofilament-200 (NF-200) for phosphorylated and non-phosphorylated protein (clone-N 52) antibody (Sigma SIH) were employed. The quantification of positively stained cells in substantia nigra region was carried out under light microscope. For quantification of positively stained cells for each of the antibody used, the brain section was screened for 100 cells and the percentage of positively stained cells was calculated. The intracellular neurofibrillary tangle bearing neurons were identified using Beilschowsky's silver stain.

Statistical evaluation

The statistical evaluation of the data was done by analysis of variance and the significance was tested by comparing the least significant differences (Snedecor and Cochran, 1967).

Results

There were no differences in the body weights and food intake between any of the treatment groups while serum calcium levels were on expected lines. Increase in brain aluminium levels was observed (Table 1) and was found to be significantly higher (5.5 ± 0.55 μg/g wet weight) in rats receiving Ca- restricted and high- aluminium diet for eighteen months when compared to rats receiving normal Ca and high aluminium diet (3.9 ± 0.36 μg/g wet weight) while low Al levels with and without Ca restriction were comparable. The light microscopic examination of brain sections showed no alteration in the gross histology (viz. granulation/vacuolation) in rats fed on Ca restricted diet with low/high

Al supplementation (Fig. 1) Our earlier short term study of six and twelve months duration of Al exposure

Table 1: Effect of dietary calcium restriction on brain aluminium levels.

SI.No.	Groups (n=6)	Al level (μg/g) wet weight
1.	Normal Ca + Low Al	2.9 ± 0.54^{a}
2.	Normal Ca + High Al	3.9 ± 0.36^{a}
3.	Low Ca + Low Al	2.8 ± 0.45^{a}
4.	Low Ca + High Al	5.5 ± 0.55^{b}

Values with different superscripts are significantly different ($p < 0.01$).

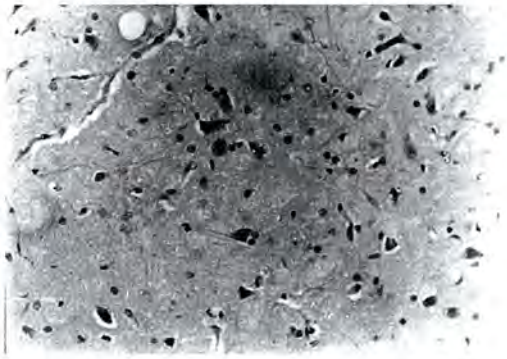

Fig. 1 : Substantia Nigra region of the brain. H & E X 250

(unpublished) showed no cytoskeletal abnormalities in rats fed on calcium restricted diet with low / high Al supplementation. However, the brain Al levels were higher in high Al fed rats and hence it was decided to continue the feeding schedule for 18 months. Ca-restricted rats receiving high Al for 18 months showed an increase in the number of tangle bearing neurons in substantia nigra, as revealed by light microscopic examination of H & E stained brain sections and also confirmed by Bielchowsky's silver stain methods (Fig. 2 and Table 2). No significant changes were observed with respect to any other group of rats.

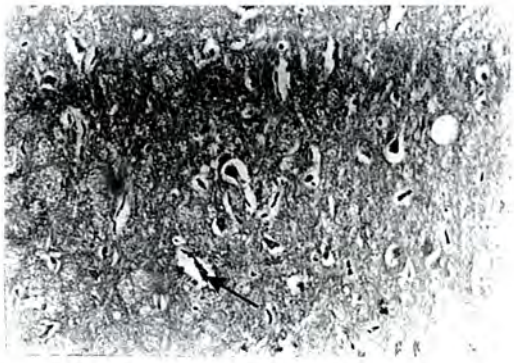

Fig. 2 : Neurofibrillary tangle bearing neurons (arrow) in the Substantia Nigra region - Beilschowsky's stain X 250.

The immunoreactivity to monoclonal antibodies against NF-200 and MAP-Tau was found to be higher in substantia nigra region of rats which received Ca-restricted diets supplemented with high Al. With NF-200

Table 2 : Number of Neurofibrillary Tangle (NFT) bearing neurons in substantia nigra region

Sl.No.	Groups (n=6)	No. of NFT bearing neurons
1.	Normal Ca + Low Al	7.0 ± 1.0^{a}
2.	Normal Ca + High Al	7.0 ± 2.0^{a}
3.	Low Ca + Low Al	5.0 ± 2.0^{a}
4.	Low Ca + High Al	23.0 ± 3.0^{b}

Values with different superscripts are significantly different ($p < 0.01$)

immunoreactivity, the number of positively stained cells in these animals was also comparatively higher than all other groups. However, with respect to MAP tau, both high & low Al exposure with low Ca diets was significantly different for positive cells as compared to normal Ca diets. (Figs. 3-5 and Table 3).

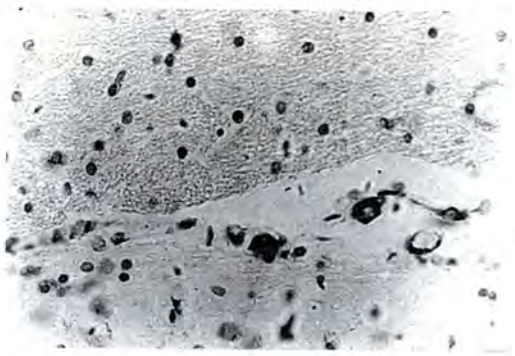

Fig. 3 : Negative control brain section for the immunohistochemical detection of NF - 200 and MAP - Tau X 400.

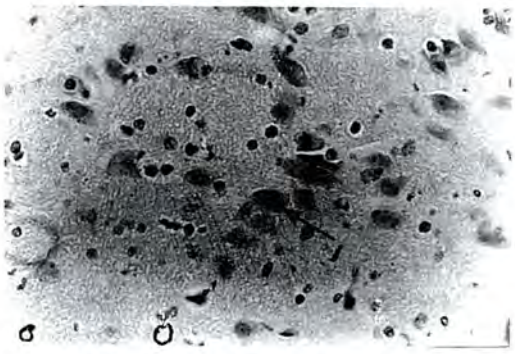

Fig. 4 : NF - 200 positive cells (arrow) in the hippocampus region X 400.

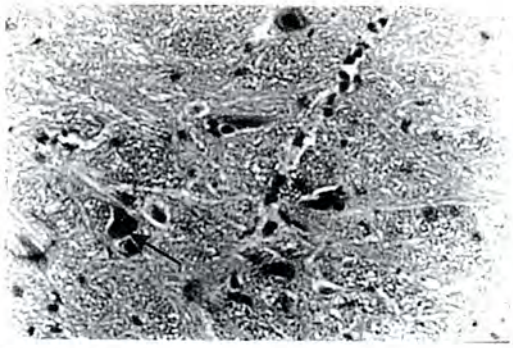

Fig. 5 : MAP - Tau positive cells (arrow) in the substantia nigra region X 400.

Table 3 : Number of Positive cells (%) representing immunoreactivity for monoclonal antibodies to MAP-Tau and NF-200

SI.No.	Groups (N=6)	Positive cells (%)
	Tau	NF - 200
1.	Normal Ca + Low Al	12 ± 3^a 10 ± 4^a
2.	Normal Ca + High Al	11 ± 3^a 9 ± 3^a
3.	Low Ca + Low Al	20 ± 4^b 11 ± 2^a
4.	Low Ca + High Al	25 ± 5^b 21 ± 4^b

Values with different superscripts are significantly different ($p < 0.01$)

Discussion

Abnormalities in the neuronal cytoskeleton in SDAT have been extensively reported in the literature. Cytoskeletal proteins are the potential targets of Al toxicity and hyperphosphorylation of tau and formation of NFTs has been observed in the Al-associated dementias (24, 25). The aggregation of both these protein components in the neurodegenerative disorders with which Al is associated, could be attributed to the specific interactions of Al with the phosphate groups of the highly phosphorylated cytoskeletal proteins-MAPs and NFPs (26, 27).

Earlier studies from our laboratory have shown that age dependent accumulation of Al in the rat brain was influenced by the levels of calcium in the diet (unpublished data). Another study reported earlier showed increased brain Al levels when fed with 200mg/kg/day of $AlCl_3$ by gavage for 8 weeks (28). Also literature evidence indicates enhancement of neurotoxicity potential of Al in states of Ca deficiency (29). Therefore, this study was conducted to understand the effect of moderately high levels of Al on the cytoskeletal components when dietary Ca was low. Earlier histochemical and immunocytochemical procedures adopted on rat brains with Al injections directly into the brain for 5 days, looked into the inflammatory response and cholinergic terminals in the hippocampus (30). We also observed that the changes seen were in line with those reported by other workers, although in different experimental models (29, 31). Moreover, this situation simulates the one widely prevalent in India where Al vessels are commonly used for cooking and storing food and the intake of essential nutrients like iron and calcium is below optimum (20,32). This issue was also stressed in an earlier work by Liu J *et al* (33).

In the present study, high number of NFTs was found mainly in the substantia nigra region, and Al being an integral part of the NFTs has been proved by a study of rabbits (34) and microprobe studies (35). Since Al enters the brain via transferrin endocytosis (36), it is likely that levels of Al could be much higher in areas such as substantia nigra and basal nuclei where the density of transferrin receptors is high. The neurodegeneration in this region is associated with certain neuronal disorders like Parkinson's dementia (24, 37). Earlier Garruto *et al* (31) obtained similar results with regard to Al induced

changes in cytoskeletal components in cyanomolgous monkeys maintained on chronic low dietary Ca supplemented with very high Al levels (150 mg/day). They have observed Al deposition and neurodegenerative changes, comparable to those seen in spinal cord, brain stem, substantia nigra and cerebrum in Amyotrophic lateral neurons. Aluminium binding to MAP-Tau and NF-200 protein and paired helical filaments (PHFs) has been shown *in vitro* also (38, 39). Decrease in total number of synapses was seen in a study done on male rats of 18 months age and fed Al at a dose of 100mg/kg/day for 100 days (40). An important observation in our study is the development of pathological changes even though the dietary Al exposure is not remarkably high as used in earlier studies. The Al-associated neuronal degeneration could probably be due to a longer period of Al exposure in vivo rather than a higher acute exposure. Therefore, Al-Ca interactions at the gut level appear to be important in determining Al-neurotoxicity as suggested by earlier workers also (41). As has been stated that select human populations are at risk of Al neurotoxicity (42), the same could be inferred from our study where long term exposure to Al caused changes in the neuronal milieu.

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Procedural Sedation in Children : An Overview

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Abstract

Modern day practice is aimed not only at treatment, but to provide the management at minimal discomfort to the patients. This entails various diagnostic and short therapeutic procedures that can be done at bedside or radiology suites or minor operation theaters. The discomfort to the patient includes the pain caused by the procedure and discomfort in lying still while the procedure is being performed. The anxiety is much more in pediatric patients that require administration of medication to be alleviated. The development of safe anesthetic medications has caused widespread use of such medications. The use of these medications is not free of complications and thus, various authorities have developed guidelines for safe use of procedural sedation in office setting. We here discuss the need for procedural sedation, the drugs available and the guidelines for their safe use.

Key words: Procedural sedation and analgesia (PSA), minimal sedation, moderate sedation, conscious sedation, deep sedation, general anesthesia, rescue.

Introduction

Various procedures in the ward and investigation suites require the patient to be calm and quiet. This may require an intervention (medication) especially so in children who by nature are more anxious and uncooperative than adults. The aims of analgesia, anxiolysis and amnesia are achieved by administering medications including hypnotics and anesthetic medication. The medication given aims at providing

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analgesia, anxiolysis and amnesia. By guidelines, the procedure should be under direct control or under supervision of anesthesia team. The paucity of qualified anesthetist, especially so in developing countries like India, mandates that physicians other than anesthetists take more responsibility in providing procedural sedation. The safety for the procedure has been addressed to by various authorities – American Academy of Pediatrics (AAP), American Society of Anesthesiologists (ASA), Joint Commission on the Accreditation of Healthcare Organizations (JCAHO), and American College of Emergency Physicians (ACEP) – by formulating guidelines for the physicians and other workers, who are authorized to provide procedural sedation. The development of short-acting sedatives, improved monitoring, and new regulatory requirements have led to the evolution of new paradigms of safe, effective, and resource-efficient systems for providing procedural sedation outside the operating rooms by anesthesiologists and non-anesthesiologists (including pediatricians and pediatric surgeons).

Definitions

Procedural sedation is defined by the ACEP as "a technique of administering sedatives or dissociative agents with or without analgesics to induce a state that allows the patient to tolerate unpleasant procedures while maintaining cardio-respiratory function. Procedural sedation and analgesia (PSA) is intended to result in a depressed level of consciousness that allows the patient to maintain oxygenation and airway control independently." (1)

AAP and ASA have jointly formulated guidelines and amended it from time to time for safe administration of procedural sedation (2).

The procedural sedation requires mild to moderate sedation. As the effect of the drugs used is a continuum and the depth of sedation can vary with different doses and blood concentration, it is vital for the personnel providing the procedural anesthesia to accurately identify the plane of sedation so as to avoid complications and associated morbidity and mortality. Thus, ASA has provided the definition for levels of sedation/ analgesia.

"*Minimal Sedation (Anxiolysis)* is a drug-induced state during which patients respond normally to verbal commands. Although cognitive function and coordination may be impaired, ventilatory and cardiovascular functions are unaffected." The patient

can maintain his airway and does not require any support.

"*Moderate Sedation / Analgesia* ("*Conscious Sedation*") is a drug-induced depression of consciousness during which patients respond purposefully to verbal commands, either alone or accompanied by light tactile stimulation. No interventions are required to maintain a patent airway, and spontaneous ventilation is adequate. Cardiovascular function is usually maintained." This is the plane required for most invasive procedures done outside the operation suites.

"*Deep Sedation / Analgesia* is a drug-induced depression of consciousness during which patients cannot be easily aroused but respond purposefully following repeated or painful stimulation. The ability to independently maintain ventilatory function may be impaired. Patients may require assistance in maintaining a patent airway, and spontaneous ventilation may be inadequate. Cardiovascular function is usually maintained." This plane if achieved needs to be identified and appropriate action taken to prevent a mishap.

"*General Anesthesia* is a drug-induced loss of consciousness during which patients are not arousable, even by painful stimulation. The ability to independently maintain ventilatory function is often impaired. Patients often require assistance in maintaining a patent airway, and positive pressure ventilation may be required because of depressed spontaneous ventilation or drug-induced depression of neuromuscular function. Cardiovascular function may be impaired." This plane of sedation/ anesthesia is used for major procedures in operation suite under controlled condition.

"***Rescue*** of a patient from a deeper level of sedation than intended is an intervention by a practitioner proficient in airway management and advanced life support. The qualified practitioner corrects adverse physiologic consequences of the deeper-than intended level of sedation (such as hypoventilation, hypoxia and hypotension) and returns the patient to the originally intended level of sedation." Any person who provides procedural sedation must be proficient with rescue.

Who should deliver "procedural sedation"?

The procedural sedation should be administered by a qualified anesthetist or any qualified non-anesthesiologist sedation practitioner (physician), or by a qualified supervised sedation professional. The qualification

requirement has been detailed by ASA and requires proficiency with procedure to take informed consent, identifying the patients who shall require specialist anesthetist, the drugs used for sedation, understanding of level of sedation, monitoring capabilities and capable of 'rescue'. The presence of professionals capable to handle emergencies is mandatory. Various studies have shown that professionals managing the emergency department and pediatricians are well-suited to provide moderate to deep sedation (3). Cote *et al* analyzed the adverse events in hospital based and non-hospital based procedural sedation in children and documented that procedural sedation provided by person other than properly trained physicians and dental surgeons resulted in increased serious adverse events including death and permanent neurological deficit (4). The factors responsible for adverse event were inadequate pre-sedation evaluation, improper dosage, lack of knowledge of drug interactions, lack of monitoring, lack of resuscitation equipment and rescue training and earlier discharge from the facility.

Indications

The procedural sedation is required for a variety of diagnostic and therapeutic procedures. This is especially true for children less than 6 years old and children with developmental delay (5).

Diagnostic:

1. Non-invasive – CT scan, MR imaging. The anxiolysis is required in children for claustrophobia and so that child does not move while the scanning is taking place.
2. Invasive – Radiological procedures like central line placement, voiding cystography, filling cystometry, fine needle aspiration cytology, biopsy, endoscopy, lumbar puncture, etc. The sedation requires anxiolysis and analgesia. The deeper plane is required.

Therapeutic:

1. Minor procedures: Suturing of wounds, abscess drainage, radiology guided intervention procedures like percutaneous nephrostomy, fracture reduction, dental procedures, wound debridement, burn dressings and other procedures done under local anesthesia.
2. Radiotherapy
3. Endoscopic shock wave lithotripsy.

The medications for procedural sedation are given with following goals:

1. guard the patient's safety and welfare;
2. minimize physical discomfort and pain;
3. control anxiety, minimize psychological trauma, and maximize the potential for amnesia;
4. control behavior and/or movement to allow the safe completion of the procedure; and
5. return the patient to a state in which safe discharge from medical supervision, as determined by recognized criteria.

Procedure to be followed while delivering procedural sedation

The patient should be evaluated pre-sedation and decide who can provide sedation to the patient. A qualified person or an anesthesiologist should be called in case of patients who are ASA grade 3 or above, child with special needs, difficult airway and tonsillar hypertrophy (higher Mallampati score). The ASA physical status classification is shown in table 1.

Other high risk factors include snoring, stridor, sleep apnea, cranio-facial malformation, history of airway

Table 1 : ASA Risk stratification system.

P1	A normal healthy patient
P2	A patient with mild systemic disease (eg, controlled reactive airway disease)
P3	A patient with severe systemic disease (eg, a child who is actively wheezing)
P4	A patient with severe systemic disease that is a constant threat to life (eg, a child with status asthmaticus)
P5	A moribund patient who is not expected to survive without the operation (eg, a patient with severe cardiomyopathy requiring heart transplantation).
P6	A declared brain-dead patient whose organs are being removed for donor purposes

All classes are given a suffix E if it is an emergency.

difficulty, vomiting, bowel obstruction, gastro-esophageal reflux, pneumonia or oxygen requirement, reactive airways disease, hypovolemia, cardiac disease, sepsis, altered mental status and history of sedation failure. One should formulate a plan and direct the personnel who shall provide the sedation. A written informed consent is to be obtained. Pre-anesthesia preparation should be followed. The decision of fasting depends on the urgency of the procedure and the kind of feeding. Rather, the need for pre-procedural fasting has been questioned in recent series showing no increase in adverse events in patients not meeting fasting guidelines (6, 7). It has been observed that it is more difficult to sedate fasting child. Although there is insufficient published evidence for supporting/ refuting safe fasting periods before anesthesia, ASA task force has formulated following guidelines (8):

1. Clear liquids – 2 hours
2. Breast milk – 4 hours
3. Infant Formula – 6 hours
4. Non-human milk – 6 hours
5. Light meal – 6 hours.

The sedation is given by the designated qualified professional with adequate monitoring. The patient is discharged by the physician with responsible adult after explaining written post-op and follow-up instructions.

Pre-anesthesia workup

Detailed history of medical status of the child should be taken. The guardian should be asked the history suggestive of major abnormalities in other major body systems. Prior history of sedation/ anesthesia, any adverse events, any drug or other allergies and current medication(s) should be asked for.

The child should be evaluated for vital signs. Heart and lung assessment and airway assessment (Mallampati score) should be done (9) (Table 2).

The procedure suite should be equipped with suction, oxygen, airway management equipment, resuscitation medication and equipment and antidotes for sedation drugs.

The child should have a functional IV access before providing sedation. The insertion of IV line itself is a painful procedure and various modalities are used to reduce the pain. These include Eutactic Mixture of Local Anesthetics – Lidocaine and procaine (EMLA) (10, 11), topical liposomal 4% lidocaine cream (12), lidocaine iontophoresis (13, 14), intradermal lidocaine and vapo-

Table 2 : Mallampati score

Class I:	Soft palate, uvula, fauces, tonsillar pillars visible (no difficulty in intubation).
Class II:	Soft palate, uvula, fauces visible (no difficulty in intubation).
Class III:	Soft palate, base of uvula visible (moderate difficulty in intubation).
Class IV:	Hard palate only visible (severe difficulty in intubation).

coolant sprays (15). In Operation Theater, the child can be induced using inhalational agents (isoflurane, sevoflurane) and the IV line established later. During the procedure the patient is to be monitored as per protocols (described later).

Drugs used in Pediatric population (Table 3)

Benzodiazepines bind to the GABA (γ-aminobutyric acid) receptors in the CNS and potentiate GABA mediated chloride influx. This potentiation of the inhibitory effect of GABA is responsible for the properties of benzodiazepines – sedation, amnesia, anxiolysis, anticonvulsant and respiratory depressant. The higher doses provide better amnesia (16).

Midazolam has a fast onset of action (1-2 min) and short half life (2.7 hours). Midazolam is hydroxylated to 1-hydroxymidazolam in liver (has 10% activity). The diazepam, on the other hand, has extremely long half-life (0.8-2.25 days) as do its metabolites – N-desmethyldizepam (1.6-4.2 days) and nordiazepam (about 8 days). Lorazepam has delayed onset of action (15-20 min) and longer half-life (6-8 hours) as compared to midazolam. Thus, midazolam is most commonly used benzodiazepine for procedural sedation. (17)(Dose: Intravenous: 0.05-0.1 mg/kg IV 3 min before procedure, not to exceed a total cumulative dose of 0.4 mg/kg or 6 mg; Intramuscular: 0.1-0.2 mg/kg IM 30-45 min before procedure; Oral: 0.25-0.5 mg/kg PO 30-45 min before procedure; Intranasal: 0.2-0.6 mg/kg/dose inhaled intranasally 10 min before procedure; Rectal: 0.3-0.5 mg/kg/dose PR 30-45 min before procedure). The main side-effect of benzodiazepines is respiratory and cardiovascular depression that is accentuated by simultaneous use of opioids (18) (reduce dose by 30-50% in combination with opioids). In case of

Table 3 : Drugs used in Procedural Sedation

Drug	Mechanism of Action	Dosage	Side Effects	Antidote	Onset of Action	Duration of Action	Contra-indication	Comments
I. Benzodiazepines								
Midazolam	Potentiates GABA receptors in CNS	*Intravenous:* 0.05-0.1 mg/kg IV 3 min before procedure, not to exceed a total cumulative dose of 0.4 mg/kg or 6 mg; *Intramuscular:* 0.1-0.2 mg/kg IM 30-45 min before procedure; *Oral:* 0.25-0.5 mg/kg PO 30-45 min before procedure; *Intranasal:* 0.2-0.6 mg/kg/ dose inhaled intranasally 10 min before procedure; Rectal: 0.3-0.5 mg/kg/dose PR 30-45 min before procedure.	Respiratory depression, cardiovascular depression. Respiratory depression more when used with opioids.	Flumazenil (0.01 mg/kg/dose IV infused over 15 sec; not to exceed 0.2 mg/dose; may repeat every min; not to exceed total cumulative dose of 0.05 mg/kg or 1 mg (whichever is lower).	1-2 min	20-30 min		Fluctuations in Vital Signs, apnea, headache, N/V, coughing, over-sedation, drowsiness, amnesia (positive effect)
Diazepam		0.04-0.1 mg/kg bolus, titrate in increments of 1-2 mg to desired effect (IM very painful)	Blood dyscrasias, thrombo-phlebitis, sedation, hypotension, bradycardia, respiratory istress, seizures		5-10 min	2-3 hours	Hyper-sensitivity, acute bronchial asthma, or airway obstruction	May initiate histamine release
Nitrazepam		5-10 mg				5-10 hours	COPD patients	Increase in nightmares and behavioral alterations
Lorazepam		Oral 50 µg/kg			15-20 min	6-8 hours		

Table 3 (Contd.)

Drug	Mechanism of Action	Dosage	Side Effects	Antidote	Onset of Action	Duration of Action	Contra-indication	Comments
Flurazepam		Oral 0.2-0.5 mg/kg			15-25 min	7-8 hours		Paradoxical stimulation, irritability and sweating
II. Opioids								
Morphine	Bind to specific receptors in CNS and increase pain threshold, alter pain perception and inhibit ascending pain pathways.	0.05-0.1mg/kg slowly over 4 min.	Respiratory depression, cardiovascular depression, pruritis, flushing	Naloxone (Post anesthetic reversal): 0.005-0.01 mg/kg IV/IM, may repeat q2-3 min; Opiate intoxication: 0.01-0.1 mg/kg dose IV/IM, may repeat every min; not to exceed 2 mg/dose)	3-5 min	3-4 hours	Hypersensitivity, acute bronchial asthma, or airway obstruction	
Meperidine		1mg/kg, titrate to desired effect max: 100mg	Hypotension, respiratory depression, truncal rigidity, seizures		3-10 min	2-4 hours	Hyper-sensitivity, MAO inhibitors	Meperidine is not used because its metabolite has neurotoxic effects.
Fentanyl		1 µg/kg/dose IV; if needed, may repeat by 1 µg/kg increments; not to exceed total cumulative dose of 4 µg/kg	Hypotension, respiratory depression, truncal rigidity, seizures		1-2 min	2-4 hours	Hyper-sensitivity	Unlike morphine, fentanyl has minimal cardiovascular depression and hypotension rarely occurs.
III. Barbiturate								
Pheno-barbital	Potentiates GABA action	*Intravenous:* 1-2 mg/kg/dose IV; if needed may repeat dose; not to exceed a cumulative dose of 6 mg/kg or 150-200 mg; *Intramuscular:* 1-6 mg/kg IM; not to exceed						

Table 3 (Contd.)

Drug	Mechanism of Action	Dosage	Side Effects	Antidote	Onset of Action	Duration of Action	Contra-indication	Comments
		100 mg/dose; *Oral:* 4-6 mg/kg PO; not to exceed 100 mg/dose		Not available				Paradoxical excitation may occur. Induce hepatic microsomal enzymes.
Metho-hexital		25 mg/kg/dose PR 15 min before procedure; not to exceed 500 mg/dose; Intravenous 1mg/kg					Porphyria, hypersensitivity	
IV. Imidazoline agonist								
Dexmedito-midine	Alpha 2 agonist	1 mg/kg IV given as a 10 minute infusion then 0.2-0.7 mg/kg/hr OR 2.5 mg/kg IV given at least over 2 minutes	Arrhythmia, cardiovascular instability, nausea, vomiting, hypoxia.	Atipamezole (3750-5000 $\mu g/m^2$ IM)	30 min	2-3 hours		
Etomidate	Increase affinity of GABA receptors for GABA	0.1-0.2 mg/kg slow IV push over 30-60 sec	Reduces seizure threshold. Reduces cerebral blood flow. Causes adrenocortical suppression, myoclonic involuntary movements, pain on injection nausea and vomiting.		<1min	3-5 min		Cardiovascular stability. Decreases intraocular tension. Causes nausea and vomiting.
V. Inhalational Anesthetics								
Halothane	Progressive depression of CNS, Exact mechanism not known.		Hepato-toxicity					Has sweet odor. An intermediate solubility in blood combined with a high potency permits rapid onset and recovery from anesthesia.

Table 3 (Contd.)

Drug	Mechanism of Action	Dosage	Side Effects	Antidote	Onset of Action	Duration of Action	Contra-indication	Comments
Sevoflurane			Compound A formation with soda lime can lead nephro-toxicity, hepatotoxicity.					Is non-pungent, has minimal odor, produces bronchodilation and causes least degree of A/W irritation among currently available volatile/ inhaled anesthetics.
Nitrous oxide	Endorphins may be involved.	As adjunct (in low concentration – 50%) or isolated hypnotic (higher concentration – 70%)					Middle ear surgery, gut surgery, air embolism.	It increases the volume of gas in space – pneumothorax, air embolus, increases pressure - sinuses, middle ear, pneumo-encephalo-graphy.
VI. Miscellaneous								
Ketamine	Dissociative anesthesia - dissociation between thalamo-cortical and limbic system	Intravenous: 1-2 mg/kg loading dose IV; 0.25-1 mg/kg IV q10-15min; administer slowly, not to exceed 0.5 mg/kg/min; Intramuscular: 2-5 mg/kg/dose IM; Oral: 6-10 mg/kg/dose PO mixed in cola or other beverage 30 min before procedure	It increases bronchial and salivary secretions. Increased skeletal muscle tone.	Not available	IV - within 1 min. IM - 3-5 min.	IV duration of action lasts about 5-10 min. IM - 20-30 min.	It is relatively contraindicated in hypertensive patients and in patients with coronary artery disease, open eye injury and raised intracranial tension.	It does not affect cough reflex or cause respiratory depression, so is favored in emergency non-fasting state.
Propofol	Unknown; ? through GABA receptors	1-1.5 mg/kg IV loading dose; 0.25-0.5 mg/kg IV q3-5min or 50-150 µg/kg/min continuous IV infusion	Respiratory and cardiovascular depression. Causes irritation and burning on IV administration.	Not available	<1min	3-10 min		Preferred because of short duration of action and short hospital stay.

Table 3 (Contd.)

Drug	Mechanism of Action	Dosage	Side Effects	Antidote	Onset of Action	Duration of Action	Contra-indication	Comments
Chloral hydrate	Exact mechanism not known.	25-75 mg/kg/dose PO/PR; not to exceed 1 g/dose (infants) or 2 g/dose (children); administer 30 min before procedure	Respiratory depression.	Not available	30-40 min	2 hours	ASA class III, Leigh encephalopathy, tonsillar and adenoidal hypertrophy, obstructive sleep apnea, severe hepatic or renal impairment.	Unpredictable result. May cause nausea and vomiting. Induces hepatic cytochrome P450, irritant to skin and gastric mucosa, burning taste.
Triclofos	Hydrolysed in body to trichloro-ethanol which is probably the active metabolite.	Upto 1 year – 25 – 30 mg/kg; 1 – 5 year – 250 – 500 mg; 6 – 12 years – 500 mg – 1 g; adults and children > 12 years – 1 – 2 g.					Cardiac disease, hepatic impairment, hypersensitivity, nursing mother and pregnancy, renal impairment.	

overdose, flumazenil may be given (0.01 mg/kg/dose IV infused over 15 sec; not to exceed 0.2 mg/dose; may repeat every min; not to exceed total cumulative dose of 0.05 mg/kg or 1 mg (whichever is lower).

Opioids bind to specific receptors in CNS and increase pain threshold, alter pain perception and inhibit ascending pain pathways. Morphine is long acting with significant respiratory depression. Fentanyl is the most favored opioid because of early onset of action (1-2 min) and short half-life (2-4 hours). Also, unlike morphine, fentanyl has minimal cardiovascular depression and hypotension rarely occurs. It should be given slow IV as rapid push may cause chest wall rigidity and apnea. (Dose: 1 μg/kg/dose IV; if needed, may repeat by 1μg/kg increments; not to exceed total cumulative dose of 4 μg/kg). Meperidine is not used because its metabolite has neurotoxic effects.

Naloxone (antidote – partial agonist) should be at hand while administering opioids so that it can be given in case of severe respiratory depression. (Post-anesthetic reversal: 0.005-0.01 mg/kg IV/IM, may repeat q2-3min; Opiate intoxication: 0.01-0.1 mg/kg dose IV/IM, may repeat every min; not to exceed 2 mg/dose).

Ketamine has dissociative and amnesic properties. It does not affect cough reflex or cause respiratory depression, so is favored in emergency

non-fasting state. It causes cardiovascular and respiratory stimulation (transient respiratory depression if injected too rapidly or in high doses) with normal or increased skeletal muscle tone. It is thus relatively contraindicated in hypertensive patients. It increases bronchial and salivary secretions, so should be pre-medicated with anti-cholinergics like glycopyrrolate. Onset of action for intravenous (IV) administration is within 1 min, and duration of action lasts about 5-10 min. If administered intramuscularly (IM), the onset of action is observed between 3-5 min, and duration of procedural conditions lasts about 20-30 min. (Dose: Intravenous: 1-2 mg/kg loading dose IV; 0.25-1 mg/kg IV q10-15min; administer slowly, not to exceed 0.5 mg/kg/min; Intramuscular: 2-5 mg/kg/dose IM; Oral: 6-10 mg/kg/dose PO mixed in cola or other beverage 30 min before procedure). The relationship between serum concentration and dissociative effect is poorly defined in children (19). Roback *et al* showed that 4mg/kg IM ketamine was more effective than 2mg/kg IV although it also had more incidence of vomiting and required prolonged post-procedural monitoring (20). It is not favored in adults as sole anesthetic due to emergence delirium (rarely seen in children below 1 year of age). As no specific antidote is available, pharmacologic effects are not reversible. The safety profile has been studied and ketamine has been recommended by various authors for procedural sedation in children (21- 23).

Propofol is a sedative with no analgesic action. The mechanism of action is unknown, although it appears to mediate through GABA receptors. It is ultra-short acting (onset <1min; duration 3-10min). As the plane of sedation rapidly progresses to deep sedation and can cause cardiovascular depression and hypotension, close monitoring is essential. (Dose: 1-1.5 mg/kg IV loading dose; 0.25-0.5 mg/kg IV q3-5min or 50-150 μg/kg/min continuous IV infusion). It causes irritation and burning on IV administration. No specific antidote is available. Propofol is favored by most anesthetists for short procedures because of faster, smoother recovery, lack of need for prolonged post-procedure monitoring and short hospital stay (24, 25). But because of transient respiratory depression and hypotension it is safe only in monitored environment (26, 27). In a cost-effectiveness study, propofol + fentanyl was the most cost-effective regimen followed by axillary block, ketamine + midazolam and fentanyl + midazolam respectively (28).

Etomidate is an ultra–short-acting nonbarbiturate hypnotic. It produces

rapid induction (onset <1min; duration 3-5min) without histamine release and with minimal cardiovascular and respiratory effects. As with ketamine or barbiturates, etomidate transiently lowers cerebral blood flow by 20-30% and slightly reduces intracranial and intraocular pressure. It has no analgesic properties. It may cause nausea or vomiting and reduces seizure threshold. (Dose: 0.1-0.2 mg/kg slow IV push over 30-60 sec).

Barbiturates have been used since long for as hypnotics. They are particularly useful for procedures requiring immobilization like radiologic procedures. Paradoxical excitation may occur. (Dose: Phenobarbital: Intravenous: 1-2 mg/kg/dose IV; if needed may repeat dose; not to exceed a cumulative dose of 6 mg/kg or 150-200 mg; Intramuscular: 1-6 mg/kg IM; not to exceed 100 mg/dose; Oral: 4-6 mg/kg PO; not to exceed 100 mg/dose; Methohexital: 25 mg/kg/dose PR 15 min before procedure; not to exceed 500 mg/dose; Intravenous 1mg/kg) (29). No specific antidote is available.

Chloral hydrate is oral sedative used earlier. It has unpredictable effect, paradoxical hyperactivity may occur, may cause nausea and vomiting, respiratory compromise has resulted in permanent neurological damage and deaths. (Dose: 25-75 mg/kg/dose PO/PR; not to exceed 1 g/dose (infants) or 2 g/dose (children); administer 30 min before procedure). It should no longer be used especially in patients with ASA class III, Leigh encephalopathy, tonsillar and adenoidal hypertrophy, obstructive sleep apnea. Hoffman *et al* documented that chloral hydrate provides inadequate sedation (esp. deep sedation) with increased incidence of side-effects (2).

Methoxyflurane has been studied in a small group of 14 patients as analgesic in emergency department delivered through a hand-held device. It was concluded from the study that methoxyflurane can be used with minimal side effects in patients after extremity trauma. It appears less useful as a procedural agent when patients are unable to anticipate and achieve a sufficient level of analgesia before painful stimulus infliction. Pre- and intraprocedure coaching is an important aspect of its use especially if initial pain scores are low.

Nitrous oxide is an inhalational agent that has been used as adjunct (in low concentration – 50%) or isolated hypnotic (higher concentration – 70%) with minimal side effects. (30).

Dexmedetomidine is a new oral drug used as pre-medication before anesthesia and procedural sedation (including IV line insertion) and has shown promising results (31).

High concentrations of sucrose have been used as adjunct in neonates. It has been shown to suppress EEG responses in brain in response to bedside procedures like heel-prick. The exact mechanism is unknown. (Dose: 0.012 to 0.12 g (0.05– 0.5 mL of 24% solution)).

For older and cooperative children, other non-pharmacological modalities, such as parental presence, hypnosis, distraction, topical local anesthetics, and guided imagery, may reduce the need for or the needed depth of pharmacologic sedation. Use of distraction techniques is effective in reducing situational anxiety in children more than 10 years old and lowering parental perception of pain distress in younger children. (32- 34).

Monitoring during Procedural Sedation

The level of sedation should be carefully monitored. It is common for children to pass from the intended level of sedation to a deeper, unintended level of sedation (5). Various scoring systems have been developed to accurately differentiate the depth of sedation objectively. These scores include Children's Hospital of Wisconsin sedation scale (Table 4) (2), Ramsay Sedation Scale Score (Table 5) and Bispectral Index Monitor (35, 36).

Table 4 : The Children's Hospital of Wisconsin Sedation Scale

Sedation Classification	Sedation Score	Description
Inadequate	6	Anxious, agitated, or in pain
Minimal–conscious	5	Spontaneously awake without stimulus
Conscious–moderate	4	Drowsy, eyes open or closed, but easily arouses to consciousness with verbal stimulus
Moderate–deep	3	Arouses to consciousness with moderate tactile or loud verbal stimulus
Deep	2	Arouses slowly to consciousness with sustained painful stimulus
	1	Arouses, but not to consciousness, with painful stimulus
Anesthesia	0	Unresponsive to painful stimulus

Table 5 : Ramsey Sedation Scale Score

Sedation score	Clinical response
1	Fully awake
2	Drowsy but awakens spontaneously
3	Asleep but arouses and responds appropriately to simple verbal commands
4	Asleep, unresponsive to commands, but arouses to shoulder tap or loud verbal stimulus
5	Asleep and only responds to firm facial tap and loud verbal stimulus
6	Asleep and unresponsive to both firm facial tap and loud verbal stimulus

All patients need continuous monitoring for the color (to assess adequacy of perfusion), respiratory excursions (adequate ventilation), vitals monitoring and pulse oximetry. The high risk patients should also have ECG monitoring and capnography. Microstream capnography is a new armamentarium for the personnel monitoring the patient given sedation that monitors pCO_2 in a non-intubated patient to pick up the hypoventilation early and intervene before hypoxemia manifests (37). In case the patient cannot be directly observed (like in MRI suite/ radiotherapy), the monitors with audible alarms are mandatory.

When to Discharge a Patient after Procedural Sedation

ASA in its guidelines on procedural sedation delegates the discharge responsibility with the physician but the exact safe timing of discharge after procedural sedation has not been studied in detail (Table 6). In a study on 1367 patients, Newman *et al* found 92% of side effects occurred during the procedure with median time 2 min after final medication dose. Post-procedural side-effects occurred only in those patients who had observed these effects during the procedure. They found that serious side-effects rarely occur after 25 minutes of last dose (38). Broadly it can be said that the child should be monitored in the recovery till he attains the level of consciousness same as pre-sedation level or that the child remains awake for 20 minutes in quiet environment (5). The discharge criteria recommended by AAP are:

1. Cardiovascular function and airway patency are satisfactory and stable.

Table 6 : Aldrete Recovery Score

Activity

- Voluntary movement of all limbs to command — 2 points
- Voluntary movement of 2 extremities to command — 1 point
- Unable to move — 0 points

Respiration

- Breathe deeply and cough — 2 points
- Dyspnea, hypoventilation — 1 point
- Apneic — 0 points

Circulation

- BP ± 20 mm Hg of preanesthesia level — 2 points
- BP ±20-50 mm Hg of preanesthesia level — 1 point
- BP ±> 50 mm Hg of preanesthesia level — 0 points

Consciousness

- Fully awake — 2 points
- Arousable — 1 point
- Unresponsive — 0 points

Color

- Pink — 2 points
- Pale, blotchy — 1 point
- Cyanotic — 0 points

Total score must be > 8 at conclusion of monitoring.

2. The patient is easily arousable, and protective reflexes are intact.
3. The patient can talk (if age appropriate).
4. The patient can sit up unaided (if age appropriate).
5. For a very young or handicapped child incapable of the usually expected responses, the pre-sedation level of responsiveness or a level as close as possible to the normal level for that child should be achieved

6. The state of hydration is adequate.

Conclusion

Procedural sedation is an advanced science in itself. The patients need to get the benefit of the availability of new drugs and advanced techniques for monitoring by properly trained personnel. The drugs when used by adequately trained personnel with adequate mentoring can alleviate pain in children during painful procedures and increase patients and parents satisfaction. This holds true especially for oncology patients who require repeated interventions. The sedation should be provided by trained physicians and monitored as per guidelines so as to ensure safety and prevent mishaps.

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