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Editorial

Understanding "Nudge" in Health Care


Kuldeep Singh

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For a long time we have been talking about literacy. Literacy means developing skills to read and write. When we compare the health statistics, we often correlate it with literacy level. We factor out the higher literacy rate in Kerala (nearly 93.9%) as an important reason for a single digit infant mortality rate. However, literacy rates are increasing in India and that is reflected in improved quality of life.[1] But the consequent improvement in health with regards to the proportionate reduction in infant mortality is not happening in our country.

This makes us ponder and forces us to think beyond literacy to the concept of education. Education is a holistic concept whereby people are enabled to think beyond understanding to development of higher analytical skills and application of the knowledge and skills. The twentieth century was devoted toward education and development of schools of education. However, do the educated people take a better decision? Perhaps not always.

Human brain takes in billions of bits of information daily. Given that much of information, it is hard to take the best decision and make good choices. Even the best-educated people including medical professionals often make different and at times poor decisions. Here comes the role of "Nudge." The final common pathway for the application of nearly every advances in medicine and technology is human behavior. The behavior may not be determined solely by the knowledge level and education as is commonly believed. The time has come that we have to go beyond emphasis on education and now think about behavior and behavioral economics.

There is a gap between the thinking of highly trained professionals and public. Despite increasing awareness, the footfall on the hospitals is increasing with underutilization of services available in the peripheral facility. A large proportion of visiting patients to the hospitals can be managed by preventive and promotive advices given by local health care worker. The public at large are also predisposed to cognitive biases and making wrong choices where there are opportunities for better choice that could be presented to them. Flagship program of Government of India *Ayushman Bharat Yojana* presents a huge opportunity to be made more attractive by following the Pareto principle (also known as the 80/20 rule, the law of the vital few, or the principle of factor sparsity) which states that, for many events, roughly 80% of the effects come from 20% of the causes. An inbuilt part of the Ayushman Bharat Yojana is to focus on creating 1,50,000 health and wellness centers (HWC) in the country with the objective to provide comprehensive health care at a facility nearest to public. In fact, this assumes a significant importance as a cost effective strategy for health care provisions to the poor. Eighty percent of the population will benefit through HWCs. Moreover, efforts required will be only 20% in creating appropriate health care interventions that may also rely on lost cost innovations. To drive the fact deeply, not much will be required in investing to create HWCs. The facilities already exist. They even do not require technically expert medical professionals as the task can very well be done through schools and existing motivated and trained health care workers in the centers. Here is the opportunities for what we can do with the help of nudges.

A "Nudge" is defined as any aspect of the choice architecture that alters people's behavior in a predictable way without forbidding any options or significantly changing their economic incentives. In other words, to be counted as a nudge the intervention must be easy and cheap to avoid. A nudge can be as simple as an advice.

The founder father of Nudge theory is Richard Thaler, the U.S. economist, who was the winner of the 2017 Nobel Prize for economics. When the "choice architecture" is designed to influence behavior in a predictable way but without restricting choice, it is called a "Nudge." Cass Sunstein and Richard Thaler in their best seller "Nudge: Improving Decisions About Health, Wealth, and Happiness" have demonstrated that despite our cognitive biases we can use human fallibility and the way we think to our advantage. The book also explored concept of "libertarian paternalism" which means that people are at their liberty for choice and at the same time, a soft advice is inbuilt. In other words how public and private organizations can help people make better choices through principles of behavioral economics.

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To drive the point in another way, people are already exposed to nudges from time immemorial. The soft push by parents in the form of advice to respect seniors and care for environment are examples of nudges. A 20-second advertisement for a noodle commercial is a nudge. However, the nudge utilized by multinational companies and by business units are manipulative nudge to promote their brands and pressurize gullible people into buying more. Similar types of commercials for positive health care are few and countable only on fingertips. Best example is "Swachh Bharat Mission (SBM)" promoted by Government of India.

Influenced by Thaler behavioral economic theory of nudge, British Prime Minister David Cameron in 2010, created a behavioral insights team, now known as the Nudge Unit, to leverage opportunities to improve his government's efficiency through behavioral science and careful testing. The unit quickly demonstrated how nudges could influence behavior. Although their initial area of focus was the problems of obesity, diet, and alcohol, but they soon expanded to many areas in administration policies.[2]

With the exception of a few countries in Africa and South America, no nudge unit exists in a developing country until now in Public Hospital as far as we are aware.

World Bank, in its report, aims to capture both the spread and form of behavioral science in 10 countries, selected based on being innovators or early adopters in the field: Australia, Canada, Denmark, France, Germany, Netherlands, Peru, Singapore, United States, and United Kingdom.[3]

In 2016, Patel et al launched the Penn Medicine Nudge Unit to systematically develop and test approaches using nudges to improve health care delivery. Health system leadership, frontline clinicians and staff, and members of the unit itself generated ideas. Their early successes and failures revealed some lessons about the role that nudge units can play in improving health care.[4]

No Nudge Unit exists in India yet. This might be due to design issues as well as state capacity in developing nudges. In September 2016, the NITI Aayog was supposed to set up a "Nudge Unit" on the lines of the Behavioral Insights Team in the United Kingdom. It was reported that NITI Aayog had tied up with the Bill & Melinda Gates Foundation to go about changing behaviors of people. The policies that were supposedly going to benefit from this nudge unit were the flagship programs of the current government—SBM, Jan Dhan Yojana, Digital India, Beti Bachao Beti Padao, and so on. The SBM requires behavioral change on a large scale; the efforts of the mission have been directed mostly toward construction of toilets. The NITI Ayog has again put an advertisement for recruiting people experts in Behavioral economics to join Aayog for the job of nudge specialists.

Final Mile, a private venture, who called themselves behavior architects, initiated nudge but the company has

Table 1 Nudge has found its way in many of the human operations. The following examples will be quite noteworthy

Situation/Condition	Problem identified	Effect when "Nudge" used	Reference and remark
Infective conditions	Branded medicines were prescribed over generic	Generic medicines were put as default and branded medicines given as other choice in Electronic health records	Patel et al, 2016[8]
Heart ailments (myocardial infarction)	Only 15% were referred for cardiac rehabilitation as process was manual	Team introduced that all patients with MI registered for cardiac rehabilitation by default increased referral	Enhancing cardiac rehabilitation through behavioral nudges—clinical trial-NCT03834155
Ordering investigations on HIS panel	Physicians tend to order low cost investigations leading to over-ordering	Study suggested to use nudge carefully and make test panel appropriately	Sedrak et al, 2017[9]
Sugar sweetened beverage (SSB) usage by adolescents	Companies encourage SSB placements by keeping in shops in front doors	Computational model of keeping non-SSB in optimal position encouraged consumption of healthy drinks compared with SSB	Wong et al, 2015[10]
Chlamydia screening among students	Type of incentive which increases uptake for screening	Financial incentives framed as a gain or loss to promote Chlamydia screening showed that £5 vouchers vs. £200 lottery weighs toward former	Niza et al, 2014[11]
Protection for HIV infection, other sexually transmitted infections, and unintended pregnancy	Poor compliance	The Empower Nudge lottery to increase dual protection use: a proof-of-concept randomized pilot trial in South Africa	Galárraga et al,, 2018[12]

Abbreviations: HIV, human deficiency immunovirus; MI, myocardial infarction.

recently been taken over by Fractal Analytics who are now promoting the model in India using artificial intelligence.

Nudge units in health care are conspicuous by their absence in India. Health care presents an opportune time to encash on the behavioral economics.

Nudge unit are also evolved form of quality improvements initiatives. Schneider et al in 2017 have shown in a cluster-randomized trial, a multistate campaign targeting hospitals and professionals involved in surgical care and infection control was associated with an increase in adherence to evidence-based practices that can reduce surgical site infections.[5] In fact, nudges offer opportunities to influence medical professionals' behavior toward overuse and underuse of health services.[6] Translating systematic reviews, meta-analysis, and Cochrane reviews into properly designed and laymen understandable language also qualifies as nudges.

The word "Nudge" has caught the fancy of many groups in India in last couple of years including journalists, economists, policy studies expert.[7] Some of the common examples of Nudges used in health care are highlighted in ►**Table 1**. This is an opportune time for Indian health care system also to go for nudging with intention of assessing the impact and improving quality of nudges. The task is not as simple as it appears. To an uninitiated person with no knowledge of economics and behavior it may appear too philosophical. To make "Nudge" in health care a reality, team similar to that, which works with advertisement industry, is required.

A word of caution—a nudge is contextual and works for the society for which it is constructed for and may not work everywhere. Simply borrowing nudges from developed countries may not work in our country or region.

Conflict of Interest

None declared.

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Do Maternal Micronutrient Deficiencies Program the Body Composition and Behavior of the Offspring? Probable Underlying Mechanisms

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Abstract

Obesity and noncommunicable diseases (NCDs) like diabetes are epidemic in India. Developmental origins of health and disease hypothesis, based on epidemiological evidence, associates maternal undernutrition and low birth weight (LBW) of the offspring with increased obesity and diabetes in their later life. Considering widespread maternal micronutrient (MN) deficiencies, LBW, and NCDs in India, we tested the hypothesis, "maternal MN deficiency per se programs the offspring for obesity and increases risk for NCDs in their later life" in rodent models. We showed in Wistar rat offspring that maternal MN (single or combined) deficiency per se: (1) increased body fat (visceral fat) and altered lipid metabolism, (2) decreased lean body and fat free mass, and (3) altered muscle function and altered glucose tolerance/metabolism and insulin sensitivity. Rehabilitation prevented vitamin but not mineral restriction-induced changes in offspring, which showed partial mitigation. Increased oxidative/steroid stress, decreased antioxidant status, and inflammatory state were the associated common mechanisms in the offspring. Our attempts to assess the role of epigenetics showed that folate and/or vitamin B12 deficiencies altered mother's body composition besides that of the offspring. Additionally, in C57BL/6 mice, B12 deficiency-induced anxiety was observed in mothers and offspring. That expressions of histone modifying enzymes in mice brain and promoter methylation of *adiponectin*, *leptin*, and *11βHSD1* genes in rat offspring were altered in MN (B12 and Mg) deficiency suggested that altered epigenetics most likely plays a role in maternal MN deficiency-induced changes in body fat/lipid metabolism and anxiety-like behavior in mothers and offspring.

Keywords
- micronutrient restriction
- body composition
- insulin resistance
- lipid and carbohydrate metabolism
- oxidative stress
- epigenetics

Introduction

The major paradoxical factor for undernutrition, overweight, and obesity is the poor dietary intake of healthy foods and improper food habits. According to United Nations (UN) Food and Agriculture Organization, the number of undernourished people worldwide increased from 777 million in 2015 to 815 million in 2016, and most live in developing countries,[1] where the number of stunted children indeed declined by 9% between 2012 and 2017. It is also reported that 46% of the global overweight children live in Asia.[2] Also, there has been a worsening of adult obesity globally.

Micronutrient (MN; vitamins and minerals) deficiency, whose symptoms are sparsely visible, is called "hidden hunger" and a large portion of global population suffer from it.[2] Inadequate consumption of macro- and micronutrients impedes fetal development and badly affects infants' and children's growth. Alternately, his/her mother's malnutrition during pregnancy and lactation, through a phenomenon called the developmental origins of health and disease

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(DOHaD), increases body fat, especially visceral adiposity (i.e., storage fat) in his/her childhood. Indeed, obesity and associated noncommunicable diseases (NCDs) constitute a leading cause of death worldwide, with increasing prevalence among children and adults.

Maternal undernutrition, the greatest risk factor for low birth weight (LBW) offspring, is proposed to result in the development of NCDs in their middle age. More alarming than type-2 diabetes mellitus, is the prevalence among Indians of other NCDs: hypertension (~30% of adults), heart attacks and most cardio vascular diseases (CVDs: 7–14%), polycystic ovary syndrome (PCOS: 2.2–26% of adolescent girls and reproductive age group women), osteoarthritis (22–39%), osteoporosis at age ≥50 years (7–50%), and chronic kidney disease (stage ≥3: 4–6%). This not only indicates the intensity of the problem but also stresses the need for immediate corrective action. Indeed, according to National Family Health Survey-4 (NFHS4) data, NCDs are estimated to account for 53% of all deaths in India,[3] and they are among the most serious public health problems of the 21st century.

In India, more than 165 million are obese and prevalence of obesity is higher among women than men. NFHS4 reported that in India, prevalence of obesity is more in high socioeconomic states like Andhra Pradesh, Punjab, Delhi, Telangana, and north eastern states compared with Bihar, Madhya Pradesh, Chhattisgarh, and Jharkhand, the low socioeconomic states.[4] India was the global diabetes capital about a decade ago[5] and with approximately 18% of Indian adults being type-2 diabetes mellitus (T2DM) at present,[6] we are soon expected to regain this dubious distinction. Overweight and obese individuals have three times greater risk to develop NCDs including metabolic syndrome, which features T2DM, CVDs and high blood pressure. Obesity can also lead to cancer, gallbladder disease, gallstones, osteoarthritis, gout, breathing problems, such as sleep apnea and asthma. The higher prevalence of obesity and overweight among Indians and its continuing increase are indeed of grave concern for India.

Interestingly, rates of obesity increase rapidly where MN deficiencies are more prevalent.[7] Epidemiological studies show that deficient levels of some MNs are associated with increased body fat deposition.[8] From a mechanistic view point, lower blood levels of vitamins and minerals increase adipogenesis leading to adipocyte dysfunction, which impairs glucose metabolism and insulin secretion further, resulting in impaired glucose tolerance, insulin resistance (IR), and T2DM.

UNICEF reported that approximately 16% of babies are born with low birth weight (<2.5 kg) globally and South Asia has the highest LBW incidence. India ranks third in having the highest percentage of LBW newborns.[9] According to the death statistics report between 2010 and 2013 by census office, India, 48.1% of infant mortality (before completing postnatal 29 days) is due to LBW and premature births.[10] Undernutrition is widespread among Indian women (nonpregnant, nonlactating, and also pregnant and lactating mothers). Abundant epidemiological evidence reports that Indian LBW babies are of the thin fat (thrifty) phenotype, perhaps as a consequence of DOHaD, due to maternal undernutrition during critical windows of development.[11] While robust international data indicates LBW children to be at the greatest risk for adiposity, IR and associated NCDs in later lives, recent Indian data have demonstrated IR among LBW children around 8 to 12 years of age.[12]

More overweight/obese the persons are, more likely they are insulin resistant and at increased CVD risk. However, substantial numbers of overweight/obese individuals remain insulin sensitive and not all insulin resistant persons are obese. It has been apparent for many years that overweight/obese individuals tend to be insulin resistant and become more insulin sensitive with weight loss. Compelling evidence indicates that CVD risk factors are present to a significantly greater extent in the subset of overweight/obese individuals that is also insulin resistant. As a corollary to this, an improvement in CVD risk with weight loss has been shown to a significantly greater extent, especially in those overweight/obese individuals who are insulin resistant at baseline. In view of these observations, it appears reasonable to suggest that IR, a multifaceted syndrome is the link between overweight/obesity and adverse clinical syndromes related to excess adiposity.[13]

According to recent reports, India has the second highest number (14.4 million) of obese children in the world.[14] It is estimated that 5.74 to 8.82% of Indian school children are obese. Alarmingly, 21.4% boys and 18.5% girls aged between 13 and 18, are either overweight or obese in urban south India.[15] Curiously, overweight and obesity rates are increasing in children and adolescents not just of high socioeconomic groups but also in low income groups, in whom underweight still remains a major concern. Indeed, overweight and obese children are likely to stay obese into adulthood and childhood obesity greatly increases the risk of developing diabetes in young adulthood and CVDs in later life. Obesity also adversely affects hormonal development of the child. For example, girls develop puberty and menarche earlier, with irregularities in menstrual cycles (e.g., polycystic ovary disease) and also develop facial hair. Boys, on the other hand, show impaired development of external genitalia and tend to have an enlargement of their "breast" region.

Given this background, continued high prevalence of LBW and thin fat Indian babies (most likely due to maternal undernutrition and the consequent programming for high body fat and IR in offspring), taken together with reports of increasing prevalence of childhood adiposity and IR among 8- to 12-year-old Indian children and the highest NCD risk of such offspring in later life, the situation is alarming indeed. Of the various in utero factors that influence diseases in adult life, maternal undernutrition is very important. By modulating the nutrients available for transfer to fetus, maternal nutrition affects fetal growth, development, and its glucose/insulin metabolism irreversibly. Further, its effects vary, if it occurs during different windows of early development. However, studies so far, have demonstrated the role of macronutrients, for example, carbohydrates, proteins, and fats[16-18] but not those of MNs, in the DOHaD.

MN are important determinants of organism's structure and metabolism.[19] Their deficiencies, common among populations of developing countries, have important public health implications that need immediate attention.[20] However, information is scanty on the role if any of widespread maternal MN deficiencies in India, in the high prevalence of obesity and NCDs among Indians. It looks reasonable that fetal programming due to rampant MN undernutrition in pregnant Indian women could underlie or be associated with high prevalence among Indians of overweight, obesity, and associated NCDs.

Several studies indicate that some vitamins and minerals influence insulin at different levels.[21] Moreover, MN deficiencies have persistent effects on many fetal tissues and organs,[22] although no clinical signs of deficiency are seen in the mother.[23] Also, consequences of MN imbalance during fetal/perinatal development may not manifest at the time of nutritional insult but appear later during development.[23] Multiple MN deficiencies during pregnancy and/or lactation that are common in developing world are associated with LBW and increased perinatal mortality and morbidity.[24]

Considering the difficulties in conducting mechanistic studies in human patients, we have performed studies in appropriate experimental animal models to validate/negate the hypothesis, "maternal MN deficiencies per se program the offspring for higher body fat/altered body composition and changes in macronutrient metabolism, which increase their risk for obesity and associated NCDs in their later lives."

This review highlights evidence from our studies in experimental animal models that maternal MN imbalances prenatal, in utero and early postnatal, modulate body composition, behavior, and macronutrient metabolism in offspring that could contribute significantly to the obesity and IR epidemic in India.

Our Studies in Rat and Mice Models

In view of shorter life span of laboratory animals and since genetic and environmental influences can be controlled in them, substantial efforts have been put on establishing animal models for developmental programming that are relevant to human situation.[25] Our initial studies in the albino, Wistar/National Institute of Nutrition (WNIN) rat models showed that chronic 50% restriction of all MNs from mothers' diet through their phases of growth, gestation, and lactation had no significant effects per se in mothers nor on the offspring's birth weight.[26,27] However, offspring born to vitamin (but not mineral) restricted mothers had lower body weight at weaning but not later. Interestingly, maternal vitamin and mineral restrictions both increased the offspring's body fat percentage (specially visceral fat) and decreased the percentage of lean body mass (LBM) and fat-free mass (FFM) but did not affect the offspring's glucose tolerance or insulin sensitivity up to 6 months of their age.[26,27] Despite their similar effects on the offspring's body composition, maternal vitamin but not mineral restriction-induced changes in the offspring were preventable by rehabilitating mothers from parturition but not weaning,[26,27] indicating the importance of maternal MN status during pregnancy and lactation. It was therefore of interest to delineate the mineral(s) whose restriction in the mother showed effects in the offspring.

Iron and folate deficiency are widespread among Indian women. Around 20% of Indian babies are born with LBW despite the mandatory iron and folate supplementation to all pregnant Indian mothers.[28] Minerals like Mg and Zn are deficient among Indian women[29] and Mg, Zn, Mn, and Cr modulate insulin,[30] the sole anabolic hormone that regulates macronutrient metabolism and is vital for fetal growth. Therefore, we assessed next, the effect of maternal dietary restriction of these minerals singly as per the study protocol presented in ►**Fig. 1**. The diet was restricted in the individual mineral by not adding its salt in the mineral mixture that was mixed with the diet. We observed no significant effects in mothers (body composition, reproductive performance, and offspring's birth weight), except that maternal Zn restriction decreased offspring's body weight at birth and later. Nevertheless, maternal dietary restriction of all four minerals affected the offspring's body composition (increased body fat percentage, especially visceral adiposity and decreased the percentage of LBM and FFM),[31-39] similar to those seen in offspring of rat dams on 50% restriction of all minerals.[27]

Adipogenesis that begins in utero and accelerates neonatally, is considered a prime candidate for developmental programming. Robust literature suggests that changes in body adiposity and lipid metabolism are the earliest to occur, much before tissue IR manifests.[40,41] In fact, IR is hypothesized to originate in impaired adipogenesis and/or fat metabolism.[42,43] In line with these reports, chronic 50% restriction of all vitamins/minerals[26,27] or restriction of Mg, Mn, Cr, and Zn singly from the mothers' diet, significantly increased body fat percentage, visceral adiposity, and plasma triglycerides in offspring, albeit the earliest time point at which these changes manifest was different among different MN restrictions.[32,35,36]

Normally, body fat percentage increases to compensate a decrease in the mass of muscle, another insulin sensitive tissue which is most important in postprandial glucose clearance.[44] In line, we observed that 50% restriction of all vitamins/minerals or restriction of individual trace elements from mothers' diet during maternity persistently decreased the percentage of LBM and FFM (representing muscle and bone) in offspring. Taken along with our observation that despite significant increase in body fat percentage and visceral adiposity, the offspring born to MN restricted rat dams had no change in their tissue-associated fat percentage (TAF%),[36,37] seems to indicate that decreased LBM and FFM percentage in them could indeed be due to decreased muscle mass per se. Interestingly further, our observations that expression of myogenic genes/transcription factors *Pax3, MyoD, Myf5*, and *MyoG* was decreased in chromium restricted (CrR) offspring[37] not only corroborate the above inference but also indicate that decreased myogenesis and muscle development could be underlying the decrease in their percentage of LBM and FFM.

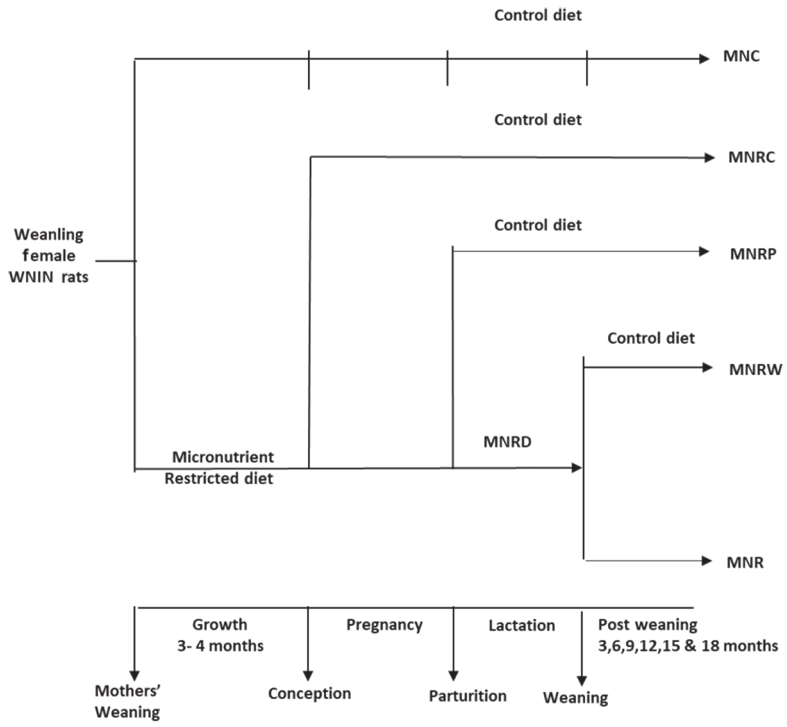

Fig. 1 Study protocol to assess the effects of maternal micronutrient restriction in Wistar rats and their offspring. MNC, micronutrient control diet; MNR, micronutrient restricted; MNRC, micronutrient rehabilitation from conception; MNRD, micronutrient restricted diet; MNRP, micronutrient rehabilitation from parturition; MNRW, micronutrient rehabilitation from weaning; WNIN, Wistar NIN.

It is known that skeletal muscle fibers are formed prenatally and the total number of fibers and/or relative proportions of different fiber types are determined mostly during fetal and early postnatal development. In line, preconceptional nutrient restriction (~50%) has been shown to decrease the total myofiber number in the fetal semitendinosus muscle.[45] Also, 50% maternal undernutrition has been shown earlier to decrease total myofiber number and increase the fast myosin type-IIb isoform in the longissimus dorsi of sheep offspring, while it decreases the proportion of fast-twitch myofibers in the vastus lateralis of 14–day-old sheep.[45,46] Since muscle is the major site of postprandial glucose disposal, it is reasonable that changes in muscle mass, fiber type, growth patterns, and functional characteristics of muscle fibers during perinatal period are important in programming the offspring for IR and T2D. Taken together, it is evident from our observations that maternal MN restriction during growth, pregnancy, and/or lactation-induced changes in the offspring's body composition which suggest their predisposal to IR and associated NCDs in later life.[39] Our finding that rehabilitation from as early as parturition could reverse changes only in gene expression but not the phenotype of the offspring (except in Mg and Zn restrictions) further reiterates the importance of maternal MN status in determining the offspring's body composition and their predisposal to adult onset NCDs.[43]

Effects of Maternal MN Restriction on Adipose and Muscle Functions in Offspring

Adipose tissue is not just a store of excess energy but is a major endocrine organ, secreting a wide range of protein factors and signals called adipokines, besides fatty acids and other lipid moieties.[47] Adipokines modulate adipose tissue function, lipid metabolism, and also insulin sensitivity/resistance. Therefore, we assessed next in the offspring, effects if any, of maternal MN restriction on adipose tissue function (besides those on weight and distribution), by determining changes in plasma lipid profile and levels of adipokines (associated with high body fat) in plasma and adipose tissue. We observed dyslipidemia in micronutrient restricted (MNR) offspring as evident from altered plasma lipid profile[43] and in line with earlier reports, they had high plasma leptin levels which corroborated their high body fat percentage. However, hypoleptinemia seen in 50% mineral restriction (MR) and magnesium restriction (MgR) offspring,[27,32] despite their high body fat percentage, is at variance with earlier reports showing a positive relation between them. Nevertheless, the observed hypoleptinemia is in agreement with leptin deficiency reported earlier in genetically obese rodent models and in types 1 and 2 DM patients. Thus, further studies are warranted to delineate the role if any, of hypoleptinemia in maternal MR-induced high body fat percentage in offspring. However, vitamin restriction (VR) and MR offspring had lower plasma adiponectin levels suggesting their probable IR status

Table 1 Effects of maternal MN restriction in Wistar rat offspring

Maternal MN restriction (R)	Body composition (%)	Effects on adipose and lipid metabolism	Effects on muscle, Insulin/ glucose homeostasis & Carbohydrate metabolism	Stress mechanisms
50% Mineral R	↑Fat, ↓LBM, ↓FFM,	↓Leptin, ↓Adiponectin, ↔TNF-α, ↑TG, ↔TC, ↔HDL	↓GTT Insulin secretion	↓GSH
50% Vitamin R	↑Fat, ↓LBM, ↓FFM	↑Leptin, ↓Adiponectin, ↔TNF-α, ↑TG,↔TC,↔HDL	↔GTT Insulin secretion	↑MDA,↓GSH, ↑SOD, ↑GPx
MgR	↑Fat, ↓LBM, ↓FFM, ↑AI	↓Leptin, ↓Adiponectin, ↔TNF-α, ↔TC, ↔HDL, ↔TG, ↔FFA, ↑expression of FAS & FATP1	↑Plasma insulin, ↓GTT Insulin secretion, ↓Glucose uptake	↔MDA, ↔GSH, ↔SOD, ↔GPx, ↔catalase, ↑11βHSD1 expression
ZnR	↑Fat, ↓LBM, ↓FFM	↓TG, ↓TC, ↓FFA,↔FFA	↓Plasma insulin,↓ GTT Insulin secretion	
CrR	↑Fat, ↓LBM, ↓FFM, ↑AI	↑plasma Leptin and ↑leptin expression, ↔ PPAR ϒ & SREBP2 expression, ↓Adiponectin, ↑PAI, ↑TNF-α, ↑TG,↑FFA,	↑Plasma glucose, ↑Plasma insulin, ↑GTT Insulin secretion, ↑Glucose uptake, ↑Glucose intolerance, ↓expression of myogenesis genes MyoD, Myf5, MyoG, ↓expression of muscle atrophy genes atrogin and *MuRF* genes	↑MDA, ↓catalase ↓SOD, ↓GPx, ↑11βHSD1 expression
MnR	↔ Fat, ↔ LBM, ↔FFM, ↑AI	↔Leptin,↔IL6, ↔IL1 β, ↑TNF-α, ↔TG, ↔TC, ↓HDL	↑Plasma glucose, ↓Plasma insulin, ↓GTT Insulin secretion, ↑Glucose intolerance, ↓Glucose uptake	
Folate R	↑Fat, ↓LBM, ↓FFM, ↑AI	↔Leptin, ↓Adiponectin, ↑TNF-α, ↔IL6, ↔IL1 β, ↑TG ↑Fattyacidsynthase, ↑Acetyl-CoA-carboxylase	↑Plasma glucose, ↑Plasma insulin, ↑GTT Insulin secretion, ↑Glucose intolerance, ↔Glucose uptake, ↑PEPCK, ↔FBPase, ↑G6Pase, ↓PyK, ↔GK	↔MDA, ↓catalase, ↔GPx, ↓GSH, ↑GSSG, ↑Protein carbonyls, ↑cortisol
Vitamin B12R	↑Fat, ↓LBM, ↓FFM, ↑AI	↓Leptin, ↓Adiponectin, ↑TNF-α, ↑IL6, ↓IL1 β, ↑TG, ↑TC, ↑Fattyacidsynthase, ↑Acetyl-CoA-carboxylase	↑Plasma glucose, ↑Plasma insulin, ↑GTT Insulin secretion, ↑Glucose intolerance, ↔Glucose uptake, ↑PEPCK, ↑FBPase, ↔G6Pase, ↓PyK, ↔GK	↑MDA, ↓catalase, ↔GPx, ↓GSH, ↑GSSG, ↑Protein carbonyls, ↑cortisol
Folate R + B12R	↑Fat, ↓LBM, ↓FFM, ↑AI	↔Leptin, ↔Adiponectin, ↔TNF-α, ↑IL6, ↓IL1β, ↔TG, ↑TC, ↑Fattyacidsynthase, ↑Acetyl-CoA-carboxylase	↑Plasma glucose, ↑Plasma insulin, ↔GTT Insulin secretion, ↑Glucose intolerance, ↔Glucose uptake, ↑PEPCK, ↔FBPase, ↔G6Pase, ↔PyK, ↔GK	↔MDA, ↓catalase, ↑GPx, ↔GSH, ↑GSSG, ↔Protein carbonyls, ↑cortisol

Abbreviations: ↑, increase; ↓, decrease; ↔, no change; *11βHSD1*, 11-beta-hydroxysteroidehydrogenase1; AI, adiposity index; CrR, chromium restriction; FAS, fatty acid synthase; FATP1, fatty acid transport protein 1; FBPase, fructose-1, 6-bisphosphotase; FFA, free-fatty acid; FFM, fat-free mass; G6Pase, glucose-6-phosphotase; GK, glucokinase; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GTT, glucose tolerance test; HDL, HDL cholesterol; IL1β, Interleukin 1 β; IL6, Interleukin 6; LBM, Lean body mass; MDA, malondialdehyde; MgR, magnesium restriction; MN, micronutrient; MnR, manganese restriction; PAI, plasminogen activator inhibitor; PEPCK, phosphoenolpyruvate-carboxykinase; PPAR ϒ, peroxisome proliferator activated receptor gamma; PyK, pyruvatekinase; R, restriction; SOD, superoxide-dismutase; SREBP2, sterol regulatory element binding protein 2; TC, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor α; ZnR, zinc restriction.

(►**Table 1**).[26,27] Interestingly further, changes in circulating adipokine levels were mitigated only in those offspring rehabilitated from birth but not weaning, confirming the importance of maternal vitamin and mineral nutrition during gestation and lactation in modulating these changes (►**Table 2**). On the other hand, maternal CrR modulated adipose tissue adipokine levels (adiponectin, leptin, and tumor necrosis factor [TNF]-α) only in male but not female offspring, while circulating adipokine levels were influenced variably in offspring of both genders.[34,36] Also, rehabilitation affected different adipokine levels variably among male and female offspring. Notwithstanding that vitamin and mineral restrictions in the mother increased body fat in offspring, it appears that probable underlying/ associated mechanism(s) leading to adiposity and functional changes could be different at least to some extent.

As for the effects of maternal MN restriction on muscle function in offspring, although fold stimulation of muscle glucose uptake by insulin was unaffected, basal glucose uptake by muscle (diaphragm) was significantly and irreversibly decreased in MgR offspring, whereas it was increased in CrR offspring, which could be prevented by rehabilitation albeit in male offspring.[31,37] Since, both MgR and CrR offspring had lower percentage of LBM and FFM (muscle mass), the present results not only suggest altered basal capacity of muscle to clear glucose but also their differential sensitivity to different maternal MN restrictions, mitigation by rehabilitation, in addition to the gender differences in the effects. On the other hand, maternal Mn restriction diversely affected basal muscle glucose uptake in male and female offspring, but decreased the insulin

Table 2 Effect of rehabilitation on chronic maternal dietary micronutrient restriction induced changes in Wistar rat offspring

Maternal MN restriction (R)	Effect of rehabilitation on chronic maternal dietary restriction induced changes in offspring
50% mineral R	Mineral rehabilitation from parturition or weaning had little effect on birth weight, body composition, and plasma triglycerides.
50% vitamin R	Rehabilitation from parturition or weaning prevented the changes in body fat percent, lean body mass, fat-free mass, and oxidative stress.
MgR	Rehabilitation regimes (parturition and weaning) partially/could not corrected the changes in body composition, but not the changes in glucose metabolism. While changes in FAS and FATP1 were not correctible by rehabilitation, those in leptin and TNF-α were corrected by rehabilitation from parturition but not from weaning. *11βHSD1* expression was corrected by both rehabilitation regimes.
ZnR	Rehabilitation regimens (parturition and weaning) corrected the body weights of male but not female offspring. Rehabilitation from parturition or weaning partly corrected the changes in the percentage of body fat but had no such effect on other parameters of lipid and carbohydrate metabolisms.
CrR	Rehabilitation regimes (conception, parturition, and weaning) did not correct body adiposity but restored the circulating levels of lipids and adipocytokines and, in general, corrected the changes albeit partially in glucose metabolism and stress parameters, Rehabilitation partly corrected myogenic and atrophic gene expression but had no effect on LBM percentage or FFM percentage or glucose uptake by muscle.
MnR	Rehabilitation regimes (conception, parturition, and weaning) partially corrected the increase in TNF-α. Total cholesterol levels were lower in rehabilitation regimes by 18 months of age, while HDL levels were improved in rehabilitation from parturition.
Vitamin B12R	Altered body composition, lipid profile, adipocytokine levels, increased insulin secretion, impaired glucose tolerance, altered lipid and carbohydrate metabolism, etc., seen with maternal vitamin B12R were reversible by B12 rehabilitation from conception but partially by rehabilitation from parturition and weaning.

Abbreviations: B12R, B12 restriction; CrR, chromium restriction; MgR, magnesium restriction; MN, micronutrient; MnR, manganese restriction; FATP, fatty acid transport protein; FAS, fatty acid synthesis; FFM, free-fat mass; HDL, high density lipoprotein; TNF, tumor necrosis factor; ZnR, zinc restriction.

stimulated glucose uptake in both (unpublished observations). Interestingly, rehabilitation corrected the changes variably among the offspring of the two genders. It was further interesting that MnR offspring fed high fat diet in later life, had higher basal and insulin stimulated muscle glucose uptake than those fed normal fat diet or the manganese control diet (MNC) offspring fed high fat diet.[48] It appears from these observations that maternal MN restriction affected muscle mass and function (basal and/or insulin stimulated glucose uptake), in addition to modulating their susceptibility to high fat feeding and may thus predispose offspring to develop hyperglycemia in later life (►**Tables 1** and **2**). Overall, our results indicate that maternal MN restriction not only affected body composition (muscle and fat) of the offspring but also the function of these two important insulin sensitive tissues.

Effects on Macronutrient Metabolism in the Offspring

Carbohydrate Metabolism

Fifty percent restriction of maternal vitamins or minerals per se or their postnatal continuation did not affect fasting plasma glucose and insulin levels in offspring till 6 months of age.[26,27] Although in 6 months, old MgR offspring, fasting plasma insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) index were significantly higher,[31] their insulin secretion to a glucose challenge (area under curve of insulin [AUC insulin] during the oral glucose tolerance test [OGTT]) was significantly lower and irreversible[31,32] by rehabilitation. In zinc restriction (ZnR) offspring, all these parameters were decreased almost irreversibly.[33,35] On the other hand, maternal Cr restriction raised fasting plasma glucose, insulin, HOMA IR, and also AUC of glucose and insulin during OGTT,[38] albeit the effects were seen late in their life and some of them were indeed of transient nature.[34,38] Interestingly, rehabilitation mitigated these changes only partly. In line with consistent fasting hyperglycemia, moderate increase was seen in phosphoenolpyruvate carboxykinase (PEPCK) expression in liver, suggesting increased gluconeogenesis (unpublished results). Maternal Mn restriction, however, induced consistent, fasting hyperglycemia along with hypoinsulinemia in the offspring.[48] Interestingly, maternal Mn restriction increased the offspring's susceptibility to impaired glucose metabolism, especially when fed high fat diet in later life, as indicated by the increase in AUCs of glucose and insulin during OGTT.[48] In general rehabilitation, alleviated changes due to maternal Mn restriction, while those due to maternal Mg and Cr restrictions were irreversible or partly reversible at the best. These findings (except in CrR) are in agreement with earlier literature showing decrease in glucose-stimulated insulin secretion in the offspring of protein deficient mothers, which was proposed to be due to β-cell exhaustion or a decrease in β-cell numbers/mass similar to that in T2DM.[49] It thus appears that maternal MN restriction and its postnatal continuation modulated insulin secreting capacity of the offspring under fasting and fed states, which in turn influenced glucose uptake and metabolism.

Lipid Metabolism

Hormones that affect glucose/lipid metabolism modulate the activities of enzymes and proteins involved in adipogenesis/lipid transport, for example, fatty acid synthase (FAS) and fatty acid transport proteins (FATPs)[50-52] and obesity is

reported to affect FAS and FATP1 expressions variably. That FAS and FATP1 expressions were higher in liver and adipose tissue of MgR offspring at 6 and 18 months of age,[32] suggests that increased fatty acid synthesis and transport may underlie the increased body fat in MN restricted offspring. However, comparable levels of plasma total cholesterol, high density lipoprotein (HDL) cholesterol, free-fatty acids, and triglycerides (TGs) among control and MgR offspring at 18 months of age and no effect of rehabilitation, do not appear to corroborate the increased FAS and FATP1 expression in MgR offspring.[32] Interestingly, plasma lipid profile was comparable among groups of male CrR offspring, while CrR female offspring had higher plasma TGs and free-fatty acids than controls and Cr rehabilitation modulated these changes.[34] While changes in plasma lipid profile were inconsistent in ZnR offspring,[33] MnR offspring (both genders) had high-plasma total cholesterol but varied HDL cholesterol levels.[48] Interestingly, maternal Mn restriction increased circulating TGs and free-fatty acids in offspring chronically fed high-fat diet later in life compared with those fed normal fat diet or MNC offspring fed high-fat diet.[48] Thus, it is evident from the results that maternal MN restriction not only affected the adipose tissue content and functions but also altered lipid metabolism.

Considered with available literature, our observations indicate that diverse nutritional insults in mothers (macro- or micronutrient restrictions) cause similar phenotypic characteristics in the offspring at least with respect to their body composition and insulin secretion. While programming for obesity is undoubtedly a multifactorial process, the diversity of maternal nutrient deficiencies which increase adiposity in offspring appear to suggest some common mechanisms/pathways underlying/associated with these effects. Apparently, any common mechanism for developmental programming of an obese/insulin resistant adult phenotype must explain how early environmental stress (maternal deficiency of diverse nutrients) can set in motion persistent biochemical and molecular changes which cause pervasive, damaging effects in their later life.

Deducing the Role of Stress, Epigenetics, and 1-Carbon Metabolism

Literature search indicates stress (oxidative/steroid), an inflammatory status, changes in taurine metabolism and epigenetics to be some common mechanisms associated with developmental programming of offspring to obesity and adult onset diseases, especially due to maternal malnutrition during pregnancy and/or lactation.[53-57]

Epigenetics, a mechanism proposed to underlie developmental programming involves the following: (1) methylation/demethylation of CpG islands in promoter regions of target genes which suppress/activate gene expression respectively; (2) methylation/deacetylation and demethylation/acetylation of histones, which activate or suppress the associated gene expression, respectively; and (3) micro-RNAs which modulate the expression of specific genes.[55,56] Indeed, modulation of gene expression is dependent on interaction/balance among the above three constituent mechanisms. Interestingly, constituents 1 and 2, which form an important portion of epigenetic mechanisms, require a methyl donor (e.g., S-adenosyl methionine [SAM]), which is supplied chiefly by the 1-carbon metabolic pathway[58] and folic acid, vitamin B6 and B12 are the important MNs that regulate this pathway. Indeed, robust literature indicates that deficiency of any of these MNs modulates 1-carbon metabolic pathway (i.e., SAM availability) and, in turn, the epigenetic regulation of gene expression.[58]

Folate and vitamin B12 deficiencies are common in Indians, especially among women of reproductive age group, pregnant, and lactating mothers.[59,60] All pregnant Indian women receive the mandatory iron and folate supplements regardless of their deficient vitamin B12 status and recent observations suggest the greater importance of the ratio of folate and vitamin B12 than their actual levels per se.[61] It was hence considered essential to assess whether or not maternal deficiency of folate and/or B12 (the two MNs that regulate SAM availability directly, by modulating 1-carbon metabolic pathway) alter the body composition (body fat/visceral adiposity) and LBM/FFM of the offspring and, if so, evaluate whether changes in epigenetic mechanisms underlie/are associated with these changes in offspring. Therefore, we evaluated in a Wistar rat model, whether or not dietary folate and/or vitamin B12 deficiency affect the body composition and reproductive performance of mothers, as also the effects of maternal folate and/or vitamin B12 deficiencies on body composition, development, and function of adipose/muscle in the offspring. We also determined the modulation if any, of the 1-carbon pathway/production of SAM in mother/offspring, which is essential for epigenetic regulation of gene expression changes associated with offspring's body composition.[62-66]

Unlike the other MN deficiencies studied earlier, it was interesting that dietary folate and/or B12 restriction per se increased body weight, body fat (visceral adiposity), and altered lipid profile in female Wistar rats (mothers) before mating, albeit the differences were significant with only B12 restriction.[62] Also, only the offspring born to vitamin B12-restricted dams had LBW, while those of folate and/or vitamin B12 restricted dams weighed higher at/from weaning.[62] Indeed, the offspring had higher body fat (especially visceral fat) from 3 months and were dyslipidemic at 12 months, at which time they had high levels of tumor necrosis factor α, leptin and interleukin 6; and low levels of adiponectin and interleukin 1β in circulation and also in adipose tissue.[64] All vitamin-restricted offspring had higher activities of hepatic fatty acid synthase and acetyl-CoA-carboxylase and higher plasma cortisol levels.[62] On the other hand, three enzymes in the β-oxidation pathway, hydroxyacyl-coenzyme A dehydrogenase, medium-chain specific acyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase were down regulated in pups born to vitamin B12 deficient mothers (►**Table 1**). Interestingly an age-dependent differential expression of peroxisome proliferator activated-receptors (PPAR) α and ϒ was observed

in B12 deficient pups.[65] Indeed, enriched/differential expression of 27 proteins involved in pathways that regulate amino acid, lipid, and carbohydrate metabolism was observed, and this was restored to control levels after B12 rehabilitation of restricted mothers from parturition.[65,66] In conclusion, maternal and peri- or postnatal folate and/or vitamin B12 restriction increased visceral adiposity (perhaps due to increased corticosteroid stress), altered lipid metabolism in rat offspring probably by modulating adipocyte function resulting in inflammatory status.

Although dietary vitamin B12 restriction increased the mother's body weight and fat, it decreased LBM percentage and FFM percentage but not the percentage of tissue associated fat (TAF)[62] indicating that the increase was only in the storage form (visceral) but not functional fat. Similarly, maternal B12R also decreased LBM percentage and FFM percentage in only male offspring, but their TAF%, basal, and insulin stimulated muscle glucose uptake were unaffected. Nevertheless, at 12 months of age, B12R offspring had significantly higher fasting plasma glucose, insulin, HOMA-IR and also impaired glucose tolerance.[63] In line, hepatic gluconeogenisis was high in them as evident from increased expression of gluconeogenic enzymes PEPCK, fructose-1, 6-bisphosphatase, and pyruvate kinase.[63] Unlike maternal mineral restrictions which altered body composition in offspring of both genders,[35,36] folate and/or vitamin B12 restriction altered body composition in only male but not female offspring.[62] That changes in body composition, glucose, and lipid metabolism in offspring were prevented by rehabilitation from conception,[64] whereas rehabilitation from parturition and weaning corrected them only partially,[63] not only indicate their causal relationship but also highlight the importance of vitamin B12 during pregnancy and lactation with particular reference to growth, muscle development, glucose tolerance, and metabolism in the offspring (►**Table 2**). Interestingly, altered body composition was intergenerationally transferred from F1 offspring to F2; however, the effects were seen in only male offspring but not in females suggesting the probable gender differences in the effects of maternal vitamin B12 and/or folate restriction in the offspring (unpublished data).

Maternal folate and/or vitamin B12 restrictions not only induced similar changes in body composition, carbohydrate, and lipid metabolism in the offspring as did maternal single-mineral restriction or 50% restriction of all vitamins and minerals from the mother's diet but were also associated with altered expression of relevant genes that could be mitigated by folate and/or vitamin B12 rehabilitation.[65] Therefore, it appears that modulation of epigenetics and other common mechanisms could underlie/be associated with the maternal MN (folate and/or vitamin B12) restriction-induced phenotypic changes in the offspring.[65,66]

Mouse genome is closer to human genome and is rich in CpG islands[67] that undergo methylation/demethylation affecting gene expression and hence mouse is considered a model better suited to assess epigenetic changes. Therefore, studies were conducted in C57BL/6 mice to decipher the underlying/associated epigenetic changes if any.

In partial agreement with our findings in Wistar rat models, we observed that severe but not moderate vitamin B12 deficiency impaired lipid profile, induced adiposity, and caused adverse gestational outcome in mothers.[68] On the other hand, both severe and moderate maternal vitamin B12 deficiencies caused negligent care of their pups and anxiety in them in addition to the effects on the body composition (increased visceral adiposity), dyslipidemia, fasting hyperglycemia, and insulin resistance in the offspring,[69] besides catch up growth and most of these changes commenced early in the offspring's life. However, only severe vitamin B12 deficiency-induced anhedonia/depression in mothers and was associated with the thinning of interneuronal connections in prefrontal cortex and hippocampus.[70] Offspring of both severe and moderately B12 deficient dams also showed pronounced anxiety behavior whereas offspring of severely B12 deficient dams developed depression (►**Fig. 2**).[70] Rehabilitation from parturition but not weaning was beneficial albeit in only delaying the onset of deleterious effects in offspring whereas rehabilitation from parturition and weaning could both prevent onset of depression but not anxiety in the offspring.[70]

That alterations in epigenetic mechanisms may be associated with changes in body composition[71] and macronutrient metabolism in offspring is evident from the methylated DNA immunoprecipitation (MeDIP) sequencing of liver in control and B12 restricted rat offspring. A total of 214 hypermethylated and 142 hypomethylated sites were observed in the 10 kb region upstream of transcription start site (TSS), which is enriched in genes involved in fatty acid metabolism and mitochondrial transport/metabolism.[65] Interestingly, behavioral changes in severely B12 deficient female mice were associated with epigenetic changes as evident from the overexpression of both writer (histone-methyl-transferase, SUV420H1) and eraser (histone deacetylase, HDAC4) classes of histone modifying enzymes.[70] Thus vitamin B12 restriction appears to alter promoter DNA methylation and/or modulate histone modifying enzyme expression, which in turn regulate the expression of genes involved in important metabolic processes influencing the offspring's phenotype.[66,69,70] That B12 rehabilitation from conception reversed methylation of many of these regions to control levels probably suggests their causal relationship with the metabolic phenotypes.[65,66] Whether or not similar epigenetic alterations underlie the behavioral changes seen in the offspring, remain to be deciphered.

In line with our observations that offspring born to MN restricted dams had increased stress (oxidative/glucocorticoid) and inflammation (high levels of inflammatory and/or low levels of anti-inflammatory cytokines in circulation and/or adipose tissue), we observed that chronic dietary Mg restriction increased inflammation (↑leptin, MCP1, interleukin 1β[IL1β], PAI active, IL6, and TNF-α) and glucocorticoid stress in pregnant mothers[72,73] who intriguingly had high-adiponectin levels. Indeed changes in inflammatory status and steroid stress were observed even in placenta and embryo on gestational day 15 and in the offspring at 6 months of their age.[73] Interestingly,

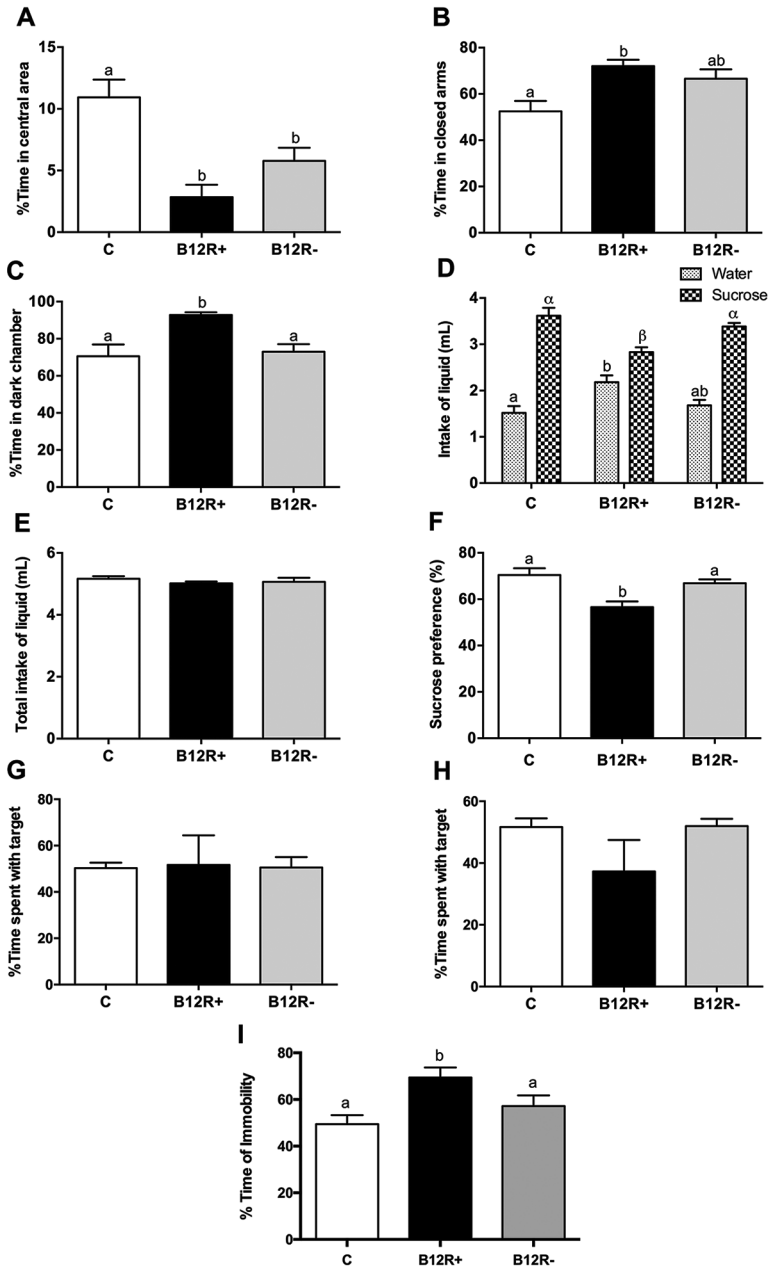

Fig. 2 Effect on behavior (evaluation of anxiety, panels A, B, C), evaluation of depression/anhedonia (panels D, E, F) and Effect on social interaction behavior (evaluation of depression panels G, H, I). Values are mean ± standard error of mean (SEM) (n = 5–7). Bars with different superscripts (a/b or α/β) are significantly different from one another at $p < 0.05$ by one way ANOVA/Bonferroni's post hoc test. (**A**) Percentage time spent in central area in open field test; (**B**) percentage time spent in closed arms in elevated plus maze test; (**C**) percentage time spent in dark chamber in light-dark exploration test. (**D**) Differential intake of water and sucrose. (**E**) Total intake of liquid. (**F**) Sucrose preference (%). (**G**) Social interaction with unfamiliar female mouse. (**H**) Social interaction with unfamiliar male mouse. (**I**) Percentage immobility time in tail suspension test. ANOVA, analysis of variance.

increased steroid stress and inflammatory status were associated with appropriate changes in the expression of *11βHSD1* and *11βHSD2*, the enzymes which modulate the formation of the biologically active steroid (►**Fig. 3**) and adiponectin.[72,73] Altered adiponectin and 11βHSD1 expression on GD15 appeared to be epigenetically regulated in pregnant mothers, placenta, and embryo, but not in the offspring, as indicated by the hypomethylation of CpG loci in the promoter region of corresponding genes (unpublished observations). Although expression of *11βHSD1*, leptin, and adiponectin genes was increased in the adipose tissue of MgR offspring, it was intriguing that methylation of CpG loci in their gene promoters did not show corresponding change (unpublished observations). Considering that epigenetic regulation of gene expression is the effect of the net balance among different epigenetic mechanisms (methylation/demethylation of gene promoters and histones, acetylation/deacetylation of histones, and micro-RNAs), it appears premature to conclude on the role of epigenetics in these gene expression changes from

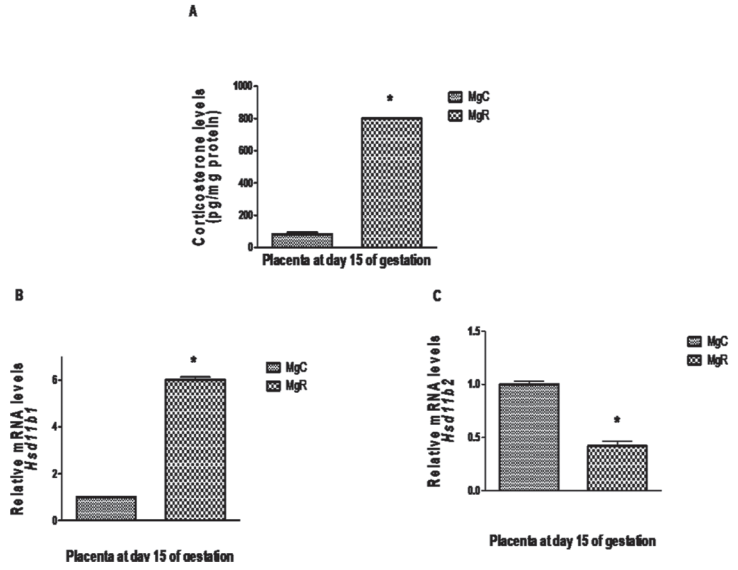

Fig. 3 Effect of chronic dietary magnesium restriction on placental (**A**) Corticosterone levels and expression of the genes (**B**) *11β HSD1* and (**C**) *11β HSD2* on gestational day 15 in WNIN female rats. Values represent mean ± standard error of mean (SEM) ($n = 6$). MgC, control diet; MgR, magnesium restriction. Values with "*" represents significantly different by Student's *t*-test ($p < 0.05$).

our limited observations on the promoter methylation of a few genes or the expression of a few histone modifying enzymes in isolation.

Insulin resistance is often associated with increased oxidative stress and/or decreased antioxidant status.[53,54] Indeed, IR and oxidative stress have been suggested to be causally related. In line with such reports suggesting a causal role for oxidative stress in IR, we observed that VR offspring, despite having markedly higher activities of antioxidant enzymes, such as superoxide-dismutase (SOD) and glutathione peroxidase (GPx) in liver, had increased oxidative stress,[26] perhaps suggesting that vitamins, the nonenzymatic antioxidants act as the primary line of defense against oxidative stress in the VR offspring. Alternately, they could mean that the role of antioxidant enzymes in maintaining the VR animal's antioxidant status is limited. Increased oxidative stress and decreased activity of antioxidant enzymes were also observed in the offspring born to folate and/or vitamin B12 restricted Wistar rat dams which had high body fat/visceral adiposity, low LBM, and FFM percentage, IR, impaired glucose tolerance/metabolism, and changes in lipid profile/metabolism later in their lives.[62-64] Our findings thus appear to suggest that antioxidant enzyme activities in offspring were modulated by maternal vitamin restriction, to cope up with increased oxidative stress.

Chronic maternal mineral or Mg restriction on the other hand, had no effect on oxidative stress and/or antioxidant defense (enzymatic and nonenzymatic),[32] while maternal CrR increased plasma malondialdehyde (MDA) levels and decreased hepatic SOD and Gpx activities, which curiously were mitigated variably by rehabilitation in male and female offspring.[38] Increased oxidative stress was observed even in the offspring of Zn and Mn restricted rats.[35,48] It appears that changes in oxidative stress/antioxidant status may be associated with maternal mineral restriction-induced changes in body fat, glucose tolerance, and impaired insulin response to glucose challenge, in addition to changes in corticosteroid stress, inflammation, and epigenetic alterations.

Conclusion

In conclusion, our studies in rodent models reported here, have demonstrated for the first time, to the best of our knowledge, that maternal MN deficiencies per se program the body composition and behavior in the offspring. Modulation of inflammation, oxidative, and/or glucocorticoid stress and epigenetics in mothers, placentae, and fetuses during gestation appears to underlie the alterations in relevant gene expression that are associated with altered body composition and behavior of the offspring. Although our findings in the rodent models are in line with the fetal programming for adult diseases hypothesis of Barker, they appear to differ from the "thin fat" phenotype suggested by Barker, in that changes in body composition and macronutrient metabolism were seen in the offspring even though they had normal birth weight.

That maternal MN restriction-induced changes in the offspring were most often, not prevented/reversed by rehabilitation from as early as conception/parturition appears to be in disagreement with the preventability/reversibility of changes by the concept "intervention during the first thousand days of the offspring's life." From our observations in the experimental animal models, it looks essential that we should ensure optimal MN status of adolescent girls, nonpregnant, and nonlactating woman and the mothers to be to enable us prevent the long-term consequences of maternal MN deficiencies in the next generation, for example, prevalence of overweight, obesity, and associated NCDs, such as T2DM, hypertension, CVDs, etc.

Conflict of Interest
None declared.

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Treating Hereditary Ataxias—Where Can We Help?

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Abstract

Hereditary ataxias comprise a group of neurological disorders which affect different levels of the neurological axis including the cerebellum, peripheral nerves, cognition, and the extrapyramidal system. These are categorized by the mode of inheritance as autosomal recessive, autosomal dominant, X-linked, and mitochondrial cerebellar ataxia. Definitive curative therapy is not available for these disorders. However, a wide array of emerging treatment options, especially in terms of symptomatic therapy, rescues this group from therapeutic nihilism. Several drugs have been assessed including riluzole, valproate, lithium, etc., as well as rehabilitative, and neuromodulatory strategies. In addition, symptomatic therapies for ancillary symptoms, such as seizures, movement disorders, spasticity, dystonia, etc., should also be targeted. Lastly, molecular therapeutic possibilities are also being explored in animal studies. In this review, we elucidate on the current treatment options available for hereditary ataxias.

Keywords
- ataxia
- treatable
- cerebellar
- hereditary ataxia

Introduction

Hereditary ataxias (HAs) are a group of neurodegenerative disorders with variable and multiple neuraxial involvement, including cognition, seizures, movement disorders, extrapyramidal systems, and peripheral neuropathy.[1] The implications of a diagnosis of HAs include not only an inexorably progressive course but also a lack of curative therapies. In the absence of the same, management remains essentially supportive and symptomatic. We review the current therapeutic options available in this group of disorders.

Approach to Treating Hereditary Ataxias

HAs are categorized based on the pattern of inheritance into autosomal recessive and dominant ataxia, mitochondrial ataxia syndromes, X-linked HAs, as well as episodic, and congenital ataxias. There are no U.S. Food and Drug Administration–approved medications till date for the treatment of HAs. Most of the drug usage is based on case series and small trials. Such studies often have limited clinical translation. Also, the treatment duration is usually brief, and therefore long-term treatment effect is uncertain.[2]

A presumptive diagnosis about the cause is imperative, since the treatment depends upon it. However, this is challenging because of the myriad of etiologies that can present similarly.[3]

Autosomal Recessive Cerebellar Ataxias

Autosomal recessive cerebellar ataxias (ARCA) occur due to functional impairment of proteins involved in the lysosomal or mitochondrial pathways.[4] This opens up potential therapeutic avenues that target the pathogenic pathway in autosomal recessive ataxias. ARCAs are not just ataxia syndrome but involve a huge range of symptomatology including peripheral neuropathy, intellectual disability, dementia, extrapyramidal syndrome, oculomotor apraxia, etc.[5] ►**Table 1** summarizes treatment options of autosomal recessive ataxias. We also discuss below the management of neurological and nonneurological features in this group.

Treatment of Ataxia

Ataxia, secondary to a metabolic mechanism, responds to and slows down in response to appropriate supplementation. The disorders amenable to this form of therapy include

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Table 1 Treatment options in autosomal recessive cerebellar ataxias

Disorder	Drug	Dose
Friedreich ataxia	Idebenone Coenzyme Q10	5–20 mg/kg/day 30 mg/kg/day
Ataxia with vitamin-E deficiency	Vitamin E	800–1,200 mg per day
Abetalipoproteinemia	Vitamin E Vitamin A low-fat diet Medium-chain triglyceride	150 mg/kg
Refsum's disease	Low phytanic acid diet Plasma exchange	Below 10 mg per day
Niemann–Pick type C	Miglustat	200 mg thrice daily
Cerebrotendinousxanthomatosis	Chenodeoxycholic acid	750 mg/day
Ataxia with CoQ10 deficiency	CoQ10 supplementation	30 mg/kg/day
Ataxia with glut-1 deficiency	Ketogenic diet, modified Atkin's diet	

Abbreviation: Co, coenzyme.

ataxia with vitamin-E deficiency (AVED), Refsum's disease, Niemann–Pick disease type C (NPC), cerebrotendinous xanthomatosis (CTX), ataxia with Coenzyme Q10 deficiency, and ataxia with glut-1 deficiency.

Friedreich's ataxia (FRDA) is an autosomal recessive guanine, adenine, adenine (GAA) triplet repeat expansion disorder on the frataxin gene on chromosome 9q13.[6] It is the most frequent form of inherited ataxia. Frataxin, a mitochondrial protein, is involved in iron–sulfur cluster biosynthesis. Multiple therapeutic strategies have been used to address the primary mechanism of injury that includes increasing frataxin levels by histone deacetylase (HDAC) inhibitors or by recombinant human erythropoietin; use antioxidants, such as coenzyme Q10 and vitamin E; lowering mitochondrial iron stores with deferiprone; and improving energy metabolism by supplementation of L-carnitine.[7] However, despite promising results in some earlier studies, controlled studies have failed to demonstrate halting of disease progression with these approaches. Several open-label studies have demonstrated beneficial effects of idebenone on cardiac hypertrophy in FRDA.[7] Treatment with it should therefore be individualized to a subgroup of patients with severe hypertrophic cardiomyopathy and those with early disease.

AVED is an autosomal recessive disease caused by mutations on chromosome 8q13 in the α-tocopherol transfer protein (*TTPA* gene). It presents as a slowly progressive spinocerebellar ataxia (SCA) syndrome, closely resembling Friedreich's (FRDA) ataxia. Cardiomyopathy is less common in AVED, whereas titubation and dystonia are more specific for it.[8] They also have a slower course, with milder neuropathy compared with patients with FRDA. Daily, divided, high doses of vitamin E (800 mg/day) usually lead to cessation of disease progression and neurological improvement, although recovery may be slow and often incomplete. The results of vitamin-E supplementation are more beneficial if started before 15 years of disease duration; the earlier, the better. In a group of 24 patients with AVED, oral vitamin-E supplementation (doses of 800–1,200 mg/day) for 1 year led to symptomatic improvement.[9]

Abetalipoproteinemia is a rare metabolic disease caused by mutations in the gene encoding for the large subunit of microsomal triglyceride transfer protein (*MTTP* gene), on chromosome 4q22–24. This mutation leads to absence of plasma apolipoprotein B containing lipoproteins, which in turn leads to the impaired utilization of fat and fat-soluble vitamins leading to deficiency states. Symptoms usually begin before the age of 20 years.[10] Retinitis pigmentosa also may be seen. The "gold standard" diagnostic test is by sequencing the *MTTP*. Dietary modification consists of a low-fat diet and vitamin replacement of fat-soluble vitamins, such as vitamins E and A. Ataxia in abetalipoproteinemia may also respond to vitamin-E supplementation in large doses (30–88 mg/kg/day), along with vitamins A and D.[8] However, this may require prolonged supplementation (up to 15 years) to bring about stabilization of the ataxic syndrome.

Refsum's disease is a rare autosomal recessive disorder of fatty acid metabolism, caused by mutations of the gene encoding for the peroxisomal enzyme phytanoyl-CoA hydroxylase, on chromosome 10. This enzyme catalyzes the first step in the α oxidation of phytanic acid.[11] Impaired fatty acid oxidation of phytanic acid (found predominantly in dairy products, meat, and fish) leads to accumulation in the body. Analysis of serum phytanic acid levels is done to confirm the diagnosis. Dietary restriction halts disease progression and the goal of therapy is reduction of normal daily intake of phytanic acid to a maximum of 10 mg/day.[12] This is sometimes not sufficient to prevent acute attacks and stabilize the progressive course. Plasma exchange or chronic lipid apheresis can be done in such cases.[12]

Niemann–Pick type-C disease (NPC) is a rare autosomal-recessive lipid storage disorder, characterized by unique abnormalities of intracellular transport of endocytosed cholesterol along with sequestration of unesterified cholesterol in lysosomes. *NPC1* gene (chromosome 18q11) mutations occur in 95% and *NPC2* gene on chromosome 14q24.3 occur in the remaining. These result in accumulation of glucosylceramide, lactosylceramide, and GM2 and GM3 gangliosides in the brain. This may be a factor contributing to its neurological manifestations. Neurological manifestation predominates in the adult and juvenile forms of the disease, the most common features ones being cerebellar ataxia, vertical supranuclear ophthalmoplegia, dysarthria, dysphagia, intellectual impairment, and movement disorders. Splenomegaly and psychiatric disorders are common accompaniments. Vertical supranuclear palsy is usually present early in the disease but also occasionally develops later in the disease. Miglustat, a glucosylceramide synthase inhibitor which prevents glycolipid accumulation, dosed at 200 mg thrice daily, stabilizes disease progression in most patients treated for

1 year or more, based on a composite assessment of parameters: horizontal saccadic eye movement velocity, ambulation, swallowing, and cognition. The overall benefits are generally modest, suggesting that miglustat may slow, but not halt, the progression of neurological abnormalities.[13,14] Cyclodextrin, a cholesterol-sequestering agent, has also shown some possible therapeutic value in preliminary studies in NPC, and clinical trials are underway for the same.[13]

CTX is a rare, autosomal recessive disorder of lipid storage caused by a mutation of the enzyme 27-sterol hydroxylase (*CYP27* gene) on chromosome 2, which forms a part of the hepatic pathway for bile-acid synthesis. Reduction in their synthesis leads to an increase in levels of serum cholestanol and urinary bile alcohols. They get deposited as xanthomatous lesions in various tissues, particularly the brain, ocular lenses, and tendons, resulting in a variable clinical phenotype, which consists of both neurological and systemic manifestations. Neurological symptoms usually start around 20 years of age and predominantly include cerebellar ataxia, spastic paraparesis, sensorimotor peripheral neuropathy, extrapyramidal signs, seizures, psychiatric problems, and cognitive impairment. Juvenile cataracts, progressive neurological dysfunction, along with mild pulmonary insufficiency, are unique symptoms distinguishing CTX from ataxic disorders. CTX can be diagnosed by testing serum cholestanol and urinary bile alcohol levels. MRI brain reveals global atrophy and parenchymal lesions, nerve conduction studies show an axonal neuropathy, delayed central conduction times are seen on evoked potentials(visual, brainstem auditory, and somatosensory), and electroencephalography typically shows diffuse slowing with paroxysmal discharges. It is treatable by supplementation with oral chenodeoxycholic acid (CDCA), the recommended dose being 250 mg thrice a day. Side-effects include diarrhea, restlessness, and irritability, although infrequent. A combination of CDCA and statins has also been studied but this did not improve ataxia. LDL apharesis has also been used to reduce the cholestanol levels but this did not lead to an improvement in ataxia.[15]

Ataxia with coenzyme Q 10 (CoQ10) deficiency may be due to primary or secondary CoQ10 deficiency. Primary CoQ deficiency occurs due to mutations of genes involved in the coenzyme Q pathway (CoQ2, CoQ9, etc.).[16] Secondary deficiency is due to other genetic mutations (e.g., aprataxin). High doses of CoQ10 (30 mg/kg/day) are shown to be effective in the treatment.[17]

Ataxia with glut-1 deficiency is treated with ketogenic diet and modified Atkin's diet. Alphalipoic acid facilitated glucose transport and may also be of benefit in this condition.[18]

Treatment of Peripheral Neuropathy

Autosomal recessive ataxias are often associated with peripheral neuropathy. Neuropathy in AVED patients responds to vitamin E supplementation. In patients with abetalipoproteinemia, vitamins A and E supplementation improve sensory examination. In Refsum's disease, several reports describe stabilization of peripheral neuropathy with low phytanic acid and plasma exchange.[19] Although patients with CTX may not exhibit much improvement in ataxia, CDCA supplementation may lead to improvement in peripheral neuropathy in a subset of patients.

Treatment of Epilepsy

Glut-1 deficiency is strongly associated with seizures which are highly responsive to diet modifications described above.[20] Cataplexy in NPC patients may respond to antidepressant and central stimulants instead of miglustat.[14]

Treatment of Cognitive Impairment

Miglustat in NPC and CDCA in CTX patients have been shown to improve cognition.[13,15]

Treatment of Movement Disorders

AVED patients often have head tremor and dystonia, the latter being responsive to vitamin-E therapy but not the former. Dystonia in CTX may be treated with miglustat. Dystonia in glut-1 deficiency responds to ketogenic and modified Atkin's diet. Other symptomatic measures including anticholinergics for dystonia, β-blockers/primidone for postural tremor, botulinum toxin therapy for focal dystonia, and levodopa therapy for parkinsonism should also be added.

Treatment of Visual Abnormalities

Although autosomal recessive ataxias exhibit characteristic visual involvement, the response to therapy is poor. AVED and abetalipoproteinemia have retinitis pigmentosa which does not respond to vitamin E. NPC patients develop cataracts unresponsive to dietary therapy and plasma exchange.[21]

Autosomal Dominant Ataxias

Autosomal dominant cerebellar ataxias are classified into SCAs and episodic ataxias (►**Table 2**). These disorders are managed as follows.

Treatment of Ataxia

Riluzole is a potassium channel opener and regulates the activity of deep cerebellar nuclei leading to reduction in neuronal hyperexcitability. In a study of 40 patients with various cerebellar ataxia, 100 mg per day of riluzole led to a reduction in the scale for the assessment and rating of ataxia (SARA) compared with placebo.[22] These findings were also replicated in another study on different forms of cerebellar ataxia in which 50% of patients in the riluzole arm showed improvement compared with placebo arm (11%) in the SARA scale.[23] Although a promising drug, further studies are essential to evaluate its efficacy in HAs.

Lithium carbonate has also been studied in patients with SCA type 3 (SCA3) by a phase-II clinical trial. The outcome scales used were the mean neurological examination score

Table 2 Treatment options in autosomal dominant cerebellar ataxias

Therapy	Disorder	Dose
Riluzole	SCAs and other HAs	100 mg/day
Varenicline	SCA3	1 mg twice daily
Buspirone	SCAs	30 mg twice daily
Zinc	SCA3	50 mg twice daily
Insulin-like growth factor 1	SCA3	50 mcg subcutaneously twice daily
Acetazolamide	EA2	250–1,000 mg per day
4-amino pyridine	EA2	5 mg thrice daily
Mexiletine and carbamazepine	SCA3	For cramps
Botulinum toxin type A	SCA3	For dystonia and spasticity

Abbreviations: EA, episodic ataxias; HA, hereditary ataxia; SCA, spinocerebellar ataxia.

for the assessment of spinocerebellar ataxia (NESSCA) which did not show a difference between groups.[24]

Zinc therapy (50 mg per day) was also evaluated in 36 Cuban patients with SCA type 2 (SCA2) in a randomized double-blind trial.[25] A small benefit in terms of decrease in ataxia scores and saccadic latency on the SARA scale was reported. Zinc therapy was also tolerated well by study participants.

Varenicline, a partial α4β2 agonist at the neuronal nicotinic acetylcholine receptor used for smoking cessation, has also been studied in SCA3 in 20 patients.[26] There was a trend toward improvement of SARA scores in axial and rapid alternating movements in patients with SCA3 at the end of 8 weeks.

Serotonin deficiency has been hypothesized to play a basis in the development of ataxia. Buspirone was shown to be not superior to placebo when administered over 3 months.[27] However, citalopram, a selective serotonin reuptake inhibitor, was shown in a SCA3 mouse model to reduce ataxin deposits, as well as improve motor symptoms.[28] This could be a promising therapy.

The insulin-like growth factor-1 (IGF-1) is a central nervous system (CNS) neuromodulator. It has been studied in patients with SCA3 and SCA type 7 (SCA7) in a 2-year prospective uncontrolled clinical trial. Administration of IGF-1 in the doses of 50 µg/kg/twice a day subcutaneously have been found to improve ataxia in SCA3 patients over 8 months.[29]

The second group of autosomal dominant cerebellar ataxias includes episodic ataxias (EA). EA1 may respond to various drugs, such as acetazolamide, valproate, and carbamazepine- and lamotrigine in EA1. EA2 is managed with acetazolamide and 4-aminopyridine, a potassium channel blocker.[30]

As per the American Academy of Neurology (AAN) comprehensive systematic review summary published in 2018,[31] in episodic ataxia type 2, 4-aminopyridine at 15 mg/day probably reduces ataxia over 3 months. In ataxia of mixed etiology, riluzole probably improves ataxia over 8 weeks. For FRDA and SCA, riluzole probably improves ataxia at 1 year. For SCA3, valproic acid at 1,200 mg/day, possibly improves ataxia at 12 weeks. Thyrotropin-releasing hormone possibly improves some ataxia signs over 10 to 14 days. For ambulatory, SCA3 patients, lithium probably is not effective over 48 weeks.

Treatment of Movement Disorders

Patients with SCA often have movement disorders. SCA3 patients may exhibit Parkinsonism that responds to levodopa therapy to some extent.[32] Other medications for symptomatic benefit include anticholinergics, benzodiazepines, baclofen, and carbamazepine, as well as botulinum toxin therapy.

Treatment of Sleep Abnormalities

Sleep disorders including restless leg syndrome, rapid eye movement sleep behavior disorder, excessive daytime sleep (EDS) predominate among the nonmotor manifestations in patients with SCA.[33] These are managed with appropriate therapy as in any other condition.

Treatment of Other Motor Symptoms

Pain in SCA patients may be musculoskeletal, or secondary to dystonia and spasticity. Pain may respond to baclofen and amitriptyline. Cramps may be treated with carbamazepine and mexiletine.[34] Sulfamethoxazole-trimethoprim and baclofen have been reported to benefit spasticity and rigidity in patients with SCA3.[35] Botulinum toxin injection may also be used for the treatment of dystonia.

Treatment of Psychiatric Issues

Patients with SCA may have associated anxiety and depression. These issues should be screened for and treated appropriately in all patients with SCA.

X-Linked Cerebellar Ataxias

These disorders have an onset during childhood to early adulthood and are associated with cerebellar dysgenesis.[36] Clinically, these disorders present with ataxia, hypotonia, and cognitive dysfunction. These chiefly include oligophrenin, calcium/calmodulin-dependent serine protein kinase, Solute Carrier Family 9 Member A6, and ABC-binding cassette transporter B7. Management of these disorders is supportive and symptomatic in the absence of any specific curative therapy.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a form of late-onset cerebellar ataxia associated with intention tremors. It is a neurodegenerative disorder that occurs due to expanded cytosine guanine guanine triplet repeats in the *FMRI* gene.[37] There is no specific therapy for FXTAS. Propranolol and primidone are used for intention tremor. Memantine in one trial was shown to impart some benefit in verbal memory.[38] Bilateral deep brain stimulation in the zona incerta/VoP has recently shown to be of benefit in tremor, as well as ataxia, to some extent in recent reports.[39]

Mitochondrial Ataxias

Mitochondrial disorders affect the respiratory chain, leading to impairment of tissues highly dependent on aerobic metabolism. Neurological mitochondrial disorders include ataxia,

dementia, epilepsy, stroke-like episodes, encephalopathy, and movement disorders. Although a Cochrane review did not identify any specific therapeutic benefit, several vitamins, and other cofactors have been used in management of mitochondrial disorders.[40] As such, the treatment is largely supportive, including cataract surgery, pacing for cardiac arrythmias, and medical management of endocrinopathies, such as diabetes mellitus.

Disease Modifying Therapies

These therapies target the genetic abnormality in HA to circumvent the syndrome. FRDA has been evaluated extensively in this regard. One approach has been to increase frataxin expression by histone deacetylase inhibition. High-dose nicotinamide (2–8 g/day) has been studies in ten patients for 2 weeks. Patients in this study had increased frataxin expression. However, these were not associated with clinical improvement.[41] RG2833 is a drug in phase-I trial that leads to increased frataxin expression.[42] These limited studies support the potential role of epigenetic interventions in FRDA.

Diseases with polyglutamine repeat, such as SCA have "toxic gain of function" in the related protein expression. Hence, therapies for downregulation of pathogenic gene expression are potentially beneficial.

Gene silencing strategies administered to SCA3 transgenic mice led to motor and pathological improvement. Intracerebral injection in SCA2 transgenic mice of antisense oligonucleotides against *ATXN2* also led to improved motor function.

Recently, trehalose, a chemical chaperone protective against cell toxicity has been tested in SCA3.[43] It prevents pathological protein aggregation within cells. A trial is currently underway (ClinicalTrials.gov Identifier: NCT02147886).

Neurorehabilitation in Hereditary Ataxias

Strategies for rehabilitation in patients with HA include physical therapy, speech therapy, and occupational therapy. Physical therapy incorporates conventional physical therapy, treadmill exercises, biofeedback therapy, as well as computer-assisted training. A combination of intensive physical therapy with occupational therapy may be of most benefit.[44] In a systematic review of rehabilitation in degenerative ataxias, 17 studies met the inclusion criteria. Fifteen of these 17 studies showed an improvement in at least one outcome of gait, ataxia, balance, and function.[45] The other conclusions of this review were that greater intensity (60 minutes or more at least thrice weekly) had improved effectiveness and 4 weeks of rehabilitation was needed to see benefit on ataxia, and three for benefit on balance. Also, multifaceted programs may have greater effect. Various rehabilitation strategies may be employed, personalized to the patient. For initial ataxia stages, sport-based exercises, such as tennis and badminton, may be preferred as they challenge the coordination system. Virtual reality systems, such as XBOX games could also be used in addition.[46] In patients with moderate ataxia, physiotherapy, in addition to falls, training should be employed. In advanced ataxia, physiotherapy may not be of great benefit, but treadmill training may be of benefit. The role of speech therapy is less certain. A Cochrane review concluded that evidence so far is insufficient to support a role of speech therapy in HA.[47]

As per the AAN systematic review,[31] among nonpharmacologic options for degenerative ataxias, a 4-week inpatient rehabilitation probably improves ataxia and function. Transcranial magnetic stimulation possibly improves cerebellar motor signs at 21 days.

Genetic Counseling

Genetic counseling is the process of education of patients and family members about a genetic disorder to assist them in making medical and personal decisions. If a proband is afflicted with a specific ataxia syndrome, he or she should be provided appropriate counseling about it. For autosomal dominant cerebellar ataxias, most probands have an affected family member. Family history may be negative in the event of early parental death, late onset of the disease in the parent, incomplete penetrance of the disease, or de novo mutations. The risk to the proband's siblings is 50% if one parent is afflicted. The offspring of the proband also has a 50% risk of inheriting the pathogenic mutation. For proband with autosomal recessive cerebellar ataxia, the parents are obligate heterozygotes and are asymptomatic. The sibling has a 25% chance of being affected, 25% chance of being unaffected and 50% chance of being a carrier. The offspring of these probands are obligate heterozygotes. In X-linked ataxias, the father of a male proband is neither affected nor a carrier. The mother with an affected male relative is an obligate heterozygote. Male siblings will be affected; female siblings will be carriers.

For at-risk adult asymptomatic individuals, genetic testing should be offered in the context of genetic counseling and only after the genetic diagnosis is confirmed in the proband.[48] For at-risk asymptomatic individuals below the age of 18 years, genetic testing for a disorder that does not have treatment is not considered to be appropriate and may have debilitating personal and social implications.[48] Recent advances have made possible preimplantation genetic diagnosis (PGD).[49] Once the pathogenic mutation has been identified in an affected individual, prenatal testing, as well as PGD, is possible. In this procedure, genetic testing of ova during in vitro fertilization is conducted, and embryos free from the pathogenic mutation undergo implantation to ensure disease-free progeny.

Neuromodulation

Noninvasive cerebellar stimulation using anodal transcranial direct current stimulation (tDCS) have shown some benefit in ataxia in terms of posture and gait.[50,51] In one randomized trial, a combination of cerebellar anodal tDCS in combination with spinal cathodal tDCS has been studied in 21 patients with neurodegenerative ataxia. In this study, 2 weeks of cerebellospinal tDCS showed a significant

improvement in SARA, International Cooperative Ataxia Rating Scale, 9-Hole Peg Test, and 8-m walking time compared with sham stimulation.[52] Transcranial magnetic stimulation (TMS) has also been assessed in one randomized double-blind sham controlled trial with 74 patients with mixed ataxias. Patients undergoing TMS showed some improvement in 10-minute walk time, number of steps in 10 m walk, and standing capacities.[53]

Molecular Therapeutics

With advances in genetics, antisense oligonucleotides (ASO) have already become available as molecular therapies in a range of neurodegenerative disorders, such as nusinersen for spinal muscle atrophy, eteplirsen for Duchenne's Muscle Dystrophy and Inotersen for familial amyloid polyneuropathy. In two complimentary mouse models of SCA3, ASOs that targeted human ATXN3 were targeted. ASOs were shown to suppress ATXN3 in the Q84 model but not in the second model (Q135 cDNA).[54] Such studies are paving the way forward in the development of genetic therapies.

Conclusion

HAs are a group of neurodegenerative syndromes without any curative therapy. However, a wide range of supportive and symptomatic therapies are available which should form the backbone of management in these patients. Riluzole has the best evidence for ataxia treatment so far. However, studies that have assessed riluzole have been small and clinical benefits modest. Rehabilitation also has a role with some evidence for multiple modality rehabilitation strategies and increased intensity, neuromodulation through transcranial magnetic simulation, and transcranial direct current stimulation have a possible role and demand more exploration. Several trials are ongoing targeting treatment of HA which may yield fruitful results in the future. As in several neurological disorders, perhaps molecular therapeutics, hold the key to the treatment of hereditary ataxias in coming times.

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Conflict of Interest
None declared.

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Brain Edema: Newer Concept of Treatment

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Abstract

Brain edema is excess accumulation of water in intracellular or extracellular spaces of the brain. It may be due to traumatic brain injury, neoplasm, infection, or following surgery. Advent of electron microscope and molecular pathophysiology of fluid transport through blood–brain barrier has elucidated the mechanism of edema formation, that is, ion channels and transport of fluid into extracellular space. Currently approved treatments, such as decompressive craniectomy and osmotherapy, controlled hyperventilation, and administration of diuretics, were developed prior to any knowledge of modern cerebral edema pathophysiology. These therapies attempt to manage downstream end-stage events without directly attenuating the underlying molecular mechanisms of cerebral edema. Next few years will yield new knowledge of how particular proteins drive edema influx, paving the way for rationally designed therapeutics that directly target key steps in cerebral edema formation, thereby achieving what currently approved therapies do not. Pharmacological agents which can block edema formation are being tried experimentally and clinically. Development in imaging, that is, computed tomography and diffusion tensor magnetic resonance imaging, has helped in antemortem assessment of evolution and resolution of brain edema as a dynamic pathophysiology. Animal studies shows release of vasoactive substances, that is, histamine, serotonin, adrenaline, nitric oxide, substance P, prostaglandins, tumor necrosis factor-α, and cytokines, in the injured brain results in activation of inflammatory cascade, which is the important cause of brain edema.

Keywords

- cerebral edema
- blood–brain barrier
- inflammation
- corticosteroid
- ion transport

Introduction

Brain edema is an excess accumulation of water in the intracellular and/or extracellular spaces of the brain. Brain edema after traumatic brain injury is a frequent finding. Brain edema is often associated with neoplasm, infection, that is, abscess and granulomas, and following surgery. Grossly, in older German terminology of brain edema, the cut surface oozes fluid (Hirn edem). In brain swelling, the cut surface is dry (Hirn swellung). With the advent of light and electron microscope and molecular basis of fluid transport through blood–brain barrier (BBB), further understanding of the structure and function of the barrier and the mechanism of edema formation has evolved in the last few decades, that is, ion channels and transports, so pharmacological agents which can block edema formation is being tried experimentally and clinically.[1,2] New developments in imaging, that is, computed tomography and diffusion tensor magnetic resonance imaging, has helped in antemortem diagnosis, evolution, and resolution of brain edema as a dynamic pathophysiology.[3,4]

Types of Brain Edema

Vasogenic brain edema is caused by disruption of the BBB. Intravascular fluid escapes through the endothelium (pinocytosis), or leaky capillary tight junction, for example, through trauma, tumor, hemorrhage, and granuloma.

Cytotoxic edema (oncotic cell swelling) is characterized by accumulation of water inside the neurons, microglia, and astrocytes. Sometimes they bloat or rupture and fluid

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escapes into extracellular space, for example, infarction and neurotoxic agents. Ionic edema, an extracellular edema that occurs in the presence of an intact BBB, forms immediately following cytotoxic edema.

Osmotic edema occurs when plasma dilution decreases serum osmolality, resulting in a higher osmolality in the brain compared with the serum. This creates an abnormal pressure gradient and movement of water into the brain, which can cause progressive cerebral edema, resulting in a spectrum of signs and symptoms from headache and ataxia to seizures and coma, for example, water intoxication and hepatic failure.

Hydrocephalic edema (*interstitial*) occurs in obstructive hydrocephalus due to a rupture of the cerebrospinal fluid (CSF)–brain barrier. This results in transependymal flow of CSF, causing CSF to penetrate the brain and spread to the extracellular spaces and the white matter. Interstitial cerebral edema differs from vasogenic edema as CSF contains almost no protein.

Experimental/Clinical Studies

Since the release of vasoactive chemicals in the injured tissue of live human being is difficult to demonstrate, we used animal head injury models by direct demonstration of vasoactive substances done in the injured brain. Samples of edematous brain were collected during surgery, and blood samples of head injured patients were collected serially demonstrating rise of vasoactive substances. Prognosis following head injury patients correlated well with the decrease and normalization of vasoactive agents. Interestingly, immunological study in severely head injured patients shows rebuilt of anti-brain antibodies possibly due to escape of cerebroproteins to circulation.[5-10]

Inflammatory substances mediating vasogenic brain edema has been studied in rats, dogs, rabbits, and cats. Trauma was produced by stab wound of the brain in the former and fall of weight in the later animals. Intracerebral hemorrhage was created by injecting blood into the brain of rats.[8] Important finding was the release of vasoactive substances resulting in inflammation, that is, histamine, serotonin, adrenaline, nitric oxide, substance P, prostaglandins, tumor necrosis factor-α and cytokines, in the injured brain.[5-8,10] Recent studies reveal important role of inflammation as a cause of edema formation.[7,11] All those substances play an important role in opening the endothelial junction and efflux of fluid, leukocytes, and platelet into the extracellular space. They also help in the escape of intravascular fluid through endothelial cells by pinocytosis. Increased levels of biogenic amines and tissue enzymes in the blood/CSF is an indirect evidence of increased BBB permeability and related to prognosis.[12-19]

Pathogenesis

Disruption of the BBB is the most important prerequisite for edema formation. Several mediators have been discovered to act at the BBB either passively or actively. Serum which escapes into the extracellular spaces ultimately increase tissue volume and raise intracranial pressure (ICP). Both vasogenic and cytotoxic edema results in increased ICP and eventually decreased cerebral perfusion pressure (CPP). This is in line with the Monro–Kellie hypothesis which states that "the sum of the intracranial volumes of blood, brain, CSF, and other components is constant and that an increase in any one of these must be offset by an equal decrease in another." Elevated ICP and diminished cerebral perfusion can lead to tissue ischemia. Ischemia in turn activates autoregulatory mechanisms designed to restore cerebral perfusion. However, vasodilation increases cerebral blood volume, which in turn increases ICP, lower CPP, and provokes further ischemia.[17]

After traumatic brain injury, cerebral blood flow (CBF) autoregulation is impaired or abolished in most patients. When pressure autoregulation is impaired or absent, ICP decreases and increases with change in CPP. Also, autoregulatory vasoconstriction seems to be more resistant compared with autoregulatory vasodilation which indicated that patients are more sensitive to damage from low rather than high CPPs. Molecular biologic studies recently reveals trance endothelial passage of fluid into the extracellular space resulting in brain edema by active transporter and aquaporins.[2,12]

Pitfalls of Using Animal Models (Factors of Laboratory to Bedside Treatment Translational)

Commonly used animal model of acute central nervous system injury do not accurately reflect human disease. Fault with experimental design may lead to false positives. Clinical trials often do not replicate promising results in experimental studies.

Treatment of Brain Edema

The goal of medical management of cerebral edema is to maintain optimal ICP, ensure regional and global CBF to meet the metabolic requirements of the brain, and prevent secondary neuronal injury from cerebral ischemia.[17]

Standard medical management of cerebral edema involves using a systemic approach, from general measures, that is, optimal head and neck positioning for facilitating intracranial venous outflow, proper airway, avoidance of dehydration, and systemic hypotension, and maintenance of normothermia, to specific therapeutic interventions like controlled hyperventilation, administration of diuretics, osmotherapy, and pharmacological cerebral metabolic suppression.[17,20,21] Some of the drugs clinically used and others under experimental studies are listed in ►**Table 1**.

Future treatment is possibility of drug cocktail which will be useful to prevent secondary brain injury and protect the neurons.

Comparison of vasogenic edema associated with trauma and tumor:Dexamethasone and mannitol are very effective in cases of tumor edema (►**Figs. 1** and **2**),[4,17] whereas it is not very effective in trauma edema. Thus, traumatic vasogenic edema is multifactorial involving BBB leakage, cytoplasmic

Table 1 Drugs reducing vasogenic brain edema

Factors increasing edema	Inhibitory/Blocking substances
Ion channel cotransporter-Na^+-K+-2Cl- cotransporter suri regulator NC C8-ATP	Bumetanide Glibenclamide
• Vasopressin (V1 A and V2 receptor) antagonist	Conivaptan
• Inflammation mediators • Oxidative mediators • Adhesion mediators • Cytokines, IL-1α, 1β, TNFα, IL-6 • Chemokines • Catecholamines	Anti-inflammatory drugs, i.e., indomethacin steroids Pentoxifylline pCPA H2-blockers-ranitidine/cimetidine Ibuprofen
• Enzymes (increases blood and CSF) LDH, ALD, MDH, GPT, GOT, cyclooxygenase-2	Cortisone, CRF Metaraminol Acetazolamide Dextran Urea SC236 and dexamethasone rofecoxib
• Hemoglobin degradation product(free iron)	Iron chelation
• Free fatty acids	Endogenous inhibitors (long chain fatty acids)
• Prostaglandins mitochondrial permeability damage cerebral anaerobic metabolism polyamines free radicals endothelin chloride transport carbonic anhydrase kappa opioid aquaporin 4	Indomethacin Cyclosporin A Citicoline lactate NMDA receptor antagonists–ifenprodil Scavengers–vitamin C and E 21 aminosteroids Edaravone N-acetyl cysteine Citicholine Endothelin antagonists–patent EPO 838223 CL transport inhibitor–torase CA inhibitors–acetazolamide agonist, niravoline, dexamethasone, and HCRF

Abbreviations: CA, carbonic anhydrase; CL, chloride; CRF, corticotropin-releasing factor; HCRF, human corticotropin-releasing factor; IL, interleukin; TNF, tumor necrosis factor.

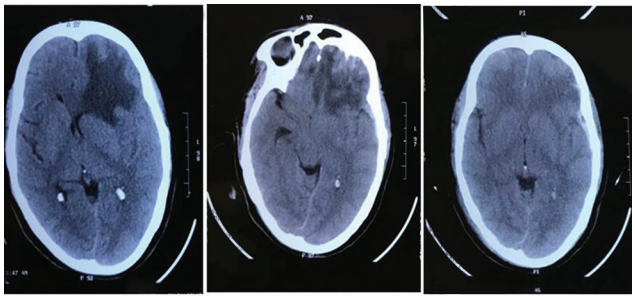

Fig. 1 Resolution of traumatic edema (**A**–**C**). (**A**) Postinjury. (**B**) 72 hours later (20% mannitol intravenously 100 mL/8 hours). (**C**) 2 weeks later (no intravenous mannitol).

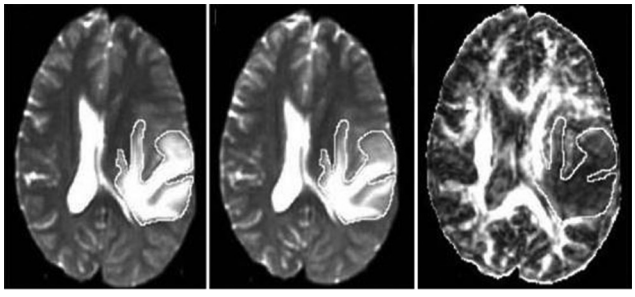

Fig. 2 (**A**–**C**)Resolution of peritumoral edema (diffusion tensor magnetic resonance imaging [DT-MRI]) with dexamethasone treatment. (**A**) Before steroid therapy. (**B**) 24 hours after steroid therapy. (**C**) 72 hours after steroid therapy.

transport, inflammatory mediators released from endothelium, platelets, and glial cells.

Surgical decompression and use of osmotherapy to reduce brain edema and its deleterious effect remains the mainstay of treatment even today. This only attenuates the primary injury but cannot abate the secondary cascade of events. Drugs which inhibit or slow the various secondary mechanisms are still in an experimental stage.

Conclusion

Currently approved treatments for cerebral edema—decompressive craniectomy and osmotherapy—were developed prior to any knowledge of modern cerebral edema pathophysiology. These therapies attempt to manage downstream end-stage events without directly attenuating the underlying molecular mechanisms of cerebral edema.[2]

The water movements involved in cerebral edema are dependent upon ionic fluxes, which are ultimately mediated by individual channels and transporters. The study of cerebral edema is essentially the study of maladaptive ion transport. While significant gaps still remain in our understanding of how specific proteins contribute to cerebral edema, the fields of cerebral edema and brain CSF dynamics are robust and productive. Doubtlessly, the next few years will yield new knowledge of how particular proteins drive edema

influx, paving the way for rationally designed therapeutics that directly target key steps in cerebral edema formation, thereby achieving what currently approved therapies do not.

Conflict of Interest
None declared.

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Acute Stroke Imaging: Current Trends

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Abstract

The management of acute ischemic stroke has witnessed a paradigm change in the last few years with the advent of mechanical thrombectomy. Imaging plays a key role in evaluation and patient selection. Computed tomography (CT) forms the workhorse in most centers due to its widespread availability and quick performance, though magnetic resonance imaging (MRI) can also be adopted as a reasonable alternative. The key role of imaging is to rule out hemorrhage and other stroke mimics while at the same time establish early signs of ischemia and provide detailed information of cervicocranial vasculature and salvageable brain parenchyma; all in the shortest timeframe. Key imaging predictors of good clinical outcomes are good Alberta stroke protocol early CT score (ASPECTS) (greater than 6) and collateral scores. Selection of patients beyond the standard window period of 6 to 8 hours has become possible by tissue perfusion imaging with some recent trials demonstrating the utility of thrombectomy even up to 24 hours. Quick MRI-based protocols are being devised to achieve similar information as on CT with no adverse effects related to radiation and contrast effects. Research is underway to decipher the intricacies of blood flow in the brain through more sophisticated imaging methods in attempt to increase the base for mechanical thrombectomy, which will benefit more number of patients.

Keywords
- ischemic stroke
- mechanical thrombectomy
- computed tomography
- MRI
- cervicocranial vasculature
- salvage brain parenchyma

Introduction

The realization in the last couple of decades that manifestations of stroke can be reversed has completely transformed the management strategies from palliative care to immediate and prompt institution of treatment. From intravenous tissue plasminogen activator (tPA) therapy[1] to clot retrieval by endovascular mechanical means,[2] the last two decades have witnessed a paradigm shift in stroke treatment. The only hurdle that still remains is time following stroke onset at which the patient arrives and receives treatment,[3] which still proves to be a dealmaker or a deal-breaker. As in many other pathologies of the human body, so also in acute stroke, imaging and treatment strategies are closely intertwined. Imaging results often govern the choice of treatment options and many a times the reverse is also true whereby, a particular treatment modality may determine what imaging needs to be performed. Thus, imaging has an immense role to play in choosing which patients will best respond to stroke treatment. The various nitty-gritties of acute stroke imaging form the core of the following article.

Pathophysiology

The optimal functioning of neurons and synapses is heavily dependent on the availability and supply of oxygen and nutrients. Any interruption in these can lead to progressive irreversible damage of the functional neural systems over a course of few hours. This is unlike other cells of the body, which have the capacity of healing and regenerating. Time, indeed, is brain. For a better perspective, approximately 1.9 million neurons die each minute of nutrient deprivation, which extrapolates to aging by approximately 3.6 years each hour without treatment.[4] In terms of clinical interpretation,

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analysis of SWIFT PRIME and STAR trial data[5] showed that treatment initiated within 2.5 hours of symptom onset resulted in independent function in 91% of patients; 1 hour delay in treatment decreasing a positive clinical outcome by 38%.[6] Beyond 3.5 hours after symptom onset, every 60-minute delay results in a 20% lower chance of regaining function independence.

The pathological connotation of acute stroke can be understood based on a three-compartment model of brain parenchyma following arterial occlusion. The central area of "infarct core" is the first to be involved and represents nonviable brain that cannot be salvaged even with very prompt treatment. The immediately surrounding "ischemic penumbra" represents brain with reduced blood flow that has potential for survival if blood flow is rapidly restored. The outermost affected zone represents brain tissue that is likely to survive even without such treatment. Biochemically, tissue damage occurs by the influx of Ca^{2+} into cells, the release of excitatory amino acids, and the activation of receptors and receptor-operated ion channels.[7] Protein synthesis reduction is the earliest and most sensitive metabolic response to ischemia that may be reversible in the penumbra but not in the core.[8] The penumbra thus forms the target volume of brain reperfusion therapy.[9]

Aim of Imaging

Intravenous tPA was the only Food and Drug Administration-approved treatment of acute ischemic stroke within 4.5 hours, for a long time. After the success of various thrombectomy trials, stentrievers were subsequently approved in suitably selected patients, the main determinant of which is brain imaging. The aim of imaging is multifold, beginning from choosing the most appropriate patients for treatment, to excluding those who are unlikely to benefit, to the extreme subset of the ones who will potentially deteriorate following reperfusion. In the current scenario, imaging modalities essentially involve cross-sectional imaging by either computed tomography (CT) or magnetic resonance imaging (MRI). The target of assessment is the brain parenchyma and the vessels supplying the corresponding parenchyma as well as same/distant perfusion status (►**Fig. 1**).

Broadly, the objectives of imaging are as follows:

1. Identifying infarct/hemorrhage.
2. Ruling out other potential stroke mimickers, for example, gliomas, infections, etc.
3. Detecting early signs of ischemia/infarct (parenchymal evaluation).
4. Identifying the site and extent of vascular occlusion (evaluation of pipe).
5. Attempt to prognosticate the patient's response to treatment and evaluating the ischemic penumbra (perfusion and penumbra).
6. Formulating a treatment decision based on the assessment of all the above-mentioned parameters.

The entire exercise of providing all these answers should not take more than 10 to 15 minutes.

Imaging Modalities

Computed Tomography

CT currently forms the workhorse of acute stroke imaging due to its easy availability, quick data acquisition capability, and reasonably good demonstration of findings. The first and the foremost consideration is to rule out hemorrhage so that intravenous therapy can be immediately instituted if the patient is in window of being treated. This is followed by exclusion of other pathologies (clinical mimics) and identification of signs of ischemia. The CT correlate of ischemia is hypodensity, which develops owing to cytotoxic edema and increased water content followed imminent or actual cell lysis. This hypodensity may be manifested in the form of sulcal effacement and/or loss of gray–white distinction in the ischemic or oligemic zones.[10] The various signs described are loss of insular ribbon sign (►**Fig. 2A**), obscure lentiform

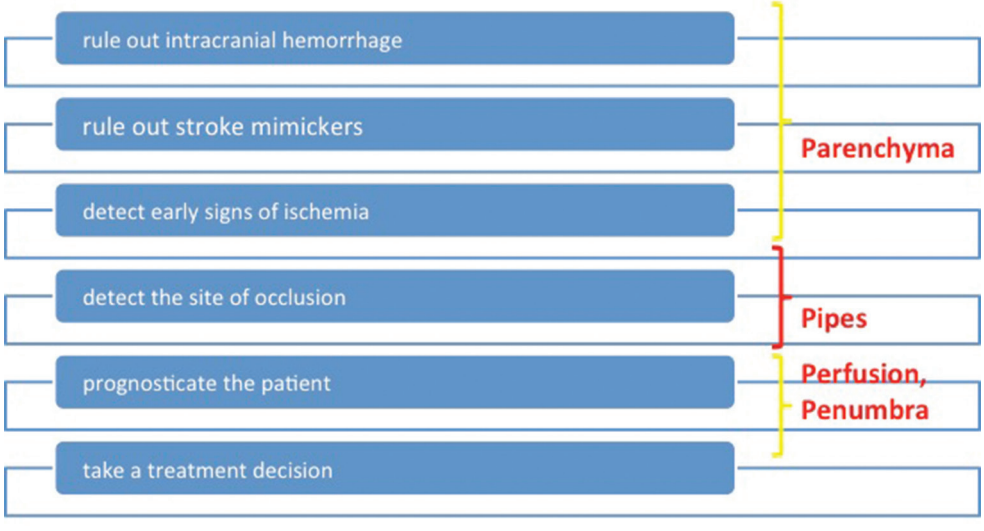

Fig. 1 Algorithmic approach to imaging in acute stroke with reference to the 4 P's (parenchyma, pipes, perfusion, and penumbra).

nucleus sign (►**Fig. 2B**), hyperdense artery sign (hyperdense middle cerebral artery [MCA]/basilar dot sign) (►**Fig. 3**), etc. Standard 5-mm thick sections are often enough, though thin (0.5–1 mm) slices are helpful for increased spatial resolution for detection of small infarcts and to resolve partial volume averaging effects. It may also help in reconstruction of the image in other planes to delineate the infarcts and arterial thrombi better.[11] Sometimes, subtle hypodensities are not discernible. It has been suggested to view all such scans on the console after changing the window settings to "stroke window," which is nothing but reducing the window width to reach a level and width to between 30 and 40 HU (►**Fig. 4**).[12]

It has been seen that the degree of early ischemic changes on CT correlates with stroke severity scores, which is a predictor of clinical outcome.[13] This can be objectively assessed using the Alberta Stroke program early CT score (ASPECTS), which can help in appropriate patient selection for endovascular therapy as well as provide a prognostic marker for treatment response.[14] ASPECTS is a 10-point score which is derived from CT images at ganglionic and supraganglionic sections (►**Fig. 5**). Each structure in the MCA territory is given a 1-point score, with maximum possible being 10. One point is deducted when hypodensity is detected in a particular area. A score of 6 or greater signifies a better response to reperfusion than score of below 6.[15]

There are limitations associated with plain CT scan. It does not accurately indicate the core and the penumbra. However,

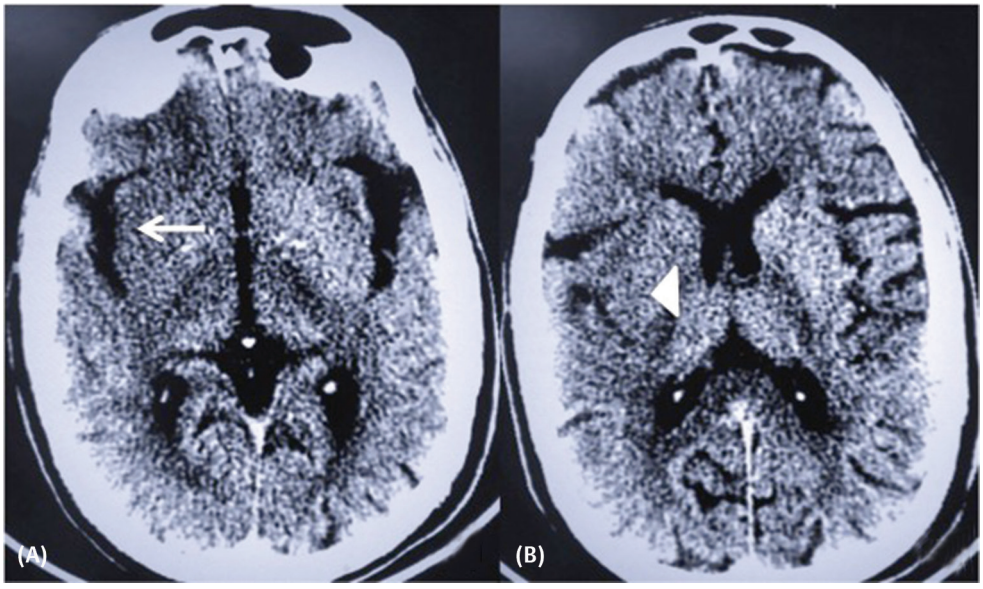

Fig. 2 Axial noncontrast computed tomography (CT) brain sections demonstrating the two early signs of stroke corresponding to loss of gray–white matter definition, namely loss of "insular ribbon" sign (arrow, **A**) and "obscure lentiform nucleus" sign (arrowhead, **B**). Note the normal attenuation of similar structures of the contralateral side.

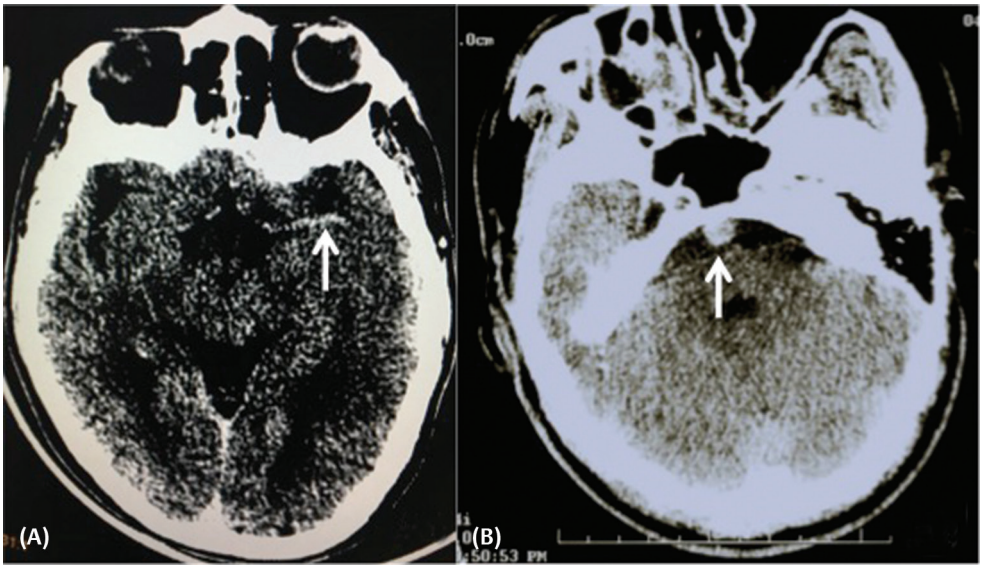

Fig. 3 Axial noncontrast computed tomography (CT) brain sections showing hyperdense arteries (arrows) in two different patients of left middle cerebral artery (MCA) thrombus (**A**) and basilar thrombus (**B**). Note the expanded hyperdense artery.

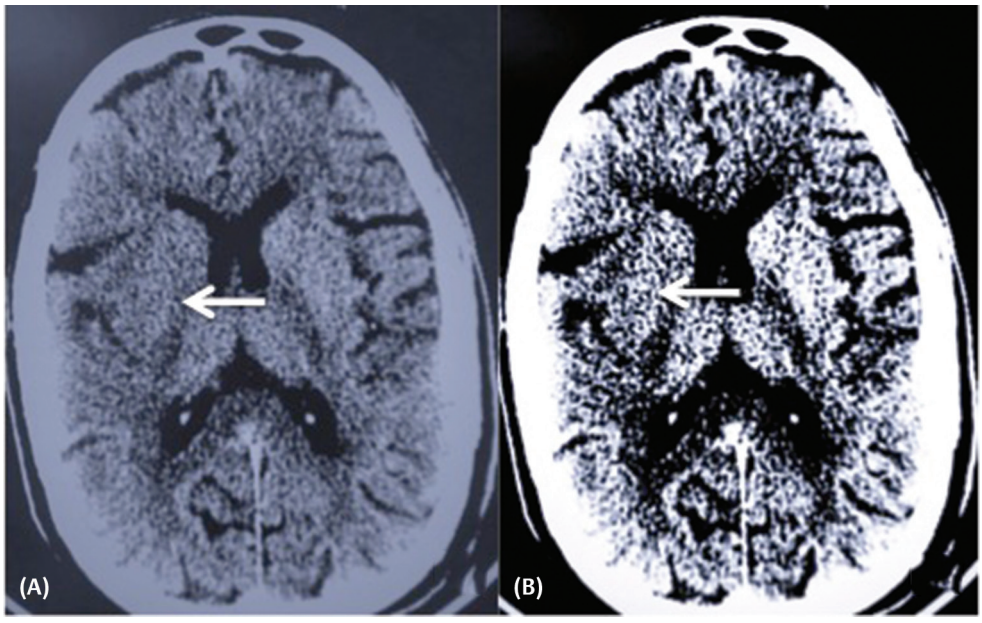

Fig. 4 Axial noncontrast computed tomography (CT) brain section at standard brain window (**A**) and narrow window width setting (**B**) with arrow indicating increased resolution of lentiform hypodensity in the latter settings.

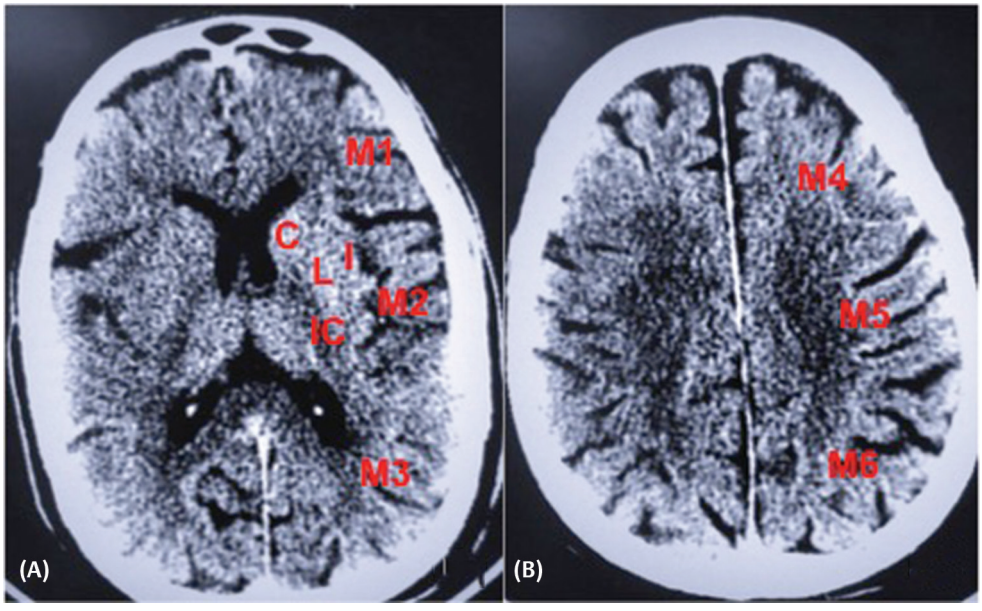

Fig. 5 Demonstration of Alberta stroke protocol early CT score (ASPECTS) at basal ganglionic (**A**) and supraganglionic (**B**) levels. C, caudate; I, insular cortex; IC, internal capsule; L, lentiform; $M^{1\text{-}6}$ respective territorial supply of middle cerebral artery (MCA) territory.

whatever information it provides is currently reasonable for taking treatment decisions. Other pitfalls include artifacts in thin slice imaging, artifacts due to calcification especially in evaluation of the vessels, and radiation hazards.

CT Angiography

CT angiography (CTA) forms an essential part of stroke evaluation providing information regarding the presence, site, and size of the thrombotic occlusion. Volumetric acquisition of the arterial tree from the aortic arch to the vertex is performed when the iodine-based contrast is within the arterial system following "bolus chase" technique. It is quick and provides images with excellent spatial resolution, which can be viewed and reconstructed (►**Fig. 6**) in any plane.[16] Proximal large vessel occlusions (LVOs) respond poorly to intravenous tPA and are indications for endovascular methods. Distal occlusions with low clinical scores respond better to intravenous tPA showing early and better response with fewer number of hemorrhagic complications. CTA may also provide information on possible source of embolism, if any, and identifies tandem lesions. It provides a roadmap to intracranial access prior to thromobectomy.[17] Another potential advantage is the capability of assessing the collateral status distal to the vascular occlusion (►**Fig. 7**). Maintained good distal perfusion has been noted to be a marker for positive

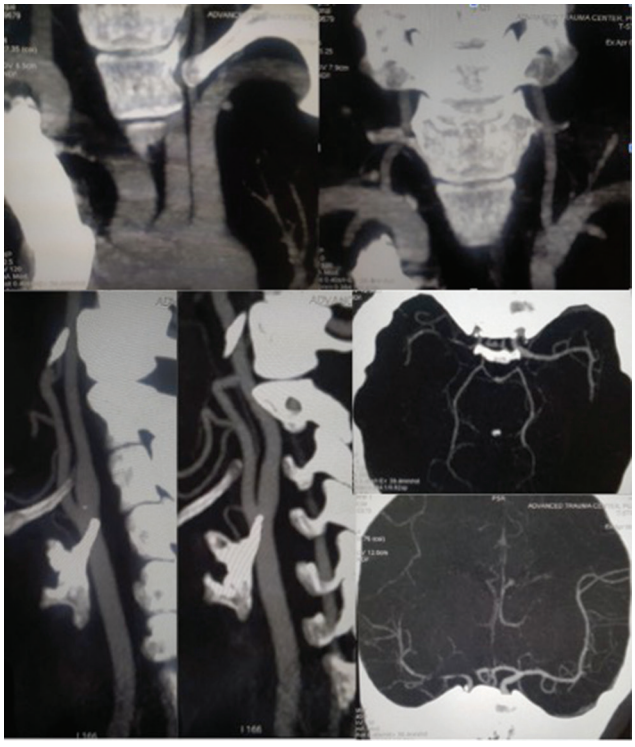

Fig. 6 Computed tomography (CT) angiography depiction of the entire cervicocranial arterial system during stroke evaluation. Note thrombus in the right middle cerebral artery (MCA).

clinical outcome.[18] Several collateral scoring systems are described, most of which classify the collaterals into poor, intermediate, and good grades. The overall essence is that the system should be easily classifiable and replicable having good interobserver agreement with accurate predictive outcomes. Single arterial phase imaging may not always be informative. Some workers have described multiphasic CTA acquisition (typically triphasic), which better brings out the distal vascular details due to opacification of the cortical branches in the delayed phase.[19] The first phase is acquired from the aortic arch to the skull vertex, whereas the subsequent two phases are acquired from the base skull to the vertex at an interval of 4 to 5 seconds each. We, at our institute, have modified the protocol to obtain first and third phase in a quest to reduce radiation dose, achieving reasonably good results. Another advantage of a multiphasic acquisition is the better delineation of intravascular thrombus and accurate measurement of its length and volume. A pseudothrombus can be distinguished from slow/static blood which tends to fill in the delayed phase (►**Fig. 8**). These parameters have been seen to correlate with reperfusion rates with intravenous tPA. Clot length of 8 mm has been described by a group of authors as the cut-off for successful recanalization of MCA occlusion with intravenous tPA.[20]

It has been seen in certain instances of internal carotid artery (ICA) terminus occlusion that slow blood flow in the cervical ICA gives the false impression of a long segment ICA "pseudo" occlusion as the contrast bolus in such cases is so much delayed that it does not reach the proximal cervical ICA in the arterial phase. Delayed phase CTA in such cases is beneficial.

CT Perfusion

CT perfusion (CTP), which initially formed an essential part of stroke imaging CT protocol, fell into disrepute due to its no significant proven role in stroke management, if the patient is in window period. Of late, however, CTP has again bounced back as a modality for demonstrating the salvageable brain tissue in select group of patients beyond the window period as highlighted in DAWN and DEFUSE 3 trials.[21,22] Data acquisition involves scanning the volume of interest (with the availability of multidetector CT scanners currently, the entire brain can be scanned) in either "helical shuttle mode" or "toggle mode" following injection of 40 to 50 mL intravenous iodine-based contrast agent at 5 mL/sec using 18 G intravenous access. CTP samples the first pass wash-in and wash-out of contrast bolus.[23] The data set is processed on the respective workstations to obtain the following maps: cerebral blood volume (CBV), cerebral blood flow (CBF), mean transit time (MTT), and time to peak. These parameters help one in

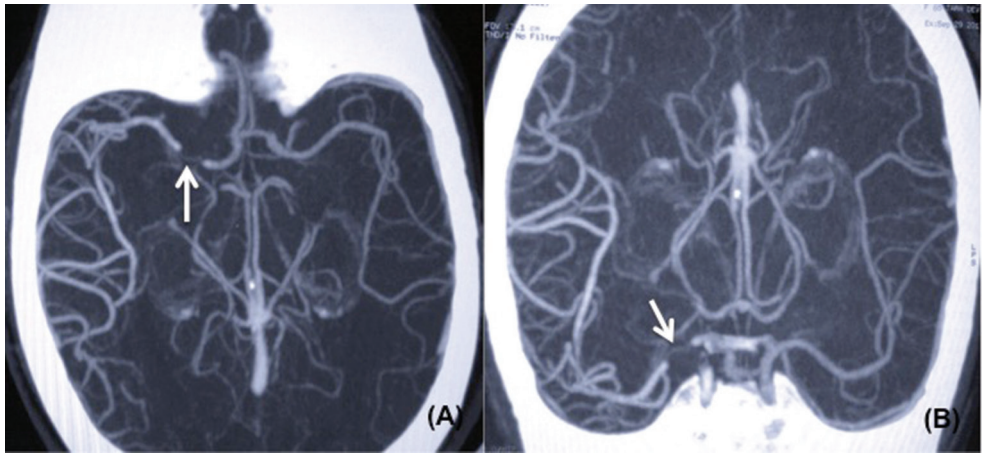

Fig. 7 Axial (**A**) and coronal (**B**) maximum intensity projection formats of computed tomography (CT) angiography demonstrating very good collateral score, depicted by complete opacification of middle cerebral artery (MCA) territory distal to the thrombus (arrow).

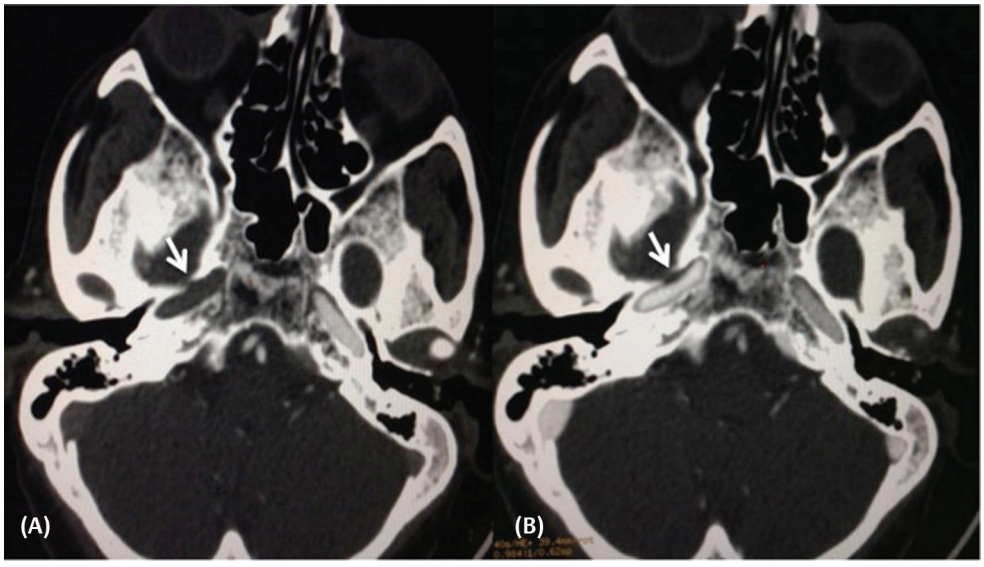

Fig. 8 Arterial (**A**) and venous (**B**) phase of biphasic computed tomography (CT) angiography demonstrating progressive opacification of the right petrous internal carotid artery (ICA) in the venous phase which was nonopacified in the arterial phase (arrow) signifying "pseudothrombus" and highlighting another use of multiphasic computed tomography (CT) angiography acquisition.

deciphering the extent of infarct core and the amount of salvageable tissue. Infarcted area (►**Fig. 9**) shows reduced CBF (< 40–50% of contralateral reference) and CBV with increased MTT (> 145% contralateral reference), while the salvageable penumbra has increased MTT, slightly reduced CBF but maintained or raised CBV. The penumbral hypothesis proposes that the higher is the mismatch between the volume of irreversible ischemia and the volume of hypoperfused but functional brain tissue, the more beneficial the revascularization would be.[24] CTP has also been used for predicting the incidence of hemorrhagic transformation in ischemic stroke and recognize stroke mimics.[17,25] The processing of CTP data sets is time consuming, potentially delaying the treatment. However, to reduce estimation times and increase objectivity for the estimation of volumes of infarcted and penumbral brain tissue, computer software are available which can automatically generate the various tissue volumes. One of these (RAPID) has been shown to significantly improve the patient care by giving accurate values.[26]

The other concerns with CTP are increased radiation dose to the patient, inaccuracies due to carotid stenosis, atrial fibrillation, or reduced cardiac output. Seizures and vasospasm may also give false positive results. Inspite of these, the benefits CTP offers far outscores its deficiencies in stroke patients presenting beyond the window period or in other scenarios where the onset is not clear, for example, wake-up strokes, etc.

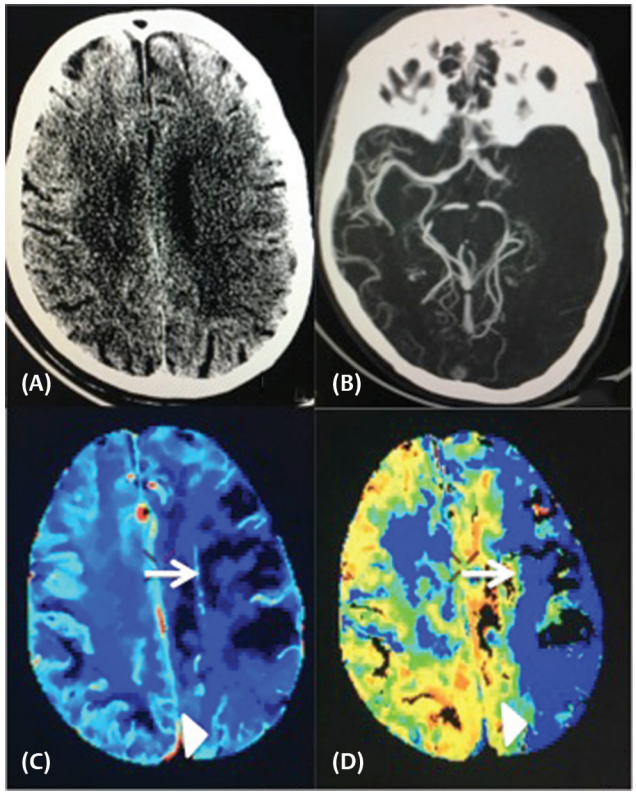

Fig. 9 Noncontrast computed tomography (CT) (**A**), axial maximum intensity projection (MIP) (**B**) CT angiography and subsequent CT perfusion-based cerebral blood volume (CBV, **A**) and mean transit time (MTT, **B**) maps demonstrating left middle cerebral artery (MCA) thrombus with a large infarct core (reduced CBV and prolonged MTT, arrow) and a small ischemic penumbra (maintained CBV but prolonged MTT) posterior to it (arrowhead).

Magnetic Resonance Imaging

MRI provides better soft tissue details than CT. However, its use is limited by scarce availability, long acquisition times, patient motion issues, and problems with metallic hardware that sometimes needs to accompanied with the patient especially in cases of acute stroke. The goals of imaging in acute stroke and the information to be sought, however, stay the same, irrespective of the modality being used, namely CT or MRI. The MR sequences employed for the purpose are T2-weighted, fluid-attenuated inversion recovery (FLAIR)-weighted, diffusion-weighted (DW), and

susceptibility-weighted (SW), perfusion-weighted images along with MR angiography (MRA). However, the entire protocol may take close to 20 to 25 minutes, which is contradictory to basic fundamental of stroke treatment—brevity and promptness. For addressing this issue, short protocols have been devised to reduce the acquisition times and to avoid delay in initiating treatments.[27] MR protocol with FLAIR, diffusion-weighted imaging (DWI), gradient recalled echo (GRE)/SW sequence, and MRA, which can be acquired in 6 to 7 minutes, is seen to provide reasonable information, on which the decision to intervene may be taken. Contrast perfusion may be added depending on the information required.

T2/FLAIR

T2/FLAIR sequences help in identification of early edema. FLAIR may detect subtle subarachnoid hemorrhage that may have been precluded on CT.

GRE/Susceptibility-Weighted Imaging

These sequences have a high sensitivity for identifying hemorrhages. Sometimes clinically silent bleeds and microhemorrhages are detected. In such cases, a dilemma arises whether to reperfuse or not. It has been seen that few bleeds do not negate reperfusion therapy. However, large bleeds may make the patient prone for large hematomas.[12]

DWI

DWI signal changes are determined based on the molecular motion of water. It should be interpreted in the context of apparent diffusion coefficient maps to rule out T2 "shine through" effect. It is the most reliable sequence for detecting cerebral ischemia and delineation of the infarct core. It can detect ischemia as early as 11 minutes after symptom onset.[28] Reversal of DW positive infarcts has been documented following reperfusion in few studies.[29] Large DW lesions have poor clinical outcomes. Some studies have identified 70 mL as the cut-off,[30] while others have found 25 mL as the threshold.[31] Contradictory reports are also available for increased incidence of hemorrhagic transformation following therapy in large sized DW lesions.[32,33]

MRA

MRA is useful for evaluating occlusions, stenosis, intraluminal clots, etc. Noncontrast time of flight method is often useful, though at times, it may falsely overestimate occlusions due to slow flow states.[34] Yet, it has proven to be of clinical benefit when MR is being used for stroke evaluation.[35]

MR Perfusion

MR perfusion (similar to CTP) is very useful for demonstrating potentially salvageable tissue. It can either be performed without contrast (arterial spin labeling [ASL]) or following gadolinium-based contrast (dynamic susceptibility contrast imaging [DSCI]). ASL, being based on water which is an endogenous contrast, may not provide accurate information on CBF.[36] DSCI is the technique of choice where MR protocol is being followed. The information, which it provides is akin to CTP. The DSCI parameters are used in conjunction with DWI to obtain a mismatch, which signifies salvageable penumbra.

Concept of DWI–FLAIR Mismatch

T2 signal on MRI progressively increases with passage of time following acute arterial occlusion on T2 FLAIR images in areas of DWI positive stroke. Immediately following acute stroke, the DW hyperintense ischemic lesion does not show any changes on FLAIR sequence. It was shown that this phenomenon best identifies the patients to be within 3-hour window in whom intravenous tPA should be recommended, who would favorably respond to perfusion.[37] This was labeled as DWI–FLAIR mismatch (►**Fig. 10**) and was initially interpreted as a sign of salvageable penumbra. It needs to be understood that it would be more appropriate to believe this parenchymal area to be a stable core based on the unstable core model.[38] In short, FLAIR negative stroke appears to be a reasonable indication for stroke therapy with reasonable results.

It is beyond doubt that the MR provides a wealth of information about acute ischemic stroke. The issue however is the appropriate utilization of the data and making it relevant to patient care immediately, which at this point is lacking. An example is the increased detection of hemorrhages not evident on CT, but inability of the investigators to

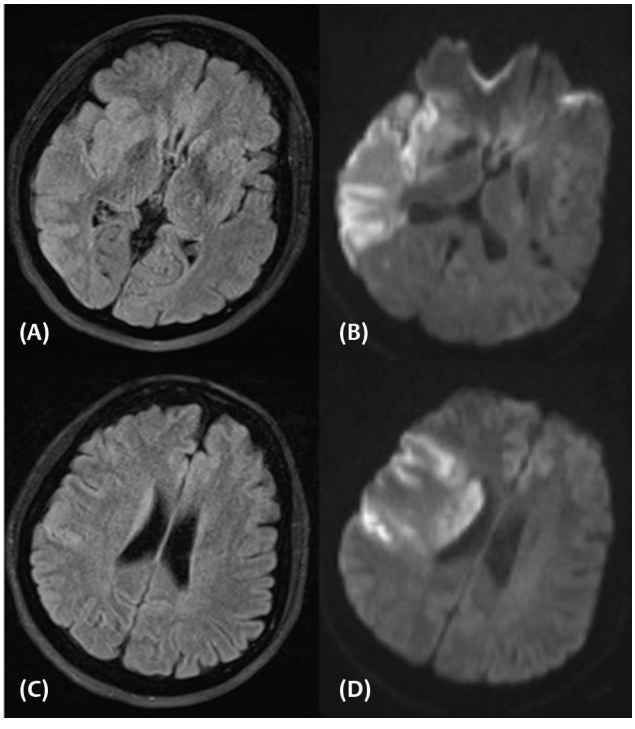

Fig. 10 Axial fluid-attenuated inversion recovery (FLAIR) weighted (**A**, **C**) and diffusion weighted (DW) (**B**, **D**) magnetic resonance imaging (MRI) images showing mismatch in the FLAIR and DW hyperintensities suggesting that the ischemic insult is within 3 to 4 hours and the patient can potentially benefit from reperfusion therapy. (FLAIR signal changes are not clearly seen while the diffusion changes are well appreciated.)

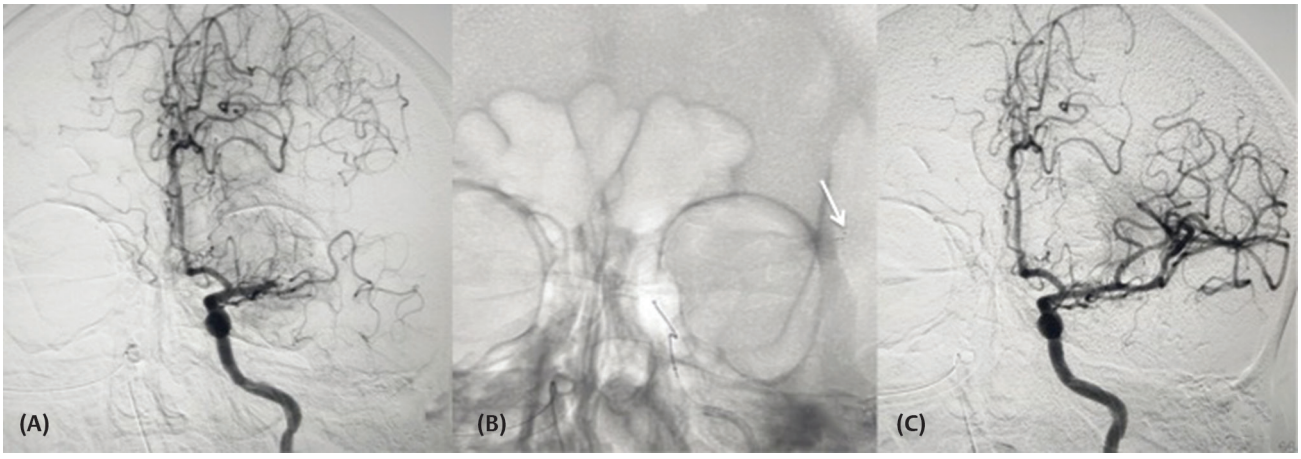

Fig. 11 Frontal digital subtraction angiography (DSA) (**A**, **C**) and radiography (**B**) image showing complete recanalization of middle cerebral artery (MCA) branches following mechanical thrombectomy using stentriever (arrow pointing to its distal aspect) in acute ischemic stroke due to MCA occlusion.

utilize this additional information.[39] It is therefore difficult to prove that increased sensitivity of MR to certain findings will translate to better clinical outcomes. The areas that MRI definitely scores, time constraints let aside, are in excluding stroke mimics, accurately identifying hemorrhages, and recognizing patients who, though rapidly improving, are likely to decline due to early recurrence, for example, in vascular stenosis. These patients may initially skip treatment, feigning improvement, on the contrary, my go down clinically in the ensuing few hours or days.[40]

CT versus MRI

This question has many perspectives to it. The main consideration in stroke imaging is to provide quick, prompt, accurate, and meaningful information with least adverse effects to the patient. One modality scores over the other in some aspects while it is versa on other fronts. CT is quick, easily available providing both qualitative and quantitative results. The two disadvantages are radiation exposure and use of iodinated contrast, which can be potentially renotoxic. MR can provide all these details with increased accuracies without radiation issues. It, however, tends to be a longer investigation (inviting motion artifacts), less easily available, expensive, and is contraindicated in patients with cardiac pacemakers, metallic prosthesis, and who are medically unstable. Practically, the choice is usually based on institutional preference, availability, and clinical information required.

Role of Catheter Digital Subtraction Angiography

Catheter digital subtraction angiography (DSA) still remains the gold standard; however, has practically fallen into disrepute due to the excellent information provided by noninvasive modalities of CT and MR. DSA, however, shows the vascular anatomy, vessel patency, and collateral supply exquisitely. The biggest benefit remains that of dynamic evaluation of blood flow in a particular arterial territory. Cerebral DSA is anyway done prior to attempting thrombectomy (►**Fig. 11**) for LVOs which can help one in confirming the vascular findings obtained previously by CT or MRI.

Conclusion

Last 5-year period has seen a paradigm shift in the management of acute ischemic stroke with literature showing tremendous results with mechanical thrombectomy in acute ischemic stroke-related LVOs. The primary aim of imaging is selection of potential candidates who are most likely to be benefitted with treatment. In resource poor setting, a plain CT is often enough in clinically appropriate patients prior to starting intravenous tPA. CT provides information regarding the presence and quantum of infarcts (ASPECTS). LVO needs to be established with CT/MRA for considering endovascular therapy. Patients presenting beyond the window period having low/borderline ASPECTS should undergo CT/MR perfusion to identify and quantify the salvageable penumbra. More studies are underway to decipher the intricacies of blood flow in the brain in attempt to increase the base for mechanical thrombectomy, which will benefit more number of patients.

Conflict of Interest
None declared.

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Orientation of Cone-Beam Computed Tomography Image: Pursuit of Perfect Orientation Plane in Three Dimensions—A Retrospective Cross-Sectional Study

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Abstract

Objective This study aimed to evaluate the reproducibility of nine reference planes used in orientation of as-received cone-beam computed tomography (CBCT) images in all three dimensions.

Material and Methods The study was conducted on CBCT images of 15 adult subjects (mean age 21.2 ± 5.8 years). The anonymized CBCT images were oriented using five different methods created from nine reference planes by two experienced orthodontists. For each subject, pitch, yaw, and roll changes with five orientation methods were recorded twice by each observer.

Statistical Analysis The inter- and intraobserver agreement was tested using intraclass correlation (ICC) and Bland–Altman plot. The intra- and interobserver error was analyzed using paired *t*-test. Analysis of variance and paired *t*-test were used to analyze the differences among the various pitch, roll, and yaw orientation planes.

Results Inter- and intraobserver agreement (ICC, 0.9) was excellent for all the nine reference planes. The interobserver reliability showed statistically significant differences for four planes namely Frankfort horizontal plane constructed on right side ($p = 0.014$) and left side ($p = 0.000$), transorbital plane ($p = 0.001$), and midsagittal plane on top view ($p = 0.036$); however, the mean differences were clinically insignificant.

Conclusion The landmark-based nine reference planes used in this study to orient CBCT images showed good reproducibility. Therefore, these reference planes can be used to orient CBCT images and can be incorporated into automated software.

Keywords
- cone-beam computed tomography
- orthodontics
- orthognathic surgery
- cephalometry
- standardization

Introduction

Standardization of head orientation is crucial in treatment planning and evaluation of treatment effects in patients with skeletal deformities. Natural head position (NHP) is recommended for two-dimensional (2D) and three-dimensional (3D) imaging/photography.[1-3] NHP is the natural position of the head in which subject rest their head habitually. It is most reproducible position for clinical photographs and cephalograms acquisition, which are important modalities in quantifying the dentofacial deformities in traditional orthodontic/orthognathic surgical planning.[4] NHP also represents the true aesthetic and functional anatomic form of the face.[5]

Three-dimensional cone-beam computed tomography (CBCT) is considered to be a modern state of the art imaging. It allows a smooth digital workflow from diagnosis to treatment planning and execution by integration with other digital technologies like digital models and 3D

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stereophotogrammetry. The virtual 3D models generated from CBCT images have been used for treatment planning and to assess the treatment and growth changes.[6-9] In recent years, various 3D software programs have been introduced for the analysis of CBCT data[10,11] and 3D cephalometric analysis.[12-14]

During CBCT acquisition, the patient's head is stabilized throughout the scanning procedure by various methods, such as the chin rest and head-positioning devices, which hold the patient's head in a stable but random orientation. Treatment planning and assessment made using a randomly orientated head is difficult and potentially inaccurate. Ruellas et al showed that the orientation of CBCT image strongly influences the quantification of growth changes assessed using serial CBCTs.[15]

Inconsistencies in the orientation plane among the samples may lead to inconsistent measurements. For example, in case of clockwise rotation of the head, the mandible would appear to be backwardly placed which is otherwise normal. Similarly, the definition of boundaries of different pharyngeal airway space can be influenced by the inaccuracies in orientation of CBCT image.[16]

Various authors suggested different methods for orienting the patient head during CBCT acquisition that includes (1) stereophotogrammetry, (2) facial markings along laser lines, (3) clinical photographs and the Pose from Orthography and Scaling with Iterations (POSIT) algorithm, (4) digital orientation sensing, (5) handheld 3D camera measuring system, and (6) laser scanning.[1,2,17-19] However, these methods are impractical in routine clinical practice.

On the other hand, reorienting the CBCT images using stable cranial landmarks and reference structures may be a practical alternative.[20] In literature, different orientation methods have been reported for the orientation of CBCT images. Most of these methods utilize the modification of common 2D planes such as Frankfort horizontal (FH) plane and focused on orienting the head in sagittal plane (pitch) only.[20,21] The evaluation of the relationship between landmark-based reference planes and NHP on 2D cephalograms showed that FH plane, Krogman–Walker Line, and Palatal plane are the close approximation of NHP.[4,22,23] However, the reliability and reproducibility of these anatomical reference planes for 3D CBCT orientation has not been evaluated.

With increasing clinical applications of CBCT imaging and growing implications of artificial intelligence (AI) such as automated 3D cephalometrics[24] and automated 3D airway analysis[16] in the craniofacial analysis, need for establishment of the correct reference plane for head orientation is required. Therefore, a study was conducted to evaluate the reliability and reproducibility of five methods of orientation of as-received CBCT images in the 3D space using landmark-based craniofacial reference planes.

Materials and Methods

Sample Collection

The study was conducted on CBCT image data obtained from 15 subjects with skeletal malocclusion who were enrolled in the orthognathic clinic at Division of Orthodontics and Dentofacial Deformities, Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi. The mean age of the sample was 21.2 ± 5.8 years (7 males, 8 females). The following inclusion and exclusion criteria were used to select the CBCT images: All the patients were diagnosed to have skeletal malocclusion and potential surgical patients. The CBCT images were screened for adequate field of view (FOV) to include the landmarks required for 3D orientation. The CBCT data was also screened for any artifacts and adequate imaging quality. The patients with cleft lip and palate and hemifacial microsomia or any significant defect that marred the identification of landmarks were excluded. The study was initiated following the approval from the institutional ethics committee.

The sample size was calculated by G*Power software using the data from Pittayapat et al.[21] For a 95% power, the minimum sample size required was calculated to be nine.

CBCT Acquisition Protocol and Data Storage

The CBCT scans were obtained using i-CAT Next Generation machine (Imaging Sciences International, Hatfield, Pennsylvania, United States) at 120kV, 5mA, 17x22cm FOV, 0.3-mm Voxel and 26-second scanning time. The data were saved in DICOM (Digital Imaging and Communications in Medicine) format with an isometric voxel size of 0.3 mm. The selected datasets were anonymized. Four anonymous datasets were created with the different random sequence. Two orthodontists (R.B., K.S.) separately performed the orientation of anonymized datasets at two different occasions with a time interval of 2 weeks between them.

Orientation Procedure

Dolphin imaging software (version 11.5, Dolphin Imaging & Management Solutions, Canoga Park, California, United States) was used to perform the orientation of 3D CBCT images. Before orientation, the hard tissue volume segmentation of all CBCT scans was done. The gray scale value was standardized between 200 and 400 voxel values.

The reorientation involved correction of head position in all the three planes of space. Five planes for sagittal plane, two for coronal, and two for axial plane were used for correction of head posture (►**Table 1**, ►**Fig. 1**). The definition of landmarks used for construction of orientation planes is given in ►**Table 2**.

Using these nine reference planes, five methods of orientation (I, II, III, IV, and V) were created with each method having reference planes in the order of sagittal, axial, and coronal axis (►**Table 3**).

Following each instance of orientation, the values of pitch, roll, and yaw were exported from the software and entered into the spreadsheet for further analysis. The changes in the pitch, roll, and yaw measurements represented the degree of rotational changes in coordinate systems of the CBCT in each plane.

Statistical Analysis

The data analyses were performed using SPSS Statistic Software Package (version 17, SPSS, Chicago, Illinois, United States).

Table 1 Three different sets of orientation planes

A. Flexion or extension (FE):
FE1: The Frankfort horizontal plane by connecting right Porion, right and left Orbitale.
FE2: The Frankfort horizontal plane by connecting left Porion, right and left Orbitale
FE3: Opisthion–Maxillion line in lateral view aligned to coincide with axial plane
FE4: Palatal plane by connecting ANS and PNS in lateral view aligned to coincide with axial plane
FE5: Opisthion–ANS plane in lateral view aligned to coincide with axial plane
B. Lateral flexion (LF):
LF1: Transorbital plane (plane passing through right and left Orbitale) in frontal view aligned to coincide with axial plane
LF2: Line passing through medial termini of right and left frontozygomatic suture landmark in frontal view aligned to coincide with axial plane
C. Lateral rotation (LR):
LR1: On top view–the crista galli, cribriform plate mid sagittal structures In frontal view–glabella, ANS were oriented to match with mid-sagittal plane
LR2: Right and left anterior margin of external acoustic meatus (AMEAM) aligned to coincide with coronal plane (line connecting right and left AMEAM)

Intraobserver reliability was calculated by the intraclass correlation coefficient (ICC) for the measurements obtained by each examiner at two different time periods (with an interval of 2 weeks). Interobserver reliability was also assessed using ICC by comparing the measurements obtained by each examiner. ICC values were estimated using a two-way mixed-effects model. Reliability was ranked according to the ICC value and considered excellent when it was above 0.9.

The intra- and interobserver agreement (reliability) was further assessed with the Bland–Altman plot (►**Fig. 2**).

The paired *t*-test was used to compare the T1 and T2 measurements of the pitch, roll, and yaw orientation methods for each observer. The mean of roll and yaw measurements of same time observation (T1 or T2) were used for comparison purpose since the same method was used more than once. The interobserver error was calculated by comparing the measurements of each method by two observers. The mean of T1 and T2 alignments for each orientation method for each observer was calculated for the comparison purpose. The paired *t*-test was used to compare the measurements between the two observers for the pitch, roll, and yaw orientation methods.

To compare the different orientation methods, the mean of T1 and T2 measurements for each orientation method was calculated by combining the measurements of both observers. The one-way analysis of variance (ANOVA) test was used to compare the five different pitch methods for each observer. The two different roll and yaw orientation methods were compared using paired *t*-test.

The significance level was set at $p < 0.05$ for all the statistical analysis used in this study.

Results

Intra- and Interobserver Reliability

The ICC values were above 0.97 for all the parameters of both intra- and interobserver assessments. These results indicate excellent reliability for both intra- and interobserver assessments.

Intra- and Interobserver Comparison

The intraobserver comparisons showed statistically significant differences in flexion or extension 2 (FE2) ($p = 0.048$) for observer 2 and FE1 ($p = 0.014$), FE2 ($p = 0.000$), lateral flexion 1 (LF1) ($p = 0.001$), and lateral rotation 1 (LR1) ($p = 0.036$) for interobserver comparisons (►**Table 4**).

Comparison of Different Orientation Methods

The results of the comparison of different pitch, roll, and yaw orientation methods showed statistically insignificant differences among the various methods used for 3D orientation of CBCT image (►**Tables 5** and **6**).

Bland–Altman Plot

In the pitch orientation, Bland–Altman plot (►**Fig. 2**) showed greater variation for FE3 and FE4 for both observers. Interobser variation was high for FE4. For the roll orientation, the Bland–Altman plot (►**Fig. 2**) showed less variation for both transorbital and transfrontozygomatic suture planes. Anterior margin of external acoustic meatus (AMEAM) line showed the least variation for the yaw orientation (►**Fig. 2**).

Discussion

The traditional 2D cephalometric analysis involves assessment of the relationship of different skeletal components to each other and in relation to the stable craniofacial reference planes.[25-27] Evaluation and planning of the position of maxillary/mandibular structures in NHP may provide a more realistic outcome in the clinical scenario. It is essential to set up the 3D coordinate system for the 3D cephalometric measurement and determine the correct pitch, roll, yaw, and translational movements of craniofacial skeletal structures.

The head orientation using cranial reference planes for recording the cephalogram is fundamental to the science of cephalometrics. The validity of cephalometric measurements and analysis is influenced by the reference planes used to reorient the radiograph.[28] Acquisition of CBCT in

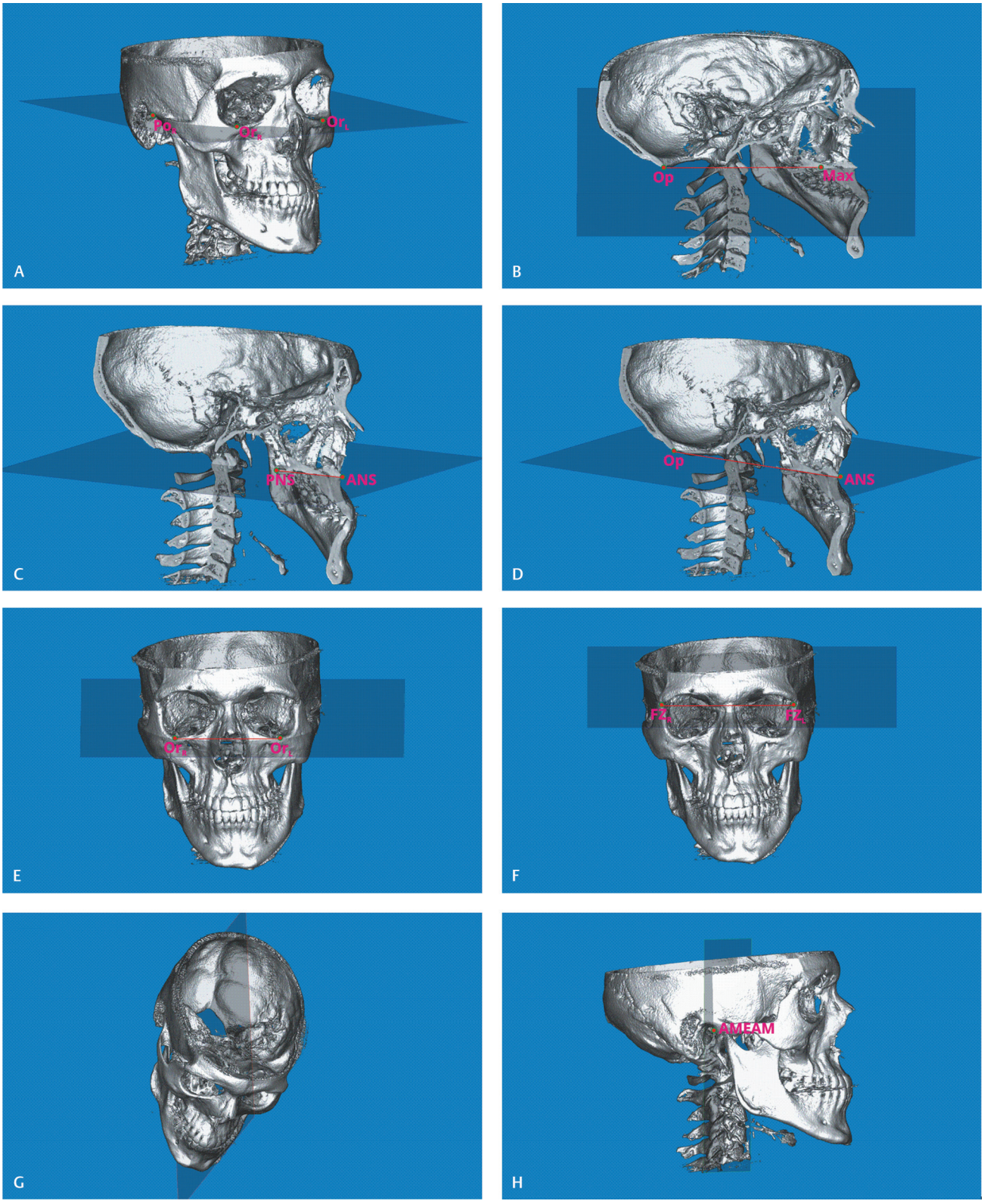

Fig. 1 Orientation planes: (**A**) FH plane; (**B**) Opisthion–Maxillion plane; (**C**) ANS–PNS plane; (**D**) Opisthion–ANS plane; (**E**) transorbital plane; (**F**) transzygomatic suture plane; (**G**) midsagittal plane passing through mid-sagittal structures; (**H**) coronal plane passing through AMEAM on both sides. AMEAM, anterior margin of external acoustic meatus; ANS, anterior nasal spine; FH, Frankfort horizontal; PNS, posterior nasal spine.

NHP or a reproducible position has been attempted with the help of additional tools. These techniques have been found impractical since it requires extra instrumentation, expertise and it is time-consuming. The use of landmark-based reference planes that are the close approximations of NHP has been proven to be a reliable alternative.[4,29]

Table 2 Definition of landmarks

S. No	Landmark	Definition
1.	Orbitale (Or)	The lowest point on the inferior margin of the orbit
2.	Porion (Po)	The most superior midpoint of the external auditory meatus
3.	Opisthion (Op)	The middle point on the posterior margin of the foramen magnum, opposite to the basion
4.	Maxillion (Max)	A point just below (occasionally above) the key ridge midway between the upper and lower border of the palate in the midsagittal plane
5.	Frontozygomatic suture point (FZS)	The medial point of the orbital rim of the zygomaticofrontal suture
6.	Anterior nasal spine (ANS)	The tip of the bony anterior nasal spine
7.	Posterior nasal spine (PNS)	The tip of the bony posterior nasal spine
8.	Anterior margin of external acoustic meatus (AMEAM)	The anterior most point on the anterior margin of external acoustic meatus

Table 3 Methods used for orientation

Methods	Combination of orientation planes
I	LF1-FE1-LR1
II	FE2-LF2-LR2
III	LF1-LR1-FE3
IV	LF2-LR2-FE4
IV	LF1-LR1-FE5

Abbreviations: FE, flexion or extension; LF, lateral flexion; LR, lateral rotation.

In the current study, the intra- and interobserver reliability of five planes used for pitch correction was excellent (ICC >0.97). The comparison between the different pitch orientation planes showed no significant variation for two observers (p = 0.613 and 0.809 for O1 and O2, respectively). The Opisthion–ANS plane (FE5) was used for the first time to orient CBCT and it showed the least variation among the five sagittal planes. This was followed by the FH plane (►**Fig. 2**). In general terms, FH plane can be a reliable plane for head orientation (CBCT orientation) that corroborates with the previous studies. Lin et al studied the reproducibility and reliability of landmark-based horizontal reference planes. They used the FH plane and lateral semicircular canal plane (LSP) in their study and demonstrated satisfactory results with different FH planes and LSP plane for the orientation of 3D skull models.[20] Daboul et al used left and right Porion (Po) and left Orbitale (Or) on the multiplanar reconstruction view for defining the FH plane and showed excellent intra- and interexaminer reproducibility of FH planes in magnetic resonance imaging.[30]

A plane can be constructed using two or many landmarks. The number of landmarks used to define a plane and the distance between them may influence the construction of craniofacial reference plane. Each landmark possesses some uncertainty in terms of their accurate identification. When the number of landmarks increase, the plotting error for each landmark may add up and contribute to the overall variation. Similarly, the distance between the landmarks may influence the construction of a plane. The plane constructed using two landmarks with the shorter distance between them is affected more by the landmark plotting error. Accordingly, the palatal plane, which has closest landmarks among the planes used in this study, showed larger variation for both intra- and interobserver comparison.

It is also important to note that the ease of identification of certain landmarks like ANS and PNS is hampered in patients with cleft lip and palate that may require use of alternative landmarks. Although the Opisthion–ANS plane (FE5) showed the least variation, one should be careful while using this plane for orientation in facial asymmetry cases.

The FH plane (FE2) defined using the combination of three landmarks showed better intra- and interobserver agreement than the Opisthion–Maxillion plane (FE3) and Palatal plane (FE4) that were defined using only two landmarks. This may be due to the structural complexity and lack of proper definition of landmarks used to define these planes. The Maxillion landmark is not well defined in the 3D volume rendered image when compared with the other landmarks used, which may lead to a subjective error. Hence, in this study, it is observed that the influence of multiple landmarks on defining a plane is not substantial. This indicates that the use of well-defined landmarks for defining the planes is more important than the distance between the landmarks.

The intraobserver error was statistically insignificant for roll. The interobserver error was statistically significant for the transorbital plane (LF1). However, the error is within the acceptable limits. The interplane comparison (LF1 versus LF2) showed statistically insignificant differences between both the planes. Landmarks used in both planes are least affected by the facial deformities. Both the methods can be used alternatively since the variation in both the methods is minimal (►**Fig. 2**).

There are no studies reported in literature evaluating the use of reference planes for yaw orientation. In this current study, the intraobserver agreement for the yaw orientation planes was excellent. The midsagittal plane (LR1) showed statistically significant interobserver error but within an acceptable limit (►**Fig. 2**). The plane constructed using AMEAM (LR2) showed a less inter- and intraobserver difference than the midsagittal plane. For the alignment of yaw, the midsagittal structures are viewed from the top. Since there is no landmark involved and due to the complex midsagittal anatomical structures, there may be subjective variation in the selection of midsagittal line. This may account to the larger variation observed in midsagittal pane (LR1) when compared with AMEAM plane (LR2).

It has been shown that the NHP is subject to change with time and after the orthognathic surgery.[31,32] Hence, in such situations the landmark-based orientation method may be a better alternative for the long-term evaluation.

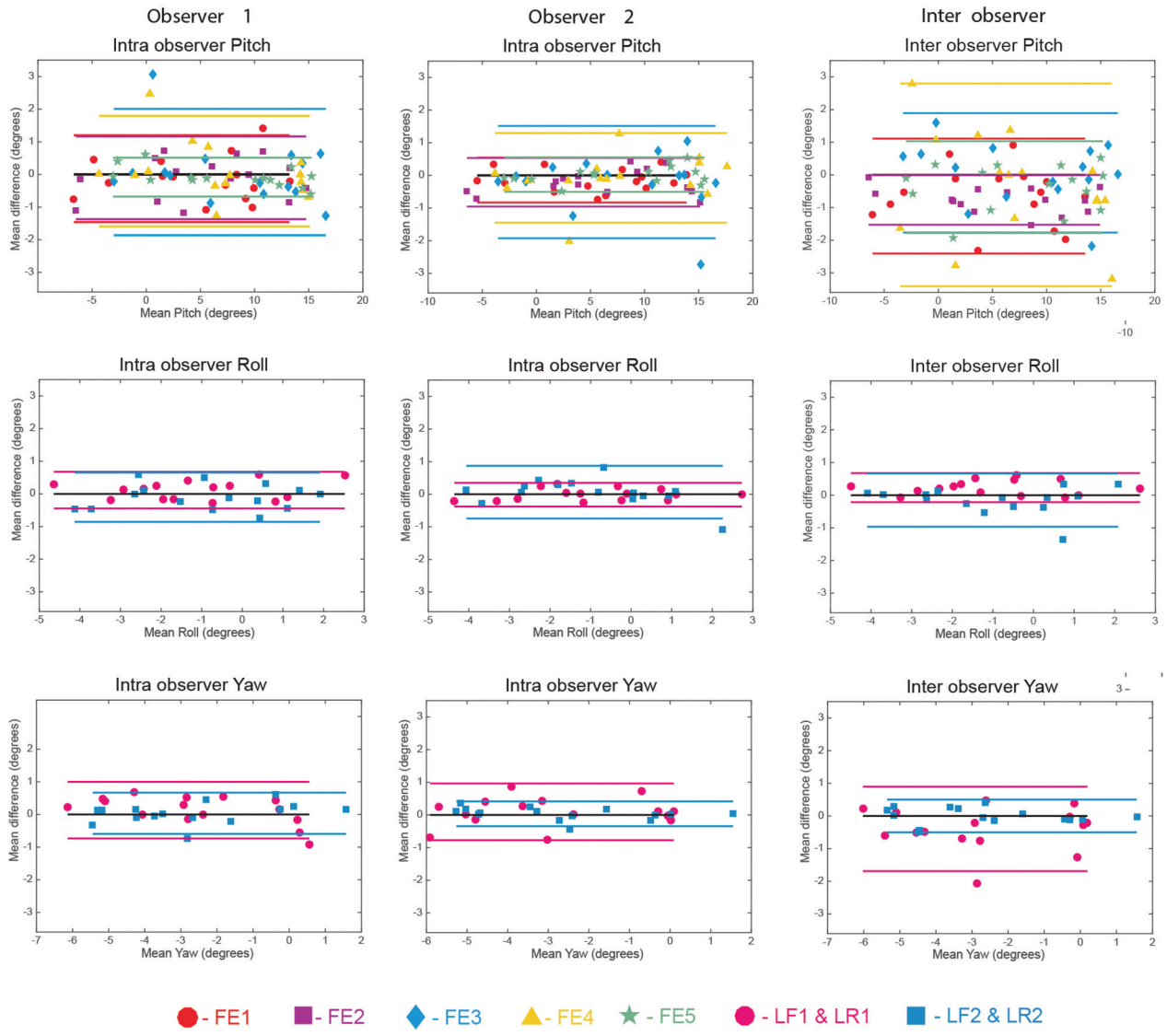

Fig. 2 Bland–Altman plots of the intra- and interobserver comparison for pitch, roll, and yaw. All the measurements errors are shown in degree. Plots show the mean and ± 1.96 standard deviation reference lines for each plane. FE, flexion or extension; LF, lateral flexion; LR, lateral rotation.

Table 4 Intra- and interobserver comparisons

	n	Observer 1			Observer 2			Interobserver			
		Mean difference	SE	*p*-Value	Mean difference	SE	*p*-Value	Mean difference	SD	SE	*p*-Value
FE1	15	−0.13	0.17	0.472	−0.14	0.09	0.137	−0.65	0.90	0.23	0.014[a]
FE2	15	−0.10	0.17	0.548	−0.21	0.10	0.048[a]	−0.76	0.39	0.10	0.000[a]
FE3	15	0.07	0.25	0.776	−0.21	0.23	0.378	0.06	0.93	0.24	0.802
FE4	15	0.10	0.22	0.659	−0.08	0.18	0.678	−0.31	1.58	0.41	0.467
FE5	15	−0.08	0.08	0.317	0.02	0.07	0.763	−0.38	0.72	0.19	0.062
LF1	15	0.12	0.07	0.130	−0.01	0.05	0.820	0.23	0.23	0.06	0.001[a]
LF2	15	−0.10	0.10	0.324	0.06	0.11	0.561	−0.15	0.42	0.11	0.184
LR1	15	0.13	0.11	0.268	0.10	0.11	0.414	−0.40	0.66	0.17	0.036[a]
LR2	15	0.04	0.08	0.669	0.03	0.05	0.499	0.00	0.26	0.07	0.996

Abbreviations: FE, flexion or extension; LF, lateral flexion; LR, lateral rotation; SD, standard deviation; SE, standard error.

[a]Significant difference.

Table 5 Comparison of different orientation methods using one-way ANOVA test

	n	Parameter	Mean	SD	*p*-Value
Observer 1	15	FE1	4.82	6.14	0.613
	15	FE2	5.37	6.59	
	15	FE3	8.03	6.71	
	15	FE4	7.02	6.40	
	15	FE5	7.44	6.59	
Observer 2	15	FE1	5.47	6.20	0.809
	15	FE2	6.14	6.73	
	15	FE3	7.96	6.97	
	15	FE4	7.32	6.96	
	15	FE5	7.82	6.53	

Abbreviations: ANOVA, analysis of variance; FE, flexion or extension; SD, standard deviation.

Table 6 Comparison of different orientation methods using paired *t*-test

	n	Parameter	Mean difference	SE	*p*-Value
Observer 1	15	LF1–LF2	–0.27	0.20	0.195
	15	LR1–LR2	0.20	0.25	0.451
Observer 2	15	LF1–LF2	0.12	0.15	0.455
	15	LR1–LR2	–0.20	0.22	0.386

Abbreviations: FE, flexion or extension; LF, lateral flexion**;** LR, lateral rotation; SE, standard error.

The major implications of this study are standardization of CBCT image for orthognathic surgical planning for more realistic treatment simulations, growth assessment, short-term and long-term evaluation of treatment outcomes, and AI technology in craniofacial imaging. The rapid progress in AI technology and with its expanding role in orthodontics and surgical discipline requires the establishment of a reliable and reproducible orientation plane, which can be effectively incorporated in future AI algorithms.

The variation in head positioning during CBCT imaging has not been reported in literature in three dimensions. The present study showed major variation in sagittal view than the axial and coronal. The sagittal plane is most commonly used for orienting the image for the purpose of treatment planning and evaluation. The methods described in the present study included the planes that involves both the maxillary and cranial landmarks, and exclusive cranial landmarks only. This may help to orient the CBCT in situations like cases with severe facial deformity, and the non-availability of cranial landmarks, like Nasion, Sella, due to the limited FOV. Another major advantage of planes used in this study is that most of the planes can be located in the CBCT obtained using medium FOV, thereby reducing the radiation exposure.

The limitation of this study is that these planes are not validated in severe craniofacial deformity cases and use of a relatively smaller sample size due to the ethical concerns arising from radiation exposure. The validity of these reference planes on complex malocclusion and facial deformities needs to be evaluated in future studies using larger sample size. The future prospective studies should be designed by strictly following ALARA principle (As Low As Reasonably Achievable) and include radiologist as an observer.

Conclusion

Three-dimensional orientation using anatomical landmarks-based planes is reproducible. The nine planes used in this study for 3D orientation of CBCT image showed good reproducibility. The significance of identification of these planes is that they can be used alternatively when one or more landmarks are not available due to any deformity or artifacts or FOV restrictions. Among the five planes evaluated for the pitch correction, Opisthion–ANS plane showed the least variation followed by the FH plane. For the roll and yaw alignment, transzygomatic suture plane and AMEAM line showed minimal variation, respectively. The impact of variation in patient positioning during CBCT imaging was high for pitch followed by yaw and roll.

Authors' Contributions

R.B.: Concept and design of the study, conducted the study, literature review, collection of data, data analysis, writing and revision of manuscript. O.P.K.: Concept and design of the study, provided samples, interpretation of data and writing of manuscript. K.S.: Data marking, interpretation of data, writing and revision of the manuscript. B.C.N.: Examined the concept, assisted in review of the study, data analysis, and writing of the manuscript.

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Conflict of Interest
None declared.

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Natal Tooth: A Histomorphologic Variant, a Rarity

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Abstract

Presence of natal or neonatal teeth in a newborn is rare, that is, 1 in 3,000 cases. Various etiological factors have been put forth explaining the presence of natal teeth but they are not very clear. Although some authors have suggested that these teeth may represent predeciduous supernumerary teeth, most of these teeth represent prematurely erupted portions of the deciduous dentition, not supernumerary teeth. They can be left untreated in some cases if they do not cause any difficulty to the mother while feeding or do not pose a risk of swallowing in the newborn. One such case of natal tooth with histological variation is presented here.

Keywords
- natal teeth
- mandibular incisors
- keratin
- hard tissue

Introduction

Natal teeth are defined as the teeth present at birth.[1] Natal teeth are also called as congenital teeth.[2] Neonatal teeth are erupted within 30 days of birth. The etiology of natal and neonatal teeth is unknown, but it is usually attributed to factors such as the hereditary transmission of a dominant autosomal gene, a superficial position of the tooth bud, endocrine disorders, osteoblastic activity in the bud, infection, hypovitaminosis, poor nutrition, and syndromes such as Hallermann–Streiff and Wiedemann–Rautenstrauch syndromes.[3-7] Superficial positioning of tooth germ increases the rate of eruption of natal and neonatal teeth due to poor nutrition of mother and fetus causing hypovitaminosis, hormonal stimulation, syphilis, and febrile incidence. Incidence of natal teeth is 1:3000 live births.[8] Most common natal teeth are lower primary central incisors.[9] Incidence of natal and neonatal teeth is 85% in mandibular incisors, 11% in maxillary incisors, 3% in mandibular canine and molars, and only 1% in maxillary posterior region. The natal teeth might occur as a familial trait as 8 to 62% of cases with positive family history have been reported.[10] Predilection for females was cited by some authors with Kates et al, a 66% proportion for female against a 31% proportion for male.[11] The presence of natal teeth can cause feeding problems, loosening and risk of aspiration, ulceration of ventral part of the tongue and frenulum.[9]

Case Report

A 24-day-old male preterm baby born to a primi mother who had underwent in vitro fertilization treatment was referred from Military Hospital with a complaint of presence of tooth in the mouth. Patient's mother complained of difficulty in feeding the baby. No other relevant medical history was recorded. Marriage of parents was nonconsanguineous. On examination, a yellowish tooth-like structure (►**Fig. 1**) was present in the lower anterior teeth region that was mobile and falls under category 1 of Hebling's classification (1997)[1,10], that is, shell-like crown loosely attached to the alveolus by a rim of oral mucosa, no roots. Plaque deposits were seen around the tooth with inflamed gingiva (►**Fig. 1**). The baby was referred to the department of pedodontics. After getting consent from the parents, the natal tooth was extracted under topical lignocaine using Spencer Wells forceps under aseptic conditions (►**Fig. 1B**). Hemostasis was achieved by asking the mother to feed the baby immediately after extraction. A sterile cotton was placed and the patient was kept under observation. Follow-up was done. Examination of the extracted tooth revealed a crown with no root that roughly measured 6 mm (►**Fig. 2**). The tooth was decalcified and subjected to histopathological study. Histopathological section of the tissue showed cellular connective tissue with inflammatory cells with adjacent mineralized tissue resembling bone (►**Fig. 3A**, **B**).

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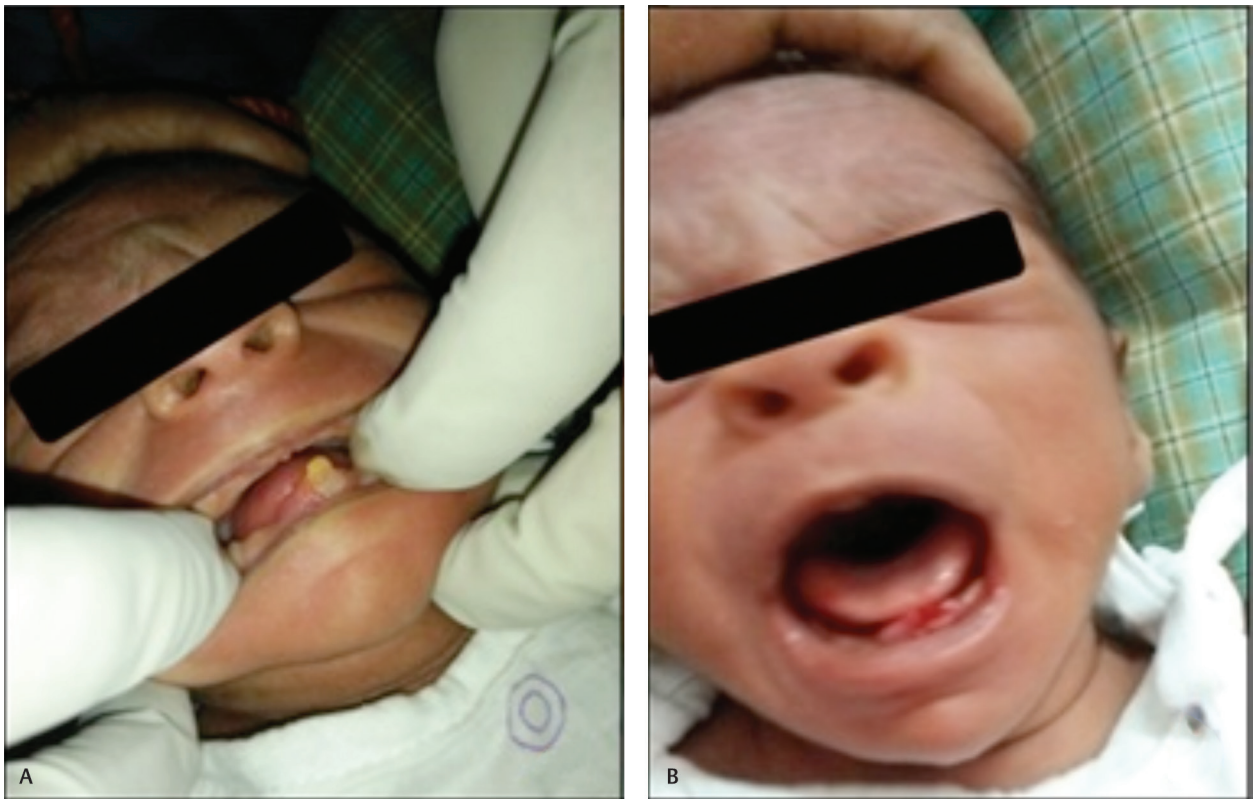

Fig. 1 (**A**) Yellowish crown appearance of the natal tooth (**B**) post-extraction.

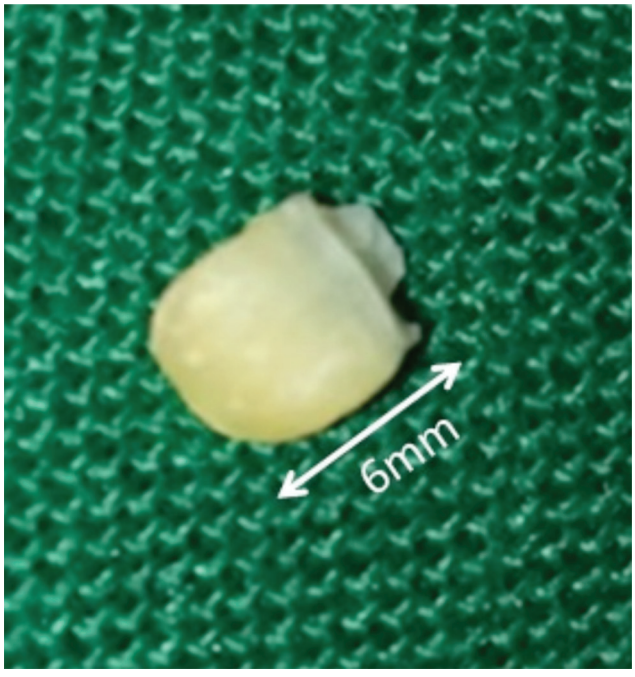

Fig. 2 Extracted tooth.

Discussion

Natal teeth are also called as dentition praecox, fetal teeth, or early infancy teeth.[2] Syndromes associated with natal teeth are cleft palate, chondroectodermal dysplasia, Hallermann–Streiff syndrome, Ellis–Van Creveld syndrome, and Riga–Fede disease. But this case was not associated with any syndrome.

Notwithstanding the normal basic structure of natal teeth, early eruption is associated with abnormal mineralization of the enamel.[12,13] Histologically, the majority of natal teeth have dysplastic or hypomineralized enamel, irregular dentin and osteodentin in the cervical portions, and interglobular dentin in the coronal regions[12,13]. The incisal edge might lack enamel. Both Hertwig's sheath and cementum might be absent.[14,15] Sometimes, histologically it resembles only thickened keratin. But in this case, the histological appearance was completely different and the hard tissue resembled bone. Knowing how to manage natal teeth is important for proper well-being of a child. In this case, even though the natal tooth caused mild problem in feeding, it was mobile. So, it was extracted to avoid aspiration of the tooth. Natal teeth must be approached individually with sound clinical judgment guiding appropriate therapy.

Conclusion

Managing a child born with natal tooth can be challenging as it poses problems in feeding, increased risk of aspiration, and causes ulceration of the tongue. Presence of supernumerary teeth should be suspected if there is a significant delay in the eruption of a localized portion of the dentition. Early diagnosis and treatment often are crucial in minimizing the aesthetic and functional problems of the adjacent teeth. Parental counseling, guidance, and proper decision making are important. Obstetricians and gynecologists and pediatricians should refer neonates born with teeth to the dental surgeons for expert management.

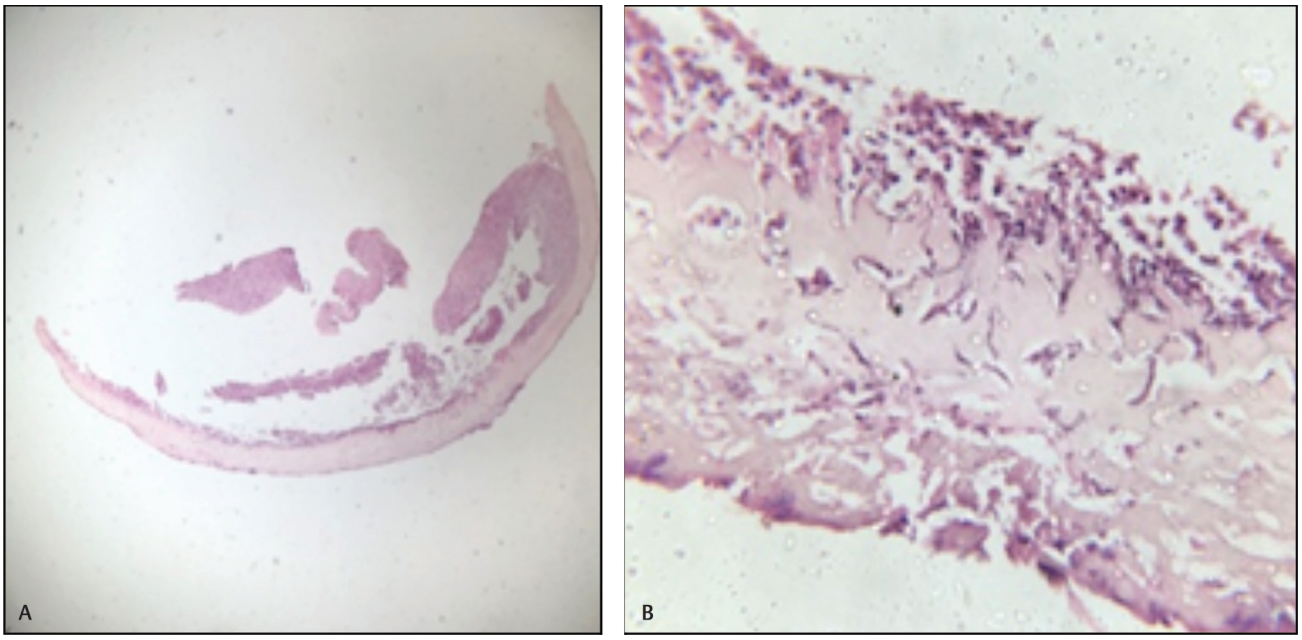

Fig. 3 (**A**) HPE section of the decalcified tooth under x4 magnification. (**B**) HPE section of the decalcified tooth under x10 magnification showing connective tissue and bone like tissue. HPE, histopathological examination.

Conflict of Interest
None declared.

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Gorlin–Goltz Syndrome with Multidisciplinary Approach of Treatment

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Abstract

Gorlin–Goltz syndrome, is an uncommon genetic condition characterized by the presence of multiple odontogenic keratocysts of jaws along with several other abnormal, cutaneous, ophthalmic, and osseous displays. This syndrome is also acknowledged by various names, such as nevoid basal cell carcinoma syndrome, jaw cyst, and bifid rib syndrome. This article illustrates about the clinical, radiological, and histological diagnostic findings and the multidisciplinary approach of treatment given to one such rare case of Gorlin–Goltz syndrome.

Keywords

- Gorlin–Goltz syndrome
- multiple odontogenic keratocyst syndrome
- nevoid basal cell carcinoma syndrome
- Gorlin's syndrome

Introduction

Gorlin–Goltz syndrome is an autosomal dominant disorder with high degree of penetrance and variable expressivity. The prevalence ranges from 1/57,000 to 1/256,000, with an equal predisposition to males and females in the ratio of 1:1.[1] For a diagnosis to be established, there should be two major and one minor criterion or one major and three minor criteria. Early diagnosis and treatment planning are mandatory as it may develop to more aggressive basal cell carcinomas (BCCs). The treatment always relies on multidisciplinary approach due to the involvement of various other systemic disorders.

Case Report

A 50-year-old female patient, who had ulcer on the scalp and was diagnosed as BCC by the Surgical Oncology Department, was referred to the Department of Oral Medicine and Radiology at Tamil Nadu Government Dental College and Hospital for a painless swelling in right side of mandible which was noticed during the treatment period. On eliciting the history of presenting illness, patient noticed the swelling on the mandible 5 months before which was insidious in onset and nonprogressive in nature. Her medical history revealed that she was on hypothyroid medication for past 1 year and her family history was noncontributory. On general examination, patient was calm, cooperative, moderately built, and nourished, her weight was 61 kg and height was approximately around 162 cm. No signs of anemia, icterus, cyanosis, clubbing and pedal edema were present. On clinical examination, facial asymmetry was present due to the presence of swelling on right side of the mandible with slight mandibular prognathism. An ulcer approximately of size 5 cm × 5 cm with raised borders was present on scalp covered by granulation tissue. Multiple cutaneous nevus was present on the face which was diagnosed as compound nevus by the dermatology department. Intraoral examination revealed that upper and lower jaws were partially edentulous and swelling was present on the right and left side of the mandible with obliteration of buccal vestibule from 44 to 47 and from 35 to 37, respectively. The overlying mucosa was normal, and the teeth present were periodontially compromised. On palpation, it was slightly tender, cystic in consistency, and buccal cortical plate expansion was felt (►**Fig. 1**).

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So, an orthopantomogram (OPG) was taken. OPG revealed three well-defined radiolucent lesions surrounded by radiopaque border present on right and left side of body of mandible and also there was pathological migration of premolars present on left and right sides. The third lesion was present in left ramus of mandible. So further for three-dimensional evaluation of the lesion cone beam computed tomography (CBCT) was taken with 10 × 5 field of view and three-dimensional assessment was done. CBCT revealed well-defined hypodense lesion on both sides of body of mandible with expansion of the lesion anteroposteriorly and buccally with perforation of the buccal cortical plate but there was no evidence of lingual cortical plate expansion. The lesion measured approximately 35.5 mm × 16.1 mm

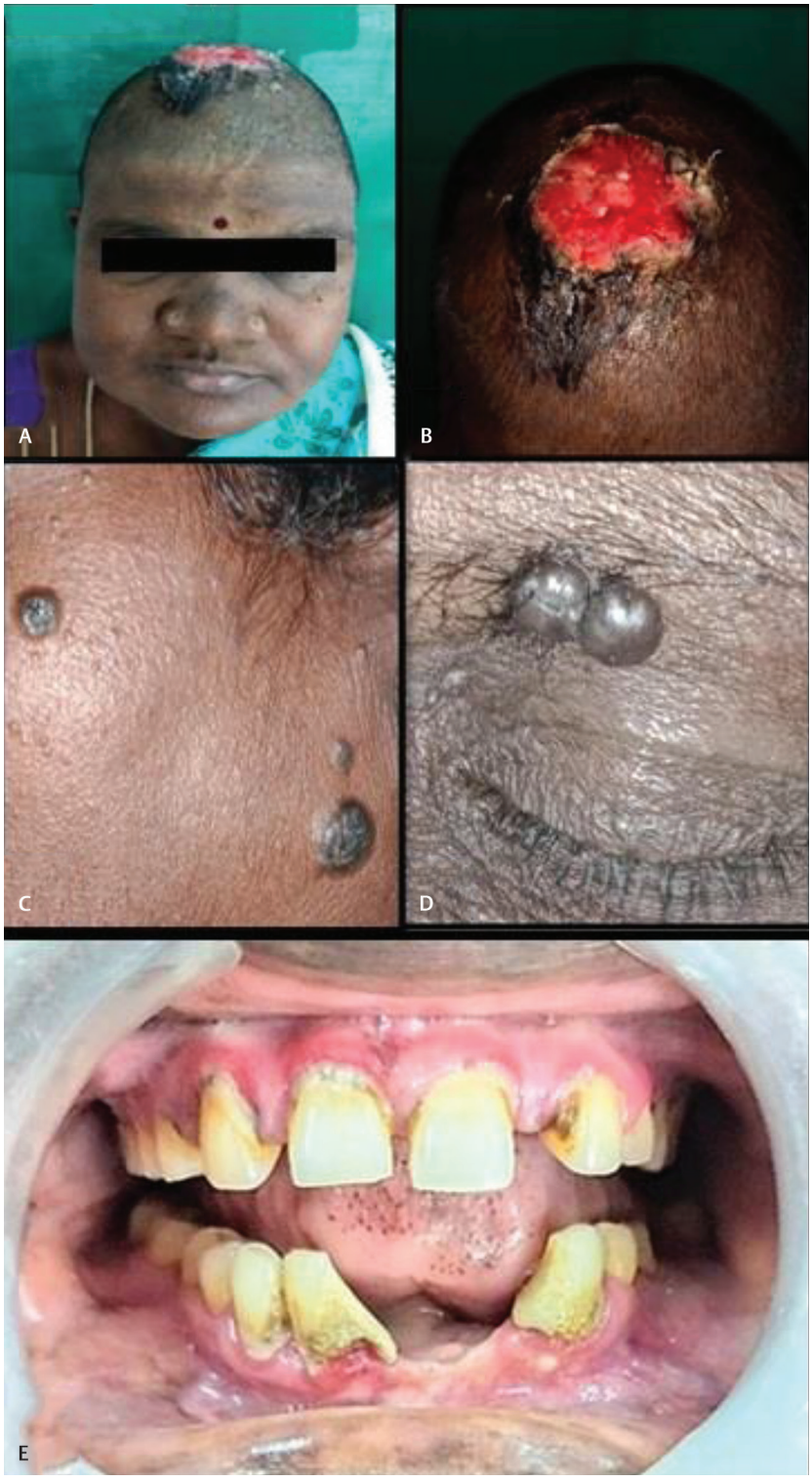

Fig. 1 (**A**) Extraoral view revealing facial asymmetry on right side; (**B**) ulcer on scalp covered with granulation tissue; (**C, D**) multiple compound nevus on face; (**E**) Intraoral examination showing obliteration of buccal vestibule from 44 to 47 and 35 to 37 with normal mucosal color and periodontially compromised teeth.

and 39.5 mm × 17.7 mm on right and left sides, respectively. The lesion in the left ramus on coronal section measured about 32.0 mm × 14.3 mm in its maximum dimension with expansion and perforation of the cortical bone (►**Fig. 2**). So, by analyzing these radiographic image findings, the lesion was interpreted as odontogenic keratocystcyst (OKC). So, by considering the multiple cystic lesions on mandible an assumption of Gorlin–Goltz syndrome was made. Later, anteroposterior view (AP) of skull was suggested which showed ectopic calcification of falx cerebri (►**Fig. 2**). Chest X-ray was taken but there was no evidence for bifid or splayed ribs.

So, considering the clinical findings, such as histopathologically proven BCC, with radiographic image analysis of multiple cystic lesions of jaws and calcification of falx cerebri, the case was diagnosed as Gorlin–Goltz syndrome and further investigations and treatment procedures were planned for the patient. Routine blood investigations were taken, which were all within the normal levels. The patient was prescribed antibiotics to treat infections in the oral cavity and the patient was planned for surgical enucleation of cysts. Under general anesthesia the cystic lesions on both sides of the mandible were enucleated along with the extraction of teeth in the involved region and send for histopathological evaluation. The histopathological examination in × 10 and × 40 magnification view revealed cyst wall lined by corrugated parakeratotic stratified squamous epithelium with basal cuboidal to columnar palisading epithelium cells, underlying fibrocollagenous stroma shows collection of inflammatory cell infiltrate composed of plasma cells, lymphocytes and local lymphoid aggregate formation, and projection of satellite cysts (►**Fig. 3**). The BCC on scalp was treated with flap surgery by the surgical oncology department. Now the patient is under regular follow-up. Postoperative OPG picture (►**Fig. 3**), and pre- and posttreated clinical pictures (►**Fig. 3**) after 1-year follow-up are shown.

Discussion

Gorlin–Goltz syndrome is a rare genetic disorder. It is believed to be caused by mutation in the human patched gene (*PTCH1* gene) that is present in the long arm of chromosome 9q22.3-q31.[2] Sahu et al reported two cases from mother and daughter.[3] The characteristic features of this syndrome was first recorded by Jarish and White in 1894, but later in the 1960s Gorlin and Goltz described them as a triad of disorders including multiple BCC, numerous keratocysts in the jaws, and skeletal abnormalities,

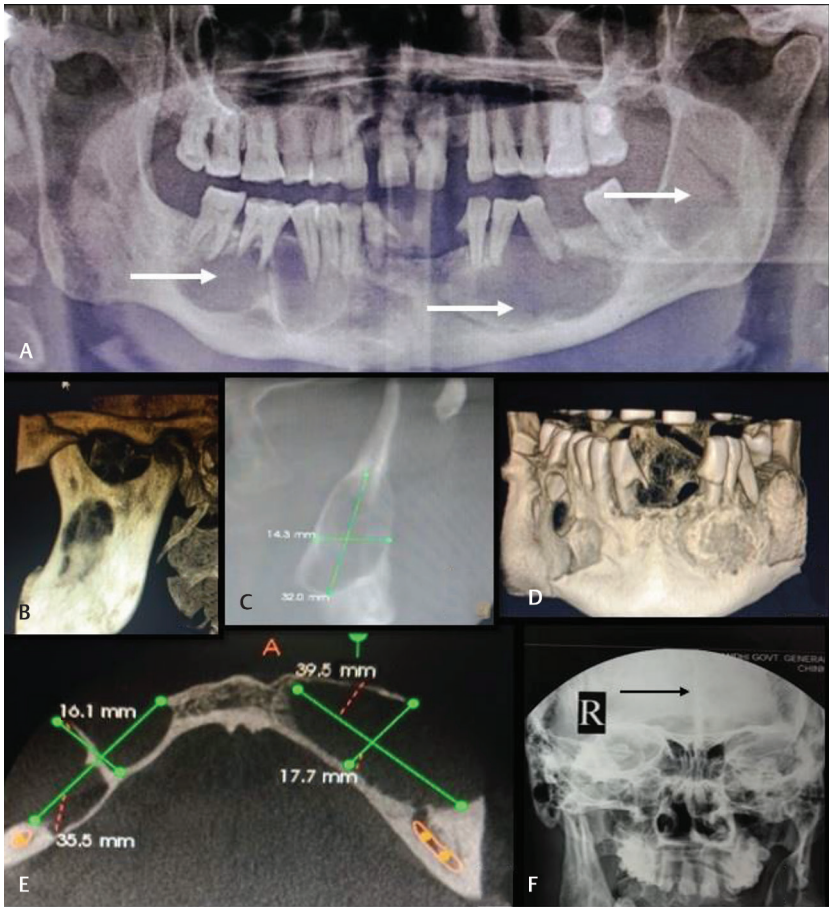

Fig. 2 (**A**) OPG shows well-defined three radiolucent lesions surrounded by radiopaque border present on right and left body and left ramus of the mandible. There is pathological migration of lower premolars on either side of mandible. No evidence of any root resorption. (**B**) Three-dimensional (3D) image reconstruction of left ramus. (**C**) Coronal section of left ramus showing expansion of lingual cortical plate with lesion measuring approximately (32.2 mm × 14 mm) in its maximum dimension. (**D**) 3D image reconstruction of mandible. **(E)** Axial section measuring approximately (35.5 mm × 16.1 mm and 39.5 mm × 17.7 mm) at its greatest dimension on right and left side respectively. **(F)** AP view skull showing ectopic calcification of falx cerebri. AP, anteroposterior; OPG, orthopantomogram.

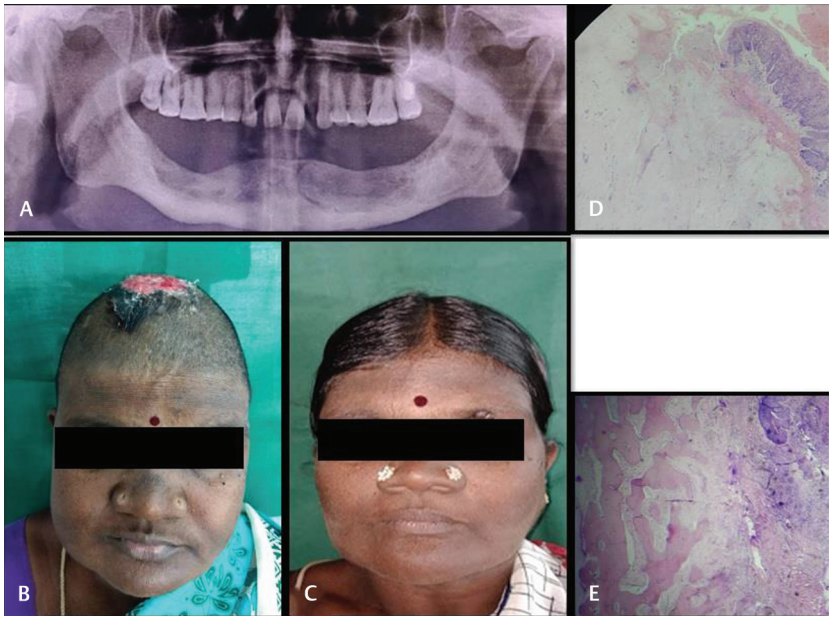

Fig. 3 (**A**) Postoperative OPG taken after 1-year follow-up. (**B, C**) Preoperative and postoperative clinical pictures. Photomicrograph of H&E stained histological section under ×10 and ×40 magnification shows (**D**) corrugated parakeratotic stratified squamous epithelium with basal cuboidal to columnar palisading epithelium cells, (**E**) underlying fibrocollagenous stroma with collection of inflammatory cell infiltrate. H&E, hematoxylin and eosin; OPG, orthopantomogram.

which gave upsurge to the Gorlin–Goltz syndrome.[4] Clinical indicators of the syndrome are grouped into the following five categories. Cutaneous anomalies include basal cell nevus, other benign dermal cysts and tumors, palmar pitting, palmar and plantar keratosis, and dermal calcinosis. Dental and osseous deformities include multiple OKCs, mild mandibular prognathism, frontal and temporoparietal bossing, kyphoscoliosis or other vertebral defects, and bifurcated ribs. Ophthalmic differences include hypertelorism, wide nasal bridge, dystopia canthorum, congenital blindness, and internal strabismus. Neurological variances include mental retardation, dural calcification, bridging of sella, agenesis of corpus callosum, congenital hydrocephalus, and medulloblastoma. Sexual malfunctions include hypogonadism and ovarian tumor-like fibrosarcoma.[5] Evans et al first ascertained the major and minor criteria for the diagnosis of the syndrome which were later revised by Kimonis et al in 2004. More than 100 minor criteria have been described. The presence of two major and one minor or one major and three minor criteria is essential to establish a diagnosis.[6] The *major criteria* include the following:

- Multiple BCCs >2, or one occurring under the age of 20 years.
- Histologically proven OKCs of the jaws.
- Palmar or plantar pits (three or more).
- Bilamellar calcifications of the falx cerebri.
- Bifid, fused, or markedly splayed ribs.
- First-degree relative with nevoid BCC (NBCC) syndrome.

Minor Criteria

- Macrocephaly (adjusted for height).
- Congenital malformation: cleft lip or cleft palate, frontal bossing, and coarse face moderate or severe hypertelorism.
- Other skeletal abnormalities: sprengel deformity, marked pectus deformity, and marked syndactyly of the digits.
- Radiological abnormalities: bulging of sella turcica, vertebral anomalies, such as hemivertebrae, fusion or elongation of vertebral bodies, modeling defects of the hands and feet, or flame-shaped hands or feet.
- Ovarian fibroma.
- Medulloblastoma.

In this case, the diagnosis was made by two major criteria, such as multiple cystic lesions of jaws and calcification of falx cerebri and three minor criteria which include multiple compound nevus with histopathologically proven BCC and slight mandible prognathism.

Skin lesions, such as cutaneous nevus, underline the early onset with BCCs up to 90% of patients by the age of 40 years. The presence of the lesion may vary from brownish colored nevi to very aggressive BCC usually in sun-exposed areas.[7] So, it is crucial to make an initial diagnosis as these patients may be shown increased risk of transformation into malignant neoplasms. They are also sensitive to ionizing radiation, mainly ultraviolet radiation. Confirmation of the diagnosis is done by DNA analysis which remains as the gold standard. Therefore, genetic counseling is anticipated for all patients and their family members with this condition.[8] The diagnostic protocol for evaluation of the patient with suspected NBCC syndrome comprises complete patient history and clinical, dermatological, radiological, dental, cardiac, and gynecological examinations.[9] Multiple OKCs alone may be confirmatory of the syndrome. Treatment usually involves removal of tumors by surgical excision, laser ablation, photodynamic therapy, or topical chemotherapy, but radiotherapy remains a contraindication.[10]

Prognosis

Most of the anomalies in Gorlin–Goltz syndrome are minor and usually not life-threatening. The prognosis depends on the behavior of skin tumors. In a few cases, aggressive BCCs have triggered the death of the patient due to tumor invasion of the brain or other vital structures. The jaw cysts are treated by enucleation but in many patients, secondary cysts will continue to develop. Varying degrees of jaw deformity may result from operations of multiple cysts and infection from the cysts is also not uncommon.[11]

Conclusion

Gorlin–Goltz syndrome is an infrequent but important entity. So, once a diagnosis is confirmed, it is essential for the patient to be appropriately managed and observed periodically. Prenatal, counseling can be given for suspected couples who are at risk. Since this syndrome is less familiar, the lack of awareness can lead to delayed diagnosis.

Conflict of Interest
None declared.

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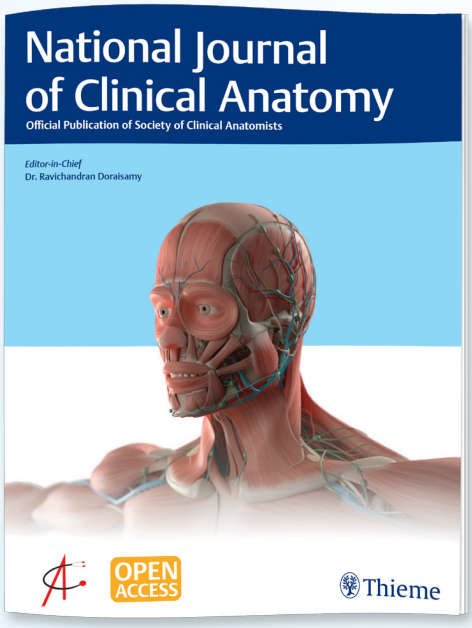

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