नाईपर हैदराबाद
NIPER HYDERABAD
HYDERABAD

राष्ट्रीय औषधीय शिक्षा एवं अनुसंधान संस्थान
औषधीय विभाग, रसायन एवं उर्वरक मंत्रालय, भारत सरकार
National Institute of Pharmaceutical Education and Research
Department of Pharmaceuticals, Ministry of Chemicals & Fertilizers, Govt. of India

Date: 02/06/2020

To,

Registrar,

BLDE (Deemed to be University)

Vijayapura, Karnataka State

We received a project proposal entitled "Molecular Phylogeny and possible drug target of SARS-CoV2 through whole genome sequence analysis" from Dr. Kusal K Das for joint submission as per our mutual MoU. Hypothesis of proposal seems novel and the research area is of immense importance in the current situation. NIPER-Hyderabad happy to accept the project with joint collaboration and mentorship Dr. Kusal K Das and Dr Shashi Bala Singh. NIPER-Hyderabad will provide all the technical and other necessary support to execute the project. Dr Pankaj Kumr Singh, Assistant Professor, Department of Pharmaceutics will be Principal investigator from NIPER-Hyderabad.

S. Gananadhamu
Registrar

Dr. Gananadhamu Samanthula

रजिस्ट्रार / Registrar
राष्ट्रीय औषधीय शिक्षा एवं अनुसंधान संस्थान (नाईपार)
National Institute of Pharmaceutical (NIPER)
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BLDE (Deemed to be University), Vijayapur& NIPER-HYD, Hyderabad

Collaboration as per MOU

PROJECT TITLE:

Molecular Phylogeny and possible drug target of Coronavirus 2 (SARS-CoV2) through whole genome sequence analysis

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Introduction:

The past two decades have seen three major highly pathogenic zoonotic outbreaks ofbetacoronaviruses. The first was Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2002, which infected over 8,000 people and killed 800 [1]. This was followed in 2012 by Middle East Respiratory Syndrome, MERS-CoV, a difficult to transmit but highly lethal virus, with 2,294 cases as of 2019, and 35% mortality[2].The third, SARS-CoV-2 is the cause of the severe respiratory disease COVID-19 [3]. It was first reported in China in late December 2019, and triggered an epidemic that rapidly spread globally to become a pandemic of devastating impact, unparalleled in our lifetimes; today's World Health Organization (WHO) situation report reads: over 2.5 million confirmed cases of COVID-19, and over 175,000 deaths; tomorrow's report will bring us new and markedly higher tallies of suffering, as the WHO continues to track the remarkable pace of expansion of this disease [4].

Although the observed diversity among pandemic SARS-CoV-2 sequences is low, its rapidglobal spread provides the virus with ample opportunity for natural selection to act upon rare butfavourable mutations[6]. This is analogous to the case of influenza, where mutations slowlyaccumulate in the hemagglutinin protein during a flu season, and there is a complex interplaybetween mutations that can confer immune resistance to the virus, and the fitness landscape ofthe particular variant in which they arise[7,8]. Antigenic drift in influenza, theaccumulation of mutations by the virus during an influenza season, provides the baselinevariation needed to enable selection for antibody resistance across populations, and thisdrift is the primary reason we need to develop new influenza vaccines every few seasons.The seasonality of influenza is likely to be dictated in part by weather patterns,longer seasonal epidemics allow selection pressure tocontinue over a more extended period, enhancing opportunities for the development ofvirus with novel antigenic surfaces that resist pre-existing immunity [9].

There is clearly an urgent need to develop an effective vaccine against SARS-CoV-2, as well as antibody-based therapeutics. Over 62 vaccine approaches are currently being explored, and a wide variety of candidate SARS-CoV-2 vaccines are in development

(Landscape of COVID-19 Variants, WHO) [10]. Most of these vaccine approaches target the trimeric Spike protein (S) with the goal of eliciting protective neutralizing antibodies. Spike mediates binding and entry into host cells and is a major target of neutralizing antibodies.Each Spike monomer consists of an N-terminal S1 domain and amembrane-proximal S2 domain, which mediate receptor binding and membrane fusion,respectively[11]. Notably, current immunogens and testing reagents are generally based on the Spike protein sequence from the index strain from Wuhan. SARS-CoV-2 is closely related to SARS-CoV; the two viruses share ~79% sequence identity and both use angiotensin converting enzyme-2 (ACE2) as their cellular receptor,however the SARS-CoV-2 S-protein has a10-20-fold higher affinity for ACE2 than the corresponding S-protein of SARS-CoV. It remains to be seen to what extent lessons learned from SARS-CoV are helpful in formulating hypotheses about SARSCoV- 2, but SARS-CoV studies suggest that the nature of the antibody responses to the Spike protein are complex[12].

Given Spike's vital importance both in terms of viral infectivity and as an antibody target, so there is an urgent need for an "early warning" pipeline to evaluate Spike pandemic evolution. The present study is focused on to identify dynamically changing patterns of mutation indicative of positive selection for Spike variants. Also, because recombination is an important aspect of corona virusevolution, we also set out todetermine if whether recombination is playing a role in SARS-CoV-2 pandemic evolution.

Aims and Objectives:

Specific Aim 1: Molecular Phylogeny study of SARS-CoV-2 using whole genome sequence study.

Specific Aim 2: Detection of variant genes and mutation site.

Specific Aim 3: Vaccine target screening SARS-CoV-derived B cell and T cell epitopes

Specific Aim 4: Designing small drug target using cheminformatics pipeline.

Objectives:

1. Intraspecies and interspecies comparative genomic of SARS-2 virus genome(Collection of SARS-2 genome sequence).
2. Phylogenetic analysis of SARS-2 virus and prediction of close related homologs of COVID-19.
3. Detection of variants and mutation sites in the genome/NGS data analysis approach.

4. Study of variant genes and proteins of SARS.
5. Identification of potential vaccine targets and small drug target.

Methodology

1. *Intraspecies and interspecies comparative genomic of SARS-2 virus genome*.
   Collection of whole genome sequences of SARS-CoV2 originated from different parts of world and various organisms. The sequences will be collected from biological database (GISAID SARS-CoV-2 sequence database). Further sequences will be processed and align using online tool.
2. *Phylogenetic analysis of SARS-2 virus and prediction of close related homologs of COVID-19.*
   To study evolution pattern phylogenetic analysis will be carried out. For this multiple sequence analysis followed by construction of phylogenetic tree using different algorithms.
3. *Detection of variants and mutation sites in the genome through NGS data analysis approach.*
   To carry out this step NGS platform will be used which involves following steps:

- *Preprocessingof data*
  Thepreprocessing step is to remove the adapter sequences indicating contamination that may be originated during quality check of the samples.
- *Alignment of datasets to Reference Genome*
  Alignment of the datasets involves, downloading the reference genome. The index will involve build to reference genome using bioinformatics tool. Sequence Alignment Mapping (SAM) format file will generated.
- *Post-alignment Processing*
  The SAM file generated in previous step was converted to BAM (Binary Alignment Mapping) format using call program of SAMtools [46]. The sorted BAM file is generated using sort program of SAMtools in turn generating index file. SAMtools manipulate the resulting alignment in SAM/BAM format with its utilities such as sort, merge, indexing. Next is to eliminate the duplicates those were supposed to be introduced during PCR uneven amplification of DNA fragments. Program MarkDuplicates of Picard tool [46] is used.

4. *Study of variant genes and proteins of SARS.*

The data generated from above step will be used for study of variant genes and proteins of SARS. Various online tools will be used to analyse the result.

5. *Identification of potential vaccine targets and small drug target.*

   For vaccine target screening SARS-CoV-derived B cell and T cell epitopes will be searched on the NIAID Virus Pathogen Database and Analysis Resource (ViPR) by querying for the virus species name: "Severe acute respiratory syndrome-related coronavirus" from "human" hosts.

   Small drug target will screening from drug bank and chemoinformatics pipeline will be followed.

Possible outcome:

The three-stage data pipeline (analysis of GISAID data, structural modeling of sites of interest, and experimental evaluation) and the identification of several sites of positive selection originated from parts of world.

1. Through genome sequence analysis will understand the sequence pattern of COVID-19 with respect to interspecies and intraspecies organism and conserved domain sequence.Changes is in sequence pattern SARS-2 at different places since outbreak.
2. The phylogenetic analysis of genomes may beacon light on closely related and under evolutionary selection in their human hosts, sometimes with parallel evolution events, that is, the same virus mutation emerges in two different human hosts. Mutation study will trace SARS-2 evolutionary paths and their ancestral genome in the human host. We can estimate genetic distances on the basis of the number of nucleotide substitutions and type of mutation.
3. The interpretation of sequence changes is a significant part of whole genome sequence analysis which involves in genetic diagnosis. Sequence conservation of a particular amino acid across multiple species tends to indicate that the amino acid is of importance, as does co-segregation of variants with disease status in family members. The absence of amino acid change in normal controls is also indicative of a variant's pathogenicity.
4. The variant genes which are obtained from above steps will be analysed for its functional part especially active site, similarity with respected other viruses and developing new drug target.

5. Screening of B cell and T cell epitopes derived from the spike (S) and nucleocapsid (N) proteins that map identically to SARS-CoV-2 proteins
6. Development of small drug target.

Duration of Work: 2 year

Budget:

In the proposed project these following Items are required

Sr No	**Items/Software's**	**Amount (INR)**
1	High Capacity Laptop (NGS Platform)	70,000.00
2.	Hard Disc drive (2 TB Capacity)	5000.00
3.	Molecular Docking software	30,00,000.00
4.	Chemical/ reagents/ Kits (Invitro analysis)	2,00,000.00
5.	Travel (Workshop/Training/Conference)	30,000.00
	TOTAL	33,05,000 (Rupees Thirty three lakhs and five thousands only)

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United Nations Educational, Scientific and Cultural Organization

Network on Research and Postgraduate Education in Biophysics, Biotechnology and Environmental Health

Life Sciences International Postgraduate Educational Center Yerevan, Armenia

UNESCO/UNITWIN NETWORK WEB SEMINAR 2020

(A Virtual Conference)

August 6-7, 2020

Theme: Current concepts of Environmental Pollution by Electromagnetic field and Corona Virus

Organizer: BLDE (Deemed to be University), Vijayapur, Karnataka, India with the support from LSIPEC, Yerevan, Armenia

A Brief Report

Entire Program of two days is recorded in YouTube – Links are provided below:-

Day1: August 6, 2020

First Session: https://www.youtube.com/watch?v=4aMZwR1FrZU

Second Session: https://www.youtube.com/watch?v=fi8v8hmUWLc

Day2: August 7, 2020

First Session: https://www.youtube.com/watch?v=e5G1m9VOgJ0&t=9s

Second Session: https://www.youtube.com/watch?v=uP4O51lcGx4

UNESCO/UNITWIN Web seminar 2020 on the theme "*Current Concepts of Environmental Pollution by Electromagnetic field and Corona Virus*" was held on 6th and 7th August 2020, jointly organized by UNESCO and BLDE Deemed to be University, Vijayapura, Karnataka.

The inauguration of the program was declared open by **Prof. Synerik Ayrapetyan**, UNESCO Chair Holder- Life Sciences, Yerevan, Armenia. **Dr. M.B. Patil**, Chancellor, BLDE (DU), Vijayapura, India, graced the inaugural function as the **Chief Guest**. The Welcome Address was delivered by Dr. Lata Mullur, Organizing Secretary,UNESCO/UNITWIN Network Web Seminar.

Guests of Honor of the inaugural ceremony were - **Dr. M.S. Biradar**, Hon'ble Vice Chancellor, BLDE (Deemed to be University), Vijayapur, India, **Dr. Shashibala Singh**, Director, NIPER, Hyderabad, India, **Dr. K.N. Sharma**, Hon'ble Vice Chancellor, Victoria University, Kampala, Uganda, **Prof. Edathil Vijayan**, President- Society for Biotechnologists of India, Kochi, India, **Prof. Chandrakant Kokate**, Former Vice Chancellor, KLE University, Belagavi and Kakatiya University, Warangal.The inaugural ceremony was presided by **Dr. Aravind V. Patil**, Dean, Faculty of Medicine & Principal, BLDE (Deemed to be University)'s Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India.

Prof. Synerik Ayrapetyan, in his inaugural speech, acknowledged the participants about the objectives of this web seminar "*The technical progress-induced exponential increase of*

electromagnetic fields (EMF) emitted from the technology and its role in the increase of the risk of generating different diseases, including virus infection, are vital problems of Public Health. At present, they are abundance of literature data on metabolic control of cell hydration, which could serve as a quantum-mechanically sensitive universal diagnostic parameter reflecting the functional state of cells, the dysfunction of which decreases the barrier function of the membrane for penetration of foreign particles, such as viruses into the cells. It has been shown that EMF could modulate the metabolic driving water efflux from the cells controlling cell hydration and the semipermeable properties of cell membrane through soluble guanylate cyclase and G proteins in the membrane. Thus, the metabolic control of cell hydration has been suggested as a biomarker for determining the hazardous and beneficial effects of EMF with different frequencies and intensities. Considering the fact that the biological impacts of major physical and chemical components of environmental mediums on cells and organisms have non-linear dose-dependent character, the fundamental knowledge in biophysics of biological effects of weak signals is necessary in order to solve modern problems of environmental health control. Therefore, the subject of this present seminar is dedicated to improve the postgraduate educational courses in Biophysics making it adequate to address the demands of environmental health control including the EMF effects on coronavirus infection through the cell membrane. This subject will elaborate a "Joint Research Project" on the basis of which the experimental work of the thesis of PhD students will be implemented." (https://unescounitwin2020.bldedu.ac.in/unesco-unitwin/)

Dr. M. B. Patil in his address, emphasized on the need of creating environmental protection awareness among public as the deterioration of the environment is in rampant progress due to lack of knowledge among public. Dr. Patil has also highlighted the green actions taken by BLDE Association to improve the quality of environment in this part of Karnataka, India.

Soon after the inaugural program, **Prof. David Carpenter** delivered the first keynote address on the topic "*Human Health effects of non- thermal electromagnetic radiation*". The 5G technology would be the next big threat for the living beings as it will cause a catastrophic damage due to spread of high frequency radiations into the environment. The use of 5G technology may cause a significant rise in blood and brain cancer, blood clots, bone problems, insomnia, heart disease, birth of disabled children, infertility problems etc. The high frequency radiation may also change

glucose metabolism and affect the DNA. Prof. Carpenter suggested using land phones instead of mobile phones and enhancing the use of message services to protect the environment and ecology. The session was chaired by Dr M. S. Biradar .Hon'ble VC, BLDE (DU), Vijayapura. India

Second Keynote address was delivered by **Prof. Synerik Ayrapetyan** on "*Metabolic driving water efflux from the cell as a quantum sensitive energy barrier for virus infection*". The chairperson for this session was Dr Aravind Patil Dean, FoM, Principal, BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India.

One invited lecture was delivered during pre-lunch session of the first day of two days web seminar. It was delivered by **Prof. Hamid Mobasheri** on the topic "*Biophysical tackle with the SARS-COV-2 virus, means for treatment of Covid-19 at atomic and molecular levels*". The session was chaired by Dr.Atul Ayare Principal, BLDEA's College of Engineering, Vijayapura. India

Post Lunch session commenced with *e-poster presentation* by Faculty and PG students. The e posters were adjudicated by three members of Scientific committee Dr. R.B. Kotanal Principal, BLDEA's S. S. M. College of Pharmacy, Vijayapur, India, Dr. Vidya Patil Dept of Anesthesiology, BLDE (Deemed to be University)'s Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India and Dr. Sumangala Patil**,** Dept of Physiology, Dept of Anesthesiology, BLDE (Deemed to be University)'s Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India. In this session One faculty, Three PG s, two PDFs and four Ph.D students presented their work.

Faculty, Ph.D., PDF and PG students were encouraged to present their scientific/ research work on the stage of UNESCO/UNITWIN web seminar to be listened by eminent scientists and professors from different parts of the world which was a lifetime opportunity. The young scientists utilized the break and the organizers had a tough time to select the best e-posters from many entries.

After e-poster presentation invited lectures were started. First talk was given by **Dr. Atanu Sarkar**, Memorial University of Newfoundland NL, Canada. "*Endocrine disrupting chemicals*

in ocean ecosystem and human health risks." The session was chaired by Dr Akram Naikwadi , Prof & Head, Dept of Pharmacology, BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India. Invited lecture 4 was delivered by **Prof. Klarskov Klaus**, University of Sherbrooke Quebec, Canada. His topic of presentation was "*Plant extract polyphenols and viral infections*". Chairperson was Dr R. V. Kulkarni Vice-Principal, BLDEA's College of Pharmacy and Research center, Vijayapura. India.Next talk was given by **Dr Sumanta Goswami** Albert Einstein Medical College NY, USA topic of presentation was "*New diagnostic assays for COVID-19* " and session was chaired by Dr Praveen Shahapur Prof, Dept of Microbiology, BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India. The first day of UNESCO UNITWIN Conference was concluded by e-poster presentation by Post doctoral fellows and Ph.D students which was judged by scientific committee. Moderator for e-poster presentation Dr. Shrilaxmi Bagali and Dr. Prachi Parvatikar. Dept of Physiology,BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India.

The second day (07-08-2020) of the web seminar started with the invited lecture of **Prof Marko S. Markov** Research International of Williamsville NY, USA on the topic "*5G Technology and Public Health Hazards*". The session was chaired by Dr Shailaja S. Patil, Prof & HOD Dept of Community Medicine, BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India

Subsequently eight invited lectures were delivered. Among that first invited lecture delivered by **Prof Sharaine Fernando** University Sri Jayewardenepura, Srilanka on "*Environmental pollution and male reproductive health*". Dr Salim Dhundasi Dean, Al Ameen Medical College, Vijayapura. India was chairperson.

Next talk was delivered by **Prof. Mary Boghosian** UNESCO/UNITWIN LSIPEC, Armenia 0n her topic of presentation was *"Innovation and Exponential Technologies in the Era of COVID 19* " and session was chaied by Dr Deepak Chauvan Assoc. Prof, Dept of Surgery, BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India.

After invited lecture total ten e-posters were presented by 10 Ph.D students from India, Iran and Armenia. The e posters were adjudicated by three members of Scientific committee Dr. R.B.

Kotanal Principal, BLDEA's S. S. M. College of Pharmacy, Vijayapur, India, Dr. Vidya Patil Dept of Anesthesiology, BLDE (Deemed to be University)'s Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India and Dr. Sumangala Patil**,** Dept of Physiology, BLDE (Deemed to be University)'s Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India. In this session Moderator for e-poster presentation Dr. Shrilaxmi Bagali and Dr. Prachi Parvatikar. Dept of Physiology, BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India.

Ten Invited lectures were delivered by **Prof. Anatoly M. Goltsev**, Prof Anatoly M. Goltsev National Academy of Science, Ukraine on "*Prospects of immune correction of anti-viral resistance with cryopreserved products of cord blood in corona virus expansion."* The session was chaired by Dr Arun C Inamdar Prof & Head, Dept of Dermatology, BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India.

After this lecture **Prof.Suleyman Dasdag** from Medeniyet University, Turkey on " *Radiofrequency radiation and distance between brain cells: could it be a biophysical approach for host cell*." Dr Surekha U Arakeri Prof & Head, Dept of Pathology,BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India chaired the session.

Prof. Iqbal Choudhary, University of Karachi delivered his lecture on "*Pakistan response to COVID-19 turning calamity into an opportunity the current state and challenges ahead*". The chairperson for this session was Dr Manjunatha Aithala, Professor, Department of Physiology, BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India. After this talk next invited lecture was delivered by **Prof. Somnath Gangopadhyay,** University of Calcutta, on "*Ergonomics and Health : Working from Home under COVID 19*" which was chaired by Dr Pradeep Malaji, Vice-Principal (Research & Academics), BLDEA's College of Engineering, Vijayapura, India

Invited lecture- 13 was given by **Prof. Iqbal Alam** of Jamia Hamdard University, India on "*Emerging Role of Nitric Oxide on the occurrence of Metabolic Syndrome and cardiovascular disease"* The session was chaired by Dr S R Mudanur**,** Dept of OBG BLDE (DU), Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India. The last invited lecture of the UNESCO/UNITWIN was delivered by **Prof. David Gee,** Brunel University London and

session was chaired by Dr. Sumangala Patil of the Department of Physiology, BLDE (Deemed to be University)'s Shri B.M. Patil Medical College, Hospital and Research Center, Vijayapura, India.

At the end of the second day of two day long web seminar, a **Round Table Discussion** was organized on "UNESCO/UNITWIN Life Sciences" AND "BLDE University" jointly under the coordination of **Prof. Synerik Ayrapetyan**, UNESCO Chair Holder- Life Sciences, Yerevan, Armenia and **Prof. Kusal K. Das**, Professor of Physiology, BLDE (Deemed to be University)'s Shri B.M. Patil Medical College, Hospital and Research Center, Vijayapura, India who is also the Dean of Environmental Health under UNESCO Chair Life Sciences (Biophysics, Biotechnology and Environmental Health). **Prof. Hamid Mobasheri**, University of Teharn, Iran, **Prof Anatoly Goltsev**, **Prof David Gee**, **Prof. Mary Boghosian**, **Prof. Iqbal Alam, Prof.Klaus Klarskov, Dr.M.S.Biradar and Dr.Aravind V.Patil** have joined in this panel discussion. The panelists also shared their views in the round table discussion for further improvement of UNESCO/UNITWIN network especially in the third world countries. The importance of biophysics as tool for life sciences including medical research development was also discussed. Three important decisions were taken 1) To establish a UNESCO Chair-Environmental Health at BLDE (Deemed to be University), India and also a UNESCO Chair at National Academy of Science, Kiev, Ukraine under 2) More research collaboration to purse PhD program under UNESCO Chair Life Sciences with various institutions under UNESCO UNITWIN network. 3) Expand UNESCO UNITWIN program with more Universities and rename it as ***UNESCO/UNITWIN Centre for Life Sciences Education & Research***.

Valedictory Session:

At the end of two days seminar the valedictory session was conducted. Prof. M.S. Biradar, Vice-Chancellor, BLDE Deemed to be University was the chief Guest of the function. UNESCO chair Life Sciences Prof.Ayrapetyan and other UNESCO coordinators were also joined in this function. The results of e-poster presentation were declared. The **Medal of Merit** Awardees were:

Faculty Category:

Dr Aruna Biradar, *India* BLDE (Deemed to be University)'s Shri B.M. Patil Medical College, Hospital and Research center, Vijayapura, India. Her presentation was entitled "*Menstrual Morbidities, Menstrual Hygiene, Cultural Practices during Menstruation, and WASH Practices at Schools in Adolescent Girls of North Karnataka, India: A Cross-Sectional Prospective Study*".

PG Category

Om Prakash Yadav, Memorial University of Newfoundland NL, Canada. His presentation was entitled "*Persistent Organic Pollutants (POPs) in the European Union: Analysis of the NORMAN EMPODAT database system*".

PDF Category

Dr. Satyajit Tripathy, from *South Africa*. His presentation was entitled "*Employment of old options to control novel corona virus: Pros and Cons".*

Ph.D. Category

1. Prerana Biswas, *India.* Topic: *"Casein fortified diet reversed the diabetes like changes induced by 4g connected mobile phone radiation in mice"*
2. Gouhar Madoyan, *Armenia.* Topic: *"Quantum-Mechanical Sensitive Na/Ca Exchange asa Target for Pain Relief Effects of 4Hz Weak"*
3. Zahar Elyasi, *Iran.* Topic: *"Biophysical insights to tackle with the corona virus SARS-COV-2 by UV"*
4. Rajat Hegde, *India.* Topic: *"Molecular Analysis of Neuronal Cell Surface Protein gene; Neuroligin 3 in Autism Spectrum Disorder".*

Vote of Thanks was proposed by Dr. Kailash S. Chadchan. The IT Team and Scientific team of the organizing committee were given special thanks for their immense supports.

Later Indian National Anthem was played.

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Article

HEALTH EDUCATION & BEHAVIOR

Health Education & Behavior
1–10

DOI: 10.1177/1090198120931040
journals.sagepub.com/home/heb
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Household Chores or Play Outdoors? The Intersecting Influence of Gender and School Type on Physical Activity Among Indian Adolescents

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Abstract

Most Indian adolescents, particularly girls and private school students, do not engage in sufficient physical activity (PA). Current understanding of these sociodemographic differences is limited by a focus on exercise, which may not fully capture PA in developing countries. We examined how gender and school type are associated with multiple PA domains and whether associations with gender differ by school type. We randomly selected an equal number of girls and boys (ages 13-16 years) from public and private schools in Southern India ($n = 395$). Cross-sectional 24-hour time-use surveys measured PA, which was categorized into three domains: chores, errands, and work; play; and transportation. Negative binomial and logistic regression modeled relative differences in domain-specific PA minutes and the probability of engaging in ≥60 minutes of moderate-to-vigorous PA (MVPA), respectively, in the prior 24 hours. Girls and boys were equally likely to meet MVPA recommendations. However, girls spent twice as much active time completing chores, errands, and work (rate ratio = 1.98, 95% confidence interval = [1.32, 2.98]), while boys spent twice as much active time playing (rate ratio = 2.11, 95% confidence interval = [1.23, 3.62]). Public and private school girls spent more active time in chores, errands, and work than boys; however, gender differences were greater among public school students (p value for interaction <.05). Although comparable MVPA levels for girls and boys are beneficial for physical health, girls may gain fewer cognitive, social, and emotional benefits associated with play. Additional research may clarify why the gendered burden of household responsibilities was greater among public school students. School-based programs to engage girls in active play may help reduce inequities.

Keywords

adolescent, gender, India, physical activity, school type

Industrialization, urbanization, and technological advancements have transformed patterns of physical activity (PA) worldwide. Mechanization has reduced the need for physical labor, inactive transportation options are widely accessible, and leisure time has become increasingly sedentary (Hallal et al., 2012; Katzmarzyk & Mason, 2009). Resulting decreases in PA have contributed to the rapidly growing burden of noncommunicable disease in developing countries (Hallal et al., 2012; Sallis et al., 2016). In India, levels of PA are particularly low among adolescents: in 2007, only 30.2% of school-going children aged 13 to 15 years met World Health Organization (WHO) recommendations for ≥60 minutes of moderate-to-vigorous PA (MVPA) per day (GSHS, 2007; WHO, 2015), although data suggest that levels of activity may be higher among adolescents living in rural areas (Bhawra et al., 2018).

Several studies have identified important sociodemographic differences in Indian adolescents' PA. Girls (Gulati et al., 2014; Swaminathan et al., 2011; Thakor et al., 2004), adolescents from higher-income backgrounds, and students

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who attend private schools (George et al., 2014; Mahaur & Badiger, 2018; Puri et al., 2008) tend to engage in less PA. However, current understanding of these sociodemographic differences is limited by a focus on exercise—planned and structured PA done to promote physical fitness (Caspersen et al., 1985)—and transportation-related PA. This may be insufficient in developing countries where adolescents commonly engage in other types of PA—any bodily movement produced by skeletal muscles resulting in energy expenditure (Caspersen et al., 1985)—such as household work and paid employment.

Examining activities beyond exercise and transportation may be particularly important for understanding sociodemographic differences in PA, as gender- and socioeconomic-based norms likely inform the type and amount of PA in which adolescents engage. Girls may be restricted in their movement outside of home due to safety concerns and notions of propriety, and expected to engage in home-centered activities that will prepare them for adult roles as homemakers (Singh & Misra, 2015; Verma & Sharma, 2003). In contrast, boys are often permitted more freedom of movement and encouraged to engage in work-related activities outside of home in preparation for roles as economic providers (Basu et al., 2017).

Adolescents who attend private schools, which are primarily accessible to wealthier families, face significant academic pressures and are expected to devote substantial time to homework and other academic activities, which may reduce time for play (Lloyd et al., 2008; Verma & Sharma, 2003). Still, school type is not only an indicator of household socioeconomic status; some poorer families send children to private school as an investment in future financial prospects. School type also indicates exposure to varying social and material supports for PA. For example, although private schools commonly provide greater access to sports equipment and play areas, a strict focus on academics may prevent extensive use of these resources (Bhargava et al., 2016). In contrast, although public school students may have fewer material supports for PA, they may be allowed more unstructured play time during school hours (Bhargava et al., 2016).

While initial evidence suggests that gender and school type independently influence PA, researchers have yet to examine whether they interact. Historically, it has been maintained that gender inequality in India is greater among women from higher social strata, whose autonomy and freedom of movement are more restricted by notions of purity and propriety (Liddle & Joshi, 1989). However, Deshpande (2002, 2011) argues that this characterization is no longer accurate and offers evidence that women in lower social strata are subject to greater socioeconomic disadvantage and less egalitarian gender norms, including more restricted decision making; whether these experiences extend to adolescents is unknown. Examining if and how the experience of gender—and its influence on PA—differs for public and private school students may help researchers successfully target and tailor PA interventions.

We examined patterns and correlates of PA among school-going adolescents in a remote district in Southern India using data collected from 24-hour time use surveys. We examined three domains of PA—(1) household chores, errands, and work; (2) play; and (3) transportation—and assessed whether gender and school type, both independently and jointly, were associated with (1) the duration of PA in each domain and (2) the probability of engaging in ≥60 minutes of MVPA.

Data and Method

Setting and Data Collection

We conducted this cross-sectional study in northern Karnataka state, Southern India. The sample was recruited in 2012 and is representative of school-going adolescents in the district capital city. We used stratified random sampling to select three private and three public schools from the city's 32 secondary schools: we divided the city into three geographic regions and selected one private and one public school from each region. We then stratified each school's roster by gender and randomly selected girls and boys 13 to 16 years of age (public school: $n = 99$ girls, 102 boys; private school: $n = 101$ girls, 105 boys). Ninety-nine percent of selected students participated (public school: 100% girls, 99% boys; private school: 100% girls, 98% boys). The institutional review boards (IRB) at Emory University and BLDE University approved all research protocols.

Outcome Variables: Physical Activity

We assessed PA using a 24-hour time-use survey modeled after the Panel Study of Income Dynamics (PSID) Child Development Supplement Weekday Time Diary (Institute for Social Research, 2007). Adolescents reported every activity in which they engaged during the previous 24-hour period. For each activity, participants were asked where the activity took place, the time it began and ended, and who else was present. Time-use surveys have demonstrated good test–retest reliability and validity compared with accelerometer data in several populations in Australia (van der Ploeg et al., 2010) and the United States (Matthews et al., 2013; Welk et al., 2014) but, to our knowledge, have not been tested in India. Time-use surveys are less subject to recall and social desirability bias as they require participants to account for all activities in which they engaged during the preceding 24 hours, unlike traditional PA questionnaires which only ask about selected activities and require estimates of time spent in each activity outside the context of the full 24-hour period (van der Ploeg et al., 2010).

Instructions for completing the 24-hour time-use survey were explained in the local language and demonstrated using a

template. Private school students completed the survey independently following the demonstration. Public school students required individual assistance from field staff to read the survey and correctly write the names of the previous day's activities; staff did not probe for additional information while assisting. All adolescents reported their activities in chronological order from midnight to midnight on the day preceding the survey; surveys were administered on Tuesdays and Fridays.

Two teams, each comprising one project coordinator and two supervisors, reviewed the 24-hour recalls and coded listed activities. Discrepancies were resolved through discussion; project coordinators made final coding decisions when needed. Listed activities were coded into 64 categories using PSID Child Development Supplement codes (Institute for Social Research, 2007). Coded activities where then grouped under 10 broader domains (sleeping; self-care; eating; household chores; errands; work outside the home; school; play and social activities [including organized sports]; transportation; child, adult, pet, and plant care). Six domains were deemed to potentially include PA: for analysis, we grouped household chores, errands, work outside the home, and child, adult, pet, and plant care together in a chores, errands, and work domain as these activities all represent responsibilities to the household; level of responsibility to the household likely varies by gender and school type making these activities important to examine as a unified domain. Consistent with prior research, play and social activities and transportation were kept as separate domains. Within the chores, errands, and work domain, we retained activities involving PA (e.g., laundry, shopping for household items, home repairs). In the play and social activities domain, we retained activities categorized as active play (e.g., playing catch with friends). In the transportation domain, we retained activities categorized as active transportation (e.g., biking).

For each activity and domain, we calculated: (1) duration (total number of minutes), (2) metabolic equivalent (MET) minutes, and (3) participation in ≥60 minutes of MVPA (yes/no), all during the previous 24 hours. To further characterize PA in this population, we also calculated activity frequency (number of bouts of PA, of any length) and participation (yes/no) in ≥1 bout of PA, both during the previous 24-hour period.

We used the Compendium of Energy Expenditures for Youth to assign each activity a MET value (Ridley et al., 2008). Three team members reached consensus on the MET value assigned to each activity. We could not identify a suitable match in the Compendium for two activities ("home repairs & outdoor chores" and "other outdoor chores"), so MET values from the Adult Compendium of Physical Activities were used (Ainsworth et al., 2011). Per WHO guidelines, we classified activities with MET values ≥3.0 as MVPA (WHO, 2011). We summed the durations of activities meeting this criterion and categorized the result as either above or below the 60-minute threshold.

Exposure Variables: Gender and School Type

Exposures of interest were adolescent's gender (girl or boy), school type (public or private), and the interaction between gender and school type.

Covariates

Multivariate analyses were adjusted for potential confounding by sociodemographic characteristics, household gender norms, and social and environmental support for PA. Sociodemographic characteristics included adolescent age, primary caregiver's highest level of education (no formal education vs. lower primary school vs. higher primary school vs. postsecondary education), religion (Hindu vs. non-Hindu), caste (general caste [most advantaged] vs. other backward class vs. scheduled caste/tribe [least advantaged]), and income (<10,000 vs. ≥10,000 Indian rupees [INR] per month). Household gender norms included whether the family only allows boys to play outside (vs. both boys and girls, only girls, or neither) and whether girls in the family are responsible for ≥1 household chore (yes/no). Measures of support for PA included whether the adolescent's friends encourage them to be active (yes/no), whether there is sports equipment available in the home (yes/no), and whether ≥1 of the adolescent's primary caregivers regularly exercises (yes/no).

Analysis

Survey weights were used in all analyses to account for the unequal probability of selection in the sampling design (by design, equal distribution of school type and gender). We excluded nine adolescents who did not have valid 24-hour recall data, yielding an analytic sample of 395. To retain the few participants missing covariate data (four covariates each missing less than 1.5% data), we used mean imputation (missing values replaced with the mean of a variable's nonmissing values). All analyses were conducted in Stata 14 (College Station, TX).

We used independent sample *t* tests to assess differences in the duration of overall, domain-specific, and activity-specific PA by gender and school type. We used Pearson chi-squared tests to assess differences in participation in ≥60 minutes of MVPA by gender and school type.

We used negative binomial regression (an extension of Poisson regression for overdispersed count data) to estimate the relative difference in minutes spent in each activity domain in the prior 24 hours. Model results are presented as adjusted rate ratios. We used logistic regression to assess adjusted associations between exposures of interest and participation in ≥60 minutes of MVPA in the prior 24 hours. Because odds ratios may overestimate prevalence ratios when the outcome is common (prevalence of participation in ≥60 minutes of MVPA = 59.75%), we converted odds ratios to prevalence ratios (Zhang & Yu, 1998).

Table 1. Weighted Characteristics of School-Going Adolescents in Southern India, by Gender and School Type.

	Total (n = 395)		Boys (n = 197)		Girls (n = 198)			Public school (n = 198)		Private school (n = 197)		
Characteristics	%	*SE*	%	*SE*	%	*SE*	*p*	%	*SE*	%	*SE*	*p*
Total			52.80	0.03	47.20	0.03		72.24	0.02	27.76	0.02	
Age (mean)	14.36	0.06	14.43	0.08	14.29	0.08		14.42	0.07	14.20	0.06	*
Mother/primary caregiver's education												***
No education	27.90	0.03	26.31	0.04	29.67	0.04		37.05	0.03	4.09	0.01	
Lower primary school	27.01	0.03	27.67	0.04	26.28	0.04		32.56	0.03	12.58	0.02	
Higher primary school	21.07	0.02	21.18	0.03	20.94	0.03		17.79	0.03	29.60	0.03	
Postsecondary education	24.03	0.02	24.85	0.03	23.11	0.03		12.61	0.02	53.73	0.04	
Religion												***
Hindu	74.65	0.02	70.27	0.04	79.55	0.03		69.57	0.03	88.36	0.02	
Non-Hindu	25.35	0.02	29.73	0.04	20.45	0.03		30.43	0.03	11.64	0.02	
Caste												***
General	18.84	0.02	17.61	0.03	20.22	0.03		11.57	0.02	37.99	0.03	
Other backward class	55.01	0.03	61.19	0.04	48.09	0.04		54.25	0.04	55.92	0.04	
Scheduled caste/tribe	26.15	0.03	21.20	0.03	31.69	0.04		34.19	0.03	6.10	0.02	
Monthly household income												***
<10,000 INR	56.02	0.03	59.07	0.04	52.60	0.04		69.33	0.03	21.65	0.03	
≥10,000 INR	43.30	0.03	40.31	0.04	46.63	0.04		30.67	0.03	75.89	0.03	

Note. All results are survey adjusted. Characteristics compared across strata using Pearson chi-squared tests, with the exception of adolescent age compared across strata using independent samples *t* test. INR = Indian rupee.
*$p < .05$. **$p < .01$. ***$p < .0001$.

We estimated two models for each outcome: a main effects model for the independent associations of gender and school type with PA, and an interaction model. We assessed interaction between gender and school type on the additive scale using the relative excess risk due to interaction (RERI) measure. An RERI > 0 indicates positive additive interaction (i.e., the effect of both exposures is greater than the sum of their independent effects), while an RERI < 0 indicates negative additive interaction (i.e., the effect of both exposures is less than the sum of their independent effects). We examined additive, rather than multiplicative, interaction because it can provide insight into the absolute excess risk attributable to the presence of both exposures, thus highlighting the most salient subgroups in which to intervene (VanderWeele & Knol, 2014).

Results

Table 1 reports weighted characteristics of school-going adolescents in the district capital city. Adolescents were 14.4 years old on average. The majority attended public schools (72%), and approximately 53% were boys. Most households were Hindu (75%), reported a monthly household income below 10,000 INR (56%), and belonged to Other Backward Classes (55%). Twenty-eight percent of primary caregivers had no formal education, 27% had completed lower primary school, 21% had completed higher primary school, and 24% had some postsecondary education. As seen in Table 1, private school students had caregivers with higher levels of education, were more often Hindu, belonged to higher castes, and lived in households with higher monthly incomes.

Total Physical Activity Duration

On average, adolescents spent 144 minutes engaged in PA during the previous 24 hours (Table 2). Average duration was similar for boys (148 minutes) and girls (139 minutes), but significantly higher among public (169 minutes) compared with private (80 minutes) school students. More than two thirds of adolescents participated in ≥60 minutes of MVPA during the previous 24 hours. Again, there were no significant differences between boys (71%) and girls (65%), but there were large differences between public (78%) and private (42%) school students. Activity frequency and the proportion of adolescents who participated in ≥1 bout of PA are presented in the appendix.

Domain-Specific Physical Activity Duration

On average, adolescents spent 53 minutes engaged in chores, errands, and work, 39 minutes in active play, and 52 minutes in active transportation in the previous 24 hours (Table 2). Girls spent significantly more time completing chores, errands, and work compared with boys (72 minutes vs. 37 minutes). Specifically, girls spent the most time doing laundry (15 minutes vs. 0.4 minutes), indoor cleaning (13 minutes vs. 2 minutes), and meal cleanup (10 minutes vs. 0

Table 2. Physical Activity Among School-Going Adolescents in Southern India, by Gender and School Type.

	Total (n = 395)		Boys (n = 197)		Girls (n = 198)			Public school (n = 198)		Private school (n = 197)		
	Mean or %	*SE*	Mean or %	*SE*	Mean or %	*SE*	*p*	Mean or %	*SE*	Mean or %	*SE*	*p*
Total duration, mean (min/day)	144.08	5.92	148.14	8.62	139.48	8.01		168.83	7.31	79.70	7.22	***
Chores, errands, work duration, mean (min/day)	53.35	4.72	36.70	6.60	72.17	6.33	***	68.35	6.24	14.35	3.32	***
Shopping: household items	5.62	1.54	7.41	2.70	3.59	1.15		6.45	2.04	3.46	1.55	
Shopping: clothes, games	0.27	0.22	0.11	0.11	0.45	0.45		0.37	0.30	0	0	
Shopping: snacks, tobacco, drinks	0.23	0.21	0	0	0.49	0.45		0.32	0.29	0	0	
Errands	4.95	1.21	4.87	1.70	5.05	1.72		5.40	1.48	3.80	2.02	
Preparing food	3.29	0.93	0.32	0.24	6.64	1.91	**	4.20	1.27	0.92	0.43	*
Serving food	2.02	0.79	0.12	0.12	4.17	1.65	*	2.67	1.08	0.34	0.25	*
Meal cleanup	4.91	1.02	0	0	10.47	2.05	***	6.70	1.39	0.26	0.26	***
Indoor cleaning	6.98	1.12	1.58	1.10	13.08	1.89	***	9.20	1.53	1.21	0.42	***
Laundry	7.01	1.36	0.36	0.22	14.52	2.72	***	9.63	1.87	0.19	0.15	***
Home repairs and outdoor chores	9.20	2.19	10.64	3.84	7.58	1.69		11.78	2.99	2.50	1.09	**
Other indoor work around house	1.03	0.33	1.03	0.47	1.03	0.46		1.43	0.46	0	0	**
Other outdoor chores	0.94	0.43	0.43	0.42	1.52	0.78		1.24	0.59	0.15	0.15	
Vehicle care	0.74	0.34	1.29	0.63	0.11	0.11		1.01	0.47	0.03	0.03	*
Paid work	5.90	2.53	8.54	4.16	2.90	2.61		7.63	3.48	1.39	1.02	
Gardening	0.02	0.02	0	0	0.05	0.05		0	0	0.09	0.09	
Playing with younger child	0.25	0.25	0	0	0.53	0.53		0.34	0.34	0	0	
Active play duration, mean (min/day)	38.65	3.40	54.46	5.53	20.79	2.99	***	45.32	4.50	21.32	3.12	***
Active play outdoors	33.48	3.24	49.26	5.30	15.64	2.69	***	39.79	4.30	17.06	2.88	***
Active play indoors	5.17	1.27	5.20	2.08	5.14	1.32		5.53	1.66	4.26	1.48	
Active transportation duration, mean (min/day)	52.07	3.18	56.98	4.16	46.53	4.82		55.17	3.94	44.03	5.07	
Biking	12.13	1.50	19.54	2.51	3.75	1.08	***	13.57	1.95	8.38	1.86	
Walking or running	39.95	3.01	37.44	3.80	42.78	4.75		41.60	3.75	35.65	4.67	
≥60 minues of MVPA (%)	68.33	0.02	70.87	0.03	65.45	0.04		78.33	0.03	42.32	0.04	***

Note. All results are survey adjusted. Minutes/day compared across strata using independent samples *t* tests. Participated in ≥60 minutes of MVPA compared across strata using Pearson chi-squared tests. MVPA = moderate to vigorous intensity physical activity.
*$p < .05$. **$p < .01$. ***$p < .0001$.

minutes). Girls spent significantly less time in active play than boys (21 minutes vs. 54 minutes), particularly in active play outdoors (16 minutes vs. 49 minutes).

Public school students spent significantly more time engaged in chores, errands, and work than private school students (68 minutes vs. 14 minutes). Public school students spent the most time engaged in home repairs and outdoor chores (12 minutes vs. 3 minutes), doing laundry (10 minutes vs. 0.2 minutes), and indoor cleaning (9 minutes vs. 1 minute). Public school students also spent significantly more time engaged in active play (45 minutes) compared with private school students (21 minutes), particularly active play outdoors (40 minutes vs. 17 minutes).

Adjusted Models of Physical Activity

Table 3 displays results for negative binomial and logistic regression models estimating the relative difference in minutes spent in each PA domain, and the probability of engaging in ≥60 minutes of MVPA, respectively (main effects only). In chores, errands, and work, girls spent 1.98 times the minutes compared with boys (95% confidence interval [CI] [1.32, 2.98]), and public school students spent 3.10 times the minutes compared with private school students (95% CI [1.79, 5.37]). In active play, girls spent approximately one third as many minutes as boys (rate ratio [RR] = 0.31, 95% CI [0.20, 0.49]), and public school students spent approximately twice as many minutes as private school students (RR = 2.11, 95% CI [1.23, 3.62]). In active transportation, girls spent three quarters as many minutes as boys (RR = 0.73, 95% CI [0.56, 0.96]), but there were no significant differences by school type. Finally, public school students were 1.85 times as likely to engage in ≥60 minutes of MVPA in the previous 24 hours compared with private school students (95% CI [1.38, 2.49]). The likelihood of engaging in ≥60 minutes of MVPA did not differ by gender.

Interaction Analysis

We observed positive additive interaction between gender and school type in the chores, errands, and work domain, indicating that adolescents who were both girls and public

Table 3. Correlates of Minutes of Physical Activity Among School-Going Adolescents in Southern India: Adjusted Negative Binomial Regression Models (Main Effects Models; $n = 395$).

	Chores, errands, work			Active play			Active transportation			≥60 minutes MVPA[a]		
	Rate ratio	95% CI		Rate ratio	95% CI		Rate ratio	95% CI		Prevalence ratio	95% CI	
		LL	UL		LL	UL		LL	UL		LL	UL
Gender (ref = boy)												
Girl	1.98**	1.32	2.98	0.31***	0.20	0.49	0.73*	0.56	0.96	0.84	0.71	1.00
School type (ref = private school)												
Public school	3.10***	1.79	5.37	2.11**	1.23	3.62	1.03	0.73	1.45	1.85***	1.38	2.49
Age (years)	0.95	0.78	1.15	0.89	0.74	1.06	1.07	0.96	1.20	0.99***	0.99	0.99
Mother/caregiver education (ref = none)												
Lower primary school	0.68	0.43	1.07	1.01	0.62	1.64	0.86	0.64	1.14	0.94	0.76	1.16
Higher primary school	0.49*	0.28	0.85	0.87	0.49	1.55	0.80	0.57	1.11	0.90	0.70	1.15
Postsecondary	0.43**	0.23	0.77	1.36	0.80	2.30	0.88	0.61	1.26	0.91	0.71	1.16
Religion (ref = non-Hindu)												
Hindu	0.62*	0.40	0.94	0.74	0.44	1.23	0.80	0.59	1.10	1.02	0.84	1.24
Caste (ref = scheduled caste/tribe)												
General	1.17	0.62	2.20	0.61	0.34	1.09	1.02	0.68	1.52	0.86	0.65	1.14
Other backward class	0.62*	0.40	0.95	0.67	0.40	1.14	0.87	0.65	1.18	0.93	0.75	1.14
Income (ref = ≥10,000 INR)												
<10,000 INR	0.80	0.53	1.22	0.82	0.56	1.22	1.11	0.88	1.40	0.93	0.80	1.08
Family only allows boys to play outside	1.13	0.76	1.69	0.76	0.49	1.19	0.72**	0.57	0.91	0.91	0.78	1.07
Girls in household responsible for ≥1 household chore	1.08	0.69	1.68	1.08	0.74	1.57	1.09	0.84	1.42	1.00	0.85	1.18
Friends encourage adolescent to be active	1.08	0.73	1.62	0.86	0.54	1.38	1.06	0.83	1.36	0.88	0.78	1.00
Family has sports equipment available in home	0.83	0.48	1.46	1.51	0.62	3.68	1.33	0.90	1.99	1.24	0.91	1.70
Parent regularly exercises	1.01	0.67	1.51	1.10	0.74	1.62	1.17	0.93	1.47	1.03	0.89	1.20

Note. All results are survey adjusted. MVPA = moderate to vigorous intensity physical activity; INR = Indian rupee.
[a]Adjusted logistic regression model.
*$p < .05$. **$p < .01$. ***$p < .0001$.

school students engaged in more minutes of chores, errands, and work than would have been predicted from the sum of the independent effects of being a girl and being a public school student (Table 4). As illustrated in Figure 1, Panel A, while girls in both public and private school spent more minutes doing chores, errands, and work than boys, the difference between girls and boys was greater among public school students. There was no evidence of significant additive interaction in active play, active transportation, or engagement in ≥60 minutes of MVPA.

Table 4. Correlates of Minutes of Physical Activity Among School-Going Adolescents in Southern India: Relative Excess Risk Due to Interaction ($n = 395$).

		95% CI		
Domain	RERI	LL	UL	*p*
Chores, errands, work	2.53	0.31	4.76	*
Active play	2.39	−0.73	5.51	
Active transportation	−0.28	−0.89	0.33	
≥60 minutes MVPA	2.39	−5.47	10.26	

Note. RERI = relative excess risk due to interaction; MVPA = moderate to vigorous intensity physical activity.
*$p < .05$. **$p < .01$. ***$p < .0001$.

Discussion

This study examined patterns and correlates of PA among Southern Indian adolescents in overall MVPA and three PA domains: chores, errands, and work; active play; and active transportation. Consistent with prior regional estimates (Shridhar et al., 2016; Swaminathan et al., 2011), the majority of adolescents (68.3%) engaged in ≥60 minutes of MVPA during the previous 24 hours. However, there were important differences by gender and school type. While girls and boys engaged in similar amounts of PA, *how* they spent their active time differed considerably. Girls spent the majority of their active time completing chores, errands, and work, while boys were primarily engaged in active play

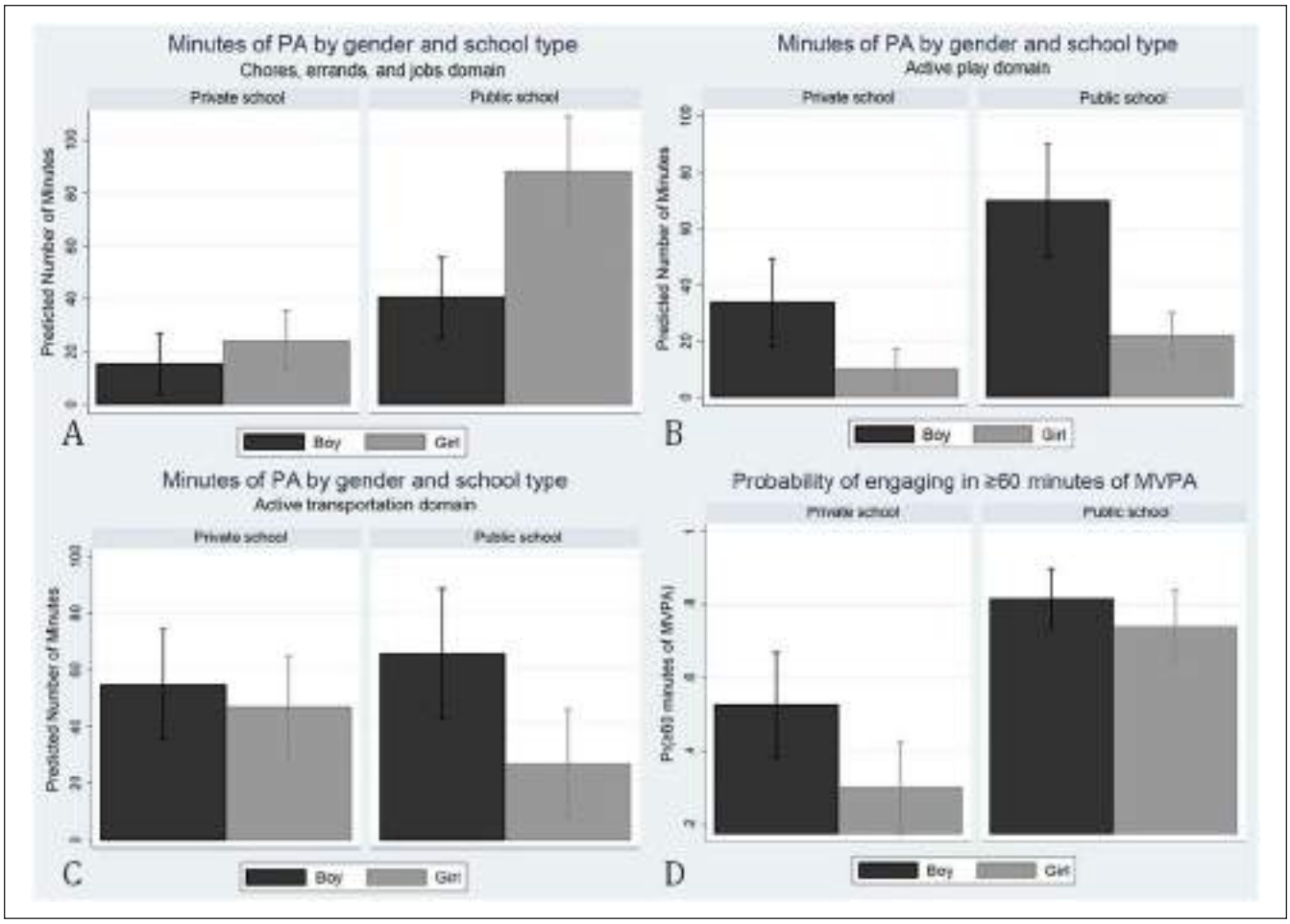

Figure 1. Interaction between gender and school type, by activity domain.
Note. Predicted values adjusted for covariates. PA = physical activity; MVPA = moderate to vigorous intensity physical activity.

and transportation. A different pattern emerged by school type: compared with public school students, private school students were less active overall *and* across domains. Notably, we found evidence of additive interaction in the chores, errands, and work domain: While girls in both public and private school spent more time in this domain than boys, the difference between girls and boys was greater among public school students.

Likely due to our approach of measuring PA across domains, rather than only in terms of exercise and transportation, we did not observe gender differences in the likelihood of achieving adequate levels of MVPA. Studies that do not measure all domains likely underestimate MVPA and may systematically underestimate the MVPA of girls. One of the only other studies to measure multiple domains of PA among Indian youth also found no significant gender differences in MVPA (Swaminathan et al., 2011). They did, however, report higher MVPA *intensity* among boys, a possibility in our sample as well: boys may have spent more time in vigorous-intensity PA, such as biking, while girls may have spent more time in moderate-intensity PA, such as indoor cleaning. While the largely comparable amount of active time for girls and boys is positive in terms of physical health, there may be other advantages and disadvantages to time spent in specific domains. Although girls may obtain physical benefits from MVPA, they may gain fewer of the cognitive, social, and emotional benefits associated with play, such as confidence, resiliency, creativity, conflict resolution skills, and learning readiness (Ginsburg, 2007; Yogman et al., 2018). Guidelines, policies, and programs may need to explicitly promote specific types of PA, like play, to ensure that these nonphysical health benefits are accessible to adolescents of all genders.

Our results are consistent with previous findings that private-school students engage in less PA than public school students (George et al., 2014; Mahaur & Badiger, 2018). Private school students spent very little time in household work, but this did not appear to translate into additional time for active recreation. In a recent qualitative study, private

school students in New Delhi discussed their academic workload as one of the most significant barriers to PA (Satija et al., 2018), and Bhargava et al. (2016) found that private schools in the northern Indian state of Uttarakhand were well-equipped with sports materials and structured play areas, but allotted little time for PA. As of 2019, India's Central Board of Secondary Education (CBSE) requires all schools to implement a daily 60-minute physical education period for students in Grades 1 to 12 (ages 5–18). However, the CBSE does not regulate all schools in the country and implementation and enforcement vary widely (Bhawra et al., 2018). Monitoring and evaluation plans as well as accountability mechanisms and funding to develop physical infrastructure in lower-resourced schools will likely be needed to achieve population-level impacts.

Our study provides the first evidence that gender differences in PA may be more pronounced among public school students. Our findings are consistent with Deshpande's (2002, 2011) assertion that gender inequality may be greater among women from lower social strata. Exploring factors that help explain greater gender disparities among women from lower social strata, including factors unique to younger women and girls, are important avenues for future research. To this end, Iyer et al. (2007) offer a useful distinction between "pure bias" and "rationing bias." The latter occurs when gender hierarchies emerge in the context of socioeconomic constraints to inform the distribution of resources and responsibilities. Such gender hierarchies become less salient as resources increase and decisions regarding distribution are no longer relevant. Rationing bias may help explain why we only observed greater gender differences among public school students in the chores, errands, and work domain. Because private school students belonged to families with higher incomes and higher social standing (Table 1), female servants and home appliances, such as washing machines, refrigerators, and mixers and grinders, may have performed the chores for which girls would have otherwise been responsible. In addition, the mothers of girls who attended private school had higher levels of education than the mothers of public school girls (Table 1) and may want their daughters to attain as much, if not more, education than themselves. As a result, they may have enabled their daughters to focus on academic extracurricular activities rather than housework.

Increasing knowledge of PA's positive effects on cognitive performance (Chang et al., 2012) may increase teacher and parent receptivity to incorporating PA into student schedules. Opportunities for play in the school environment, where students spend a substantial portion of each day, offer an immediate strategy for increasing adolescents' access to the physical and nonphysical benefits of play across gender and school type. Still, interventions will likely need to attend to physical infrastructure needs in public schools and gender-specific barriers to PA participation to avoid exacerbating existing inequities. Adolescent girls in India have reported various barriers to PA including, norms limiting "acceptable" activities, unsuitable dress codes (e.g., skirts), lack of confidence, and concerns about getting tan or sweaty (Satija et al., 2018). Programs that promote positive body image, offer examples of Indian women in sports, and offer various activity options may help facilitate equal participation (Satija et al., 2018). Formative intervention development work may also benefit from a deeper exploration of gender identity, socialization processes, and role conformity as they pertain to the type and amount of PA in which adolescents engage.

This study has several notable strengths. It extends the limited existing literature by providing a detailed picture of PA beyond the conventional domains of exercise and transportation, and highlighting the importance of gender and school type as independent and intersecting influences on PA. Using 24-hour time-use surveys allowed us to capture the range of activities in adolescents' lives and may also reduce social desirability and recall bias by requiring participants to account for all activities in the previous 24 hours, rather than select activities (van der Ploeg et al., 2010).

At the same time, it is important to note that time-use surveys do not provide an objective measure of energy expenditure, like accelerometers, and are subject to measurement error. Because accelerometers do not capture activity type, the two methods are likely best used in combination. An additional limitation is the assistance field staff provided to public school students during survey administration. While this may have contributed to small reporting differences between public and private school students, field staff were careful not to probe and limited their assistance to reading and writing. We assessed the average number of activities listed over the 24-hour period by public and private school students and observed minimal differences (21.8 and 20.8 activities, respectively). It is also important to note that our sample was restricted to school-going adolescents. Approximately 25% of adolescents in India are not enrolled in secondary school (The World Bank, 2013); these adolescents may engage in different types of PA not reported by the adolescents in our sample. Finally, the cross-sectional study design precludes discussion of the temporality or causality of associations.

Promoting PA among adolescents may help address the growing burden of noncommunicable diseases in India as elsewhere. Promoting the holistic benefits of PA, including its positive effects on cognitive performance, may be helpful in gaining buy-in from schools and families. Interventions that are tailored to the resources available in various school environments, and responsive to the norms that govern time use and notions of acceptable and expected behavior, are necessary to ensure that PA promotion efforts benefit all.

Appendix. Physical Activity Among School-Going Adolescents in Southern India, by Gender and School Type.

	Total (n = 395)		Boys (n = 197)		Girls (n = 198)			Public school (n = 198)		Private school (n = 197)		
Physical activity measure	Mean or %	*SE*	Mean or %	*SE*	Mean or %	*SE*	*p*	Mean or %	*SE*	Mean or %	*SE*	*p*
Duration (min/day) (mean)	144.08	5.92	148.14	8.62	139.48	8.01		168.83	7.31	79.70	7.22	***
Frequency (bouts/day) (mean)	3.75	0.13	3.62	0.16	3.90	0.20		4.43	0.16	1.99	0.11	***
Participated in ≥1 activity (%)	95.06	0.01	95.71	0.01	94.33	0.01		99.02	0.01	84.76	0.03	***
≥60 min of MVPA (%)	68.33	0.02	70.87	0.03	65.45	0.04		78.33	0.03	42.32	0.04	***

Note. All results are survey adjusted. Duration and frequency compared across strata using independent samples *t* tests. Participated in ≥1 activity and ≥60 min of MVPA (moderate to vigorous physical activity) compared across strata using Pearson chi-squared tests.
*$p < .05$. **$p < .01$. ***$p < .0001$.

Acknowledgments

We gratefully acknowledge Dr. MC Yadavannavar for coordinating data collection and survey supervision, the interviewers for their time and dedication, and the participating school authorities, adolescents, and caregivers, without whom this work would not have been possible.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (Award Number 3D43HD065249-03S1). IGR was supported by the National Heart, Lung, and Blood Institute (Award Numbers 5T32HL130025; 5T32HL007034).

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2 *Reviews on Recent Clinical Trials*, 2020, *15*, 2-1

REVIEW ARTICLE

COVID-19 and Pneumolysis Simulating Extreme High-altitude Exposure with Altered Oxygen Transport Physiology; Multiple Diseases, and Scarce Need of Ventilators: Andean Condor's-eye-view

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ARTICLE HISTORY

Received: May 13, 2020
Revised: August 03, 2020
Accepted: August 12, 2020

DOI: 10.2174/1574887115666200925141108

Abstract: ***Background*:** Critical hypoxia in this COVID-19 pandemic results in high mortality and economic loss worldwide. Initially, this disease' pathophysiology was poorly understood and interpreted as a SARS (Severe Acute Respiratory Syndrome) pneumonia. The severe atypical lung CAT scan images alerted all countries, including the poorest, to purchase lacking sophisticated ventilators. However, 88% of the patients on ventilators lost their lives. It was suggested that COVID-19 could be similar to a High-Altitude Pulmonary Edema (HAPE). New observations and pathological findings are gradually clarifying the disease.

***Methods*:** As high-altitude medicine and hypoxia physiology specialists from the highlands, we perform a perspective analysis of hypoxic diseases treated at high altitudes and compare them to Covid-19. Oxygen transport physiology, SARS-Cov-2 characteristics, and its transmission, lung imaging in COVID-19, and HAPE, as well as the causes of clinical signs and symptoms, are discussed.

***Results*:** High-altitude oxygen transport physiology has been systematically ignored. COVID-19 signs and symptoms indicate a progressive and irreversible failure in the oxygen transport system, secondary to pneumolysis produced by SARS-Cov-2's alveolar-capillary membrane "attack". HAPE's pulmonary compromise is treatable and reversible. COVID-19 is associated with several diseases, with different individual outcomes, in different countries, and at different altitudes.

***Conclusions*:** The pathophysiology of High-altitude illnesses can help explain COVID-19 pathophysiology, severity, and management. Early diagnosis and use of EPO, acetylsalicylic-acid, and other anti-inflammatories, oxygen therapy, antitussives, antibiotics, and the use of Earth open-circuit-astronaut-resembling suits to return to daily activities, should all be considered. Ventilator use can be counterproductive. Immunity development is the only feasible long-term survival tool.

Keywords: HAPE, tolerance to hypoxia, SARS-Cov-2, Polyerythrocythemia, EPO, open circuit Earth space suits.

1. INTRODUCTION

The whole planet is suffering the merciless attack of the recent time most aggressive virus, the SARS-CoV-2 [1]. This pandemic, simulating a "Bio-Nuclear Attack", has taken all medical centers, governments, and the population in general by surprise. The speed of the attack, the unusual clinical characteristics, the imaging findings, the fast Case Fatality Rate reaching even more than 13% of those infected [2], the intensive care units facing difficulties with an overload of patients, and the inadequate ventilator responses have indeed created havoc. Additionally, COVID-19 presents a hyper-exponential-type spreading speed. It is not a regular exponential progression ($y = A * B^x$), but rather an asymptomatic subject can initially transmit, variably, the disease to tens or more subjects in one environment [3]. It was suggested that COVID-19 could be similar to a High-Altitude Pulmonary Edema (HAPE). SARS-CoV-2 virulence was new and poorly understood, and initially, as some can develop extreme hypoxemia, word got around that it was a SARS (Severe Acute Respiratory Syndrome) pneumonia. However, in

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1574-8871/20 $65.00+.00

some countries, 88% of the patients on ventilators lost their lives [4].

Furthermore, COVID-19 signs and symptoms can vary in severity and from patient to patient. The quarantine is a temporary break, but with high uncertainty. The possibility of vaccine effectiveness remains undetermined. All SARS - Cov-2 and COVID-19 characteristics must be discussed to improve the understanding and treatment of this rapidly progressing situation that threatens all humans' psychological, economic, and general well-being.

2. WHAT IS THE MECHANISM OF TRANSMISSION OF COV-2?

It is well documented that SARS-CoV-2 can be extremely contagious as transmitted *via* droplets, aerosols, and contaminated surfaces, as well as when patients are still asymptomatic [5]. Initially, it was thought to affect the elderly more, yet it became evident that youth, children, and people with co-morbidities (overweight, diabetes, and other underlying diseases), are also included [6, 7]. This virus has a morphological shape described as a coronavirus. It has surrounding pointing spikes, as shown through electron microscopy [8]. This shape is similar to some plant seeds that are lightweight and with pointed spikes, easily transported by wind and airborne until they fall in a suitable soil for reproduction. The higher the number of organisms, the greater the probability of reproduction, thereby avoiding extinction. A 120 nm coronavirus particle with spikes can be airborne quite easily. It can, therefore, remain suspended in the air in an environment where an infected patient coughed or simply exhaled air [9]. It may travel even long distances in cloudy and windy days when the U-V index is low [10].

We suspect the virus does not travel to lung tissue through the walls of the trachea and the bronchi. It most probably arrives in the alveolar area through inhalation as suspended particles, due to its low nano-sized weight. This mechanism would explain why there is a non-productive dry cough initially with no bronchial hypercrinia (hypersecretion) reaction. Hence, it is fundamental that breathing in a contaminated environment (using masks) be carried out through the nose, avoiding deep mouth breathing. The nose plays the role of air warming, humidifying and essentially filtering, within the bugles, the dust particles, and in this case, the virus. The nasopharynx infection may not necessarily be clinically evident through rhinorrhea and nasal breathing obstruction, but merely anosmia. It would be interesting to study if those with anosmia have a better outcome.

As the center of the virus remains distant from the contact area through the spikes, this grants it a higher survival on surfaces - a brilliant and outstanding design of nature. The time of survival on surfaces has been amply reported [11]. Noteworthy is the fact that there is less survival time in copper surfaces. Copper is a metal with similar electrochemical characteristics to silver and gold in the periodic table. Why do copper surfaces reduce the survival time of coronavirus? Copper may produce viral lysis through an electric potential due to its electro-magnetic characteristics that can act as a battery, as Volta well showed [12].

3. THE COVID-19 INCIDENCE AT HIGH ALTITUDE

It seems that COVID-19's progression at high-altitude populations: China, Bolivia, Ecuador, is slower [13], and therefore, the incidence is lower. The "extreme" Ultra-Violet Index radiation at high altitude due to decreased filtering of the thinner atmosphere could play a role in slowing the initial spreading [10, 14]. Furthermore, we have recently co-authored a paper, postulating that since high altitude residents have a down-regulation of ACE2, being this the area of viral attack in cells, they could be less susceptible to infection [13]. In (Fig. **1**), it can be seen that the Bolivian cities above 3,000m, have a slower increase in reported positive cases than Bolivian lowlands [14]. Undoubtedly, many variables may play a role in the evolution of the disease: the speed of quarantine establishment, compliance of the people, the enforcement, the use of face masks and hygiene, the speed of isolation of suspected infected people, the speed of testing for coronavirus, the tests availability, the nutritional conditions, the genetic buildup, co-morbidities, immune system conditions, closed and unventilated environments, environmental conditions (temperature, dryness, U-V radiation at high altitude) and previous exposition to other coronaviral diseases, among others.

4. SARS-COV-2 REDUCES BOTH LUNG VENTILATION AND PERFUSION AND INVADES OTHER TISSUES

Covid-19 patients can suffer fast-evolving dyspnea, and this becomes an alarming "gasping" [15]. Much like fish pulled out of their natural environment: water. So, the process can evolve to an acute pulmonary insufficiency associated with tachycardia. What gives rise to this condition? In our criteria, there is a reduction of the ventilating surface area, due to the alveoli and adjacent capillary tissue destruction. Cov-2 has also been found in the blood as RNA and fecal and urine samples [16]. Initially entering through some Angiotensin-converting enzyme 2 receptors (ACE2) of mostly lung epithelial cells, it may trigger the Nuclear Factor-kappa B (NF-kB) signaling and a cytokine storm response, as shown in previous SARS-CoV infected patients. This can be assumed from the fact that COVID-19 patients presented significantly elevated proinflammatory cytokines and chemokines such as tumor necrosis factor, interferon gamma-induced protein-10, interleukin-6, interleukin-1beta, granulocyte-colony stimulating factor, monocytes chemoattractant protein-1 and macrophage inflammatory protein-1alpha [16]. Equally, an immune thrombotic syndrome has been described based on autopsy findings of inflammation, the formation of hyaline membranes, mononuclear cells, macrophages infiltrating air spaces, and diffuse thickening of the alveolar wall, along with lesions in spleen, liver, and kidney [17, 18]. So, there is a considerable reduction in both oxygenation factors: ventilation and perfusion. Hence, the rest of the still healthy and functional lung tissue tries to compensate for this deficiency, yet SARS-CoV-2 continues the invasion and multiplies. The rupture of capillaries give rise to local hemorrhages, yet we do not know if all these patients present anemia [18]. Patients suffering from anemia

can be more severely affected by CoVid-19. Some reports show that coughing can give rise to blood-stained sputum, as seen in thromboembolism and HAPE [19]. Noteworthy is that COVID-19 is not a highly sputum productive pathology. The possible explanation is that the virus does not migrate through the cilium in the trachea and bronchi with little or no affinity for those cells, even though ACE2 receptors have been found in bronchial transient secretory cells [20]. Autopsies, however, show clear bronchi [18]. It can attack the oral mucosa [21] and the olfactory system, leaving several with anosmia [22]. The virus travels to the alveoli through the inspired air and implants itself in the alveolar - capillary membrane in the ACE2 receptors [23]. As lung blood capillary vessels are damaged, there is a strong possibility that SARS-CoV-2 enters the bloodstream at this site. ACE2 receptors are also found in the endothelium in vessels [24]. We suspect that what is considered coagulation disorders may be ACE2 receptors in the endothelium being attacked by the SARS-CoV-2, thereby generating coagulum in order to "cover" the endothelial lesions [25]. Indeed, during an inflammatory response, binding sites on the endothelial membrane are more exposed [25]. The cerebrovascular accidents, myocardial heart infarctions, and other obstructive ischemic lesions would arise from these viral endothelial attacks. Atypical vascular dynamics could also be due to SARS-Cov-2 binding to ACE2 receptors producing an imbalance of ACE1 (intact) and ACE2 (interfered) in the Renin-Angiotensin-Aldosterone System.

The reduction of the pulmonary area of gas exchange, due to what we denominated "pneumolysis" (pneumo=lung, lysis=destruction), for the first time in the International Conference on Corona Viral Genomics 7-10 June, 2020, needs to be adequately understood. Patients evolve to present gasping and extreme hypoxia with cyanosis. The lung has a surface area of around 90 square meters, similar to the size of a tennis court. We previously described it as a parachute that, if intact, allows the air to sustain the weight of the subject and decelerate adequately [26]. As the parachute with holes would not be able to hold the paratrooper, the infected lungs could not carry out the efficient gas exchange in a similar manner. Initially, in COVID-19, the direct viral attack to alveolar-capillary membranes produces a gradually increasing hypoxia. Much like ascending to high altitude, hyperventilation ensues. $PaCO_2$ is maintained at sea level values or slightly below until the functioning pulmonary surface area cannot ventilate enough to reduce it. Although CO_2 is 20 times more diffusible than oxygen, ventilation becomes insufficient. Hypoxia increases and reaches a point where "gasping" begins. This condition simulates a rapid high-altitude ascent, like reaching Mt. Everest while at sea level. Mt. Everest is the highest tolerable extreme hypoxia point on Earth, where life is possible [27]. However, hypoxia becomes deadly if the gas exchange surface area is severely compromised. This results from pneumolysis, inflammation, local bleeding, transudates and exudates, edema and hypoxia induced pulmonary hypertension, creating more capillary stress failure (an atypical HAPE-type reaction with or without superinfection). Further aggravated by a sea-level hemoglobin count or anemia. The subject initially hyperventilates, and when hypoxia becomes very severe, he begins gasping for breaths in the final stages [28]. Ventilator assistance would be of limited use, and increased positive end-expiratory pressure (PEEP) would be detrimental in the brittle alveolar tissue. It can even lead to localized pneumothorax areas, thereby aggravating more the clinical condition [29]. Fever could result from cytolysis and liberation of intracellular components, including sialic acid from the rupture of red blood cells and possibly other intracellular enzymes, cytokines, and pyrogens [17].

5. SIMILARITIES AND DIFFERENCES BETWEEN COVID-19 AND HIGH ALTITUDE PULMONARY EDEMA (HAPE)

Concerning the use of ventilators in COVID-19, it has been found that previous SARS-type treatment is not very effective [29]. Some even suggested that the COVID-19 would be similar to HAPE, and hence patients could receive the same treatment [30]. The characteristics of HAPE include ascent to high-altitude, shortness of breath, coughing, cyanosis, hypoxemia, headache, tachycardia, tachypnea, discrete rales, and sometimes blood-stained sputum. Most HAPE symptoms resemble those of COVID-19. There are some in-situ high-altitude HAPE reports [31], but we have never seen it in the city of La Paz in Bolivia (4,100-3,100m). Those reports of HAPE without descent are probably a form of atypical pneumonia with different characteristics, pulmonary hypertension, and altered radiologic imaging at high-altitude. However, overall, the pathophysiology of HAPE is different from that of COVID-19.

In COVID-19, it is best to run a CAT scan as it gives high definition pathognomonic images, whereas, in HAPE, a chest X-Ray is more than enough, generally. The HAPE chest X-Rays presents unilateral or bilateral cotton-like images that are patchy, diffuse, fuzzy, and irregular in their distribution (Fig. **2**). It is edema in the alveolar sacs but with the alveolar-capillary structures unaltered except in very few spaces, due to high permeability edema and barometric pulmonary hypertension stress failure [26, 32]. In COVID-19, the lung CAT scan shows multiple, independent, atypical, irregular, clear, and delimited dense images in the initial stages, but these then evolve to be heterogeneous (Fig. **3**). These "strange images" have been referred to, following the standard X-Ray terminology, as "ground glass" [33]. Ground glass is an area of increased attenuation in the lung with preserved bronchial and vascular markings. However, it has genuinely called our attention as the images in COVID-19 are very peculiar in shape and density. One can observe that there are clear borders with no bronchial and vascular markings, and we believe this image should instead be referred to as "Sheer curtain" as it is quite uniform (Fig. **3**). Later on, it becomes more heterogeneous and a ground glass type image. These observations have significant implications for the interpretation of this new disease and require a new radiological classification. The virus destroys the alveolar-capillary tissue (pneumolysis), and there is edema [34], possibly hemolysis, hemorrhage, and in some long-lasting

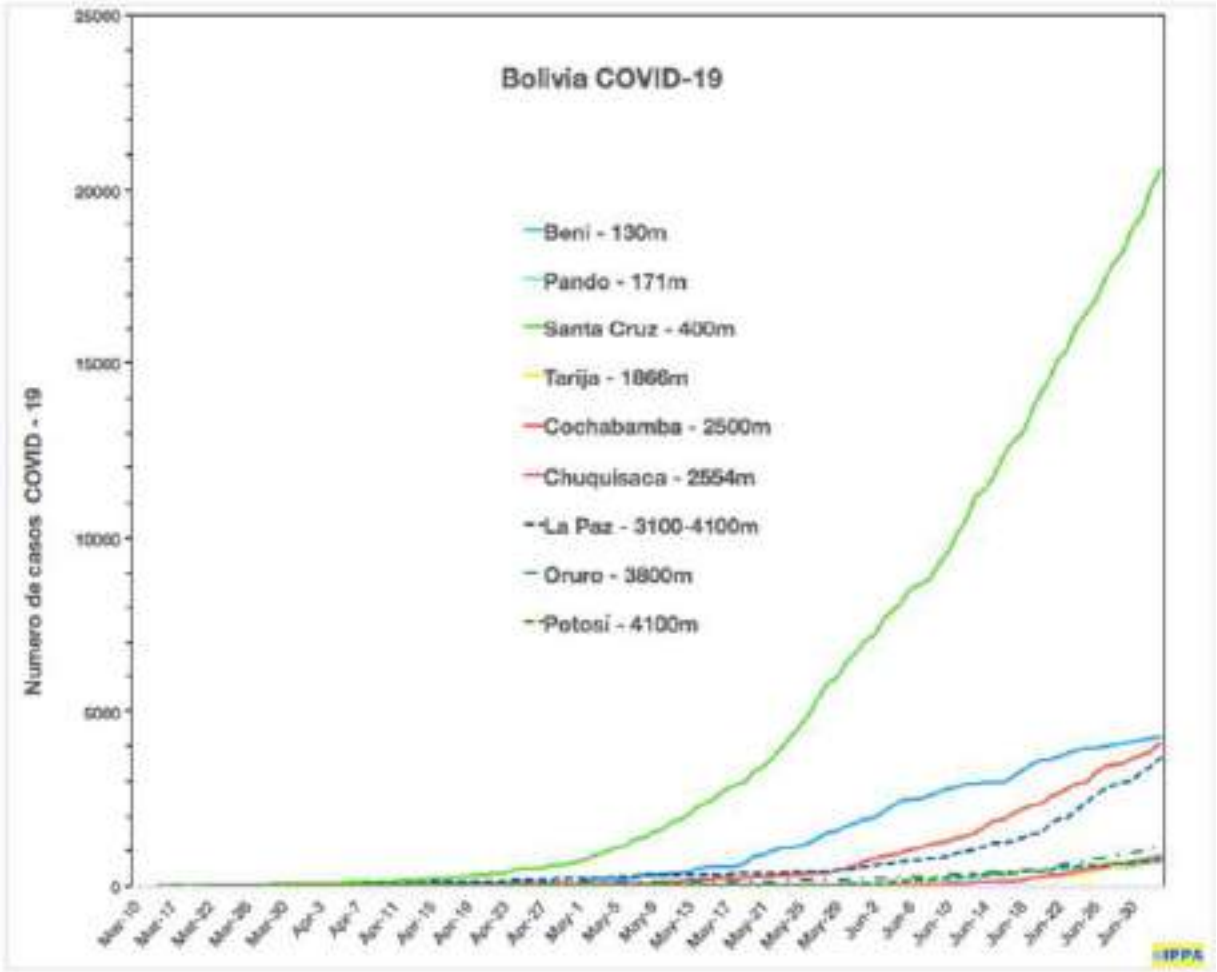

Fig. (1). Progression of the CoV-2 virus in Bolivia. The dashed blue lines correspond to the city of La Paz located above 3,000m of altitude as compared to the green line in the city of Santa Cruz in the lowlands, both infected at the same time. An updated figure until the end of the pandemic can be accessed at http://altitudeclinic.com/blog/2020/04/covid-2-bolivia/. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

cases, pus from superinfection. As hypoxia progresses, simulating a fast Mt. Everest climb, it could trigger pulmonary hypertension and consequently HAPE-type edema in the remaining intact lung tissue, further aggravating hypoxemia. In both pathologies, an increase in the FIO_2 raises blood gases PaO_2, but not as efficiently in COVID-19 as compared to HAPE [35, 36]. Above all, the HAPE process resolves quite fast in children (1-2 days) and a little longer in adults (3-5 days) with full radiologic clearance.

Nevertheless, fundamentally, although there is a dilated right ventricle and pulmonary hypertension in HAPE, once a resolution is achieved, there is a normal lung function without any residual sequelae. The same outcome is possible if the subject remains at altitude, or returns to sea level, or even if he climbs afterward to the summit of Mt. Everest [37]. Conversely, in COVID-19, the coronavirus produces alveolar-capillary cytolysis (pneumolysis), and the intense inflammatory-immunological response evolves to irreversible tissue damage and fibrosis [34]. In around 25% of the cases, there is also heart muscle compromise [38, 39]. Likewise, COVID-19 has a longer, more dramatic, and destructive evolution than HAPE. In the recovery process, it can result in a reduced pulmonary function and end up limiting the exercise capacity. At high-altitude, COVID-19 sequelae can result in a compensatory increase of the red blood cells, secondary polycythemia that would be interpreted by most as Chronic Mountain Sickness or what we call Poly-erythrocyt-hemia (Poly=many, Erythrocyt=RBC's, Hemia=in blood) [40].

In pathological studies, pulmonary thrombosis not typical in SARS has been reported [41]. There are suspicions of disseminated intravascular coagulation, *i.e.*, a severe clotting disorder. The alveolar-capillary destruction (pneumolysis) would give rise to a "fluidification" of the area that would, in our understanding, generate an intense inflammatory reaction that could alter the coagulation system. Pneumolysis could give rise to the atypical "Sheer curtain" images in well-defined areas in the CAT scan, as mentioned above. Due to

the speed of the viral attack on the lungs, there is a massive self-destructive immune response depending on the amount of inoculation of the coronavirus. Endothelial damage through the ACE2 receptors could also generate inflammation and coagulum formation.

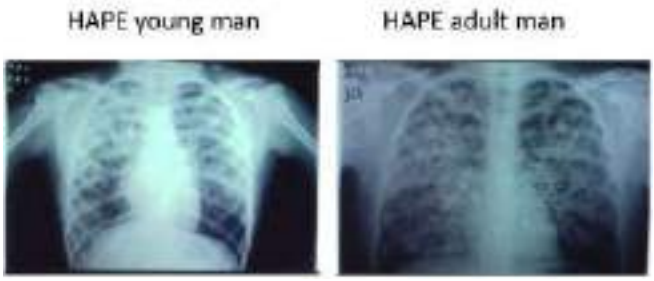

Fig. (2). HAPE chest X-Rays, from a young man (left) and an adult (right) in the city of La Paz-Bolivia (3,500m). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Fig. (3). CAT scans of COVID-19 patient at high altitude who was a permanent resident of 4,100m in the city of El Alto (Photo courtesy of Centro Especializado en Tomografias - CET). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Fig. (4). CAT scans of an 11-year-old COVID-19 patient from Arequipa-Peru, with severe lung compromise showing the "Sheer curtain" images (Photo courtesy of Dr. Diego Grajales López). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Furthermore, COVID-19 presents initially with a dry cough that is very irritating along with fever that accelerates heart rate, also aggravated by progressive hypoxia. The increased cardiac output complicates pulmonary function. COVID-19 is also associated with muscular body pain that brings along adynamia, discomfort, anorexia, and sometimes diarrhea [42]. Hyperventilation, fever, sweating, and diarrhea can give rise to dehydration, increasing thromboembolism risk.

6. THE OXYGEN TRANSPORT TRIAD AT HIGH-ALTITUDE

Fig. **4** is from a COVID-19 patient in the city of El Alto, at 4100m, a miner who had a previous lung injury several years back from toxic substance inhalation. The CAT scan showed extensive lung compromise. He was transferred to an ICU, placed on a ventilator, and after several days, passed away. However, it is interesting to note that at high altitude, the death rate is lower, and recovery more successful. Understanding high-altitude hypoxia and its adaptive mechanisms can help clarify the severe complications of SARS - CoV-2. The physiological oxygen transport system can be represented as a triad (Fig. **5**): 1) the pneumo-dynamic pump (respiratory system), 2) the hemo-dynamic pump (cardiovascular system), and 3) hemoglobin (erythropoietic system) [40]. All three function together to transport oxygen to the tissues. This triad is like a tripod. When a sea-level dweller ascends to high altitude, the Partial Inspired Oxygen Tension (PIO_2) drops, and oxygen is assimilated in the body "by diffusion and by diffusion alone", quoting August Krogh [43]. However, the metabolic processes of cellular life must adapt to a lower barometric pressure.

Fig. (5). The Oxygen Transport Triad. It is formed by 1) The Pneumo-dynamic pump (Lungs), 2) The Hemo-dynamic pump (Cardio - vascular system), and 3) The Hematopoietic System (Hemoglobin).

7. PNEUMO-DYNAMIC PUMP (RESPIRATORY SYSTEM)

The first response to high-altitude exposure is hyperventilation (an increased function of the pneumo-dynamic pump) [44], based on a higher respiratory frequency and higher tidal volume, particularly during temporary dyspneic episodes due to sudden uphill climbs or any other exercise that demands more oxygen. Hyperventilation at rest at high-altitude does not fully compensate for the decrease of the PIO_2 [26]. Let us assume a resting sea level ventilation of 4.82 L/min/m2 according to the Ideal Gas Law: PV = nRT (where P = Pressure, V = Volume, n = Avogadro's number, R = Ideal gas constant and T = Temperature). If nRT remains constant in order to expose the pulmonary area to the

same number of oxygen molecules at high-altitude (3600m) following Boyle's Law, then:

$P1*V1 = P2*V2$

Sea Level Pressure * Ventilation Volume = La Paz Pressure * V2

(760 mmHg) * (4.82 L/min/m^2) = (495 mmHg) * V2

V2 = 7.40 L/min/m^2

In reality, the average resting ventilation at 3,600m (La Paz, Bolivia) is 5.07 L/min/m^2, so the actual number of oxygen molecules exposed to the lungs is 1/3 less than at sea level, and hence a PaO_2 of 60 mmHg (instead of the sea level 95 mmHg) is also 1/3 less. High permanent hyperventilation is not possible as there would be respiratory muscle fatigue and excessive respiratory alkalosis, which is unacceptable for optimal cellular metabolism. This is confirmed in the breath-holding test performed in La Paz, where the normal SpO_2 of 90% rises after a deep breath to around 98% (sea level values) in healthy subjects [45, 46]. Artificial ventilation benefits from this physiological response, but not as efficiently when there are pulmonary destruction and fragile tissue.

8. HEMO-DYNAMIC PUMP (CARDIOVASCULAR SYSTEM)

The other functional adaptation factor is cardiac hyperactivity (hemo-dynamic pump). Under hypoxic conditions, the heart rate and stroke volume increase with pulmonary hypertension and sometimes systemic arterial hypertension. All these improve perfusion and thereby allow faster oxygen transport.

9. HEMOGLOBIN (ERYTHROPOIETIC SYSTEM)

The 3rd component is the erythropoietic system that regulates the production of hemoglobin, the iron-containing oxygen-transport. Upon arriving at a high-altitude, there is a logarithmic increase of the hematocrit until a maximal optimal plateau is reached [47].

Furthermore, for anyone at high-altitude (resident or visitor), the Tolerance to Hypoxia formula shows that paradoxically, the higher the altitude, the higher the tolerance to low oxygen levels (low PIO_2) provided there is an optimal acid-balance balance and THID=0 (BE=0) [48, 49].

Tolerance to Hypoxia = (Hemoglobin / $PaCO_2$) * 3.01

The formula has 2 fundamental variables and a constant:

A) The numerator (Hemoglobin) represents the metabolic component of the formula that takes time to develop. It is the most sustainable functional support of the oxygen transport triad. Hemoglobin increases with increasing altitude (Fig. **6**). It is not an immediate response. It follows the Formula of Adaptation = time / Altitude Δ [47]. For an altitude like that of La Paz, Bolivia (3,600m), it takes around 40 days to reach an optimal hemoglobin level, ascending from sea level (Fig. **7**). The cardio-respiratory pumps are gradually relieved of their over demand. Over those 40 days, dyspnea gradually decreases during exercise.

B) The denominator ($PaCO_2$) represents the respiratory component of the formula. It changes immediately upon arrival to high altitude through hyperventilation. For example, the normal sea level $PaCO_2$ of 40 mmHg is reduced to 30 mmHg at the altitude of 3,600m in the city of La Paz. $PaCO_2$ decreases with increasing altitude (Fig. **8**). A higher $PaCO_2$ decreases the affinity of the hemoglobin oxygen dissociation curve (the Bohr effect).

C) The constant 3.01 of the formula is derived from dividing the normal sea level Hb 13.3 gm/dl by the normal $PaCO_2$ = 40 mmHg and equating it to 1: K * 13.3 / 40 = 1.

This sets the tolerance to hypoxia at sea level as 1. As the altitude increases, the normal Hb and $PaCO_2$ values at each altitude provide the tolerance factor. For La Paz, (3,600m) and Mt. Everest (8,842m), it is 1.7 and 4.86 times more tolerant, respectively. This means a person on the summit of Mt. Everest can tolerate hypoxia nearly five times more than at sea level, without losing his life breathing a PIO_2 of 43 mmHg.

$PaCO_2$ plays a fundamental role in the acid-base status of blood, according to the Henderson-Hasselback equation. From the sea level point of view, a high-altitude low $PaCO_2$ resulting from hyperventilation is interpreted as Respiratory Alkalosis (with an elevated pH) that needs to be compensated. One way would be to decrease ventilation to increase $PaCO_2$, but that would compromise oxygen capture, as stated above. So, a metabolic decrease in balancing bicarbonates through renal compensation is the only feasible mechanism. Once a stable pH (7.4) is reached in around 1 or 2 days, the organism is in its optimal metabolic state at 3,600m. This is a normal acid-base balance for high altitude and not a chronic respiratory alkalosis. Some authors have found that a mild alkalotic pH remains for a more extended period of time in sea level travelers ascending to 5,000m, as compared to residents. However, other variables have to be considered, like pre-existent anemia [47]. Sea level acid-base interpretation charts, such as the Siggaard-Andersen or the Davenport Nomograms give the wrong interpretation at high altitude, and should not be used. Hence, we developed high altitude correction factors for the Van-Slyke equation and the Acid-Base Nomogram [49]. We also established that it is not possible for over 200 million inhabitants at high altitude to live in a permanently abnormal acid-base status [50]. In other words, the new acid-base status at high altitude is the normal condition (as at sea level) and a fundamental part of survival.

An SpO_2 of 90% with a PaO_2 of 60 mmHg are standard values for the high-altitude residents in the city of La Paz, Bolivia (3,100-4,100m) [51]. So, this is, without doubt, tolerable. However, we have the advantage of a raised compensatory hematocrit with an adequate acid-base status and normal pH.

In Chronic Mountain Sickness (or what we call Poly-erythrocyt-hemia), there is always a decreased PaO_2 with re-

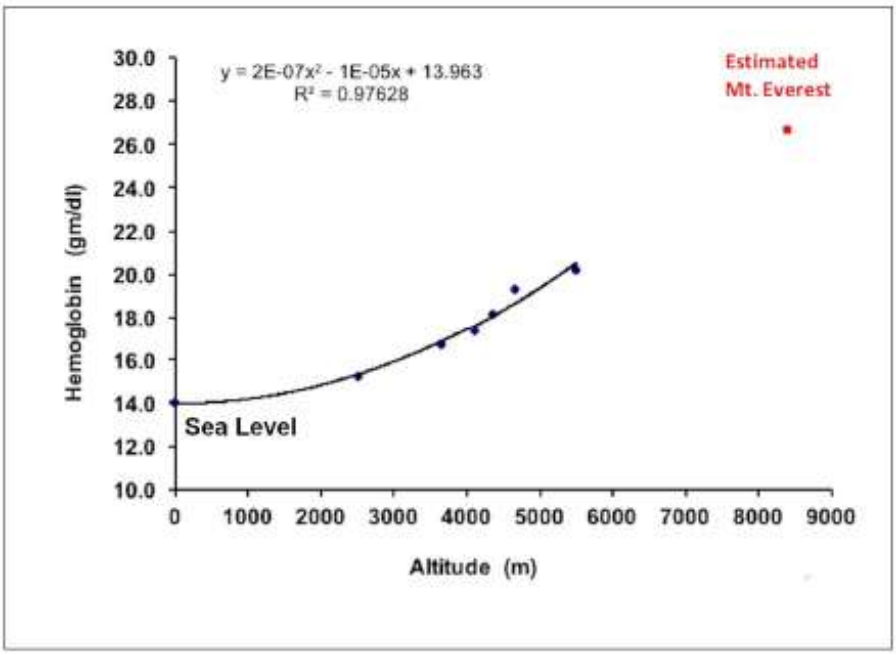

Fig. (6). The increase of hemoglobin with respect to altitude [48]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

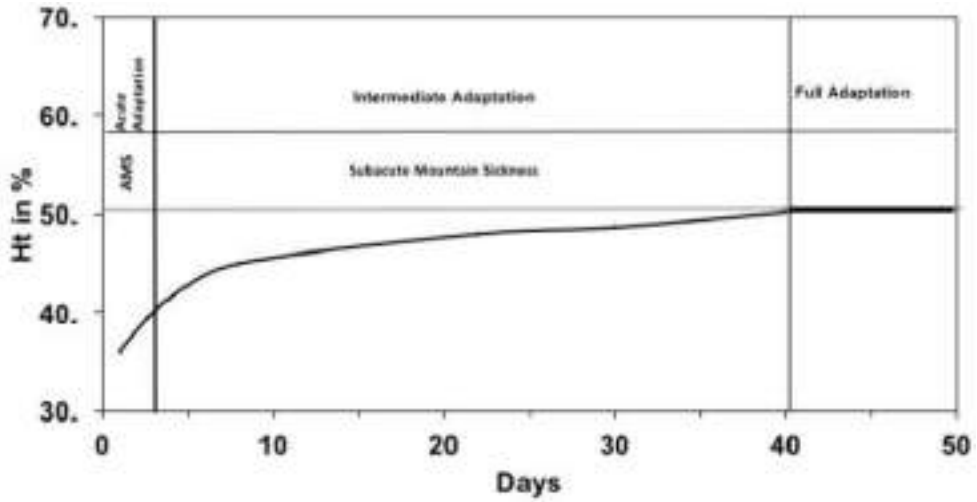

Fig. (7). The increase of hematocrit upon returning to high altitude with respect to time. It is a logarithmic curve time where hematocrit increases until it reaches the optimal plateau, for optimal oxygen transport to the tissues in this case at 3600m. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

spect to normal high-altitude residents [40]. Pulmonary function is compromised with shunts, ventilation/perfusion inequality, hypoventilation, and lung oxygen diffusion alterations. The only possible compensating mechanism to sustain an adequate oxygen tissue supply becomes the hemoglobin increase, *i.e.*, the triad's 3rd pillar [40]. Consequently, we believe there is no "excessive" increase of red blood cells but rather the only way to survive with hypoxic diseases in a chronic hypoxia environment [40]. And likewise, the most energy-efficient mechanism.

10. COVID-19 BLOOD GASES CASE REPORTS

Blood gases in a sea-level COVID-19 intubated patient-reported pH = 7.19, $PaCO_2$ = 70.1 mmHg, PaO_2 = 63.7 mmHg and bicarbonates 26.0 mEq/L. This is a truly critical condition. The diagnosis would be severe respiratory acid-

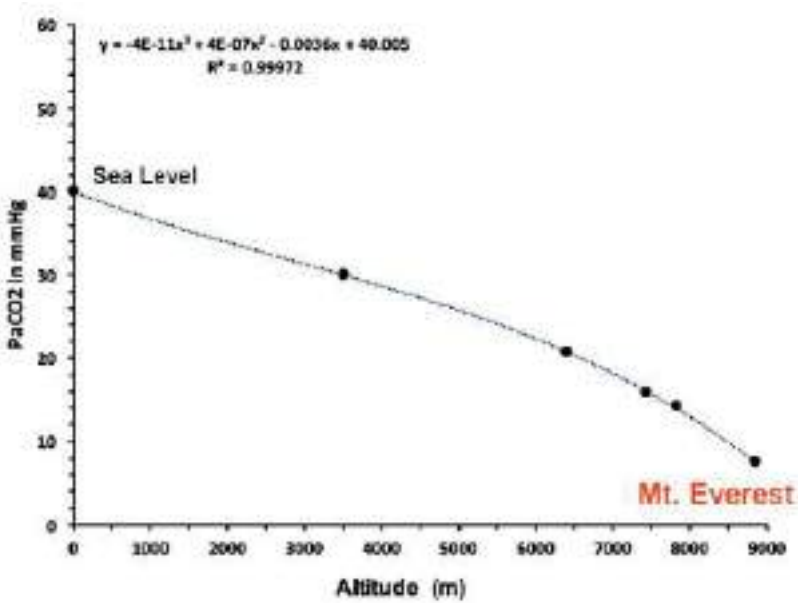

Fig. (8). The decrease of PaCO2 with respect to altitude [48]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

osis with a very high $PaCO_2$ (the denominator) and hence a very low tolerance to hypoxia leading to a fatal outcome. In another case in Arequipa-Peru (2,335m), an 11-year-old girl lost consciousness in the harvesting fields. She was found to be COVID-19 positive. Under oxygen therapy, her blood gases reported: pH = 7.00, $PaCO_2$ = 116.9 mmHg, PaO_2 = 152 mmHg, Ht = 47% and Hb 15.4 g/dl. A severely elevated $PaCO_2$ pattern, and at high altitude signifies a greater risk of low tolerance to hypoxia. She was intubated at the ICU and presented nighttime hypoxemia on FIO_2 = 100%, alternating with daytime improvement and reduction of FIO_2. The last blood gases at the moment of expiration, while being ventilated, reported: pH = 6.96, $PaCO_2$ = 30.5 mmHg, PaO_2 = 235 mmHg, Na = 135mmol/L, K = 5.36mmol/L, Ca = 0.59mmol/L, Cl = 91mmol/L. Lactic Acid=19.7 mmol/L (0.5-1.5), Ht = 12% (Data courtesy of Dr. Diego Grajales López). The diagnosis in the final stages, when she presented 20,000 leukocytes, would be severe metabolic acidosis. The PaO_2 is high, but the oxygen content would be very low associated with a cardio-vascular failure, low hematocrit, and metabolic failure. Both cases have a failure of the oxygen transport triad, as observed.

11. DISCUSSION

Based on 50 years of medical experience treating acute, intermittent and chronic hypoxia illnesses, at the High Altitude Pulmonary and Pathology Institute (IPPA) in La Paz (3,600m), Bolivia, we present a perspective review and analysis on COVID-19 semiology and pathophysiology, as well as SARS-CoV-2 in its general characteristics and transmission mechanisms under different circumstances. All this, to understand its hypoxic impact and the effectiveness of mechanical ventilators. Furthermore, a comparative analysis between COVID-19 and High Altitude Pulmonary Edema (HAPE) is performed through high-altitude oxygen transport physiology, imaging, and previous publications' review. These analyses are used to propose some COVID-19's potential short-term and long-term treatments, from the pathophysiological point of view.

12. COVID-19 AND THE OXYGEN TRANSPORT TRIAD

In COVID-19, the fundamental three components of the oxygen transport triad can all be compromised in a very short time (around 1 week). The dyspnea becomes intense, the tachycardia very important and fundamentally on a low hemoglobin count such as that of sea level, or anemia, hypoxia becomes critical, and life cannot be sustained.

If one of the oxygen transport triad components' funcionality is reduced, the other two can try to compensate. What can be done? The immediate treatment protocol strategy would be to increase the FIO_2, in an attempt to reduce hyperventilation and eventual "gasping", a final survival reflex. Although hyperventilation has been associated with excessive $PaCO_2$ removal, deep breaths associated with it can also give rise to a higher SpO_2 and hemoglobin oxygen capture [45]. The heart, the second component of the triad (the hemo-dynamic pump), can also be compromised [39, 52], and this can additionally aggravate the situation. This compromise, in our criteria, could be the result of a transfer of the virus through the capillaries (viremia) to the left ventricle or coagulation alterations producing inflammation or myocardial infarction. The hemo-dynamic pump could also be stimulated but at a higher energy cost, and possibly with low significance.

13. COVID-19' S DERIVED HYPOXIA POSES A TREATMENT CHALLENGE

Sea-level physicians fear hypoxia, which led to the confusion of when to put patients on ventilators. Monitorization of pulse oximetry early in this disease can indicate the requirement and early administration of oxygen *via* nasal prongs. This could avoid aggravating hypoxia and pulmonary hypertension resulting from HAPE-type focalized edema, further reducing gas exchange.

The management of patients with lung compromise should be focused on improving the oxygen supply while immunity overcomes the coronavirus. The initial procedure is to gradually raise the FIO_2 until an adequate pulse oximetry level is achieved: > 95% SpO_2 at sea level and > 85% at high altitude (3,600m). Nevertheless, when lung compromise is severe, FIO_2 can be increased to 100% and still be insufficient for adequate oxygen transport, and possibly giving rise to oxidative stress. If the SpO_2 drops below 85% at sea level, the condition becomes critical.

A possible coping strategy is to elevate hemoglobin levels until the critical condition is surpassed, depending on the degree of pulmonary tissue destruction (pneumolysis). This, provided there are no coagulation alterations or if coagulation is under control. Conversely, life is possible with only one lung, which is 50% of functional lung tissue, but it requires time for adjustment.

After surviving the disease, fibrosis can ensue as damaged tissue cannot regenerate itself after the aggression. This reduces the functional lung tissue surface. What becomes evident is that those who survive a severe compromise of the lung at the peak of the disease can hardly walk due to shortness of breath. However, once they recover, they gradually improve with decreasing dyspnea on mild exercise. In the long run, maximal exercise capacity could be limited, and a subsequent compensating poly-erythrocyt-hemia is likely to appear. The latter, as a last chance survival strategy. Among post-COVID-19 patients, some may have to live permanently on supplementary oxygen. Excepcionally, few could even be candidates for lung transplants, but this has several limitations aside from high costs.

14. MECHANICAL VENTILATORS' USE

Is a mechanical ventilator useful under these conditions? Ventilators are mostly necessary for central nervous system structural and functional alterations, neuromuscular disorders, obstructive and restrictive diseases (like ARDS), surgery under anesthesia, post-surgery recovery, during coma or unconsciousness, collapsed lung, brain injury, COPD, drug overdose, Guillain-Barre syndrome, myasthenia gravis, pneumonia, polio, stroke, and upper spinal cord injuries and others [53]. SARS-Cov-2 falls into none of these, with minor individual case exceptions. The fundamental role of ventilation is to move air into the lungs, allowing oxygen input and removing carbon dioxide. Risks of ventilator use include infection, vocal cord issues, lung injury from barotrauma, pneumothorax, even hyperoxia toxicity, and maybe thromboembolism. We believe ventilator use is very limited in COVID-19 patients. It has been advised that low PEEP be used in ventilators, only if they are necessary [29]. Many cases that were put on ventilators remained over three weeks, with an adverse prognosis. In New York City, 88.1% of those on ventilators died. Weaning, in those cases, becomes very difficult if not impossible [4]. The low hemoglobin and high $PaCO_2$ are linked to a deficient hemo-dynamic pump, significantly reducing "tolerance to hypoxia" and making life unsustainable. This implies that increasing the hemoglobin in the shortest time possible could allow for a better survival rate. It would simulate a high-altitude biological response to low oxygen pressure provided the pH is maintained within normal values, and $PaCO_2$ is effectively reduced by hyperventilation.

The idea of "respiratory rest" is fundamental for the treatment, if the PaO_2 can be sustained at acceptable limits, provided there is enough alveolar surface area. Unfortunately, severe cases evolve beyond that point.

15. TREATMENT PROPOSAL: IMPROVING BLOOD OXYGENATION THROUGH INCREASED HEMOGLOBIN

The most feasible solution to reduce the critical hypoxia in COVID-19 would be to increase the hemoglobin. This is based on the physiological response to chronic hypoxia (Fig. **6**). At high-altitude, due to a higher hemoglobin count, the arterial oxygen content is higher than at sea level, and oxygen therapy is more effective (Table **1**). Thus, a high hemoglobin count reduces the pneumo-dynamic and hemo-dynamic pumps' hyperactivity. Therefore, as soon as a patient presents the first COVID-19 respiratory symptoms, a wise strategy would be to inject an initial erythropoietin (EPO) dose repeating it, 3 days later, if necessary. The idea would be to increase the hemoglobin and hematocrit (the 3rd triad factor) if the lungs and heart get compromised. This could explain why the case given EPO recovered successfully [54]. The sooner EPO is given (due to the time delay of RBC production, and hematocrit rise), the better. EPO also has been shown to increase tolerance to hypoxia in the brain [55].

Some physicians question the use of erythropoietin, as there is a fear of clothing. This needs to be further investigated. At IPPA, in La Paz, we have previously used EPO in patients with pulmonary fibrosis (unpublished results). We originally proposed this in: https://www.preprints.org/-manuscript/202005.0085/v1. Other recent publications also suggest the use of EPO [56, 57].

Hemograms performed in a hospital in the city of El Alto, Bolivia (4,100m), with 1 million inhabitants, presented > 56% hematocrits in 52% of males and 26% of females. These people carry out normal lives, so there should be no fear of increasing the red blood cells [40].

Blood transfusions could also help in more acute and severe stages. As Hyperimmune plasma therapy excludes red blood cells, with adequate matching tests, whole blood transfusion should be considered. The latter would not only pro-

Table 1. CaO2 = (SpO2 x 1.36 x hemoglobin) + (0.003 x PaO2), which corresponds to (hemoglobin oxygen transport) + (dissolved oxygen in plasma), respectively. At high-altitude, healthy subjects have a higher CaO2 than those at sea level. During oxygen therapy, giving 100% oxygen to breathe at 3,600m, increases CaO2 by 3.28 ml O2/dl. This is more effective than at sea level, where it only increases by 1.98 ml O2/dl. AA = Ambient air. CaO2 = Arterial oxygen content.

	SpO_2	ml O_2 Hb capacity/g	Hb g	Hemoglobin O_2 content	Dissolved O_2 Factor	PaO_2 mmHg	Dissolved O_2 content	CaO_2
Sea Level AA	99	1.36	14	18.84	.003	100	0.3	19.14
Sea Level 100% O_2	99	1.36	14	18.84	.003	760	2.28	21.12
3600m AA	90	1.36	16	19.58	.003	60	0.18	19.76
3600m 100% O_2	99	1.36	16	21.54	.003	495	1.5	23.04

vide SARS-Cov-2 antibodies, as hyperimmune plasma therapy is currently widely used in COVID-19, but it would boost oxygen transport. This would give the immune system time to defend the organism against the SARS-CoV-2.

Furthermore, Chronic Mountain Sickness (CMS), at high-altitude, has been previously described by Dr. Gustavo Zubieta-Castillo as "poly-erythrocythemia" (poly=increase, erythrocyt=red blood cells, hemia= in blood). Unlike previous reports on these patients being hypoxic due to their prolonged stay at high-altitude, poly-erythrocytemic patients can sustain breath-holding far longer than normal hemoglobin count subjects [58] They can even survive with a Mt. Everest summit-like PaO_2=35mmHg. The RBCs increase in poly-erythrocythemia is an adaptation process of the lung, heart, and other underlying diseases to a chronic hypobaric hypoxic environment [40]. Despite increased blood viscosity, circulation time in these patients is not altered [58]. At sea level, post-COVID-19 patients with resulting lung fibrosis could also present poly-erythrocythemia [40]. Blood flow compensating mechanisms such as elevated erythropoietin production (potent vasodilator nitric-oxide stimulator) [59] and perhaps angiogenesis [60], could be responsible for facilitating the transport of increased RBCs count.

Another possibility would be the use of a heart-lung machine to oxygenate blood artificially, but this is truly limited to very few cardiology surgery centers. In fact, in order to treat COVID-19, it would have been much better to equip Intensive Care Units with heart-lung machines than ventilators.

16. OTHER TREATMENT PROPOSALS

Anti-inflammatory drugs could be used early in the treatment to avoid the excessive immune reactions compromising the triad [17], and further pneumolysis. Being tops the Acetyl-Salicylic-Acid as it is an analgesic, anti-inflammatory, anti-pyretic, and anti-platelet aggregation factor, provided there are no allergies or adverse reactions. Early diagnosis is crucial to avoid pneumolysis progression. Adequate oral hydration decreases the risk of intravascular coagulation, and antitussives alleviate upper airway irritation and protect fragile lower airway tissues. Superinfection control with antibiotics is fundamental.

17. COVID-19 ARE MULTIPLE DISEASES. IS THE "SARS-COV-2" TERMINOLOGY CORRECT?

It seems evident that the name SARS-CoV-2 only pertains to one aspect of the multiple diseases and severity induced by this virus. Because of the SARS terminology, intensive care units have rushed the ventilators' use. The term "SARS-Cov-2" should only be used when the hypoxia is severe with a SpO2 < 85%; however it would seem that ventilator requirement is limited. Furthermore, CoV-2 is coronavirus (infecting agent), and COVID-19 is a disease. Consequently, the most appropriate terminology would be to describe: 1) the compromised organ and 2) the severity. Therefore, these viral diseases should be named as follows: SARS-COVID-19 (Severe Acute Respiratory Syndrome) and/or SAHS-COVID-19 (Severe Acute Heart Syndrome), and/or SAAS-COVID-19 (Severe Acute Anemic Syndrome), and/or SACS-COVID-19 (Severe Acute Coagulation Syndrome), and/or SAIS-COVID-19 (Severe Acute Inflammatory Syndrome), and/or a SABS-COVID-19 (Severe Acute Brain Syndrome), and/or SAES-CoV-2 (Severe Acute Endothelial Syndrome) and/or SAKS-COVID-19 (Severe Acute Kidney Syndrome) or other variants. It seems that several aspects could moderate the individual response to CoV--2: genetics, lifestyle, nutrition, stress, exercise or sedentary condition, obesity, dehydration, inflammatory processes, processed food, exposure to toxic substances such as insecticides, chemical fertilizers, medication, chronic diseases, previous exposition to other viral, bacterial, parasitic or fungal diseases, viral pathogenesis.

This is why the outcomes can be so catastrophic as the diseases and their severity are not being clearly differentiated. Following pre-established protocols in all patients is questionable because it is not one disease; it is several. Within the Gauss Distribution, the diseased population falling in the extremes have the worst outcomes. We have previously postulated a similar concept concerning Chronic Mountain Sickness [40]. It is not one disease but rather multiple diseases in a chronic hypoxia environment. The Po-

ly-erythrocyt-hemia is a physiological response of survival when PaO2 is reduced by hypoxia inducing diseases. Hemoglobin function is in the steep part of the oxygen dissociation curve. Not understanding the range of diseases associated with the increase of red blood cells, determines a society where the majority of these patients are treated identically without truly resolving their underlying diseases.

18. A PRAGMATICAL STRATEGY

The fact of the matter is that CoV-2 has overtaken the planet. The main problem with COVID-19 is the irreversible, progressive tissue damage, more than hypoxia itself, if not lethal. What the world desperately needs is a way to destroy the virus. An effective vaccine is being developed and thus far ineffective viral treatments [61]. A temporary best solution is to avoid exposure, and a permanent one is to have an excellent immune system. CoV-2 is like an alien. We need to isolate ourselves from the contaminated air we breathe. To save the economy, we propose that everybody don an Earth astronaut-type suit with its open-circuit electrically filtered breathing units and go back to work. Mass production of very economical suits is possible so that poor people also have access. The good thing is that these could have an outside air exchange mechanism. In contrast, spacesuits have a closed re-breathing complicated and expensive system because of the surrounding vacuum and anoxia in outer space. Upon arrival home, people should take off the suit with safety measures such as with disinfecting showers. This way, it does not matter if somebody is susceptible to catching COVID-19, nor if someone who has it, can transmit it. To revert the pandemic, taking care of the majority of the cases is not enough. Asymptomatic, pre-symptomatic or, under-diagnosed individuals are the most dangerous who spread the disease. The risk will end when the last patient is identified and treated until full recovery. With the use of space like suits, the hospitals would have a higher chance of managing severe cases more gradually until our immune system takes over.

CONCLUSION

COVID-19 is a formidable challenge for human survival. Knowledge of high-altitude hypoxia physiological adjusting mechanisms can help understand these multiple diseases caused by CoV-2 and change therapeutic strategies. HAPE and COVID-19 are not the same, however in COVID, after progressive pneumolysis, a HAPE-type hypoxic edema superimposed on intense local inflammation can rapidly aggravate the condition. The pathogenesis and radiologic imaging have been differentiated. ACE2 location sites and their function are fundamental to understand COVID-19 pathophysiology. The Oxygen Transport Triad can help explain the resulting hypoxemia and these diseases treatment (Fig. **5**). Increasing the 3rd component of the oxygen transport triad through the use of erythropoietin and/or blood transfusions could improve outcomes while immunity develops. Anticoagulants, antibiotics, antitussives, anti-inflammatory medication, oxygen, and hydration are advised. No fixed protocols of treatment for all should be used. A possible strategy to return to work, fundamental to avoid social conflict, would be to use an economical Earth open-circuit respiratory air filtering whole-body suit. The planet, for the moment, no longer belongs to us, humans. It belongs to CoV--2 until our immune system can fully overcome it.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

The authors have no financial / non-financial arrangements or connections that are pertinent to the submitted manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors wish to thank Lucrecia De Urioste and Rafaela Zubieta De Urioste for all their valuable support. We also express our gratitude to our founding father, guide and mentor Prof. Dr. Gustavo Zubieta-Castillo (1926-2015) and all the Zubieta family members: Clotilde, Rosayda, Nancy, Luis, Gustavo Ardaya (physicians, biotechnologist and nutritionist) that worked many years at our Institution (IPPA), collaborating us in many aspects. We also thank Dr. Oscar Murillo for expressing his ideas on the treatment of patients in a Geriatrics environment and Ph.D. Jay Mehrishi, for some suggestions.

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ORIGINAL ARTICLE

Effect of L/N-type Calcium Channel Blocker (Cilnidipine) on Oxidative Stress in Nitric Oxide-deficient Hypertensive Rats

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Abstract:

Background: The sympathetic nervous system plays a major role on the renal function through the vasoactive system and the renin-angiotensin aldosterone system. Even though interest in the renal protective effects of sympathetic blocker has been increased, there are not much data to clarify this efficiency in nitric oxide deficient hypertensive rats. *Aim and Objectives:* To find out the effect of cilnidipine, L/N-type calcium channel blocker on oxidative stress of kidney in Nitric Oxide Synthase (NOS) inhibited experimental hypertensive rats. *Material and Methods:* Male Albino Wistar rats (n-24) were randomly allocated into four groups: Group 1 control received vehicle; Group 2 received Cilnidipine; Group 3 received N^G-nitro-L-Arginine Methyl Ester (L-NAME) hydrochloride; Group 4 received L-NAME and Cilnidipine; All drugs are given orally for 4 weeks. Blood pressure was measured before and after intervention and twice during intervention for all the rats. On 29th day, blood was collected and animals were sacrificed and kidneys were collected. Serum and kidney tissue Malondialdehyde (MDA) levels are estimated. *Results:* The results demonstrate that there is a significant increase in Mean Arterial Pressure (MAP) in L-NAME treated rats compared to control group. Treatment with cilnidipine significantly decreases the MAP in Group 4 rats. We also demonstrated the significant elevated serum and kidney tissue MDA levels in L-NAME treated rats. Treatment with Cilnidipine reduced serum and kidney tissue MDA levels in Group 4 rats as compared to Group 3 rats. *Conclusion:* The results demonstrate that cilnidipine has suppressive effects against progressive renal injury as evidenced by decrease oxidative stress indicator MDA levels in NO deficient hypertensive rats. This effect is explained by the L/N type calcium channel inhibition of Cilnidipine, the L-type calcium channel blocking action lowers blood pressure and N-type calcium channel blocking action leads to suppression of the sympathetic nerve activity and renin-angiotensin aldosterone system.

Keywords: Nitric Oxide Deficient Hypertension, Oxidative Stress, Mean Arterial Pressure, Malon-dialdehyde

Introduction:

Kidney is the major target organ for hypertensive complications, therefore major aims of anti-hypertensive therapy should be to reduce the progression of hypertensive renal damage[1]. In vivo, vasodilators and vasoconstrictors modulate the endothelial function. It is established that Nitric Oxide (NO) produced in vascular endothelial cells has a potent vasodilator effect and plays an important role in vascular resistance and growth. L-arginine analogues such as N^G-nitro-L-Arginine Methyl Ester (L-NAME) hydrochloride administration inhibits nitric oxide synthase activity and hence reduce nitric oxide biosynthesis, leading to hypertension [2]. Accumulation of superoxide anion in biological tissues can occur in the

condition of NO deficiency that can lead to alterations in organ function [3]. NO acts as an endogenous antioxidative agent by reacting with superoxide anion generated in the living tissues, thus it provides a protective function against the action of superoxide anion in many organs including kidney [4].

Cilnidipine, a dihydropyridine L/N type calcium channel blocker [5]. N-type calcium channels are predominantly distributed in the sympathetic nervous system, control neurotransmitter release from the nerve endings of sympathetic neurons [6]. N type calcium channel inhibitory actions of cilnidipine increase the possibility that cilnidipine may have a greater renoprotective effect than L-type calcium channel blockers, because glomerular efferent arterioles do not have L-type calcium channels [7]. Although cilnidipine is expected to suppress the renal injury by suppression of sympathetic nerve activity. Renal protective profile of cilnidipine is not much assessed in animal model of hypertension [8]. Since anti-hypertensive actions of cilnidipine has not much studied in animal model with renal injury, the present study was designed to clarify the renal protective effects of antisympathetic agent, cilnidipine in NO deficient hypertensive rats.

Material and Methods:

Experimental Animals:

Twenty four adult male Albino Wistar rats (*Rattus norvegicus*) weighing 180-250 g, brought from the animal house of BLDE (Deemed to be University). The animals were kept in a environmentally controlled room with a 12-h light/dark cycle and given standard rodent chow and tap water ad libitum. The rats were acclimated to handling. The animals were adapted to the laboratory conditions for a week before the onset of the experiment.

Ethical Considerations:

Institutional Animal Ethics Committee clearance certificate was obtained for the study (Ref: BLDE/BPC/644/2018-2019 dated 15.12.2018). All the experimental procedures were done in accordance with national guidelines (Committee for the Purpose and Control and Supervision of Experiments on Animals, Government of India).

Experimental Groups:

The experimental animals were randomly allocated to four groups as shown in Table 1.

Study Protocol: The experimental protocol followed

Table 1: Experimental Groups of Rats

Groups	No of rats	Intervention
Group 1 (Control)	6/set	Vehicle (0.5% Na CMC) orally for 28 days
Group 2 (Cilnidipine)	6/set	Cilnidipine, 2 mg/kg/day in 0.5% Na CMC orally for 28 days
Group 3 (L-NAME)	6/set	L-NAME, 40 mg/kg/day orally in distilled water for 28 days
Group 4 (L-NAME+ Cilnidipine)	6/set	L-NAME, 40 mg/kg/day orally in distilled water for 28 days, Cilnidipine 2 mg/kg/day in 0.5% Na CMC orally for 28 days

Na CMC- Sodium Carboxy Methyl Cellulose, L-NAME - N^G-nitro-L-Arginine Methyl Ester

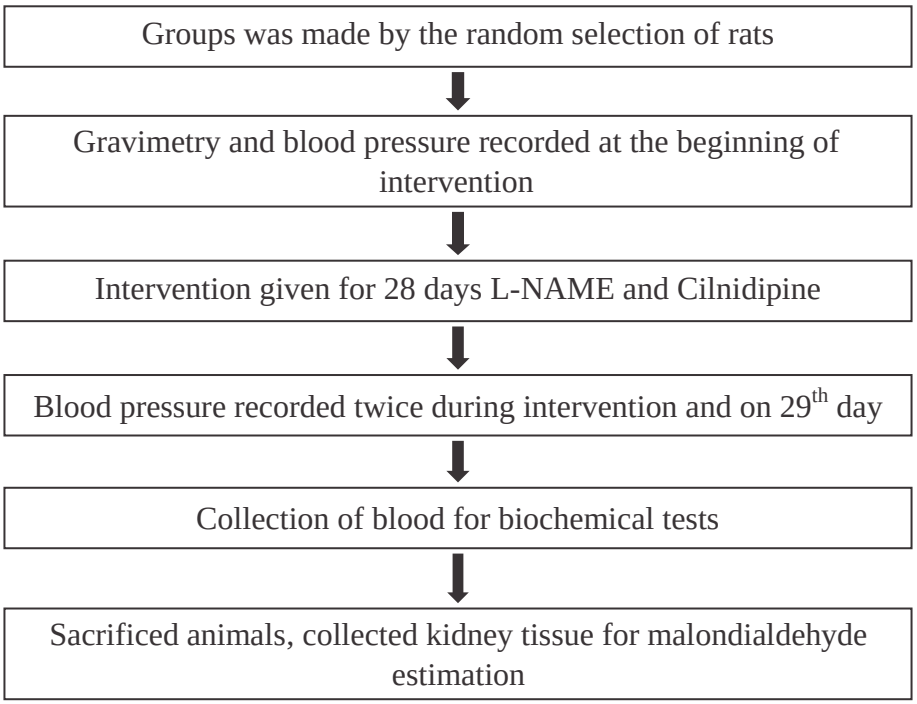

Fig. 1: Experimental Protocol

Body Weight

The body weight of all the rats was recorded on Day 1 and Day 29 using electronic balance (Practum 1102-10IN, Sartorius Lab Instruments, Germany). The weight of all the groups of rats were matched at the beginning of experiment (Table 2).

Table 2: Changes in Body Weight of the Rats

Body weight (g) (n= 6)	Group 1 Control	Group 2 Cilnidipine	Group 3 L-NAME	Group 4 L-NAME + Cilnidipine	*P*
1st day	206.5 ± 4.5	201.5 ± 5.8	209.5 ± 9.00	211.67 ± 4.08	0.191
29th day	275.75 ± 10.11	258.0 ± 0.95[a,c]	237.5 ± 7.5[a,b,d]	263.75 ± 2.62[c]	0.000*
% body weight gain	33.66 ± 2.73	32.97 ± 3.47[a,c]	13.42 ± 2.88[a,b,d]	25.02 ± 1.73[a,c]	0.000*

*L-NAME - N^G-nitro-L-Arginine Methyl Ester. Values are expressed as Mean ± SD. One way ANOVA followed by Post Hoc Tukey's multiple comparison test was done for comparison of multiple groups. Superscript a, b, c, indicate significant difference between groups. 'a' denotes comparison with Group 1, 'b' denotes comparison with Group 2, 'c' denotes comparison with Group 3. *p<0.05.*

Administration of Drug:

1. Procured L-NAME from Pro Lab Marketing PVT, Limited, New Delhi, India. L-NAME was stored in -20^{o}C refrigerator for further use. L-NAME daily dose (40 mg/kg/day) was calculated and given in the morning by oral gavage at once in distilled water to Group 3 and Group 4 rats for 28 days. [2]
2. Procured cilnidipine from Laksh Finechem Pvt. Limited, Gujarat, India. Cilnidipine was stored in the refrigerator (-4^{o}C) until further use. Cilnidipine dose for rats was calculated using the formula: Rats (mg/kg) = Human dose×0.018×5 [9]. The daily dose (2 mg/kg body weight) of cilnidipine was calculated. A suspension of cilnidipine in 0.5% Sodium Carboxy Methyl Cellulose (0.5% Na CMC) was prepared freshly every day and was administered by oral gavage once in the morning to Group 2 and Group 4 rats for 28 days.

L-NAME Induced Hypertensive Rat Model

Hypertension was induced by oral administration of L-NAME (40 mg/kg/day) in distilled water for 4 successive weeks [2].

Blood Pressure Recording

Blood Pressure (systolic and diastolic) of conscious rats was measured at the start and end of the experiment and twice during intervention. Animals were kept in the restainer for 10–20 min/day for 5 days prior to recording BP in the tail-cuff technique, and tail of the animals were warmed for 30 min for better detection of tail artery pulsations. BP was recorded using noninvasive tail cuff sensor (NIBP) Bio Pac Instrument (Bio Pac MP 100: PC windows based animal electro-physiology system) and all the parameters will be analysed by Bio Pac Student Lab 4.1 software. Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) was measured. All the measurements were made thrice and the mean of three measurements was considered for each rat. Mean Arterial Pressure (MAP) was calculated by using formula MAP = Diastolic Blood Pressure+ 1/3 Pulse Pressure [2].

Assessment of Oxidative Stress

Malondialdehyde (MDA) is a product of lipid peroxidation. Concentration of MDA are used frequently as a marker for oxidative stress. MDA concentration was estimated in the serum and kidney tissue homogenate by the method of Buege and Aust (1978) [10].10% of tissue homogenate was prepared in 0.1M phosphate buffer using tissue homogenizer (Remimotors, Bombay, India) and supernatant was used for the assay. MDA reacts with thiobarbituric acid to give a pink colour and absorbance was read at 535 nm using spectro-photometer (Schimadzu UV 800, Schimadzu Corporation, Japan).

Statistical Analysis:

Statistical analysis was done using SPSS16.0 (SPSS Inc., Chicago, USA). The values were presented as Mean ± SD. Statistical significance of multiple groups was analysed using One-way Analysis of Variance (ANOVA) followed by Post hoc Tukey's multiple comparison test. P-value < 0.05 was considered as statistically significant.

Results:

Effect of Cilnidipine on Systolic Blood Pressure and Diastolic Blood Pressure:

Hypertensive rat model by NOS3 inhibitor L-NAME was successfully developed in our laboratory (Table 3). Significant increase in SBP

in L-NAME treated rats from 9th day when compared to control while significant decrease in SBP in cilnidipine treated rats when compared to L-NAME treated was observed. Significant increase in DBP from 9th day in L-NAME treated group. There is a decrease in DBP in cilnidipine treated group on 9th and 18th day although not significant. We observed significant decrease in DBP on 29th day (Table 3).

Effect of Cilnidipine on Mean Arterial Pressure (MAP)

There is no significant difference in baseline mean arterial pressure among groups. No significant differences was observed in MAP in the control group over the 4 week experimental period. Administration of L-NAME (40 mg/kg/day) induced a progressive increase in mean arterial pressure. We found significant increase in MAP with L-NAME treated groups when compared to control group from 9th day onwards. We observed decrease in the MAP in cilnidipine treated rats on 9th day and 18th day when compared to L-NAME treated group although not significant but we found significant decrease in MAP on 29th day (Fig. 2).

Oxidative Stress

We observed significant increase in MDA levels in serum and kidney tissue of L-NAME treated rats when compared to control group. We also observed significant decrease in MDA levels in serum of cilnidipine group. MDA levels in kidney tissue of cilnidipine treated rats also decreased though not significant (Table 4).

Table 3: Effect of Cilnidipine on Systolic And Diastolic Blood Pressure

Groups	Measurement	1st Day	9th Day	18th Day	29th Day
Group 1 Control	SBP (mmHg)	107.32±3.19	112.685±8.19	99.78±8.05	111.01±9.5
	DBP (mmHg)	72.4±10.2	72.63±12.28	74.24±14.66	84.64±6.24
Group 2 Cilnidipine	SBP (mmHg)	104.20±6.43	111.21±4.09^{c}	111.71±6.43^{c}	114.37±4.87^{c}
	DBP (mmHg)	78.7±3.85	83.00±7.65	84.70±4.79^{c}	89.83±4.02^{c}
Group 3 L-NAME	SBP (mmHg)	103.33±3.32	124.11±3.96^{a,b,d}	142.73±13.42^{a,b}	152.24±8.38^{a,b}
	DBP (mmHg)	71.93±4.7	89.36±7.75^{a}	106.32±7.64^{a,b}	122.60±3.27^{a,b}
Group 4 L-NAME + Cilnidipine	SBP (mmHg)	110.29±4.14	113.46±6.71^{c}	128.25±4.23^{a,b,c}	130.24±4.03^{a,b,c}
	DBP (mmHg)	79.39±6.13	88.34±4.96^{a}	95.22±3.32^{a}	98.12±3.42^{a,b,c}
P	SBP (mmHg)	0.054	0.005*	0.000*	0.000*
	DBP (mmHg)	0.130	0.011*	0.000*	0.000*

*L-NAME - N^{G}-nitro-L-Arginine Methyl Ester. Values are expressed as Mean ± SD. One Way ANOVA followed by Post Hoc Tukey's multiple comparison test was done for multiple groups Superscript a, b, c, indicate significant difference between groups. 'a' denotes comparison with Group 1, 'b' denotes comparison with Group 2, 'c' denotes comparison with Group 3. *p<0.05. SBP-Systolic blood pressure, DBP-Diastolic blood pressure*

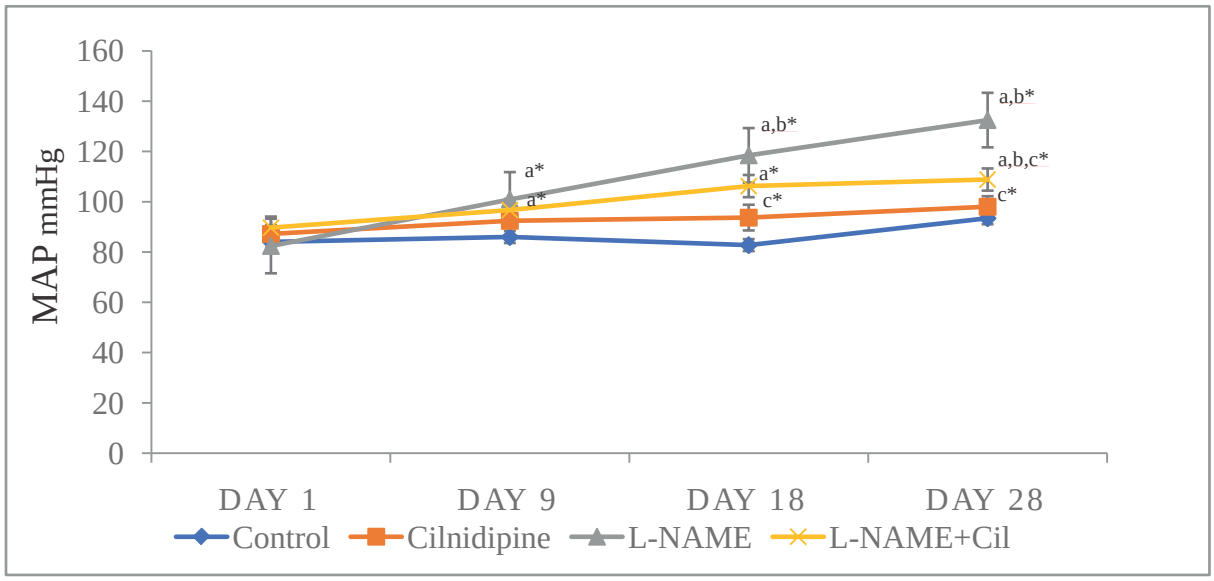

Fig. 2: Effects of Cilnidipine on Mean Arterial Blood Pressure

*Values are expressed as Mean ± SD. Oneway ANOVA followed by Post Hoc Tukey's multiple comparison test was done for multiple groups. Superscript a, b, c, indicate significant difference between groups. 'a' denotes comparison with group 1, 'b' denotes comparison with group 2, 'c' denotes comparison with group 3. *p<0.05. L-NAME - N^G-nitro-L-Arginine Methyl Ester, Cil- Cilnidipine, MAP-mean arterial pressure*

Table 4: Malondialdehyde Levels in Serum and Kidney Tissue Homogenate among Groups

Parameters	Control	Cilnidipine	L-NAME	L-NAME + Cilnidipine	*P*
MDA in serum µmoles/L	1.21 ± 0.43	1.23 ± 0.14^{c}	$1.755 \pm 0.08^{a,b}$	0.819 ± 0.11^{c}	0.001*
MDA in kidney tissue µmoles/gm	24.67 ± 0.55	24.53 ± 0.9^{c}	31.25 ± 0.54^{a}	29.95 ± 1.05	0.000*

*Values are expressed as Mean ± SD. One way ANOVA followed by Post Hoc Tukey's multiple comparison test was done for comparison in multiple groups. Superscript a, b, c, indicate significant difference between groups. 'a' denotes comparison with group 1, 'b' denotes comparison with Group 2, 'c' denotes comparison with Group 3. *p<0.05. MDA- Malondialdehyde, L-NAME - N^G-nitro-L-Arginine Methyl Ester.*

Discussion:

Cilnidipine has renoprotective effect in L-NAME-induced hypertensive rats. Chronic blockade of NO synthesis by L-NAME is a well-known model of hypertension. Although this model cannot be extrapolated to human hypertension, it provides the possibility of reducing the causes of increased blood pressure to a single factor, that is decrease in NO bio availability. Sufficient NO is needed for normal blood pressure. Thus, a failure to generate NO or an enhanced NO consumption can lead to hypertension. Deficiency of NO in the kidney might have caused vasoconstriction of the renal

artery and stimulated renin and angiotensin II production. This activation of renin angiotensin system may lead to vasoconstriction and hypertension [2]. Another mechanism of endothelial dysfunction might be NO synthase inhibition by L-NAME may have exaggerated the effect of Reactive Oxygen Species (ROS) generated by vascular NADPH oxidase [11]. Treatment with cilnidipine (Group 4) there was significant decrease in MAP observed compared to L-NAME treated rats.

In the kidney, renal sympathetic nerve activity contributes to the regulation of renal blood flow, glomerular filtration rate, electrolyte transport, and hormonal release. Sympathetic imbalance may lead to hypertension and progressive renal disease. Cilnidipine is a dihydropyridine calcium channel blocker, and it has been demonstrated to inhibit both N-type and L-type (long acting) calcium channels in various types of neurons. In one study on dogs, the increase in heart rate and plasma Norepinephrine (NE) level induced by bilateral carotid artery occlusion. This effect was blocked by cilnidipine through an inhibitory effect on sympathetic nerve overactivity. Cilnidipine has been shown to reduce NE secretion in response to renal nerve stimulation in dogs. This result was not observed by selective L-type calcium channel blocker nifedipine. Because of the N-type calcium channel blocking action of cilnidipine, there are some possibilities to suppress progressive renal injury [8].

MDA is a product of lipid peroxidation and has been used as a biomarker of oxidative stress [10]. Serum MDA levels in L-NAME treated hypertensive rats were increased when compared to control rats indicating high oxidative stress in L-NAME treated rats. It was found significant decrease in MDA level of serum of cilnidipine treated rats. We also found decrease in MDA level of kidney tissue of cilnidipine treated rats though not significant. Supporting this notion, previous studies also demonstrated that NOS inhibition enhances vascular super oxide release in rats [12] mice [13] and humans [14].

Along with L/N type calcium channel blocker, cilnidipine acts as a strong antioxidant. Cilnidipine demonstrates strongest lipophilicity and has highest antioxidant actions compare to other dihydropyridine derivatives [15]. The L/N type inhibitory actions of Cilnidipine would have a greater renoprotective effect than L-type calcium channel blockers, as there is absence of L-type calcium channels on glomerular efferent arterioles [16]. Oxidative stress can accompany hypertension in many animal studies, including Spontaneously Hypertensive Rats (SHR), angiotensin II-infused rats, renovascular hypertension and Deoxy-corticosterone Acetate (DOCA) salt hypertension.

Conclusion:

Result of our study demonstrate that the enhanced oxidative stress because of chronic NO synthase inhibition contributes to the impairment of renal function thus plays a role in the pathogenesis of NO-deficient form of hypertension. The L/N type calcium channel inhibitory actions of cilnidipine raise the possibility that cilnidipine would have a higher renoprotective effect by its strong anti-oxidant property compare to L-type calcium channel blockers.

Acknowledgements:

We acknowledge BLDE (Deemed to be University) for providing a research grant for the study (Ref No: BLDE(DU)/REG/R&D/RGC/ 2019-20/937).

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How to cite this article:

Shaikh GB, Hippargi S, Majid DSA, Biradar MS, Das KK. Effect of L/N-type Calcium Channel Blocker (Cilnidipine) on Oxidative Stress in Nitric Oxide-deficient Hypertensive Rats. *J Krishna Inst Med Sci Univ* 2020; 9(2): 73-80.

Submitted: *05-Feb-2020* ***Accepted:*** *27-Feb-2020* ***Published:*** *01-Apr-2020*

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: The WHO ACTION Trials Collaborators. Antenatal dexamethasone for early preterm birth in low-resource countries. N Engl J Med. DOI: 10.1056/NEJMoa2022398

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Statistical methods

Sample size

We estimated the sample size on the basis of the primary outcome neonatal mortality at 28 completed days with a two-sided 5% significance level test and a power of 90%. A total of about 5,416 women are needed to detect a reduction of 15.0% or more from a 25.0% deaths to 21.3%, among neonates of women who were administered ACS at <34 weeks. With 10% loss to follow-up, we estimated that about 6,018 women had to be recruited.

For the composite possible maternal bacterial infection outcome, a non-inferiority hypothesis was used. A total sample size of 5,024 women are needed (including 10% loss to follow up) to demonstrate non-inferiority within that margin of 2.5% for the increase in the maternal infection outcome, assuming equal prevalence of 10% in the two arms, with a power of 80% and a significance level of 2.5%.

Statistical analysis

For primary outcomes, intention-to-treat (ITT) analyses were to be performed. Analyses were first performed on all available data and sensitivity analyses were then performed using multiple imputation to judge the effect of missing data. Analyses of primary outcomes were corrected for multiplicity using the False-Discovery-Rate approach. For the primary outcomes, fetal or neonatal mortality and maternal severe infection outcomes pertain to the enrolled population, whereas neonatal mortality pertains to liveborn neonates only.

We also conducted a secondary “per-protocol” analysis for the maternal primary outcome, as recommended for non-inferiority analyses, excluding women with protocol violations that might affect the primary outcome.

Baseline characteristics were compared between groups to detect imbalances in prognostic variables that could bias the results. Most study outcomes are binary variables. For this type of variables, the number of participants, number of missing values and percentages by group were reported. The intervention arm was compared against the control arm for the three primary outcomes using risk ratios with 95% confidence intervals. The statistical technique used to conduct tests and obtain confidence intervals was a logistic model with a binomial distribution and the log link to obtain relative risks. The stratifying variable study site, a design variable, was included in the model, as well as a clustering feature for multiple births for neonatal outcomes. Separate models were fitted for each of the primary and secondary outcomes.

For continuous variables, the number of participants, the number of missing values, means and standard deviations or medians, quartiles and interquartile range (IQR) by group were reported. The intervention arm was compared against the control arm using mean or median differences and 95% confidence intervals. The statistical technique used to conduct tests and obtain confidence intervals for this type of variables was a general linear model including study site in the model as stratifying variable, as well as a clustering feature for multiple births for neonatal outcomes.

All models were fitted using SAS Software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Prespecified subgroup analyses

We conducted the following prespecified subgroup analyses of the primary outcomes neonatal death, stillbirth or neonatal death, and possible maternal bacterial infection:

1. Planned preterm birth: yes vs. no

2. Gestational age at first dose: 26 to <28 weeks vs. 28 to <32 weeks vs. 32 to <34 weeks

3. Number of fetus: single vs. multiple

4. Study site: Bangladesh vs. India vs. Kenya vs. Nigeria (Ibadan) vs. Nigeria (Ile-Ife) vs. Pakistan

5. Time from first dose to birth: 0 to 6h vs. >6 to 12h vs. >12 to 24h vs. >24h to 1 week vs. over 1 week

6. Mode of birth: vaginal birth vs. cesarean section

7. Any use of tocolytics: yes vs. no

We further analysed the effect of time of first dose to birth on treatment effect using a logistic model, including gestational age (GA) at first dose and number of doses in the model.

Assessing confounders and effect modifiers of neonatal primary outcomes

The effect of treatment, gestational age, time from first dose to birth and number of doses on the probability of stillbirth or neonatal death and neonatal death was assessed using a logistic model.

Variables:

- Response: stillbirth or neonatal death, or neonatal death
- Treatment (randomized): dexamethasone and placebo
- Site: (randomization was done within sites)

- Covariates: gestational age (weeks), time from first dose to birth (hours), number of doses.

Model

$$y = \log\left(\frac{p}{1-p}\right) = \mu + treat + site + exposure\,(treat) + exposure^2(treat) + ndoses(treat) + ga(treat)$$

where

A(B) means A within B,

p=proportion of events for binary neonatal outcome,

$$p = \frac{1}{1+e^{-y}}$$

y=logit for binary neonatal outcome (stillbirth or neonatal death, or neonatal death)

treat=treatment

exposure= time from first dose to birth (hours)

ndoses=number of doses

ga=gestational age at first injection (weeks)

site=study site

Gestational age at first injection was used instead of gestational age at birth because the latter is confounded with time from first dose to birth. The time interval between trial entry and birth is thus split in two non-overlapping time intervals (gestational age at first injection and time from first dose to birth).

Models were considered including terms for interactions, and the final model was selected excluding interaction terms that were not significant at 5%. Significance is assessed by p-values, in raw format and also expressed as logWorth, a logarithmic transformation of the P-value:

$$logWorth = -\log_{10}(p) = \log_{10}(1/p)$$

Goodness of fit of the model was assessed by the difference between the log-likelihood of the saturated model and that of the fitted model.

The effect of treatment was calculated in terms of relative risk (RR) from the model and plotted against time from first dose to birth by categories of gestational age at first injection.

Results

Neonatal death

The following table shows, for **the neonatal death outcome**, the significance for the different terms in the model described above. The most important effect by far is gestational age at first injection. Time from first dose to birth, study site, number of doses and treatment are significant at 1% level. The effects of gestational age, time from first dose to birth and number of doses are significantly different for each treatment.

Source	LogWorth	p-value
ga(treat)	97.784	0.00000
exposure(treat)	7.795	0.00000
site	4.187	0.00006
ndoses(treat)	3.042	0.00091
treat	1.873	0.01339
exposure*exposure(treat)	0.816	0.15267

The following table shows statistics of goodness of fit. The P-value for goodness of fit is 1, suggesting that the model fits the data well.

Source	**DF**	**-LogLikelihood**	**p-value**
Saturated model	2803	5.5452	
Fitted model	14	1141.4016	
Lack of fit	2789	1135.8564	1.0000

Stillbirth or neonatal death

The following table shows, for **the stillbirth or neonatal death outcome**, the significance for the different terms in the model described above. The effects are very similar to those described for the neonatal death outcome.

Source	LogWorth	p-value
ga(treat)	116.999	0.00000
exposure(treat)	8.226	0.00000
site	3.438	0.00036
ndoses(treat)	2.632	0.00233
treat	1.073	0.08459
exposure*exposure(treat)	0.326	0.47224

The following table shows statistics of goodness of fit. The P-value for goodness of fit is 0.9785, suggesting that the model fits the data well.

Source	DF	-LogLikelihood	p-value
Saturated model	3028	6.9315	
Fitted model	14	1436.3927	
Lack of fit	3014	1429.4613	0.9785

Data safety monitoring

A Data Safety Monitoring Board (DSMB) was appointed to monitor accruing trial data, in strict confidence, and three interim analysis were planned. The DSMB terms of reference were that they should inform the steering group chair if, in their view, there was proof beyond doubt that treatment with dexamethasone is indicated or contraindicated based on statistical considerations, practical issues, clinical considerations or new external information. The DSMB considered the Haybittle-Peto stopping rule on the primary infant mortality outcomes, as the statistical guidance for their recommendation. Using this rule, a two-sided test of hypothesis to assess superiority of one of the groups (intervention or placebo) was conducted. If the result was significant at α=0.001, the DSMB would consider recommending stopping the trial for superiority of one of the groups.

Two interim analyses were conducted by both the trial statistician (blinded) and the DSMB statistician (unblinded on request) and results were presented at DSMB meetings. The DSMB could be unblinded to the study groups if and when needed. The first interim analysis was conducted when 874 women and 972 infants (including 894 liveborn neonates) had been recruited and their complete data entered in the database. At their meeting on 19-20 November 2019, after review of 2304 women and 2536 infants (including 2337 liveborn neonates) with complete follow-up of primary outcomes, the DSMB decided to unblind the trial and recommended the trial to be stopped for mortality benefits, supported by evidence of a graded dose-response effect. Recruitment was stopped across all sites on 21 November 2019 and all ethics committees and regulatory authorities were informed of the decision to stop.

DSMB rationale for stopping the trial

The DSMB decided to recommend that the trial be stopped because they decided after a lengthy debate that the evidence of benefit was so strong that they judged it unethical to continue.

They recognized that this was a deviation from the stopping rule. However, it was in line with Section 3.4.4 of the trial protocol, which specified that the DSMB decision to stop the trial following an interim analysis was to be guided not only by statistical considerations, but also by practical issues (adverse events, ease of treatments administration, unanticipated costs), as well as clinical considerations or new external information. Likewise, in Section 8.2 of the DSMB charter for the trial (**The role of formal statistical methods, specifically which methods will be used and whether they will be used as guidelines or rules**), it is stated that: "The statistical stopping rules should not be taken as the only criterion for a recommendation to stop the trial. Safety results from the trial as well as external information should be considered. A recommendation to discontinue recruitment, in all patients or in selected subgroups, will be made only if the result is likely to convince a broad range of clinicians, including those supporting the trial/s and the general clinical community."

The decision to stop the trial was driven by:

1) New external information from sheep studies became available during the conduct of the trial about strong effect of duration of fetal exposure to glucocorticoids on fetal lung maturation[1,2]
These studies concluded that the duration of materno-fetal glucocorticoid exposure, not total dose or peak drug exposure, is a key determinant for a sustained fetal lung maturation and antenatal glucocorticoid efficacy. Evidence of fetal lung maturation was observed with at least 24 hours of glucocorticoid exposure, with exposure of 48 hours providing more sustained effect.

On account of this external information, the DSMB decided to carry out a planned pre-specified sensitivity analysis excluding women giving birth less than 24 hours during their second interim analysis and to include the findings in their decision making. This decision was made blinded to treatment allocation.

On completion of the second interim analysis of 2304 women and 2536 infants using the database closed in November 2019, the DSMB noted a clear evidence of reduction in both neonatal mortality and in stillbirth or neonatal death, the two primary outcomes, in the dexamethasone intervention arm compared to the control (placebo) arm. At that time, the overall result was a relative reduction of 18% (95% CI: 5% to 29%; p=0.008) in neonatal death with dexamethasone, and a relative reduction of 13% (95% CI: 2% to 23%; p=0.02) in stillbirth or neonatal death in the dexamethasone arm, compared to placebo. However, the results of the planned pre-specified sensitivity analysis on account of external new information described above, excluding women who delivered within 24

hours of the first injection of trial medication (whose babies would not be expected to benefit because of a short exposure to glucocorticoid) showed a 30% reduction in neonatal death (P=0.0017) and a 24% reduction in stillbirth or neonatal death (P=0.0015). This analysis further showed that dexamethasone effects strengthened for both neonatal primary outcomes as women with varying degrees of shorter intervals between first injection and birth were excluded, reaching the z=3 level after those who could only have received one dose (i.e. up to 12 hours) are removed. These findings were indicative of graded dose-response relationship and efficacy of dexamethasone.

2) Considering the evidence from the trial in the context of the existing evidence of the benefits of antenatal glucocortioids (from the Cochrane review meta-analysis), well beyond the stopping boundary

While acknowledging the fact that the P-values from these analyses were very close to but did not strictly attain the 0.001 specified by the Haybittle Peto rule, the DSMB noted that these findings were consistent with the results of the Cochrane review (involving 7774 women and 8158 infants) that largely included studies from high-income countries, which showed overall reduction of 31% in neonatal death, and concluded that it would be unethical to further expose more women (and babies) to placebo given the existing body of knowledge from high-income setting. The DSMB was not only sensitive to these individual ethics but also considered the findings of these analyses convincing to influence policy and clinical practice (collective ethics), according to the DSMB charter.

Based on these considerations, the DSMB recommended that all recruitment be stopped, and this recommendation was unanimously accepted by the Technical Advisory Group, ACTION Trial Investigators, and WHO. The funder had no role in the deliberations and in the decision to stop the trial.

Primary and secondary outcome definitions

PRIMARY OUTCOMES	OPERATIONAL DEFINITION AND MEASUREMENT
1. Neonatal death	Death of a live birth within 28 completed days of life.
2. Stillbirth or neonatal death	Any death of a fetus (post randomization) or death of a live birth within 28 completed days of life.
3. Possible maternal bacterial infection	Occurrence of maternal fever or clinically suspected or confirmed infection, for which therapeutic antibiotics were used. *Suspected or confirmed infection could be an obstetric infection (chorioamnionitis, postpartum endometritis, or wound infection) or non-obstetric infection, as defined below. Captured during hospital admission/s only*
SECONDARY OUTCOMES	
A. For the neonate	
A1. Mortality outcomes	
1. Stillbirth	Any death of a fetus (post randomization).
2. Early neonatal death	Death of a live birth within 7 completed days of life.
A2. Morbidity outcomes	
3. Severe respiratory distress*‡	Clinical features are the presence of fast breathing (respiratory rate ≥ 70 breaths per minute) AND at least one of the following clinical signs: 1. Marked nasal flaring during inspiration, 2. Expiratory grunting audible with naked ear 3. Severe chest in drawing. AND SpO2 less than 90%, or use of supplemental oxygen.
4. Neonatal sepsis*	Defined as the presence of at least two (or more) of the following signs: • Stopped feeding well • Severe chest in-drawing • Fever (body temperature of 38 °C or greater) • Hypothermia (body temperature less than 35.5 °C) • Movement only when stimulated or no movement at all • Convulsions
5. Severe Intraventricular haemorrhage (sIVH)	Defined as a Papile's intraventricular hemorrhage classification grade 3 or 4, as per transcranial ultrasound assessment. Liveborn neonates <34 weeks at birth will be routinely screened with transcranial ultrasound. Liveborn neonates ≥ 34 weeks at birth will receive transcranial ultrasound if indicated. Transcranial ultrasound assessment will be performed at day 7 postnatal or discharge (if discharge occurs before 7 days after birth).
6. Neonatal hypoglycaemia*§	Neonatal hypoglycemia is defined as blood glucose measure less than 45 mg/dl (2.6mmol/l). All liveborn newborns in hospital will have glucose levels recorded at 6 and 36 hours (before feeding or IV fluids). Any documented hypoglycaemia will also be recorded.

7. Apgar score at 5 minutes	Assessment of neonatal vitality at 5 minutes after birth. Reported as Apgar score, and proportion of babies with Apgar <7.
B. For the Woman	
B1. Mortality outcomes	
8. Maternal death	Any maternal death in a trial participant, from time of randomization to 28 completed days postpartum.
B2. Morbidity outcomes	
9. Maternal fever	Maternal fever ≥38.0 C since randomization (on any one occasion, during hospital admission/s only).
10. Chorioamnionitis	Chorioamnionitis (suspected or confirmed) based on clinical assessment by obstetric care physician. Clinical or laboratory features may include: • Maternal fever ≥38.0 C • Maternal and/or fetal tachycardia • Purulent or foul smelling vaginal discharge • Uterine tenderness • Maternal leukocytosis • Bacterial culture indicative of infection measured during hospital admission only (from randomization until birth)
11. Postpartum endometritis	Postpartum endometritis (suspected or confirmed) based on clinical assessment by obstetric care physician. Clinical or laboratory features may include: • Maternal fever ≥38.0 C • Maternal and/or fetal tachycardia • Purulent or foul smelling vaginal discharge • Uterine tenderness • Maternal leukocytosis • Bacterial culture indicative of infection measured during hospital admission/s only
12. Wound infection	Infection of a wound or incision site (including perineal tear, episiotomy incision or cesarean section abdominal incision), suspected or confirmed by obstetric care physician Measured during hospital admission/s only
13. Non-obstetric infection	Acute non-obstetric infection (suspected or confirmed) based on clinical assessment by obstetric care physician. This includes: • respiratory tract infection (including pneumonia, pharyngitis, sinusitis or similar) • Urinary tract infection (excluding pyelonephritis) • Pyelonephritis • Acute cholecystitis • Other systemic infection *Malaria is specifically excluded from this outcome* Measured during hospital admission/s only

C. Process of care outcomes	
C1. Measures of care given to neonate	
14. Major neonatal resuscitation at birth	The use of positive pressure ventilation for more than one minute
15. Timing of breast milk feeding initiation*	Timing of initiation of breast milk feeding in hours after birth (breastfeeding, cup or tube feeding).
16. Time to full enteral feeding*	Timing to full enteral feeding (in days)
17. Use of oxygen therapy*	Defined as any use of oxygen therapy, using any method
18. Length of oxygen therapy*	This is defined as the total number of days of oxygen use during hospital stay. The total number of days will be counted, even if use was intermittent.
19. Use of continuous positive airway pressure (CPAP) ventilation*	Defined as any use of CPAP during admission to neonatal special care unit/ward
20. Length of use of continuous positive airway pressure (CPAP) ventilation*	Total number of days used will be counted, even if use is interrupted for hours or days.
21. Use of mechanical ventilation (MV)*	Any use of MV during admission
22. Length of use of mechanical ventilation (MV)*	Total number of days used will be counted, even if use is interrupted or intermittent
23. Any use of parenteral therapeutic antibiotic therapy for 5 or more days *	Any use of therapeutic antibiotics (intravenous or intramuscular) for 5 or more days, even if interrupted, excluding neonates who died before 5 completed days
24. Length of use of parenteral therapeutic antibiotic therapy*	Total number of days of use of parenteral antibiotic therapy
25. Use of surfactant treatment*	Any use of surfactant
26. Number of doses of surfactant treatment*	Total number of doses of surfactant treatment
C2. Health service utilization (newborn)	
27. Length of hospital stay after birth	Length of stay in hospital after birth in complete days (initial postnatal hospitalization only)
28. Admission to a special care unit (SCU)	Admission to special neonatal care unit or neonatal intensive care unit after birth (initial postnatal hospitalization only)
29. Length of admission to	Length of admission to special neonatal care unit or neonatal intensive care unit in days

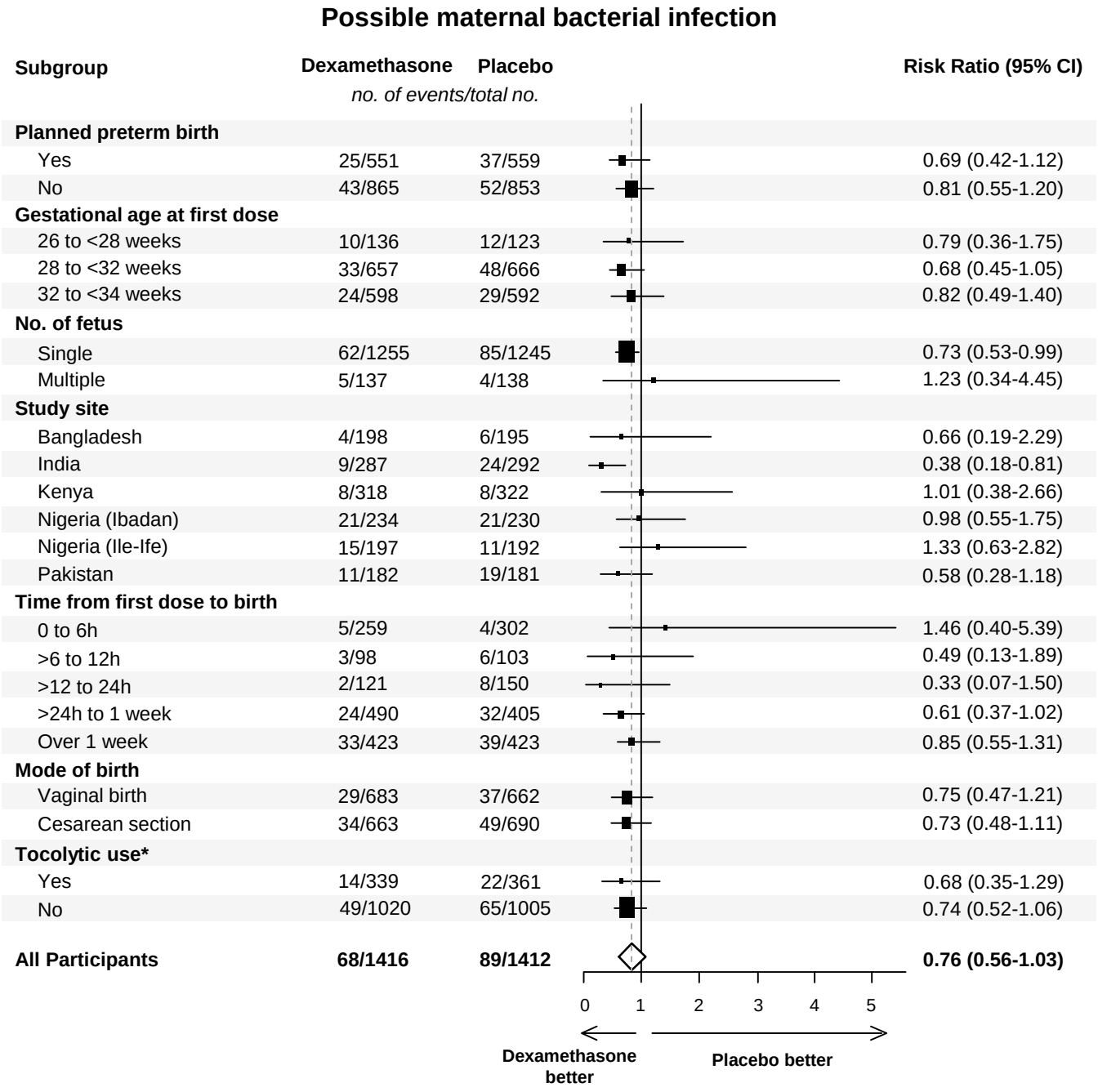
special care unit (days)	
30. Newborn readmission for health care at facility	Any readmission to a health care at facility, for any reason.
31. Length of stay for newborn readmission	Length of readmission stay in facility in days
32. Number of newborn readmission for health care at facility	Number of readmissions for health care at facility, for any reason.
33. Cause of neonatal readmission for health care at facility	All causes of neonatal readmission to health care at facilities will be recorded as per clinical diagnosis
C3. Measures of care given to woman	
34. Therapeutic antibiotics	Therapeutic antibiotics for suspected or confirmed infection (obstetric or non-obstetric). *Use of antibiotics for prophylaxis is not included in this outcome.* Measured during hospital admission/s only
35. Number of days of therapeutic antibiotic use	Number of days of use of therapeutic antibiotics for suspected or confirmed infection (obstetric or non-obstetric). Use of antibiotics for prophylaxis is not included in this outcome. Measured during hospital admission/s only
36. Any antibiotic use	Any use of antibiotics in a randomized participant (maternal) while in facility (prophylactic or therapeutic) Measured during hospital admission/s only
C3. Health service utilization (woman)	
37. Length of total maternal hospitalization for birth (days)	number of days which women are hospitalized for birth (i.e. the admission in which birth occurs). Measured from day of admission to day of official discharge from facility, in days
38. Any postpartum maternal readmission to facility	Any postpartum readmission of the woman to hospital for any reason up to 28 completed days postpartum
39. Length of stay for postpartum maternal readmission	Length of readmission stay in facility in days
40. Number of maternal readmissions to facility	Number of postpartum readmissions of the woman to hospital for any reason up to 28 completed days postpartum
41. Cause of maternal readmission to facility	All causes of maternal readmission to hospital will be recorded as per clinical diagnosis

42. Any referral of woman to another facility for treatment of complications	Any referral of woman to another hospital for treatment of complications
Measures of compliance	
43. Compliance with study allocation	Defined as the proportion of women who complete the entire course, as per the allocation
44. Use of repeat course	Total number and proportion of women who received a repeat course of dexamethasone or placebo
45. Total number of treatment doses received	Total number of treatment (dexamethasone or placebo) doses received (initial and repeat)
46. Time from initiation of first dose until birth	Defined as the time from initiation of first dose (dexamethasone or placebo) to birth, measured in hours

** Measured during initial postnatal hospitalization only, until death, discharge or completed day 7 (whichever comes first); ǂ overall, and at 24 hours; § overall, and at 6 and 36 hours*

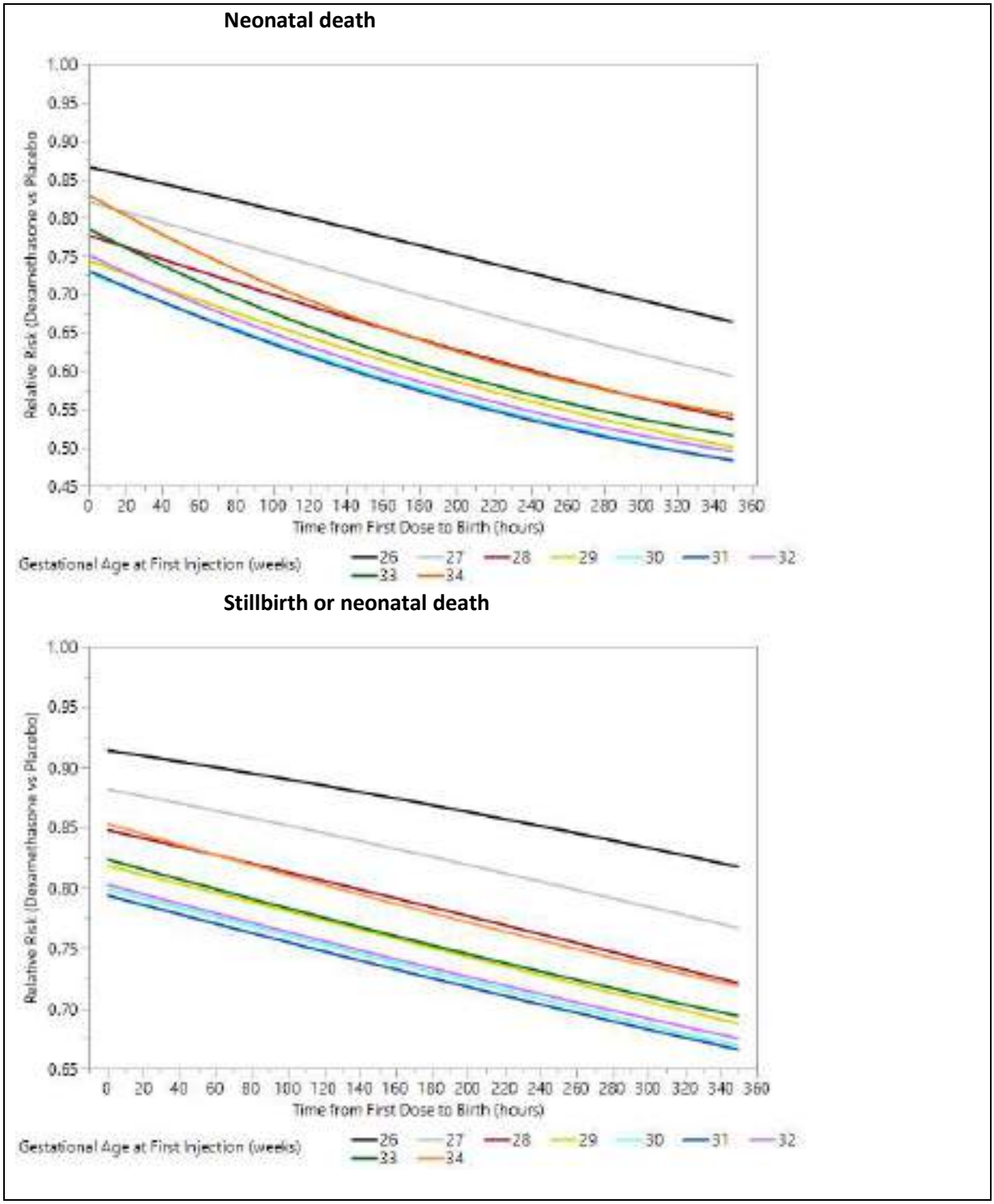
Supplementary figures and tables

Figure S1: Prespecified subgroup analyses of possible maternal bacterial infection



*Maternal use of tocolytic agent before birth. Shown are the results of the analysis of possible maternal bacterial infection (maternal primary outcome) in prespecified subgroups. The widths of the confidence intervals were not adjusted for multiplicity, so the intervals should not be used to infer definitive treatment effects. The size of each black square is proportional to the total number of women in the subgroup.

Figure S2. Relative risks of dexamethasone vs. placebo according to time from first dose to birth and gestational age at first dose



Shown are the relative risks (RR) as a function of the time from first dose to birth in hours, for different gestational ages at first injection for the two neonatal primary outcomes. There is a significant trend for the relative risk to decrease with time from first dose to birth, suggesting that dexamethasone is more protective as time of fetal exposure increases. It appears that the effect of dexamethasone is more protective as the gestational age at first injection increases from 26 until 32 weeks. However, this trend is not sustained as gestational age at first injection increases above 32 weeks. There might be confounding of time from first dose to birth with gestational age at birth that might mask or modify the effect of the intervention.

Table S1. Characteristics of ACTION-I trial hospitals

SITE	**BANGLADESH**					
FACILITY	**Facility 1**	**Facility 2**	**Facility 3**	**Facility 4**	**Facility 5**	**Facility 6**
Hospital location	Peri-urban	Peri-urban	Peri-urban	Peri-urban	Urban	Urban
Hospital level	Secondary	Tertiary	Tertiary	Tertiary	Tertiary	Tertiary
Number of births in 2016	1197	3000	5640	3624	3360	9180
Usual lower limit of gestational age for viability (i.e. active measures)	28 weeks 0 days	29 weeks 0 days	28 weeks 0 days	28 weeks 0 days	28 weeks 0 days	28 weeks 0 days
OBSTETRIC CARE						
All comprehensive obstetric care signal functions available	Yes	Yes	Yes	Yes	Yes	Yes
Number consultant obstetricians	9	7	14	24	7	12
Availability	Available during day time only	Available 24x7	Available during day time only	Available 24x7	Available 24x7	Available 24x7
What % of obstetricians are trained to perform ultrasound?	80%	100%	100%	80%	60%	5%
Number of beds:						
Admission area/s	55	46	100	180	47	42
Labor ward/s	5	2	4	30	27	42
Delivery ward/s	2	16	35	4	3	5
Postnatal ward/s	7	8	6	30	16	0
Maternal ICU	0	5	0	0	0	0

Maternal Special Care Unit	0	6	4	6	0	0
Post-operative ward/s	7	6	6	10	0	16
How soon after birth are women (without complications) routinely discharged?	24 hours	24 hours	8 hours	24 hours	24 hours	24 hours
NEONATAL CARE						
NICU available	No	Yes	Yes	Yes	Yes	No
If yes, how many beds:	-	20	50	6	12	-
Neonatal Special Care Unit available	No	No	Yes	Yes	No	No
If yes, how many beds:	-	-	85	4	0	-
Thermal control in newborn ward:						
N° of functioning incubators available:	1	10	5	3	3	4
N° of functioning radiant warmers available:	1	2	0	2	2	8
N° of functioning cradles available:	4	10	0	0	0	4
Shared use of the thermal control device	No	No	No	No	No	Yes
Antibiotics administration						
Intramuscular	No	No	No	Yes	Yes	Yes
Intravenous	Yes	Yes	Yes	Yes	Yes	Yes
Per oral	Yes	Yes	Yes	Yes	Yes	Yes

Exogenous surfactant	Not available	Always available when indicated	Not available	Always available when indicated	Not available	Not available
Respiratory support						
N° of functioning CPAP available:	1	3	4	3	3	0
N° of functioning Mechanical ventilators available:	0	2	0	6	2	0
Number Consultant Neonatologists	3	4	2	6	1	2
Availability	Available during day time only (after 8pm they are available over phone)	Available during day time only (after 8pm they are available over phone)	Available 24x7	Available 24x7	Available 24x7	Available during day time only (after 8pm they are available over phone)

Number Consultant Paediatricians	11		7	1	6	5	6
Availability	Available during day time only (after 8pm they are available over phone)		Available during day time only (after 8pm they are available over phone)	Available 24x7	Available 24x7	Available during day time only (after 8pm they are available over phone)	Available 24x7
Diagnostic equipment							
X-ray	Routinely available		Routinely available	Routinely available	Routinely available	Routinely available	Routinely available
Ultrasound for IVH	Not available		Routinely available	Routinely available	Routinely available	Routinely available	Available upon request

SITE	INDIA			
FACILITY	**Facility 1**	**Facility 2**	**Facility 3**	**Facility 4**
Hospital location	Urban	Urban	Urban	Urban
Hospital level	Tertiary	Tertiary	Tertiary	Tertiary
Number of births in 2016	6,116	4,012	10,082	2,405
Usual lower limit of gestational age for viability (i.e. active measures)	28 weeks 0 days	28 weeks 0 days	28 Weeks 0 Days	27 weeks 0 days
OBSTETRIC CARE				
All comprehensive obstetric care signal functions available	Yes	Yes	Yes	Yes
Number consultant obstetricians	18	18	28	8
Availability	Available 24x7	Available 24x7	Available 24x7	All available during day time. One available 24x7, all can be called if needed
What % of obstetricians are trained to perform ultrasound?	80%	90%	100%	80%
Number of beds:				
Admission area/s	36	60	40	7
Labor ward/s	12	8	12	8
Delivery ward/s	8	6	13	10
Postnatal ward/s	76	20	100	0
Maternal ICU	3	3	5	0
Maternal Special Care Unit	14	1	20	0
Post-operative ward/s	6	15	10	10
How soon after birth are women (without complications) routinely discharged?	72 hours	48 hours	24-48 hours	72 hours
NEONATAL CARE				
NICU available	Yes	Yes	Yes	Yes
If yes, how many beds:	14	30	22	40

Neonatal Special Care Unit available	No	Yes	Yes	No
If yes, how many beds:		30	24	
Thermal control in newborn ward:				
N° of functioning incubators available:	0	30	0	3
N° of functioning radiant warmers available:	14	30	10	40
N° of functioning cradles available:	0	0	0	10
Shared use of the thermal control device	No	No	No	No
Antibiotics administration				
Intramuscular	Yes	Yes	Yes	Yes
Intravenous	Yes	Yes	Yes	Yes
per oral	Yes	Yes	Yes	Yes
Exogenous surfactant	Available if parents can afford	Always available when indicated	Always available when indicated	Always available when indicated
Respiratory support				
N° of functioning CPAP available:	2	2	2	2
N° of functioning Mechanical ventilators available:	0	4	0	12
Number Consultant Neonatologists	2	0	0	1
Availability	0	N/A	N/A	1
Number Consultant Paediatrician	2	12	8	10
Availability	Available during day time only	Available 24x7	Available during day time only	Available 24x7
Diagnostic equipment				
X-ray	Available upon request	Routinely available	Available upon request	Routinely available
Ultrasound for IVH	Available upon request	Routinely available	Available upon request	Routinely available

SITE	KENYA			
FACILITY	**Facility 1**	**Facility 2**	**Facility 3**	**Facility 4**
Hospital location	Urban	Urban	Urban	Urban
Hospital level	Tertiary	Secondary	Secondary	Secondary
Number of births in 2016	10094	10544	7941	10334
Usual lower limit of gestational age for viability (i.e. active measures)	28 weeks 0 days	30 weeks	28 weeks 0 days	28 weeks 0 days
OBSTETRIC CARE				
All comprehensive obstetric care signal functions available	Yes	Yes	Yes	Yes
Number consultant obstetricians	2	2	3	2
Availability	Available 24x7	Available 24x7	Available 24x7	Available 24x7
What % of obstetricians are trained to perform ultrasound?	100%	50%	0	0
Number of beds:				
Admission area/s	5	2	7	1
Labor ward/s	14	35	3	12
Delivery ward/s	0	3	3	6
Postnatal ward/s	60	20	16	18
Maternal ICU	0	0	0	0
Maternal Special Care Unit	0	0	0	6
Post-operative ward/s	0	10	16	24
How soon after birth are women (without complications) routinely discharged?	24 hours	24 hours	24 hours	24 hours
NEONATAL CARE				
NICU available	Yes	No	Yes	Yes
If yes, how many beds:	1	-	16	40

Neonatal Special Care Unit available	Yes	No	No	Yes
If yes, how many beds:	44 cots 10 incubators			12
Thermal control in newborn ward:				
N° of functioning incubators available:	10	6	6	14
N° of functioning radiant warmers available:	2	2	4	0
N° of functioning cradles available:	0	10	5	10
Shared use of the thermal control device	Yes	Yes	Yes	Yes
Antibiotics administration				
Intramuscular	No	No	No	No
Intravenous	Yes	Yes	Yes	Yes
Per oral	Yes	Yes	Yes	Yes
Exogenous surfactant	Not available	Not available	Not available	Not available
Respiratory support				
N° of functioning CPAP available:	0	0	0	0
N° of functioning Mechanical ventilators available:	1	0	0	0
Number Consultant Neonatologists	0	0	0	0
Availability	-	-	-	-
Number Consultant Paediatrician	2	2	2	2
Availability	Available 24x7	Available 24x7	Available 24x7	Available 24x7
Diagnostic equipment				
X-ray	Routinely available	Routinely available	Routinely available	Available upon request
Ultrasound for IVH	Routinely available	Not available	Routinely available	Available upon request

SITE	NIGERIA-IBADAN						
FACILITY	**Facility 1**	**Facility 2**	**Facility 3**	**Facility 4**	**Facility 5**	**Facility 6**	**Facility 7**
Hospital location	Urban	Urban	Urban	Urban	Urban	Peri-urban	Peri-urban
Hospital level	Secondary	Tertiary	Secondary	Secondary	Tertiary	Secondary	Secondary
Number of births in 2016	3000	3000	2750	2000	2580	3000	2653
Usual lower limit of gestational age for viability (i.e. active measures)	26 weeks 0 days	28 weeks 0 days	26 weeks 0 days	26 weeks 0 days	26 weeks 0 days	26 weeks 0 days	26 weeks 0 days
OBSTETRIC CARE							
All comprehensive obstetric care signal functions available	yes	yes	yes	yes	yes	yes	yes
Number consultant obstetricians	8	10	2	5	20	2	3
Availability	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7
What % of obstetricians are trained to perform ultrasound?	100%	0%	100	60%	25%	100%	67%
Number of beds:							
Admission area/s	36	30	10	0	44	35	0
Labor ward/s	17	15	4	8	5	3	8
Delivery ward/s	8	26	4	14	5	3	0
Postnatal ward/s	62	30	25	26	46	15	6
Maternal ICU	7	0	0	4	0	4	0

Maternal Special Care Unit	8	6	1	10	8	3	0
Post-operative ward/s	14	26	15	26	10	19	14
How soon after birth are women (without complications) routinely discharged?	24 hours	24 hours	8 hours	24 hours	2 days	24 hours	6 hours
NEONATAL CARE							
NICU available	Yes	Yes	Yes	No	Yes	No	No
If yes, how many beds:	6	12	6	-	12	-	-
Neonatal Special Care Unit available	Yes	Yes	Yes	Yes	Yes	Yes	Yes
If yes, how many beds:	12	20	12	14	26	9	7
Thermal control in newborn ward:							
N° of functioning incubators available:	12	3	4	3	6	3	5
N° of functioning radiant warmers available:	3	3	3	4	3	2	3
N° of functioning cradles available:	10	1	15	14	16	4	4
Shared use of the thermal control device	No	No	No	Yes	Yes	Yes	No

Antibiotics administration							
Intramuscular	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Intravenous	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Per oral	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Exogenous surfactant	Available for babies with severe illnesses	Not available	Not available	Not available	Not available	Not available	Not available
Respiratory support							
N° of functioning CPAP available:	3	3	2	2	3	1	2
N° of functioning Mechanical ventilators available:	0	0	0	0	0	0	0
Number Consultant Neonatologists	1	2	2	1	3	2	1
Availability	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7
Number Consultant Paediatricians	1	0	2	4	3	2	2
Availability	Available 24x7	-	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7
Diagnostic equipment							
X-ray	Routinely available	Routinely available	Routinely available	Routinely available	Not provided	Routinely available	Available upon request
Ultrasound for IVH	Routinely available	Routinely available	Routinely available	Routinely available	Not provided	Routinely available	Available upon request

SITE	NIGERIA- ILE IFE					
FACILITY	**Facility 1**	**Facility 2**	**Facility 3**	**Facility 4**	**Facility 5**	**Facility 6**
Hospital location	Urban	Peri-urban	Peri-urban	Urban	Urban	Urban
Hospital level	Tertiary	Tertiary	Secondary	Tertiary	Tertiary	Tertiary
Number of births in 2016	2256	1829	1590	2210	2056	1874
Usual lower limit of gestational age for viability (i.e. active measures)	27 weeks 0 days	26 weeks 0 days	27 weeks 0 days	26 weeks 0 days	26 weeks 0 days	24 weeks 0 days
OBSTETRIC CARE						
All comprehensive obstetric care signal functions available	yes	yes	yes	yes	yes	yes
Number consultant obstetricians	14	13	2	7	14	20
Availability	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7
What % of obstetricians are trained to perform ultrasound?	100%	65%	50%	80%	100%	50%
Number of beds:						
Admission area/s	1	4	3	4	16	18
Labor ward/s	10	14	6	5	10	12
Delivery ward/s	10	8	6	0	4	8
Postnatal ward/s	30	49	42	16	32	Included in admission area
Maternal ICU	4	7	0	2	4	Included in General ICU 6 beds

Maternal Special Care Unit	0	8	2	1	0	Included in admission area and labor ward
Post-operative ward/s	30	34	21	11	25	Included in admission area
How soon after birth are women (without complications) routinely discharged?	24-48 hours	36-48 hours	48 hours	24 hours	24 - 48 hours	24 - 48 hours
NEONATAL CARE						
NICU available	No	No	No	Yes	Yes	Yes
If yes, how many beds:	-	-	-	8	25	N/A
Neonatal Special Care Unit available	Yes	Yes	Yes	Yes	Yes	Yes
If yes, how many beds:	32	33 cots, 15 incubators	22	15	25	50
Thermal control in newborn ward:						
N° of functioning incubators available:	13	15	6	4	6	21
N° of functioning radiant warmers available:	3	2	3	5	6	10
N° of functioning cradles available:	18	33	none	4	30	50
Shared use of the thermal control device	yes	yes	yes	no	sometimes	no
Antibiotics administration						
Intramuscular	yes	yes	yes	no	yes	yes
Intravenous	yes	yes	yes	yes	yes	yes
Per oral	yes	yes	yes	yes	yes	yes

Exogenous surfactant	Not available	Not available (unless patient procures it)	Available for babies with severe illnesses	Not available	Not available	Always available when indicated
Respiratory support						
N° of functioning CPAP available:	1	1	3	2	1	15
N° of functioning Mechanical ventilators available:	0	1	0	0	1	6
Number Consultant Neonatologists	2	2	2	2	3	4
Availability	9	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7
Number Consultant Paediatricians	9	14	2	1	15	20
Availability	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7	Available 24x7
Diagnostic equipment						
X-ray	Routinely available	Available upon request	Routinely available	Routinely available	Routinely available	Routinely available
Ultrasound for IVH	Routinely available	Available upon request	Routinely available	Available upon request	Routinely available	Available upon request

SITE	PAKISTAN	
FACILITY	**Facility 1**	**Facility 2**
Hospital location	Urban	Urban
Hospital level	Tertiary	Tertiary
Number of births in 2016	16245	15000
usual lower limit of gestational age for viability (i.e. active measures)	26 weeks	28 weeks 0 days
OBSTETRIC CARE		
All comprehensive obstetric care signal functions available	yes	yes
Number consultant obstetricians	40	18
Availability	Available 24x7	Available 24x7
What % of obstetricians are trained to perform ultrasound?	15%	70%
Number of beds:		
Admission area/s	72	8
Labor ward/s	6	32
Delivery ward/s	0	32
Postnatal ward/s	62	80
Maternal ICU	0	4
Maternal Special Care Unit	2	4
Post-operative ward/s	62	80
How soon after birth are women (without complications) routinely discharged?	6 to 12 hours	12 to 24 hours
NEONATAL CARE		
NICU available	No	Yes
If yes, how many beds:	-	16

Neonatal Special Care Unit available	Yes	Yes
If yes, how many beds:	20	10
Thermal control in newborn ward:		
N° of functioning incubators available:	7	8
N° of functioning radiant warmers available:	8	10
N° of functioning cradles available:	8	30
Shared use of the thermal control device	Yes	Yes
Antibiotics administration		
Intramuscular	No	No
Intravenous	Yes	Yes
Per oral	Yes	Yes
Exogenous surfactant	Not available	Not available
Respiratory support		
N° of functioning CPAP available:	0	4
N° of functioning Mechanical ventilators available:	0	8
Number Consultant Neonatologists	no	7
Availability	On call rosters	Available 24x7
Number Consultant Paediatrician	8	9
Availability	Available 24x7	Available 24x7
Diagnostic equipment		
X-ray	Routinely available	Routinely available
Ultrasound for IVH	Not available	Not available

Table S2. Characteristics of women at trial entry

Characteristic	Dexamethasone (N=1429)		Placebo (N=1423)	
Clinical assessment of imminent preterm birth at trial entry – no. (%)				
Spontaneously-initiated preterm birth	874	(61.2)	858	(60.3)
Preterm prelabor rupture of membranes	455	(31.8)	388	(27.3)
Spontaneous preterm labor	419	(29.3)	470	(33.0)
Provider-initiated preterm birth	555	(38.8)	565	(39.7)
Gestational age at trial entry – no. (%)				
26 weeks 0 days to 27 weeks 6 days	130	(9.1)	114	(8.0)
28 weeks 0 days to 31 weeks 6 days	654	(45.8)	679	(47.7)
32 weeks 0 days to 33 weeks 6 days	643	(45.0)	628	(44.1)
34 weeks 0 days to 36 weeks 0 days	2	(0.1)	2	(0.1)
Mean (± SD) gestational age at trial entry	30.8	(2.0)	30.7	(2.0)
Maternal age (yr) – mean (SD)	27.5	(5.8)	27.5	(5.9)
Missing – n (%)	1	(0.1)	0	(0.0)
Educational level completed – no. (%)				
No education	174	(12.2)	163	(11.5)
Primary education only	373	(26.1)	412	(29.0)
Secondary education only	549	(38.4)	501	(35.2)
Post-secondary/tertiary education	329	(23.0)	342	(24.0)
No answer	4	(0.3)	5	(0.4)
Marital status – no. (%)				
Married/Cohabiting	1380	(96.6)	1372	(96.4)
Single/Separated/Widowed/Divorced	49	(3.4)	51	(3.6)
Fetuses in the current pregnancy – no. (%)				
Single	1295	(90.6)	1290	(90.7)
Twin	125	(8.7)	129	(9.1)
Higher-order multiples	9	(0.6)	4	(0.3)
Parity – no. (%)				
0	529	(37.0)	549	(38.6)
1-2	646	(45.2)	630	(44.3)
3-4	217	(15.2)	195	(13.7)
5 or more	37	(2.6)	49	(3.4)
History of preterm birth – no. (%) *				
Yes	177	(12.4)	188	(13.2)
Unknown	28	(2.0)	21	(1.5)
Maternal weight (kg) – mean (SD)	65.4	(15.9)	64.2	(15.2)
Missing – n (%)	71	(5.0)	70	(4.9)

Maternal height (cm) – mean (SD)	156.0	(7.7)	155.7	(7.6)
Missing – n (%)	102	(7.1)	90	(6.3)
Maternal midarm circumference (cm) – mean (SD)	28.3	(4.9)	28.1	(4.9)
Missing – n (%)	53	(3.7)	61	(4.3)
Medical conditions currently present – no. (%) **				
Chronic hypertension	64	(4.5)	71	(5.0)
Diabetes mellitus (non-gestational)	13	(0.9)	14	(1.0)
HIV or AIDS	33	(2.3)	32	(2.2)
Tuberculosis	1	(0.1)	2	(0.1)
Pyelonephritis	5	(0.3)	13	(0.9)
Anaemia (hematocrit ≤26% or haemoglobin ≤9g/dL)	100	(7.0)	128	(9.0)
Malaria	48	(3.4)	55	(3.9)
Obstetric conditions currently present – no. (%) **				
Gestational diabetes	22	(1.5)	15	(1.1)
Preeclampsia or eclampsia	275	(19.2)	326	(22.9)
Gestational hypertension***	75	(5.2)	68	(4.8)
Known or suspected oligohydramnios	336	(23.5)	310	(21.8)
Known or suspected polyhydramnios	19	(1.3)	30	(2.1)
Known or suspected intrauterine growth restriction	94	(6.6)	95	(6.7)
Abruptio placentae	49	(3.4)	40	(2.8)
Placenta previa	115	(8.0)	110	(7.7)
Other obstetric hemorrhage	66	(4.6)	42	(3.0)
No obstetric condition	616	(43.1)	592	(41.6)
First date of last menstrual period known – no. (%)				
Certain	844	(59.1)	826	(58.0)
Uncertain	173	(12.1)	166	(11.7)
Unknown	412	(28.8)	431	(30.3)
Trimester of pregnancy when ultrasound for gestational age estimate was performed – no. (%)				
1st trimester (up to 13 weeks 6 days)	156	(10.9)	147	(10.3)
2nd trimester (14 weeks 0 days to 27 weeks 6 days)	344	(24.1)	329	(23.1)
3rd trimester (28 weeks 0 days and beyond)	929	(65.0)	947	(66.5)
Medication administered prior to randomization – no. (%)				
Tocolytic agent	251	(17.6)	267	(18.8)
Magnesium sulfate for neuroprotection	141	(9.9)	179	(12.6)

**This category was assessed only among women with a previous pregnancy; **Women may have had more than one condition; ***This category excludes preeclampsia and eclampsia*

Table S3. Primary outcomes with multiple imputation of missing values*

Outcome	Relative risk (95% CI)	P-value§
Neonatal death	0.84 (0.72-0.97)	0.02
Stillbirth or neonatal death	0.88 (0.78 – 1.00)	0.04
Possible maternal bacterial infection	0.76 (0.56 – 1.03)	<0.001

**20 imputations; § P-value for superiority for neonatal death and stillbirth or neonatal death, and P-value for non-inferiority for possible maternal bacterial infection; adjustments for multiplicity resulted in P=0.03 for neonatal death, P=0.04 for stillbirth or neonatal death, and P=0.002 for possible maternal bacterial infection.*

Table S4. Cause-specific neonatal mortality

Final cause of death	Dexamethasone (N=1417)	Placebo (N=1406)	Relative risk (95% CI)
Perinatal asphyxia – no. (%)	61 (4.3)	78 (5.5)	0.78 (0.56-1.07)
Respiratory distress syndrome – no. (%)	113 (8.0)	156 (11.1)	0.72 (0.57-0.90)
Neonatal sepsis – no. (%)	77 (5.4)	74 (5.3)	1.03 (0.76-1.41)
Other specific causes – no. (%)	18 (1.3)	12 (0.9)	1.49 (0.73-3.16)
Indeterminate – no. (%)	9 (0.6)	11 (0.8)	0.81 (0.33-1.96)

95% CIs are not adjusted for multiplicity and should not be used to infer definitive treatment effects

Table S5. Other secondary maternal and neonatal outcomes

Neonatal outcome	Dexamethasone		Placebo		Mean or Median Difference (95% CI) [§]
	N	Mean (± SD) or Median (IQR)	N	Mean (± SD) or Median (IQR)	
Mean birth weight* – g	1495	1819 (623)	1482	1805 (624)	14.47 (-30.36 to 59.29)
Mean head circumference* – cm	1388	30 (3)	1378	30 (3)	0.10 (-0.12 to 0.32)
Mean body length* – cm	1387	42 (5)	1379	42 (5)	0.07 (-0.29 to 0.42)
Median gestational age at birth* – weeks	1544	33 (31-34)	1526	33 (31-34)	0.00 (-0.19 to 0.20)
Median duration of oxygen therapy – hours	726	36 (18-96)	756	48 (12-93)	-12.00 (-15.59 to -8.42)
Median duration of CPAP ventilation – hours	265	48 (24-96)	337	48 (24-84)	0.00 (-8.38 to 8.38)
Median duration of use of mechanical ventilation – hours	83	18 (12-48)	103	18 (12-60)	0.00 (-6.84 to 6.84)
Median duration of parenteral therapeutic antibiotic use – hours	864	144 (63-168)	894	132 (48-168)	11.85 (2.17 to 21.53)
Median length of hospital stay after birth – days	1320	8 (3-17)	1301	8 (3-17)	0.17 (-0.58 to 0.92)
Median duration of admission to special care unit – hours	905	168 (72-168)	897	162 (60-168)	6.00 (-4.99 to 16.99)
Median time until breast milk feeding initiation – hours	1126	24 (2-60)	1049	24 (2-60)	-0.14 (-4.02 to 3.73)

Median time to full enteral feeding – hours	667	12 (6-72)	628	12 (6-84)	0.00 (-0.20 to 0.20)
Median number of newborn readmission	39	1 (1-1)	48	1 (1-1)	-
Median length of stay during newborn readmission – days	37	5 (3-7)	37	4 (3-6)	1.00 (-1.13 to 3.13)
Maternal outcome					
Median number of days of therapeutic antibiotic use – days	64	4 (1-6.5)	81	5 (2-7)	-1.40 (-2.92 to 0.13)
Median length of total maternal hospitalization for birth – days	1323	8 (4-20)	1322	8 (4-19)	0.30 (-0.54 to 1.15)
Median length of maternal re-admission – days	13	5 (3-11)	13	4 (1-9)	0.00 (-8.60 to 8.60)

**All babies were assessed, outcome not prespecified; [§]Adjusted for study site; Median number of doses of surfactant not presented because few participants received surfactant; 95% CIs are not adjusted for multiplicity and should not be used to infer definitive treatment effects*

Table S6. Adverse events

Adverse event	Dexamethasone	Placebo	Total
Maternal adverse event			
Antepartum haemorrhage	0	2	2
Dyspnea	0	1	1
Gastrointestinal upset	1	0	1
Hyperglycemia	0	1	1
Leucocytosis	0	1	1
Migraine (unspecified)	1	1	2
Postpartum haemorrhage	3	3	6
Pyrexia (unspecified)	0	1	1
Seizure	2	0	2
Total	**7**	**10**	**17**
Maternal serious adverse event			
Antepartum haemorrhage	1	2	3
Cerebrovascular accident	1	0	1
Dyspnea	0	1	1
Intrapartum hemorrhage	1	0	1
Maternal death*	5	4	9
Pleural effusion	0	1	1
Postpartum haemorrhage	4	4	8
Seizure	2	2	4
Uterine rupture	2	0	2
Wound hematoma	0	2	2
Total	**16**	**16**	**32**
Neonatal adverse event*			
Birth asphyxia	1	1	2
Neonatal death	4	1	5
Neonatal sepsis	0	1	1
Total	**5**	**3**	**8**

**Also captured as part of secondary outcome measures*

Summary of the procedures to determine the final cause of neonatal death

An exercise was undertaken to determine the final single cause of neonatal death in the trial. Neonatal death is reported in the perinatal cause of death (PCD)form for all deaths that occurred in the facility and in the verbal autopsy form for deaths that occur outside the study facilities. The WHO Newborn Health team reviewed all 609 neonatal deaths based on the forms completed at each site. Each neonatal death was assigned one underlying cause of death based on the following processes:

- All causes of death were classified into one of the following main causes of death: respiratory distress syndrome, neonatal sepsis, perinatal asphyxia, other specific cause or indeterminate.
- Verbal autopsies were reviewed where PCD form was not available and a cause of death was assigned.
- ICD principles were followed in assigning the cause of death.
- Where no valid cause of death was available in the PCD form, all available forms for the infant were reviewed to assign a valid cause of death.
- The site-specific list of cause of death was reviewed by the neonatal Principal Investigators at the respective sites and compared with the source documents. The changes suggested by the PIs were made.

The list of final cause of death was shared with the statistical analysis team to determine cause specific mortality by study groups.

Procedures relating to ultrasound assessments

All participating hospitals were provided with the following ultrasound equipment:

- 1 x Philips HD5 ultrasound system
- 3 x probes – transabdominal, transcranial and intravaginal
- 1 x Uninterruptible Power Supply (UPS) device

This equipment was expressly for the purposes of facilitating assessment and recruitment of women to the ACTION trials and assessment of intraventricular haemorrhage in neonates (hereafter referred to as the ACTION Trial ultrasound systems). It was intended to augment existing ultrasound systems at participating hospitals, and (to the extent possible) minimize ultrasound access issues for trial participants.

Obstetric ultrasound for gestational age assessment

There are several considerations for performance of dating ultrasounds in low resource settings:

- Accurate estimated gestational age (EGA)/expected delivery date (EDD) assignment is limited by multiple factors:
    - Late initiation of antenatal care;
    - Uncertain last menstrual period;
    - No prior ultrasound evaluation (estimated gestational age has been assigned by a referring care provider based on fundal height only);
    - Third trimester fetal biometric variance (+/- 21 days at >28 weeks estimated gestational age);
    - Prior scans performed by sonographers outside of the hospital with varying/unknown levels of experience or expertise; and
    - Use of biometric nomograms derived from a different (often higher resource) populations.

Furthermore, many tertiary-level maternity facilities in low-resource countries do not always have routine or 24/7 access to obstetric ultrasound services.

In order to optimize the assessment of gestational age in routine care settings, the following procedures were developed and applied:

- For women to be eligible for the trial, the gestational age must be based on the earliest available obstetric ultrasound of reasonable quality. In the event an ultrasound was available from earlier in the pregnancy, the obstetric physician determined whether this ultrasound was of acceptable quality. If it was not available (or no ultrasound assessment was available), a dating ultrasound was performed at the participating hospital.
- The study Manual of Operations provided a gestational age estimation algorithm that was adapted from American College of Obstetrics and Gynaecology (ACOG) Committee Opinion on Method for Estimating Due Date (October 2014).[3] These procedures were reviewed by two independent experts from the International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG) (Dr Lynn Coppola and Dr Sandhya Maranna).
- Individuals at participating hospitals who were involved in performing obstetric ultrasound (varied by site, but generally involved ultrasonographers, radiologists and/or obstetricians)

underwent a standardized training provided by an ISUOG expert trainer (LC or SM). This training included use of ISUOG teaching modules as well as hands-on practice. Completion of 3 to 5 obstetric ultrasounds of acceptable quality was required to demonstrate proficiency.

The following measures were implemented for quality assurance:

- During the trial, the nominated Lead for obstetric ultrasound assessment at each hospital or study site conducted periodic internal peer-review of ultrasound scans performed, as well as any refresher training on an as-needed basis.
- For those women where the ACTION Trial ultrasound system was used to identify the gestational age, scans were digitally saved (using anonymized participant ID numbers) and logged in a standard logbook. The Manual of Operations pre-specified that approximately 5% of saved scans would be randomly sampled for quality assurance purposes.
- A random sample of scans for 175 participants (6.1% of the 2852 women randomized) were selected, reviewed and scored by an ISUOG expert. The scoresheet was pre-designed to assess whether the scan had been performed correctly from a technical standpoint, and was based on criteria of Salomon et al [4]. Based on available images and scores, scans were rated by the ISUOG expert as "acceptable" or "not acceptable" to be utilized by the sites for accurate estimation of gestational age.
- This sample was not evenly distributed across countries, as some hospitals (particularly those in India and Bangladesh) had a high proportion of women who had a dating ultrasound from the first trimester of pregnancy.
- Images of sufficient quality were available for 156 participants cases (5.5% of 2852 randomized women). Of these, 145 were rated "acceptable" (93%) and 11 were not acceptable.

Neonatal transcranial ultrasound intraventricular haemorrhage assessment

- **Equipment/Machine:** Philips HD5 scanner with a sector probe (5-8 MHz)
- **Protocol for obtaining neonatal CUS**: Transcranial ultrasound was performed routinely for newborns delivered at < 34 weeks by a trained provider at 7 days postnatal age or discharge, whichever occurred first. For babies born at ≥ 34 weeks, transcranial ultrasound was performed only when specifically requested by a clinician.
- **Presence and grading of intraventricular hemorrhage (IVH)**: The presence of IVH and its grading was evaluated as below:

- Any echogenicity at the level of caudothalamic groove (extending anterior to Foramen of Munro) is suggestive of IVH
- IVH was graded according to the grading proposed by *Papile* given below
    - Grade 1 – Sub-ependymal haemorrhage without ventricular extension
    - Grade 2 – Intraventricular Haemorrhage without ventricular dilatation
    - Grade 3 – Intraventricular Haemorrhage with ventricular dilatation
    - Grade 4 – Intraventricular haemorrhage with associated parenchymal involvement

- **Data/record maintenance:** The CUS scans at each site were digitally saved using anonymized participant ID numbers and logged in a standard logbook at each site.
- **Training and quality assurance:** The following measures were implemented for quality assurance:
    - Standard operating procedures were developed describing the CUS technique including the views required and other technical requirements, interpretation and grading of IVH.
    - Prior to trial initiation, all site sonologists were trained on standard operating procedures and interpretation and reporting by an expert. Around 70 staff were trained across all sites (including neonatologists, radiologists and sonologists, though staff cadre varied by site). The training involved a presentation on the basics of ultrasonography, hardware, CUS techniques and interpretation. The trainees were then trained on neonates under the supervision of the expert.
    - A sample of all positive scans (grade 1-4; as reported by sites) and a 5% random sample of all negative scans (grade 0; as reported by sites) were reviewed and graded independently by an external expert, blinded to the grading reported by sites. Any discrepancies in grading between the site sonologists and the external expert were reviewed and resolved by mutual discussion between the two.
    - Images were available for 108 of 137 (79%) positive scans and for 58 of 65 (89%) randomly selected negative scans:
        - Severe (grade 3-4), n=15 available of total 17 (88%): 5 graded same, 1 “downgraded” by expert but site maintained as “severe”
        - Non-severe (grade 1-2), n=93 available of total 120 (78%): 3 “upgraded” by expert (3%)
        - No IVH (grade 0), n= 65 available of total 65 (89%): all graded same by expert

References

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BLDE (DEEMED TO BE UNIVERSITY) IN PARTNERSHIP WITH EMORY UNIVERSITY PRESENTS :

Issue 3

June - 2020

Inside this Issue:

B.E. Impactful

A SOCIAL DETERMINANTS NEWSLETTER

UPDATE 2019 - 2020

HEALTH CHANGES IN VIJAYAPURA

India continues to experience high levels of under-nutrition, together with increasing levels of overweight and obesity. This dual burden of malnutrition is contributing to sustained levels of infectious and chronic disease. The emergence of obesity alongside underweight has been attributed to the 'nutrition transition', specifically changes in food availability, food choice and food intake occurring with urbanization and economic growth. Understanding the relationship between health and socio-economic environment in the context of globalization has been a focus of our Emory-BLDE collaboration over the past 8 years.

In this current issue, we first highlight the findings from our 2012-2014 projects, that was established first through an NIH-funded training program and then supported by Emory University's Global Health Institute, Emory University's Global Field Experience Program and BLDE (Deemed to be University (DU). Through collaborative projects since 2012 in Vijayapura, we have shown that, in this population, underweight affects 40% of adolescents aged 13-16 years, while obesity affects 40% of their mothers. In addition, we have shown that processed and packaged foods are increasingly available, but that consumption is still low. We have documented the sources of non-local, energy-dense foods in youths' diets and the ways in which youths conceptualize of global and local food items.

Next, we also describe in this issue our new study on Drivers of Food Choice in Indian Households in the Context of the Nutrition Transition and the next steps of this collaboration including a new research collaboration and training of students from BLDE (DU) and visiting students from Emory University.

In the next issue, we will present the preliminary results from the new studies.

Dr. Shailaja S. Patil, MD, BLDE (DU)

Dr. Solveig Cunningham, PhD, Emory University

Underweight and obesity — double burden in Vijayapura

India faces a dual burden of increasing obesity and persistent underweight. In 2012, our study of 400 families in Vijayapura measured the weights of mothers and children ages 5-19 years. We found 11% of families experiencing a dual burden of underweight and overweight, as majority of the children had normal weight while majority of the mothers were overweight (26% overweight & 40% obese). The study 'Influence of Home Environment on Adolescent Unhealthy Weight was funded by a grant from NIH in the US.

Household member	Underweight (%)	Normal weight (%)	Overweight (%)
Girls	17	76	7
Boys	29	64	8
Mother	5	29	66

How do children and adolescents view foods in Vijayapura?

In another study, adolescents in Vijayapura believed non-traditional foods such as pizza, noodles, cold drinks, ice cream and cake were most prestigious along with non-local foods from both foreign countries and other regions of India. Adolescents identified curd, pulses, rice, roti, and holige as least prestigious or traditional foods. There was little overlap in the foods adolescents mentioned that they ate at home versus outside the home. This study was led by Amanda Maxfield, PhD, a student at Emory University, during a summer practicum at BLDE University in 2014 (Maxfield et al. Ecology of Food and Nutrition 2016).

Adolescents also ascribed healthfulness and modernity to food and beverage items and were aware of their availability across supermarkets & kiraana stores.continuing to eat meals at home, but now snack with friends outside the home more frequently.

***(Source:** Maxfield A, Patil S, Cunningham SA. Globalization and Food Prestige among Indian Adolescents. Ecology of food and nutrition. 2016;55(4):341-364).*

Going global — adolescents eating patterns

According to a study carried out by our team, adolescent eating patterns in Vijayapura reflect a combination of global or non-local and traditional foods and preferences. A total of 399 adolescents aged 13-16 years old who attended 3 private and 3 public schools in 2013 completed a nutrition survey. Adolescents' food intake can be compared to the recommended dietary guidelines.

The adolescents ate energy-dense food more frequently than recommended but ate fruit, vegetables and dairy less frequently than recommended. Girls reported more frequent consumption of global packaged and ready-to-eat foods, vegetables, and added oil/ghee to foods while boys reported more frequent consumption of eggs and street foods.

Healthy eating can be challenging for adolescents in the context of new and trendy packaged and processed foods. This study provided new insights on the eating patterns of adolescents. "As global foods continue to appear in low– and middle-income countries such as India, understanding dietary patterns and preference can inform efforts to improve dietary diversity and healthfulness of foods," commented team nutritionist and lead author Dr. Nida Shaikh, Assistant Professor, Georgia State University.

***(Source:** Shaikh NI, Patil SS, Halli S, Ramakrishnan U, Cunningham SA. Going global: Indian adolescents' eating patterns. Public Health Nutrition. 2016)*

Grandmother's perspectives on changing context of health in India

We engaged ten grandmothers from Vijayapura to get their perspective on globalization and health. Grandmothers provided insights about the changing context of dietary patterns and family roles arising with globalization that may be contributing to the rise in chronic diseases. The grandmothers completed a structured questionnaire that consisted of 27 close-ended questions and 2 open-ended questions. Grandmothers were asked to describe and to compare characteristics of their current and past household, including details of eating, activity practices and daily tasks.

Grandmothers indicated that household chores and food preparation are less labor-intensive and time-consuming due to mechanization and the availability of prepared foods compared to a generation earlier. Families are more often eating food out, bringing prepared food home, and/or using ready-made food mixes. In addition, adolescents are continuing to eat meals at home, but now snack with friends outside the home more frequently

***(Source:** Cunningham S, Gloor S, Patil S. Grandmothers' perspectives on the changing context of health in India. BMC Research Notes. 2017)*

Innovative 'snapshot' of private school children's physical activity and food habits

In an ancillary study led by visiting MPH students from Emory University, photo journals were used to explore children's perceptions of their food and activity habits in Vijayapura. A total of 30 boys and girls studying in 8th and 9th standard in July 2013 were given a Kodak disposable camera and a notebook for 4 days.

Children expressed interest in active pastimes such as sports and playing outside, learning and health, and indicated traditional, modern, local, and global influences in their lives. Some described how much they enjoyed their favorite sports. An eighth grade girl stated, "I have basket-ball net in my house ……..
I have taken this photo because I like basket-ball and I go to level in basket-ball."

This picture shows an adolescent girl eating her dinner in front of TV We published an article on this study :

***(Source:** Staab, E.M., Cunningham Solveig A., Thorpe, S., and Patil, S.S. (2016). A "snapshot" of physical activity and food habits among private school children in India. Childhood. 23(4): 537–553).*

Doctors chime in

We also conducted a study in which we explored physicians' perceptions on the development of overweight and how to manage it in the developing sectors of India in which underweight seems to be the established health problem among children as well as adolescents. Twenty-five physicians participated in this study by completing a semi-structured questionnaire which included 35 close-ended and 3 open-ended questions. The close-ended questions served to gain insight about causes of obesity, gender differences, and treatment options, while the open-ended questions investigated the fundamentally understood rationales for obesity.

Although physicians treated adolescents more for underweight than overweight, most acknowledged that overweight has increased in frequency over the past 5 years while underweight has been steadily decreasing. Due to risk factors such as urban dwelling, high socioeconomic status, male gender and parental obesity, physicians agree that overweight is increasing as a problem. Factors such as eating habits, activity levels, and certain environmental levels are also influential.

Most physicians do desire more training on treating patients for overweight as well as under-nutrition.

*(**Source:** Patil, S.S., Ports, J., Yadavannavar, M. C., and Cunningham, Solveig A. (2016) Physicians' Perceptions about the Emergence of Childhood Overweight in India. Journal of Krishna Institute of Medical Sciences University. 5(1):37-44.)*

Our New Study:

Drivers of Food Choice in Indian Households in the context of Nutrition Transition

The goal of this 2018-20 project, funded by the Drivers of Food Choice (DFC) competitive grants program, is to quantify aspects of women's and men's food choices relevant for addressing the dual burden of malnutrition in India through data collection and analysis based in Vijayapura, a remote district in Southern India that globalization is just reaching. The objectives are to: 1) Quantify the importance of factors including price, satiety, taste, reputation, and subsidies as proximate drivers of food choice in the context of globalizing food markets; 2) Identify the conditions under which women and men select global vs. traditional foods and how variations in these conditions can alter selections; 3) Assess the role of the public distribution system (PDS) as a driver of food choices and its implications for intake and for advancing nutrition transition. In a representative sample of 265 urban and 222 rural households, we conducted interviews with women, men and youths. Data instruments included experimental methods to elicit drivers of food choice in different scenarios and to valuate PDS bundles and a food frequency questionnaire to measure intake and nutrition transition. We used quantitative and qualitative methods to explore gender differences and communication relating to food choices and food intake. The research has real-world applications and is sustainable. This project will generate data on food choices in a remote but urbanizing region in Southern India, on the key drivers of food choices within and across households, and on the implications of the PDS. More information about the study is available at **https://www.driversoffoodchoice.org/research/project-descriptions/food-choice-in-Indian-households/**

Funded by :
This research has been funded by the "Drivers of Food Choice (DFC) Competitive Grant Programs, which is funded by the UK Government's Department for International Development and the Bill & Melinda Gates Foundation and managed by the University of South Carolina, Arnold School of Public Health, USA".

Study team practicing interviews

Pilot study data collection

Drivers of Food Choice Project Study Team

Principal Investigator

Solveig A. Cunningham, PhD, Msc.,
Associate Professor, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Co-Principal Investigator

Dr. Shailaja S. Patil, M.D.,
Professor and Head, Department of Community Medicine, Sri B. M. Patil Medical College, BLDE (Deemed to be University (DU)) India.

Co-Investigators

Ashlesha Datar, PhD.,
Senior Economist, University of Southern California. Her research focuses on the influence of neighborhoods on obesogenic behaviors and obesity in families.

Nida I. Shaikh, PhD, RD Assistant Professor, Department of Nutrition, Georgia State University, Atlanta, GA, USA.

Research Associate

Chandrika Doddihal, M.D.,
Assistant Professor, Department of Community Medicine at BLDE (DU).

Project Coordinator

Manjunath Marad, PhD Scholar, Department of Community Medicine, BLDE (DU)

Collaborative Visit from Colleagues from Emory University, Atlanta, USA

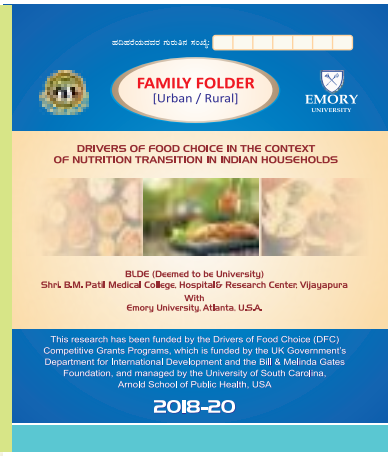
Since the start of the new collaborative study in 2018, Dr. Solveig Cunningham visited BLDE (DU) twice. In November 2018, Dr. Cunningham participated in the inauguration of the new research study 'Drivers of Food Choice in Indian Households in the Context of the Nutrition Transition'. The study was inaugurated by the chief guest Dr. M S Biradar, Honorable, Vice Chancellor of BLDE (DU). She also trained field staff to administer the study consent and survey modules to study participants.

On a second visit in June 2019, Dr. Cunningham reviewed the project progress and visited the project field areas in rural Ukkali and urban Vijayapura City. She participated in the distribution of sports kits to children in participating schools in Ukkali.

Inaugural Function, November 2018, BLDE)(DU)

Principle Investigator with Project team November 2018, BLDE (DU), India

DFC FIELD DATA COLLECTION GLIMPSES



← Interview with adolescent in rural school

Interview with Adolescent's Care taker (Urban) →

← Survey instrument cover file

Gift distribution at Ukkali

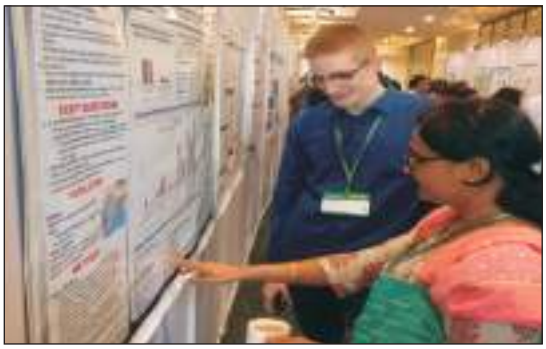
Distribution of Sports Kits in Schools, June2019, Ukkali, India

Interview at garden/farmhouse

DFC Study Team at the 2018 & 2019 Agriculture, Nutrition and Health Academy Week

Our team participated in the 3rd and 4th annual meetings of the Agriculture Nutrition and Health (ANH) Academy Week between 2018-19. In the 3rd annual ANH meeting in Accra, Ghana in 2018, Dr. Cunningham gave an oral presentation on 'Pocket money spending patterns among adolescents in India'. In the 4th annual ANH meeting in Hyderabad, India in 2019, using pilot data from a hospital-based sample of the ongoing DFC study, Dr. Cunningham gave an oral presentation on 'Understanding Food Choices in the Context of Globalizing Food Options' and Dr. Patil presented the poster 'The Public Distribution System as a Driver of Food Choice in Addressing Food Security and Food Diversity in Southern India.'

Other members of the DFC team, Dr. Doddihal and Mr. Marad, participated in learning labs at ANH 2019 that were designed to use DFC project case studies to engage participants in the science of food choice research is used to identify leverage points from which programmatic and policy actions can be derived.



Dr. Patil with her poster, ANH 2019

DFC team at ANH 2019, Hyderabad, India

VISITING STUDENTS FROM EMORY UNIVERSITY, USA

Shifts in the food environment are considered a key driver of the nutrition transition in India. These shifts in the food environment maybe facilitated by environmental cues (nudges) in food retail stores but little is known about such nudges beyond western settings. **Atsu Ishizumi,** an MPH student from Emory, participated in a summer practicum under the supervision of Dr. Shailaja Patil at BLDE (DU) from June to August 2018, where he developed and carried out an ancillary study titled 'Using nudge theory to understand the food retail environment of a globalizing South Indian city'. He conducted observational audits in 17 food retails stores including local kirana stores and supermarkets in Vijayapura City and measured four nudges; items within reach of the cash register or waiting area; items visible from the front entrance; items placed at the average adult consumer's eye level, and shelf space allocation of different foods and beverages. Preliminary findings suggest, processed foods such as savory snacks and sweets were most frequently promoted through the four nudges irrespective of the type of food store.

In addition, Atsu updated the team's growing database of food items available in Vijayapura, which includes over 1000 foods and beverages, and assisted in the preparatory phase of the ongoing new ' Drivers of Food Choices in Indian Households in the context of Nutrition Transition' study.

Philip Dollard, an MPH student from Emory University visited BLDE (DU) for his summer practicum from July to August 2017. Under the guidance of Dr. Shailaja S. Patil, Phil participated in the preliminary fieldwork of the new ongoing study. His practicum was funded by the Global Field Epidemiology program at Emory University.

CURRENT STUDENTS & INTERNS

Asha Nadabar is a Master of Public Health student in the Hubert Department of Global Health at the Rollins School of Public Health. She is currently working with Dr. Solveig Cunningham and Dr. Nida Shaikh on the drivers of food choice in India project for her MPH Thesis.

John Pothen is a 7th year MD/PhD student at Emory University in the Sociology Department. John spent the summer (June to July 2019) in Vijayapura helping with analysis, study design and data management aspects of Drivers of Food Choice Project. He is currently helping with a manuscript examining sedentary activity and pedometer counts in adolescents and with a paper regarding temporary labor migration and diet.

Manjunath Marad is a PhD student in the Department of Community Medicine (Allied Health Sciences) at BLDE (Deemed to be University), Shri B M Patil Medical College, Hospital and Research Centre, India. He is the Project coordinator of the ongoing study 'Drivers of Food Choices in Indian Households in context of Nutrition Transition' funded by the Drivers of Food Choice (DFC) competitive grants program. His dissertation is titled 'Dietary patterns and determinants of food choices in the context of nutrition transition' and is being supervised by Dr. Shailaja S Patil, Professor and Head of the Department of Community Medicine at BLDE (Deemed to be University).

Future Directions:

Dr. Shaikh Receives an Award from 2019 Academy of Nutrition and Dietetics Foundation

Dr. Nida Shaikh, Assistant Professor of Nutrition at Georgia State University, has been awarded the 2019 Amy Joye Memorial Research Award by the Academy of Nutrition and Dietetics Foundation to test a nutrition instrument called the Nutrition-Transition Food Frequency Questionnaire for adults in Southern India. Nutrition transition, the shifts in dietary patterns accompanying globalization and urbanization, are believed to be contributing to the emergence of chronic diseases such as obesity and diabetes among adults in low- and middle-income countries including India. Unfolding of the nutrition transition among adults could be measured by assessing dietary changes, but to date there are no validated dietary assessment instruments for adults. As part of the larger ongoing 'Drivers of Food Choice' (DFC) study at BLDE (DU), which seeks to understand the drivers of food choices and food intake among Asian Indian adults in the context of nutrition transition, Dr. Shaikh and colleagues at BLDE (DU) and Emory University have developed the 71-item NT-FFQ. She will test the NT-FFQ over the next year for its reliability and validity. A validated NT-FFQ would be the first tailored dietary assessment instrument to measure nutrition transition among Asian Indian adults. Further applications of this instrument would include use in research and practice.

List of Recent Publications

1. Ilana G. Raskind, PhD, Shailaja S. Patil, MD, Nikhil Tandon, MBBS, MD, PhD, Sharanya Thummalapally, MPH, Michael R. Kramer, PhD and Solveig A. Cunningham, PhD. "Household Chores or Play Outdoors? The Intersecting Influence of Gender and School Type on Physical Activity Among Indian Adolescents". Health Education & Behavior. 2020;1-10. **(Article first published online: June 9, 2020)**
2. Ilana G Raskind ,Shailaja S Patil, Michael R, Kramer Solveig A Cunningham. "Physical Activity Pattern among Indian Adolescents: Independent & Joint Association of Gender & Socio-Economic Status". American Heart Association EPI| Lifestyle, 2019; 139,Page No. 322, conference abstract published Online.
3. Ilana G. Raskind, Shailaja S. Patil, Regine Haardorfer, Solveig A. Cunningham. Unhealthy Weight in Indian Families: The Role of the Family Environment in the Context of the Nutrition Transition. Population Research and Policy Review.2018; 37 (2): 157-180.
4. Shaikh NI, Maxfield A, Patil S, Cunningham S. Healthfulness, Modernity, and Availability of Food and Beverages: Adolescents' Perceptions in Southern India. Ecology of Food and Nutrition. 2017;1-17.
5. Shaikh NI, Frediani J, Patil SS, Ramakrishnan U, Yount KM, Martorell R, Narayan KM, Cunningham SA. Development and evaluation of a Nutrition Transition-Food Frequency Questionnaire for adolescents in South India. Public Health Nutrition. 2017;20(7):1162-1172.
6. Cunningham S, Gloor S, Patil S. Grandmothers' perspectives on the changing context of health in India. BMC Research Notes. 2017; 10(1).
7. Halli S, Ramakrishnan U, Cunningham SA. Going global: Indian adolescents' eating patterns. Public Health Shaikh NI, Patil SS, Nutrition. 2016;19(15):2799-2807.
8. Shaikh NI, Frediani J, Ramakrishnan U, Patil S, Yount K, Cunningham S. Development and Evaluation of a Nutrition Transition FFQ for Adolescents in South India. The FASEB Journal. 2016; 30(1): 1153.
9. Shaikh, N, Ramakrishnan, U, Patil, S, Yount, K, Martorell, R, Narayan, KM., & Cunningham, S. Development and Validation of a Nutrition Transition-Diet Score for Adolescents in India. The FASEB Journal, 2016. 30 (1 Supplement), 43.7.
10. Shaikh, N, Patil, S, Ramakrishnan, U, Yount, K, & Cunningham, S. Development and Evaluation of a Food Behavior Survey to Assess Nutrition Transition Among Adolescents in South India. The FASEB Journal, 2016. 30 (1 Supplement), 33.7.
11. Staab, E.M., Cunningham, Solveig A., Thorpe, S., and Patil, S.S. (2016). A "snapshot" of physical activity and food habits among private school children in India. Childhood. 23(4): 537–553.
12. Maxfield, A. Patil, S. and Cunningham, Solveig A. (2016) Globalization and Food Prestige among Indian Adolescents, Ecology of Food and Nutrition, 55(4): 341-364.
13. Patil, S.S., Ports, J., Yadavannavar, M. C., and Cunningham, Solveig A. (2016) Physicians' Perceptions about the Emergence of Childhood Overweight in India. Journal of Krishna Institute of Medical Sciences University. 5(1):3744.
14. Shaikh N, Maxfield A, Ramakrishnan U, Patil S, Cunningham S. Perspectives of Food Availability, Healthfulness and Modernity Among Adolescents in India. FASEB Journal, 2015. 29: 898.30.
15. Shaikh N, Patil S, Ramakrishnan U, Cunningham S. Food consumption of Indian adolescents in a globalizing world. The FASEB Journal, 2014. 28 (1 Supplement), 1014.5.

Interested in working with us/learning more? Contact us @

India: Dr. Shailaja S. Patil, MD, Head, Department Of Community Medicine, BLDE (deemed) University, Vijayapura, Karnataka, India. **Phone:** +91-8352-262770 Extn: 2155, 2154 (office) **Email:** shailaja.dr@gmail.com

USA: Dr. Solveig A. Cunningham, PhD. Associate Professor, Department of Global Health and Sociology, Emory University, Atlanta, GA, USA. **Email:** sargese@emory.edu

What are social determinants?

The social determinants of health are the economic and social conditions including those in which people are born, grow, live and work that influences their health status and risk for disease.

Supporting hands for Extending works in Centre for Social Determinants of Health at BLDE(DU)

Our previous projects were among the initial steps in developing center for Social Determinants of Health. The goal of this center is to both understand the emerging health challenges in our changing world and to work with leaders in our community and more broadly to develop effective ways for ensuring a healthy future for the underdeveloped region Northern Karnataka.

To further strengthen our center and invest in understanding the social determinants of nutrition in our community in terms of food choices made and also various factors influencing the choices, a new study was taken up.

This collaborative research journey would not have been possible without the support of our Honorable Chancellor **Dr. M. B. Patil,** Present Hon. Vice-Chancellor **Dr. M. S. Biradar** and Dean faculty of Medicine **Dr. Aravind V Patil.**

We express our gratitude to previous administrators **Dr. B G Mulimani** (Ex-Vice-Chancellor) & Ex-Dean's **Dr. R.C. Bidari, & Dr. S. P. Guggarigoudar** for their guidance and support in our endeavor to carry out International research projects and in hosting various International students (till now 9) for their research work in collaboration with Emory University .

Our sincere gratitude to **Dr. K. M. Venkat Narayan,** Professor and Ruth and O.C. Hubert chair, Global Health department, Emory University, who visited BLDE (DU) twice and initiated the idea of developing Center for Social Determinants of Health and promoted this research collaboration. We are also grateful to globally renowned Doctors, **Prof. D. Prabhakaran** M.D., DM (Cardiology), Msc, FRCP, FNASc. Director, Center for Control of Chronic Conditions (CCCC)-Gurgaon, Vice President - Research & Policy, Public Health Foundation of India (PHFI), **Dr. Nikhil Tandon** M.D., Head of the Department of endocrinology, metabolism and diabetes at the AIIMS, New Delhi, **Dr. Shiva S. Halli** Professor, Department of Community Health Sciences Faculty of Medicine University of Manitoba, Winnipeg, Canada, for their guidance & motivation in our journey so far.

We extend our thanks to **BLDEs V.P. Dr. P. G. Halakatti** College of Engineering and Technology and Department of Civil, whose faculty helped in mapping and locating our Urban cohort houses in Vijayapura through GPS technique.

The Commissioner of Food, Civil Supplies and Consumer Affairs of Vijayapura district (Vijayapura and B.Bagewadi taluka) by providing their valuable inputs in framing our survey instrument on Public Distribution System. We will be sharing our research findings with concerned officials. Our acknowledgements to **The District Education Dept. (DDPI) and Block Education officer** for their permission to involve school children in our Drivers of Food choice study and Home environment and Adolescent unhealthy weight status in school going adolescents of Vijayapura study. We appreciate the cooperation and support extended by **Rural participant school staff** (4 private & 3 government) in conducting our DFC research project in 2019-2020. We are grateful to our participant Adolescents and their parent/caretakers from Ukkali village and Vijayapura city for their time and cooperation without whom these studies would not have been completed. Last but not the least we thank our dedicated team of Field Interviewers and Supervisors.

Acknowledgement of Funders for our research studies :

"Drivers of Food Choice (DFC) Competitive Grant Programs, funded by the UK Government's Department for International Development and the Bill & Melinda Gates Foundation and managed by the University of South Carolina, Arnold School of Public Health, USA".(2018-2020)

"Fogarty International Centre and Eunice Kennedy Shriver National Institute of Child health and Human Development at National Institute of Health, USA "(Grant ID-D43HD065249-S1) .

Market in Rural Area

Kirana Shop in Urban Area

Super Market in Urban Area

Drivers of Food Choice
Competitive Grants Program

Research Brief

OCTOBER 2020

Food Choice in Indian Households in the Context of the Nutrition Transition

Investigators: Solveig Cunningham (MSc, PhD), Shailaja S. Patil (MD), Ashlesha Datar (PhD), Nida I. Shaikh (RD, PhD)

Key Takeaways

- Accessibility, taste, and health perceptions are important drivers of food choice
- In remote parts of India, non-traditional foods were less favored than local foods and, consequently, infrequently consumed
- India's Public Distribution System may contribute to equalizing frequency of intake of staple food items (rice and pulses) for poor and non-poor households
- Urban residents had higher dietary diversity than rural residents
- Among urban residents, wealthier households consumed healthier foods such as fruits and dairy products while poorer households consumed more energy-dense items such as meats and snacks
- Rural adolescents were less likely to have ever seen or consumed global items compared to urban adolescents

Objectives

Grounded in the context of globalizing food markets, we have several project objectives. One was to identify the most salient considerations for food choice (e.g.: price, taste, reputation, etc.). A second was to quantify the role of food subsidies, specifically through the Public Distribution System, as drivers of food choice. A third objective was to identify conditions under which people select local vs. non-local Indian or global foods, examining differences between men and women and between adolescents and adults. A fourth objective was to measure how variations in food choice considerations relate to dietary intake.

Background

The globalization of food markets has facilitated the widespread availability of processed and packaged foods in nearly all parts of the Global South. Considering that India is home to nearly one-fifth of the world's population and may be experiencing a double burden of malnutrition, understanding decision-making around food in India is a high priority. This study aimed to generate data on drivers of food choice in a remote but urbanizing region in South India. This includes assessing associations between access to subsidized staple foods and dietary patterns, as these subsidies may sway diets toward the consumption of less nutritious staple grains, particularly white rice. There may be differences in patterns of food preferences and intake between men and women and between adolescents and adults, and so these are quantified in this project. These approaches provide a fuller assessment of the implications of economic, cultural, and normative components of decision-making within households around food choice.

Methods

A bespoke quantitative data collection instrument was developed. The instrument included a picture-card component asking participants about food choices in place-of-origin categories (local foods, non-local Indian foods, and global foods) that hold similar roles in diets (snacks, fruits, condiments, etc.). A 71-item food frequency questionnaire was designed to measure nutrition transition among adults. Anthropometric measurements were taken.

Representative sampling was used to create a sample of households containing adolescents in urban and rural communities. Data were collected from three members of each household to allow for comparison across men and women and across adolescents and adults within and across households. Qualitative interviews were conducted to add depth and context to various components of the quantitative work, including PDS officials, shopkeepers, and beneficiaries and migrant families. These discussions add nuance to understanding people's perceived constraints, responsibilities, and decisions.

Results

Respondents universally consumed local foods on a daily basis and global foods infrequently. Dietary diversity and frequency of eating basic staples – rice and pulses - was similar between those who do and do not receive government food subsidies through the Public Distribution System. Urban residents had significantly higher dietary diversity than rural residents. Among urban residents, wealthier households had healthier diets, consisting of fruits, vegetables, and nuts, while poor households consumed more animal-source foods, sugar-sweetened beverages, energy drinks, and snacks.

The most salient considerations in food choice were access, taste and perception of healthfulness for adults; for adolescents, price and taste were most salient. Adult and adolescent respondents showed strong preferences for local foods over non-local items. Few adults showed interest in replacing local foods with comparable non-local foods under differing conditions of food choice. Those who did show interest in trying non-local foods did so most frequently with respect to cereal and pulses. Adolescent were somewhat more interested in selecting non-local foods, especially if there were to have extra money and when looking for something tasty.

More Information

- "Food subsidies, nutrition transition & dietary patterns in a remote Indian district" By Cunningham S, Shaikh N, Datar A, Chernishkin A, Patil S. 5th Agriculture, Nutrition & Health (ANH) Academy Week, June 30 – July 2, 2020. https://www.youtube.com/watch?v=s5gV_cuaII8&feature=youtu.be
- "Food Vendors in India's Changing Food Environment" By Doddihal C, Patil SS, Marad M, Shaikh NI, Cunningham SA. 5th Agriculture, Nutrition & Health (ANH) Academy Week, June 30 – July 2, 2020. https://www.youtube.com/watch?v=kcR9gTihmPE&feature=youtu.be
- "Understanding Food Choices in the Context of Globalizing Food Options."By Cunningham, S.A. 4th Agriculture, Nutrition & Health (ANH) Academy Week, Hyderabad, India, 24–28 June 2019. https://driversoffoodchoice.org/wp-content/uploads/2020/07/8B_Solveig-Cunningham.pdf
- "Public Distribution System as a Driver of Food Choice: Evidence from a District in South India." By Doddihal C, Marad M, Datar A, Cunningham SA. 4th Agriculture, Nutrition and Health (ANH) Academy Week, Hyderabad, India, 24-28 June 2019.
- "Pocket money spending patterns among adolescents in India." By Cunningham SA. 3rd Agriculture, Nutrition & Health (ANH) Academy Week, Accra, Ghana, 25-29 June 2018.
- **Project Page** - https://driversoffoodchoice.org/research/project-descriptions/food-choice-in-indian-households/

This research has been funded by the Drivers of Food Choice (DFC) Competitive Grants Program, which is funded by the UK Government's Foreign, Commonwealth & Development Office and the Bill & Melinda Gates Foundation, and managed by the University of South Carolina, Arnold School of Public Health, USA; however, the views expressed do not necessarily reflect the UK Government's official policies.

BLDE (Deemed to be University)
Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura

BLDE (DU) WEBINAR - 05

Sept 10, 2020
Thursday, 11 AM (IST)

Dr. Prakash Kabbur

Consultant Neonatologist
President,
Train & Help Babies Organization (TaHB), Texas, USA

Topic:
PPHN in Neonates-Novel Therapeutic Modalities.

Dr. Siddu Charki

Chief Consultant Neonatologist
Asst. Prof, Dept of Paediatrics,
BLDE(DU), Shri B. M. Patil Medical College, Hospital & RC, Vijayapura

Topic:
Patent Ductus Arteriosus- A Dilemma of DOGMA

REGISTRATION LINK

HOSTED BY: DEPT OF PAEDIATRICS

Note: Zoom Link will be shared to the registered participants one day before the Webinar. Visit: www.bldedu.ac.in

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SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

In collaboration with

MORARJI DESAI NATIONAL INSTITUTE OF YOGA CENTRE, NEW DELHI

INVITATION

Virtual Inauguration of

"Advance Centre of Yoga for Cardiac Prevention and Rehabilitation"

Centre for Yoga and Exercise Science Department of Physiology

Chief Guest :

Dr. M. B. Patil

Honorable Chancellor, BLDE (Deemed to be University),
Shri B M Patil Medical College Hospital and Research Centre, Vijayapura

Guest of Honor :

Dr. Ishwar V. Basavaraddi

Director, Morarji Desai National Institute of Yoga

Dr. M. S. Biradar

Honorable Vice-Chancellor, BLDE (Deemed to be University),
Shri B M Patil Medical College Hospital and Research Centre, Vijayapura

President :

Dr. Aravind V. Patil

Dean Faculty of Medicine BLDE (Deemed to be University)
Shri B M Patil Medical College Hospital and Research Centre, Vijayapura

Date: 10-11-2020, Time: 10-11am Day : Tuesday

Venue: Academic Council Hall, University Building

This Centre will provide Health, Harmony and Happiness for community of this part of Karnataka, India.

Dr Aravind Patil	Dr. Sumangala Patil	Dr. Jyoti Khodnapur
Principal	HoD, Physiology	Co-ordinator

"PRA" YOGA (Prevention, Research and Academics YOGA Centre

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SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

In collaboration with

MORARJI DESAI NATIONAL INSTITUTE OF YOGA CENTRE, NEW DELHI

"Advance Centre of Yoga for Cardiac Prevention and Rehabilitation"

Centre for Yoga and Exercise Science Department of Physiology

Program Schedule

Welcome Address and Briefing Of Advance Centre of Yoga for Cardiac Prevention and Rehabilitation	**Dr. Sumangala Patil** HOD, Department of Physiology
Introduction of Guests	**Dr. Pallavi Kanthe** Assistant Professor, Department of Physiology
Inauguration	**Lighting the Lamp**
Guest of Honor Speech	**Dr. Ishwar V. Basavaraddi** Director, Morarji Desai National Institute of Yoga
Speech by our Guest of Honor	**Dr. M. S. Biradar** Honorable Vice Chancellor, BLDE (Deemed to beUniversity)
Chief Guest speech	**Dr. M. B. Patil** Chancellor, BLDE (Deemed to beUniversity)
Presidential Remarks	**Dr. Aravind Patil** Principal, Dean Faculty of Medicine, Chairman Centre for Advance Centre of Yoga for Cardiac Prevention and Rehabilitation, BLDE (Deemed to be University), Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura
Vote of Thanks	**Dr. Jyoti Khodnapur** Co-ordinator Centre for Advance Centre of Yoga for Cardiac Prevention and Rehabilitation

BLDE (Deemed to be University)

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

In collaboration with

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INVITATION

Virtual Inauguration of

"Advance Centre of Yoga for Cardiac Prevention and Rehabilitation"

Centre for Yoga and Exercise Science Department of Physiology

Date: 10-11-2020, Time: 10-11am Day : Tuesday
Venue: Academic Council Hall, University Building

Book Post

To,

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In collaboration with
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REPORT

Virtual Inauguration of
"Advance Centre of Yoga for Cardiac Prevention and Rehabilitation"
Centre for Yoga and Exercise Science
Department of Physiology

The virtual inaugural ceremony of the Advance Centre of Yoga for Cardiac Prevention and Rehabilitation commenced by praying Vignahartha Lord Sri Ganesh followed by lighting of lamp by a group of dignitaries of BLDE (Deemed to be University) and Morarji Desai National Institute of Yoga (Ministry of AYUSH)- Dr. M. B. Patil (Chancellor, BLDE (Deemed to be University) and Chief Guest of the ceremony), Dr. Ishwar V. Basavaraddi (Director, Morarji Desai National Institute of Yoga and Guest of Honor of the ceremony), Dr. M. S. Biradar (Vice Chancellor BLDE (Deemed to be University) and Guest of Honor of the ceremony), Dr. Aravind Patil (Principal, Dean Faculty of Medicine, Chairman Centre for Advance Centre BLDE (Deemed to be University) and President of the ceremony) and Dr. SumangalaPatil (HOD, Physiology).

This centre is the first private University yoga centre which is recognised by Morarji Desai National Institute of Yoga Centre, New Delhi, Ministry of AYUSH, and Government of India. The center aims to provide inter-disciplinary researches and Ph.D in Allied health sciences (Yoga) and Faculty medicine, under joint PhD supervision program. To promote inter-disciplinary academic activities, certificate courses, diplomas, skill development etc. to promote institutional and individual contacts among scholars, students and personnel of both the institutions. To provide opportunities for both faculty, scientists, staff and students to make optimal use of the expertise and facilities available in both the organizations through training o faculty/students/staff and through exchange of thoughts and ideas by brain storming sessions/workshops/seminars/conferences and meetings etc. To work jointly for the common research interest at a national and international levels. This includes preparation of proposals and their implementation as per the National Health

Priorities. To support the exchange of academic, research and training material. To share experiences, expertise and best practices concerning institutional administration and management. To encourage any other activities that both the institutions/parties agreed upon for mutual benefits.

Dr. Sumangala Patil, HOD, Physiology formally welcomed all the dignitaries present on the dais, virtually and participants the inaugural gathering.

Dr. Jyoti Khodnapur, presented preamble for the Initiative of the Advance Centre of Yoga for Cardiac Prevention and Rehabilitation.

Dr. Ishwar V. Basavaraddi (Director, Morarji Desai National Institute of Yoga and Guest of Honor of the ceremony) enlightened about the collaboration and impact of yoga at present era of medicine for Cardiac Prevention and Rehabilitation.

Dr. M. B. Patil (Chancellor, BLDE (Deemed to be University) and Chief Guest of the ceremony), described the importance of yoga and motivated everyone to get benefited.

Dr. M. B. Biradar, (Vice Chancellor BLDE (Deemed to be University) and Guest of Honor of the ceremony) thanked the MDNIY, Ministry of AYUSH for MOU with BLDE(DU). He said, he will continue to support the programs of the centre for growth of the centre as one of the centre of excellence.

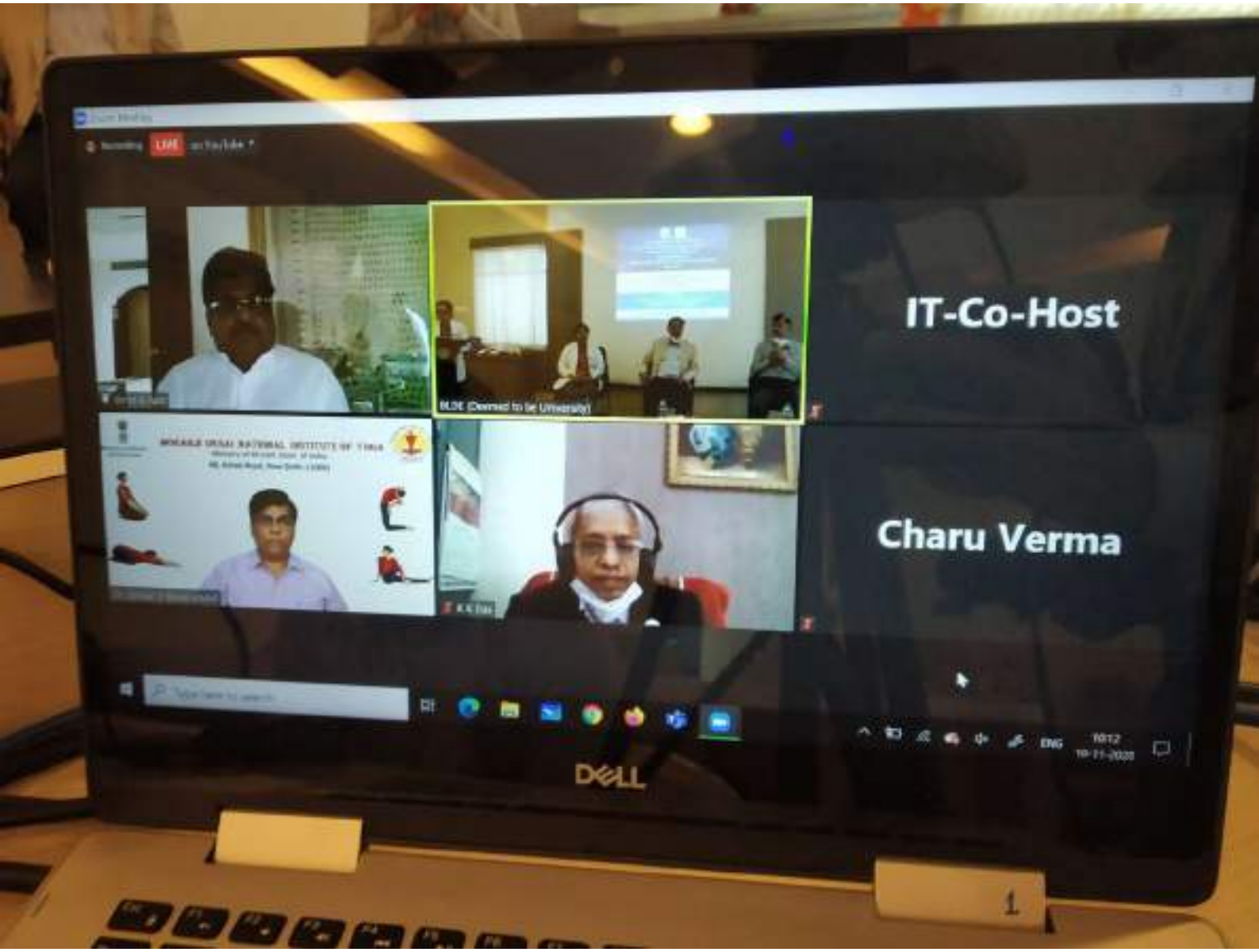
Dr. Aravind Patil (Principal, Dean Faculty of Medicine, Chairman Centre for Advance Centre BLDE (Deemed to be University) presided and in his presidential address praised the collaboration and offered all the assistance required.

In the end, Dr. Jyoti Khodnapur (Co-ordinator, Centre of Yoga for Cardiac Prevention and Rehabilitation) offered a vote of thanks to Dr. M. B. Patil (Chancellor, BLDE (Deemed to be University) and Chief Guest of the ceremony), Dr. Ishwar V. Basavaraddi (Director, Morarji Desai National Institute of Yoga and Guest of Honor of the ceremony), Dr. M. S. Biradar (Vice Chancellor BLDE (Deemed to be University) and Guest of Honor of the ceremony), Dr. Aravind Patil (Principal, Dean Faculty of Medicine, Chairman Centre for Advance Centre BLDE (Deemed to be University) and President of the ceremony). She thanked all the invited guests and participants for gracing the occasion by their solemn presence. She also thanked all staff members of MDNIY and IT department.

Inaugural ceremony concluded with National anthem.

Dr Jyoti Khodnapur

Dr. Sumangala Patil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In collaboration with

MORARJI DESAI NATIONAL INSTITUTE OF YOGA CENTRE, NEW DELHI

"Advance Centre of Yoga for Cardiac Prevention and Rehabilitation"

Centre for Yoga and Exercise Science

Department of Physiology

Introducing

Foundation Course in Yoga Science for Wellness

Preamble: Yoga and meditation practices are recognized as holistic approaches to physical and mental wellness of human being. It has been recognized that medical education and practice are very intensive and stressful. Medical teachers and medical practitioners need support emotional, intellectual and physical well being to lead happy and peaceful life. BLDE (DU) in collaboration with Moraji Desai National Institute of Yoga, New Delhi an Institution of Ministry of AYUSH, Govt of India conducting Foundation Course in Yoga Science for Wellness.

Number of Candidates: **Maximum 30**	**Instruction Mode:** **Online**
Resource person: **from MDNIY**	**Course duration:** **50 hours for one month**
Fees:Promotional Course Fee: **750=00**	**Certificate/Course material/Prospectus :250=00**

For Registration contact:

Dr. Jyoti Khodnapur, Department of Physiology (Cell no:8792544340)

Last date for registration:

07.12.2020 before 5.00pm

Course resumes from 10/12/2020 Monday to Friday (10/12/2020 to 09/01/2021)

Time: Evening 6 to 8 pm

पेटेंट कार्यालय
शासकीय जर्नल

OFFICIAL JOURNAL
OF
THE PATENT OFFICE

निर्गमन सं. 41/2020	शुक्रवार	दिनांक: 09/10/2020
ISSUE NO. 41/2020	FRIDAY	DATE: 09/10/2020

पेटेंट कार्यालय का एक प्रकाशन
PUBLICATION OF THE PATENT OFFICE

(12) PATENT APPLICATION PUBLICATION
(19) INDIA
(22) Date of filing of Application :20/09/2020
(21) Application No.202041040723 A
(43) Publication Date : 09/10/2020

(54) Title of the invention : PHARMACEUTICAL FORMULATIONS OF ELECTRO-RESPONSIVE SMART HYDROGEL FOR TRANSDERMAL DRUG DELIVERY

(51) International classification	:A61K 9/70
(31) Priority Document No	:NA
(32) Priority Date	:NA
(33) Name of priority country	:NA
(86) International Application No	:NA
Filing Date	:NA
(87) International Publication No	: NA
(61) Patent of Addition to Application Number	:NA
Filing Date	:NA
(62) Divisional to Application Number	:NA
Filing Date	:NA

(71)**Name of Applicant :**
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1)Dr. Raghavendra V. Kulkarni
2)Mrs. Sudha B. Patil
3)Dr. Krishnamachari G. Akamanchi
4)Dr. Kusal. K. Das

(57) Abstract :
The present invention relates to the development of electro-responsive transdermal delivery systems (ETDDS) with smart hydrogel for transdermal delivery of the drugs. It particularly relates to the pharmaceutical formulations of electro-responsive smart hydrogel for transdermal drug delivery. More particularly it relates to the synthesis of electro-responsive polyacrylamide-grafted-inulin (PAAm-g-INU) copolymer by free radical polymerization under the nitrogen atmosphere and then development of ETDDS by utilizing synthesized PAAm-g-INU. The membrane-controlled ETDDS were developed utilizing drug-loaded PAAm-g-INU hydrogel as the reservoir and cross-linked inulin-poly(vinyl alcohol) films as rate controlling membranes (RCM). It specifically relates to the development of membrane-controlled ETDDS utilizing drug-loaded PAAm-g-INU hydrogel as the reservoir and cross-linked inulin-poly(vinyl alcohol) films as RCMs for the transdermal delivery of rivastigmine tartarate at the requisite amount, requisite time and at requisite site in the human body that reduces the adverse effects, dose, and improves drug efficiency and patient compliance. The study revealed that the novel electrically responsive PAAm-g-INU is a useful copolymer for transdermal drug delivery triggered by an electric stimulus for on-demand dug release.

No. of Pages : 36 No. of Claims : 10

(12) PATENT APPLICATION PUBLICATION
(19) INDIA
(22) Date of filing of Application :30/09/2020

(21) Application No.202041042648 A
(43) Publication Date : 09/10/2020

(54) Title of the invention : PHARMACEUTICAL COMPOSITION OF ELECTRICALLY-SENSITIVE POLYACRYLAMIDE-GRAFTED-GUM TRAGACANTH COPOLYMER FOR ELECTRO-MODULATED TRANSDERMAL DRUG DELIVERY

(51) International classification :C07D271/07
(31) Priority Document No :NA
(32) Priority Date :NA
(33) Name of priority country :NA
(86) International Application No :NA
Filing Date :NA
(87) International Publication No : NA
(61) Patent of Addition to Application Number :NA
Filing Date :NA
(62) Divisional to Application Number :NA
Filing Date :NA

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2)Mr. Ravindra P. Birajdar
3)Dr. Krishnamacharya G. Akamanchi
4)Dr. Mallanagouda S. Biradar
5)Dr. Kusal. K. Das

(57) Abstract :
PHARMACEUTICAL COMPOSITION OF ELECTRICALLY-SENSITIVE POLYACRYLAMIDE-GRAFTED-GUM TRAGACANTH COPOLYMER FOR ELECTRO-MODULATED TRANSDERMAL DRUG DELIVERY ABSTRACT: The present invention relates to development of drug loaded electro-modulated transdermal delivery systems (EMTDS) by using polyacrylamide-grafted-gum tragacanth (PAAm-g-GT) copolymer for transdermal delivery of the drugs. A copolymeric hydrogel of PAAm-g-GT was used as drug reservoir and glutaraldehyde cross-linked blend films of poly(vinyl alcohol) and GT were used as rate controlling membranes (RCMs). More particularly it relates to the synthesis of electrically-sensitive polyacrylamide-grafted-gum tragacanth copolymer by free radical polymerization technique in the nitrogen ambience to synthesize an electrically-sensitive PAAm-g-GT. Quetiapine fumerate loaded electro-modulated transdermal delivery systems (EMTDS) were developed using polyacrylamide-grafted-gum tragacanth (PAAm-g-GT) copolymer. Drug permeation experiments in the absence of electric stimulus resulted in miniature quantity of drug release against increased permeation as observed in the presence of electric stimulus. An increased flux value of three times was recorded under application of electric stimulus. The quetiapine permeation was noticeably increased upon increase in electric stimulus. Increased and decreased drug permeation was reported for on• and off• electric stimulus respectively in a pulsatile pattern. The skin histopathology findings revealed the changes in the skin structure after application of electrical stimulus. The PAAm-g-GT copolymer is a competent biomaterial that can be used for development of electro-modulated transdermal delivery systems for on-demand drug delivery.

No. of Pages : 29 No. of Claims : 10

(12) PATENT APPLICATION PUBLICATION
(19) INDIA
(22) Date of filing of Application :27/01/2020

(21) Application No.202041003507 A
(43) Publication Date : 25/09/2020

(54) Title of the invention : ENZYME MEDIATED CELL DISRUPTION BASED EFFICIENT EXTRACTION OF CAMPTOTHECIN FROM NATHAPOETIDA NIMMONIANA PLANT

(51) International classification	:C07D0491220000, C12N0001060000, G01N0001400000, C12N0009500000, C12P0007100000
(31) Priority Document No	:NA
(32) Priority Date	:NA
(33) Name of priority country	:NA
(86) International Application No	:NA
Filing Date	:NA
(87) International Publication No	: NA
(61) Patent of Addition to Application Number	:NA
Filing Date	:NA
(62) Divisional to Application Number	:NA
Filing Date	:NA

(71)**Name of Applicant :**
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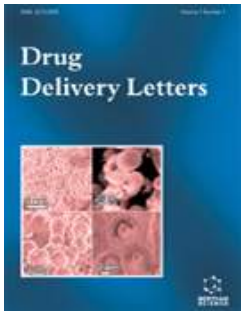
Address of Applicant :BLDEA™s SSM College of Pharmacy & Research Centre, Vijayapur, India 586 103 Karnataka India
2)Dr. Dhiraj M. Patil
3)Dr. Krishnacharya G Akamanchi
(72)**Name of Inventor :**
1)Dr. Dhiraj M. Patil
2)Dr. Krishnacharya G Akamanchi
3)Dr. Raghavendra V. Kulkarni

(57) Abstract :
Abstract: In the present invention enzyme cell disruption assisted highly efficient extraction of Camptothecinis disclosed. Camptothecin is an anticancer alkaloid present in many plant species, however, nathapoetida nimmoniana plant is a very attractive commercial source. Camptothecin is present in a very small quantity moreover the plant is slow growing tree and is classified under endangered species. Therefore efficient extraction of camptothecin is very important not from a commercial point of view but also from preserving the plant specifies. Camtothecin is mainly present in plant stem and is distributed from periphery to deep inside. Therefore disruption of the cell to facilitate deep penetration of solvent to effect efficient extraction of camptothecin is very crucial. In the present invention, cell disruption is achieved by enzymatic hydrolysis of the cell wall is effected by using enzymes cellullase and xylinase and highly efficient extraction are disclosed.

No. of Pages : 14 No. of Claims : 6



Research Article

Functionally Tailored Electro-Sensitive Poly(Acrylamide)-g-Pectin Copolymer Hydrogel for Transdermal Drug Delivery Application: Synthesis, Characterization, In-vitro and Ex-vivo Evaluation

Author(s): Sudha B. Patil, Syed Z. Inamdar (http://orcid.org/0000-0003-2448-6917), Kakarla R. Reddy, Anjanapura V. Raghu (http://orcid.org/0000-0003-0299-7409), Krishnamachari G. Akamanchi (http://orcid.org/0000-0001-7804-049X), Arun C. Inamadar (http://orcid.org/0000-0002-8877-3723), Kusal K. Das (http://orcid.org/0000-0002-3641-2117), Raghavendra V. Kulkarni*

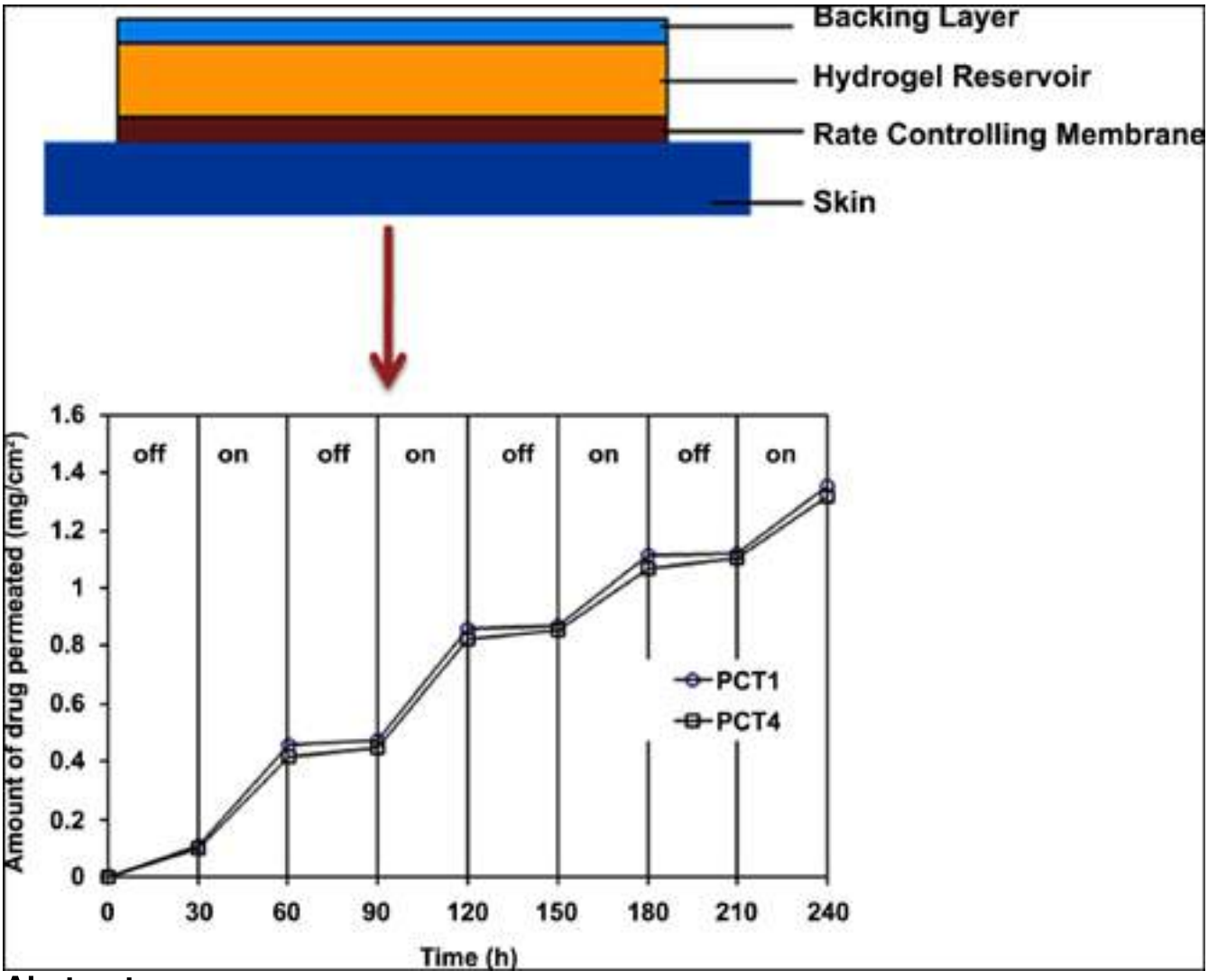
Journal Name: Drug Delivery Letters

Volume 10 , Issue 3 , 2020

DOI : 10.2174/2210303110666200206114632 (https://doi.org/10.2174/2210303110666200206114632)

Journal Home (/node/696)

Graphical Abstract:



Abstract:

Background: To develop electro-sensitive transdermal drug delivery systems (ETDDS) using polyacrylamide-grafted-pectin (PAAm-g-PCT) copolymer hydrogel for rivastigmine delivery.

Methods: Free radical polymerization and alkaline hydrolysis technique was employed to synthesize PAAm-g-PCT copolymer hydrogel. The PAAm-g-PCT copolymeric hydrogel was used as a reservoir and

cross-linked blend films of PCT and poly(vinyl alcohol) as rate-controlling membranes (RCMs) to prepare ETDDS.

Results: The pH of the hydrogel reservoir was found to be in the range of 6.81 to 6.93 and drug content was 89.05 to 96.29%. The thickness of RCMs was in the range of 51 to 99 μ and RCMs showed permeability behavior against water vapors. There was a reduction in the water vapor transmission rate as the glutaraldehyde (GA) concentration was increased. The drug permeation rate from the ETDDS was enhanced under the influence of electric stimulus against the absence of an electric stimulus. The increase in flux by 1.5 fold was recorded with applied electric stimulus. The reduction in drug permeability observed when the concentration of GA was increased. Whereas, the permeability of the drug was augmented as an electric current was changed from 2 to 8 mA. The pulsatile drug release under “on– off” cycle of electric stimulus witnessed a faster drug release under ‘on’ condition and it was slow under ‘off’ condition. The alteration in skin composition after electrical stimulation was confirmed through histopathology studies.

Conclusion: The PAAm-g-PCT copolymer hydrogel is a useful carrier for transdermal drug delivery activated by an electric signal to provide on-demand release of rivastigmine.

Keywords: Electro-sensitive, hydrogel, grafting, transdermal drug delivery, Alzheimer`s disease, rivastigmine.

References: ❯

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Article Details

VOLUME: 10
ISSUE: 3
Year: 2020
Published on: 10 September, 2020
Page: [185 - 196]
Pages: 12
DOI: 10.2174/2210303110666200206114632
(https://doi.org/10.2174/2210303110666200206114632)
Price: $25

1 Total citation
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Drug Delivery Letters

Functionally Tailored Electro-Sensitive Poly(Acrylamide)-g-Pectin Copolymer Hydrogel for Transdermal Drug Delivery Application: Synthesis, Characterization, In-vitro and Ex-vivo Evaluation (https://www.eurekaselect.com/node/179060/?tracking-code=4)
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Drug Delivery Letters

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(https://www.projectcounter.org/counter-user/bentham-science/)

ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Experimentally Validated QSAR Model for Surface pK_a Prediction of Heterolipids Having Potential as Delivery Materials for Nucleic Acid Therapeutics

Dinesh M. Dhumal, Pankaj D. Patil, Raghavendra V. Kulkarni, and Krishnacharya G. Akamanchi*

Cite This: *ACS Omega* 2020, 5, 32023−32031

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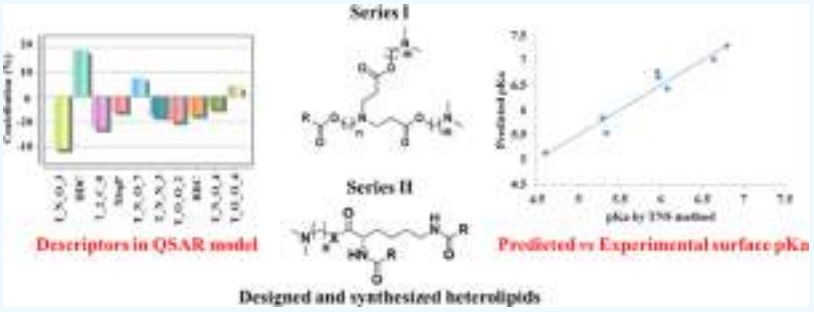
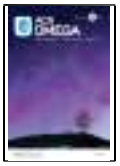


ABSTRACT: The application of lipid-based drug delivery technologies for bioavailability enhancement of drugs has led to many successful products in the market for clinical use. Recent studies on amine-containing heterolipid-based synthetic vectors for delivery of siRNA have witnessed the United States Food and Drug Administration (USFDA) approval of the first siRNA drug in the year 2018. The studies on various synthetic lipids investigated for delivery of such nucleic acid therapeutics have revealed that the surface pK_a of the constructed nanoparticles plays an important role. The nanoparticles showing pK_a values within the range of 6−7 have performed very well. The development of high-performing lipid vectors with structural diversity and falling within the desired surface pK_a is by no means trivial and requires tedious trial and error efforts; therefore, a practical solution is called for. Herein, an attempt to is made provide a solution by predicting the statistically significant pK_a through a predictive quantitative structure−activity relationship (QSAR) model. The QSAR model has been constructed using a series of 56 amine-containing heterolipids having measured pK_a values as a data set and employing a partial least-squares regression coupled with stepwise (SW-PLSR) forward algorithm technique. The model was tested using statistical parameters such as r^2, q^2, and pred_r^2, and the model equation explains 97.2% (r^2 = 0.972) of the total variance in the training set and it has an internal (q^2) and an external (pred_r^2) predictive ability of ∼83 and ∼63%, respectively. The model was validated by synthesizing a series of designed heterolipids and comparing measured surface pK_a values of their nanoparticle assembly using a 2-(*p*-toluidino)-6-napthalenesulfonic acid (TNS) assay. Predicted and measured surface pK_a values of the synthesized heterolipids were in good agreement with a correlation coefficient of 93.3%, demonstrating the effectiveness of this QSAR model. Therefore, we foresee that our developed model would be useful as a tool to cut short tedious trial and error processes in designing new amine-containing heterolipid vectors for delivery of nucleic acid therapeutics, especially siRNA.

1. INTRODUCTION

Drug delivery systems aim at improving biopharmaceutics features, such as stability, bioavailability, and targeting, and facilitate controlled delivery to maximize drug potency while minimizing side effects and toxicity. Among drug delivery systems, lipid-based drug delivery systems (LBDDS) are extensively studied for bioavailability enhancement and the technologies developed are utilized in a number of the United States Food and Drug Administration (USFDA) approved drugs.[1] Inspired by the success of LBDDS, similar efforts have been dedicated toward the development of lipid-based vectors particularly for siRNA delivery and gene therapy.[2] Among lipid-based vectors, cationic lipid-based vectors have proven to be the most successful candidates and have been widely implemented in clinical use. However, the cationic lipids being permanently charged entities suffer from toxicity.[3] Recent investigations on ionizable amine-containing heterolipids as drug carriers have successfully delivered siRNA in vivo. The surface pK_a, exhibited by the heterolipids in the nanoparticle

Received: October 9, 2020
Accepted: November 17, 2020
Published: November 30, 2020



assembly environment, in the range of 6.0−7.0 was found to be an important feature to convey their efficacy.[4−9] For example, Patisiran (Alnylam Pharmaceuticals) as the first siRNA drug approved by the USFDA has used an ionizable heterolipid-based delivery system.[10,11] Mechanistically, the ionizable and hydrophobic moieties in the heterolipids encapsulate the naked anionic siRNA through electrostatic interactions and impart overall lipophilicity to facilitate passage through the cell membranes. Heterolipids containing unsaturated fatty acid chains were found to perform better as a transporter across the membrane. This finding is explained by hypothesizing that the unsaturation in the hydrophobic tail of the heterolipids introduces "kink" in their structure resulting in the tail adopting a cone shape geometry to promote the formation of an inverted non-bilayer hexagonal H_{II} phase. The hexagonal H_{II} phase induces intercellular fusion leading to destabilization of the biological membrane and facilitation of the transport.[12] In addition, there are electrostatic interactions between carrier lipids and naturally occurring anionic membrane phospholipids. These interactions play a significant role in the successful transport of siRNA—lipid complex system. After entering into the cytoplasm of the target cells, the complex overcomes the acidic endocytic pathway through the proton sponge effect, leading to endosomal escape.[13] Despite the success of the ionizable heterolipid systems to deliver siRNA, clinical applications are hindered due to their undesired immunostimulatory effects and poor pharmacokinetics.[2,14,15] Hence, efforts are ongoing to find better candidates by synthesizing and screening new ionizable heterolipids.

The surface pK_a is the pK_a of the functional heterolipid in its nanoparticle assembly, which is different from the conventional solution-phase pK_a, and it defines the ionization behavior of the functional heterolipid in the nanoparticle assembly. The surface pK_a along with other structural features of the heterolipid vectors plays a crucial role in the entire process, including the encapsulation, transport, and endosomal escape of siRNA. The surface pK_a of heterolipids in its relevant nanoparticle environment is determined by adopting a fluorescence-based (2-(*p*-toluidino)-6-napthalenesulfonic acid (TNS)) assay method.[16] Currently, the TNS method is very effective in determining the pK_a of lipid nanoparticles (LNPs) but suffers major drawbacks like the formulation of nanoparticulate assembly and is tedious to applying the screening of a large number of samples. To reduce the number of synthesized and tested compound knowing a priori the surface pK_a of the amine-containing heterolipids, a structure-property-based theoretical model could be of great utility. This would not only help in introducing structural diversity in heterolipids and at the same time retaining the desired surface pK_a but also in curtailing the number of heterolipids to be synthesized.

Our group has been involved in designing and developing new amine-containing heterolipid-based carriers for delivery of therapeutic molecules.[17−22] In the present work, by employing a data set from the literature, a quantitative structure−activity relationship (QSAR) model has been developed using a partial least-squares regression (PLSR) technique for prediction of surface pK_a.[4] The developed model has been further validated by synthesizing selected newly designed heterolipids and comparing their predicted versus experimentally determined pK_a values. The outcome of the work offers a new perspective on the design and development of new amine-containing heterolipids with desired surface pK_a for drug delivery applications.

2. EXPERIMENTAL SECTION

2.1. Materials. Oleic acid (technical grade, 90%), 2-(dimethylamino)ethanol, 3-(dimethylamino)-1-propanol, cholesterol, 6-(*p*-toluidino)-2-naphthalenesulfonic acid sodium salt (TNS), cholesterol distearoylphosphatidylcholine (DSPC), and MPEG-2000-DSPE sodium salt were purchased from Sigma-Aldrich. Acryloyl chloride was obtained from Alfa Aesar. 3-Amino-1-propanol, ethanolamine, 4-(dimethylamino)-pyridine (DMAP), and 1-hydroxybenzotriazole (HOBt) were obtained from Spectrochem (India). Triethylamine (TEA) and thionyl chloride were obtained from SD Fine Pvt. Ltd. (India). 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDAC) was purchased from Sisco Research Laboratories Pvt. Ltd (India). Dichloromethane (DCM), tetrahydrofuran (THF), and other solvents used were of analytical grade. Precoated silica-gel $60F_{254}$ plates used for thin-layer chromatography (TLC) to monitor reactions were obtained from Merck. Water used in the entire study was obtained from the Milli-Q water purification system of Millipore Corporation (Bedford).

2.2. Development of the QSAR Model. The molecular modeling studies were performed on an Acer computer having Intel core i3-2310M Processor and Windows 7 operating system using VLife MDS (molecular design suite) 4.3 molecular modeling software supplied by VLife Sciences Technologies Pvt. Ltd., Pune, India.

A data set of 56 nitrogen-containing heterolipids reported by Jayaraman et al. with surface pK_a determined by the TNS method was used for model development.[4] The cleaned and three-dimensional (3D) optimized structures of all heterolipids were constructed in ChemSketch version 12.0 (Table S1). The 2D-QSAR study requires the calculation of molecular descriptors. Accordingly, a large number of two-dimensional (2D) physiochemical descriptors were calculated by QSAR Plus module within VLife Molecular Design Suite. For the development of the model from a total of 56 molecules, 50 molecules were selected and the remaining six molecules, 2, 5, 43, 50, 51, and 52, were eliminated as statistical outliers because of the non-optimum *Z* score.[23] The 50 molecules were divided manually into two sets, a training set of 38 molecules and a test set of 12 molecules. The QSAR model was developed using the partial least-squares regression (PLSR) technique by the forward variable selection process with pK_a activity fields as dependent variables and the calculated 116 physicochemical descriptors having a cross-correlation limit of 0.5 as independent variables.[24,25] The developed QSAR model was evaluated using the statistical measures: r^2—squared correlation coefficient, q^2—cross-validated r^2 (by leaving one out), which is the relative measure of the quality of fit, pred_r^2—r^2 for the external test set, r^2_se—standard error of the squared correlation coefficient, q^2_se—standard error of cross-validation, pred_r^2se—standard error of external test set prediction, Fischer's value *F*—a test that represents the *F* ratio between the variance of the calculated and observed activities, *N*—number of observations (molecules) in the training set, *Z* score—the score calculated by q^2 in the randomization test, best_*r* and_q^2—the highest q^2 value in the randomization test, and alpha_*r* and_q^2—the statistical significance parameters obtained by the randomization test.

The calculated value of the *F*-test when compared with the tabulated value of the *F*-test shows the level of statistical significance (99.99%) of the QSAR model. The low standard

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ACS Omega 2020, 5, 32023−32031

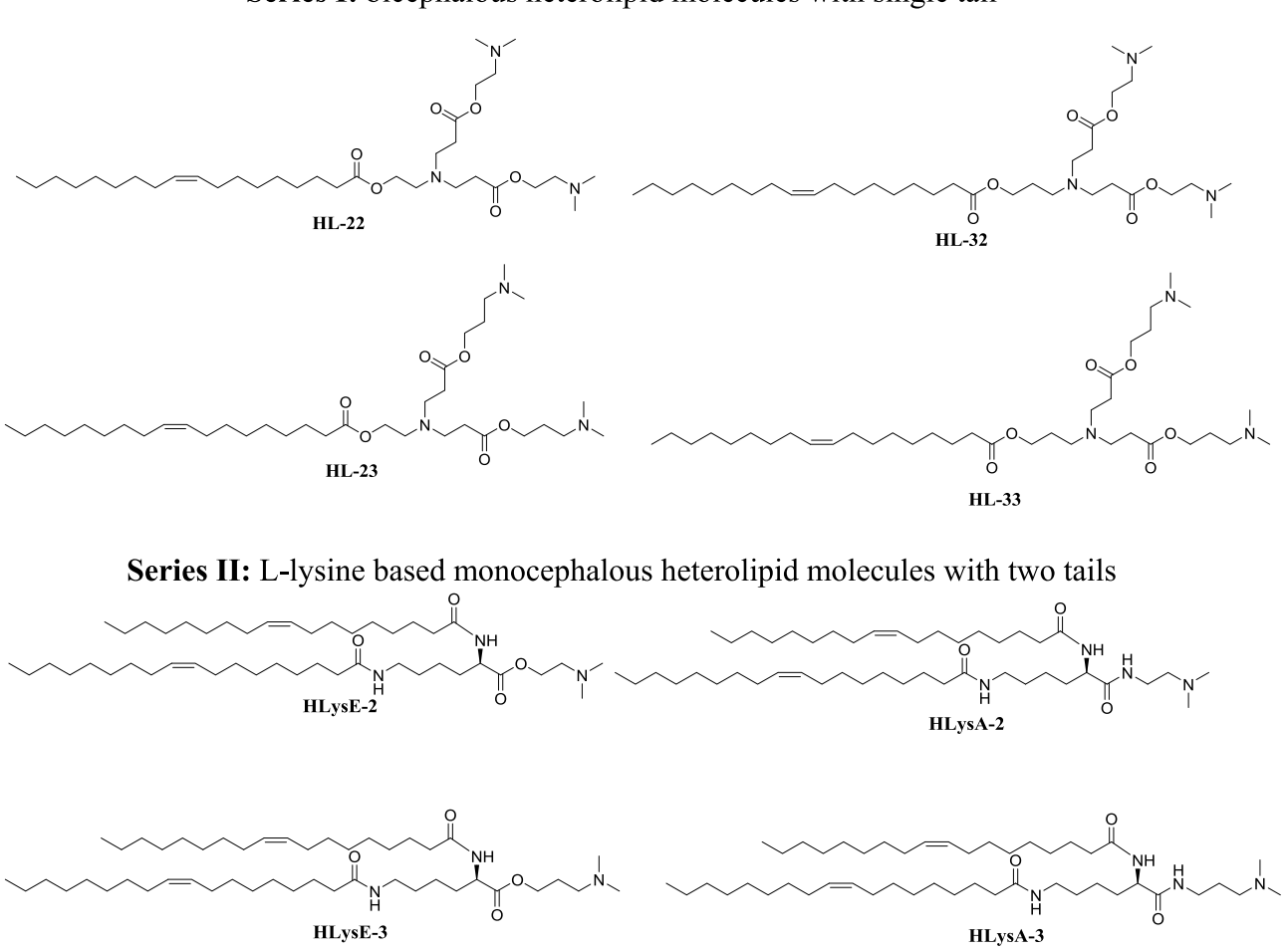

Figure 1. Designed heterolipid molecules selected for synthesis.

errors of pred_r^2se, q^2_se, and r^2_se show the absolute quality of fitness of the model. The generated QSAR model was validated for the predictive ability inside the model using cross-validation (LOO) for q^2. External validation, which is a more robust alternative method for validation, was performed by dividing the data into a training set and a test set and calculating pred_r^2. The high pred_r^2 and low pred_r^2_se would imply the high predictive ability of the model. For selecting the optimal model, r^2, q^2, and pred_r^2 values were used as deciding factors.

2.3. Design of Heterolipids for the Validation of the QSAR Model. Several molecules based on chemistry developed in our lab, and literature data were designed and few selected were synthesized for validation and evaluating the predictive ability of the QSAR model.[22,26] Two series of heterolipid molecules were designed consisting of: series I bicephalous with a single tail and series II monocephalous with two tails. The four designed molecules from series I, designated as HL-22, HL-23, HL-32, and HL-33, and the four from series II, designated as HLysA-3, HLysA-2, HLysE-3, and HLysE-2, having a widespread range of predicted pK_a of 4.64−6.80 were selected for synthesis (Figure 1). From a chemical structure standpoint, all of the molecules are homologous with a varying number of methylene groups in the head moiety and carry oleic acid chain(s) having a cis-double bond. Other distinguishing structural features of the molecules are that series I has ester linkers and three basic tertiary amino moieties, one at the branching point and two at the periphery in the head groups, whereas series II with amide linkers has only one basic tertiary amino moiety at the periphery in the head groups.

2.4. Synthesis of Heterolipids. *2.4.1. General Schemes for the Synthesis of Series I Bicephalous Single-Tailed Heterolipids. 2.4.1.1. Step-I: Synthesis of Aminoalkyl Acrylates (Scheme 1, Step I).* To a stirred solution of *N*,*N*-(dimethylamino) alcohol (**2a**/**2b**) (1.0 equiv) in 100 mL of tetrahydrofuran (THF) with triethylamine (TEA) (3.0 equiv) cooled to 0 °C was added acryloyl chloride (**1**) (1.2 equiv) dropwise under nitrogen and stirred for 3 h maintaining the reaction temperature at 0 °C. After completion of the reaction (monitored by TLC), the reaction mixture was filtered and the filtrate was concentrated to obtain the crude product as residue. The residue was purified by column chromatography using neutral alumina and dichloromethane (DCM) as stationary and mobile phases, respectively, to obtain pure aminoalkyl acrylate (**3a**/**3b**) as a light yellowish liquid (Scheme 1).

*2.4.1.2. Step-II: Synthesis of Heterodendrons **5a**−**5d** (HD-22, HD-32, HD-23, and HD-33) (Scheme 1, Step II).* A Michael addition reaction between amino alcohol (**4a**/**4b**) (1.0 equiv) and aminoalkyl acrylate **3a**/**3b** (4.0 equiv) was carried out as follows: **4a**/**4b** was added dropwise to aminoalkyl acrylate (**3a**/**3b**) at 0 °C under constant stirring and allowed to stand till the temperature of the reaction mass

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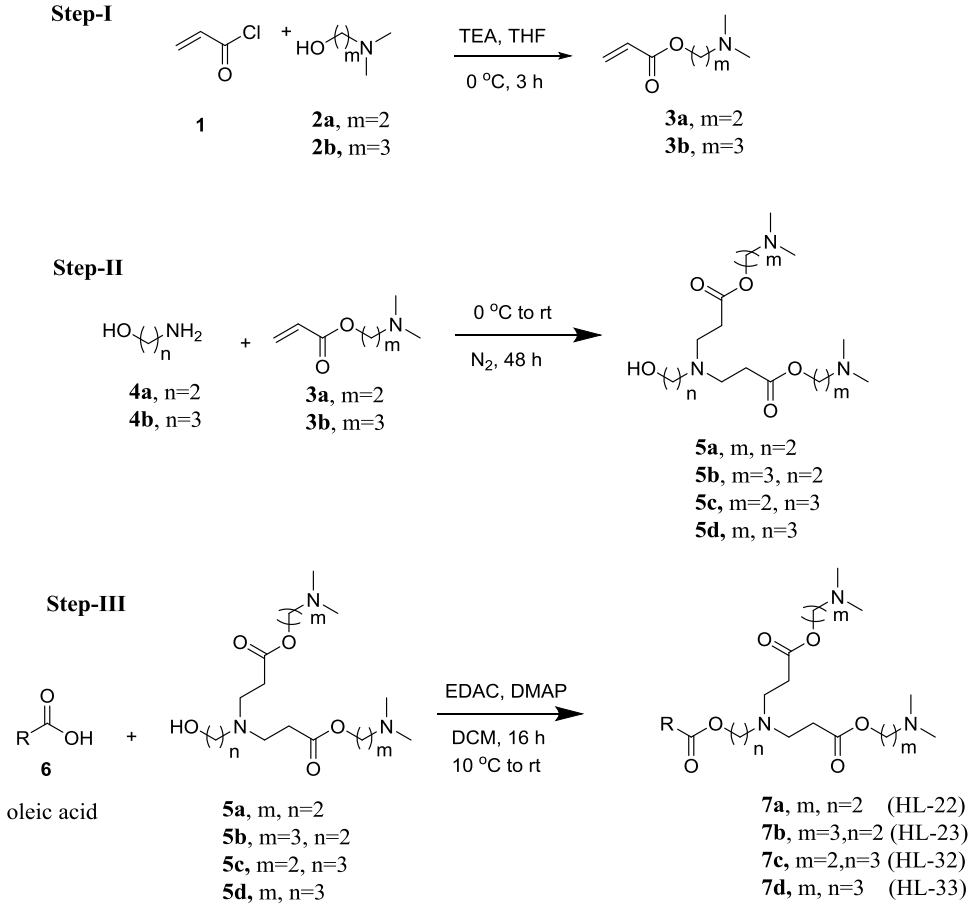
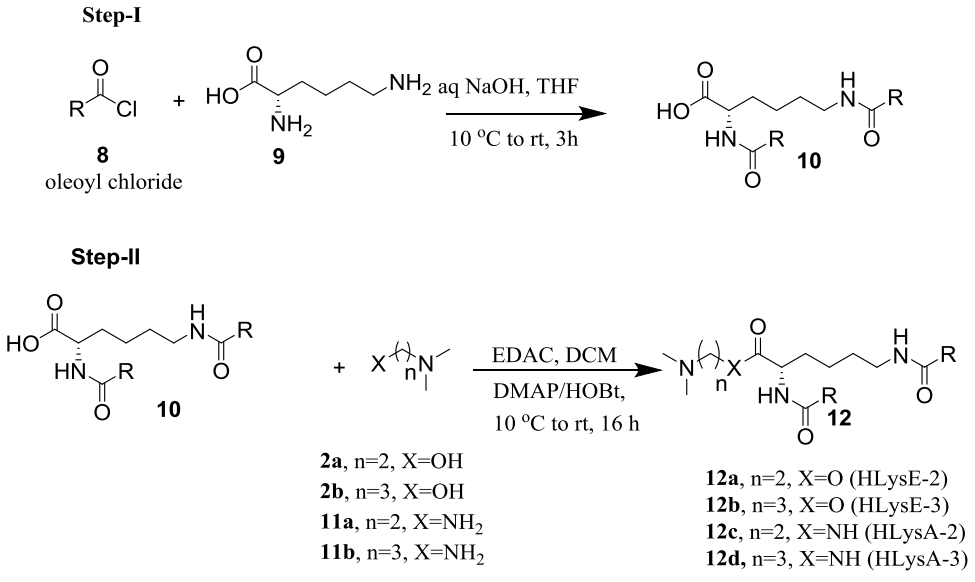
ACS Omega 2020, 5, 32023−32031

Scheme 1. General Scheme for the Synthesis of Series I Bicephalous Single-Tailed Heterolipids



Scheme 2. General Scheme for the Synthesis of Series II L-lysine-Based Monocephalous Two-Tailed Heterolipids



increased to room temperature (RT) and was again stirred continuously for 48 h. The reaction mass was subjected to rota evaporation in vacuo to remove volatiles and to obtain pure heterodendrons **5a−5d** (HD-22/HD-32/HD-23/HD-33) as a light yellowish liquid residue.

2.4.1.3. Step III: Synthesis of Heterolipids 7a−7d (HL-22, HL-32, HL-23, and HL-33) (Scheme 1, Step III). A solution of oleic acid (**6**) (1.0 equiv) in DCM along with EDAC (1.0 equiv) and a catalytical amount of DMAP was stirred at 10 °C for 30 min. To this cold solution was added heterodendron (**5a**/**5b**/**5c**/**5d**) (1.0 equiv) and the solution was again stirred for a further 30 min. The cooling bath was removed and the reaction mass was stirred for 16 h. After the completion of the reaction (by TLC), the solvent was removed from the reaction

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ACS Omega 2020, 5, 32023−32031

mixture under vacuo, and the residue obtained was purified by column chromatography (SiO_2 #60-120) using DCM/methanol (MeOH), 10:2, as the eluent to afford **7a−7d** (HL-22/HL-23/HL-32/HL-33) as a light yellowish sticky mass.

2.4.2. General Schemes for the Synthesis of Series II Lysine-Based Monocephalous Two-Tailed Heterolipids. 2.4.2.1. Step I: Synthesis of N,N′-Dioleoyl-L-lysine 10 (Scheme 2, Step I). L-Lysine (**9**) (1.0 equiv) was added to 100 mL of a 10 mM aq NaOH solution (pH 8) followed by 100 mL of THF. Oleoyl chloride (**8**) (2.0 equiv) was added dropwise to the mixture at 10 °C, and the pH was adjusted to around 8 by adding aliquots of dilute NaOH/HCl. After completion of addition, the temperature of the reaction mass was allowed to rise to room temperature under stirring for 3 h. After completion of the reaction, the reaction mass was neutralized by the addition of dilute HCl. The organic layer was separated, washed with water, dried over anhydrous sodium sulfate, concentrated in vacuo, and the residue obtained was column chromatographed (SiO_2 #60-120) using DCM/MeOH, 10:1, as the eluent to provide *N,N′*-dioleoyl-L-lysine (**10**) (Scheme 2).

*2.4.2.2. Step II: Synthesis of Monocephalous Two-Tailed Heterolipids (HLysE-2, HLysE-3, HLysA-2, and HLysA-3) (Scheme 2, Step II). 2.4.2.2.1. Synthesis of **12a/12b** (HLysE-2/ HLysE-3).* *N,N′*-Dioleoyl-L-lysine (**10**) (1.0 equiv) was dissolved in 50 mL of DCM along with EDAC (1.0 equiv) and a catalytical amount (0.1 equiv) of DMAP at 10 °C and stirred for 30 min. To the stirred mass, *N,N*-dimethylamino alcohol (**2a**/**2b**) (1.0 equiv) was added maintaining the temperature at 10 °C and continued stirring for 30 min. The temperature of the reaction mass was slowly allowed to rise to room temperature and stirring was continued for 16 h. The reaction mass was concentrated under reduced pressure, and the residue obtained was purified by column chromatography (SiO_2 #60-120) using DCM/MeOH, 10:1, as the eluent to afford **12a**/**12b** (HLysE-2, HLysE-3) as a slightly yellowish waxy solid.

*2.4.2.2.2. Synthesis of **12c/12d** (HLysA-2/ HLysA-3).* *N,N′*-Dioleoyl-L-lysine (**10**) (1.0 equiv) was dissolved in 50 mL of DCM in presence of EDAC (1.0 equiv) and HOBt (1 equiv) at 10 °C followed by *N,N*-dimethyldiamine **11a**/**11b**. The reaction mixture was stirred for 30 min at 10 °C, and then the temperature was allowed to rise slowly to room temperature and stirring was continued for 16 h. The reaction mass was concentrated under reduced pressure, and the residue obtained was purified by column chromatography (SiO_2 #60-120) using DCM/MeOH, 10:1, as the eluent to afford **12c**/**12d** as a white solid (HLysA-2/HLysA-3).

2.5. Determination of the Surface pK_a of the Heterolipids in Lipid Nanoparticle (LNP) Assembly by the Anionic Fluorescent Probe TNS. To determine the surface pK_a of the heterolipids, a literature procedure was followed.[4] LNPs consisting of heterolipids were prepared by the dry film method. Heterolipids/DSPC/cholesterol/poly-(ethylene glycol) (PEG)-lipids (40/10/40/10 mol %) were dissolved in equal volumes of the chloroform/methanol mixture, and the solvents were removed using a rotary evaporator to obtain a dry film. The dry film was hydrated in phosphate buffer to achieve a final concentration of ∼6 mM of total lipids. A TNS stock solution of 100 μM was prepared in distilled water. The LNPs were diluted by 2 mL of buffer solutions with pH in the range of 2.5−11 containing 10 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES), 10 mM mesityl(2,4,6-trimethylphenyl) (MES), 10 mM ammonium acetate, and 130 mM NaCl, and an aliquot of the TNS solution was added to give a final concentration of 1 μM. The solutions were vortexed and allowed to equilibrate at room temperature for 30 min. The surface charge of the LNP was monitored at room temperature by determining the TNS fluorescence at each pH using excitation and emission wavelengths of 321 and 445 nm, respectively. A sigmoidal best fit analysis was applied to the fluorescence data and the pK_a was measured as the pH giving rise to the half-maximal fluorescence intensity.

3. RESULTS AND DISCUSSION

3.1. Development of QSAR Model. Selection of molecules in the training set and the test set is a key and important feature of any QSAR model. We chose all those molecules whose activities lie within the range of maximum and minimum pK_a values of 8.12 and 4.17, respectively. Unicolumn statistics for the training and test sets was generated to check the correctness of the selection criteria (Table 1). The maximum and minimum pK_a values in the training and test sets were compared in a way that the maximum value of pK_a of the test set should be less than or equal to the maximum value of pK_a of the training set. Similarly, the minimum value of pK_a of the test set should be higher than or equal to the minimum value of pK_a of the training set.[27] It was found that the test set was interpolative and derived within the range of maximum and minimum pK_a values of 8.12 and 4.17, respectively, of the training set, and average values and standard deviations values of pK_a of the training and test sets provided insights into the relative difference in the mean and point density distributions of the two sets. The mean pK_a value of 7.05 of the test set was higher than the mean pK_a value of 6.61 of the training set, indicating the presence of relatively more active molecules as compared to the inactive molecules. Similarly, a relatively higher standard deviation value of 0.83 of the training set indicates that the training set has widely distributed activity between its molecules as compared to the test set.

Table 1. Unicolumn Statistics for the Training and Test Sets

	pK_a				
	average	maximum	minimum	standard deviation	sum
training	6.6124	8.1200	4.1700	0.8315	251.2700
test	7.0567	8.1100	6.2100	0.5511	84.6800

3.1.1. QSAR Equation. Various QSAR models were developed using the partial least-squares (PLS) technique and a particular equation was selected by optimizing the statistical results generated along with the variation of the descriptors in these models. The statistical significance of the selected QSAR model was further supported by the "fitness plot" obtained. The fitness plot is experimental versus predicted activity of the training set of the molecules, which provides an idea about how well the model was trained and how well it predicts the activity of the external test set (Figure 2).

The frequency of the appearance of particular descriptors in a population of equations indicates the extent of contributions of the descriptors. The contribution chart for the significant

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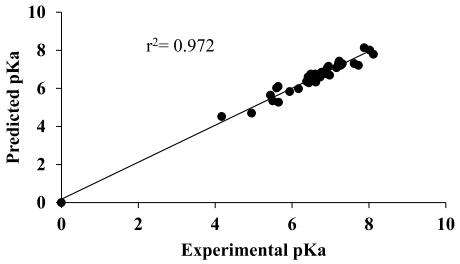

Figure 2. Graph of predicted versus experimental pK_a values for the training set.

model is presented in Figure 3, which gives the percentage contribution of each of the descriptors in the model.

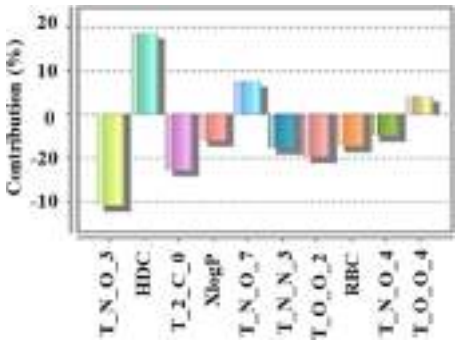

Figure 3. Percentage contributions of the descriptors in the model (descriptors explanation is given in the Supporting Information (SI)).

The best regression equation (QSAR model) obtained is represented as 1

$$pK_a = -1.0138\,(T\_N\_O\_3) + 1.1903\,(H\text{−}donor\ count) - 0.3652\,(T\_2\_C\_0) - 0.2017\,(X\log P) + 0.5584\,(T\_N\_O\_7) - 0.8980\,(T\_N\_N\_3) - 0.5408\,(T\_O\_O\_2) - 0.0711\,(rotatable\ bond\ count) - 0.1755\,(T\_N\_O\_4) + 0.1639\,(T\_O\_O\_4) + 15.6633 \quad (1)$$

3.1.2. Statistical Evaluation of the QSAR Model. The statistical results of the PLSR model are shown in Table 2. The equation explains 97.2%, r^2 = 0.972 of the total variance in the training set as well as it has internal q^2 and external pred_r^2 predictive ability of ∼83 and ∼63%, respectively. The values of internal q^2 and external pred_r^2 predictions are greater than the minimum recommended values, hence they are significant.[28] The value of *F*-test = 103.15 shows the statistical significance of 99.99% of the model, which means the probability of failure of the model is 1 in 10 000. In addition, the randomization test shows a confidence of ∼99.9%, signifying that the generated model is not random.

Table 2. Statistical Results of the QSAR Equation 1

sr. no.	statistical parameters	QSAR results
1	*n*	38
2	degree of freedom	33
3	r^2	0.972
4	q^2	0.83
5	*F*_test	103.158
6	r^2_se	0.2396
7	q^2_se	0.363
8	pred_r^2	0.6328
9	pred_r^2se	0.4366
10	*Z* score_q^2	2.88
11	best_r and_q^2	0.08616
12	alpha_r and_q^2	0.0100

The descriptors selected in the present study for QSAR modeling are defined and summarized in Tables S2 and S3. The correlation matrix between the physicochemical descriptors and alignment of independent descriptors influencing the pK_a activity is presented in Table S4.

3.1.3. Analysis of the QSAR Model. Using the QSAR model, pK_a values for the training set were predicted (Table S5). A plot of experimental versus predicted pK_a shows a higher r^2 value of 0.972 indicating the accuracy of the results (Figure 2). No significant difference was observed in the predicted and experimental pK_a values, and the *F*-test value of 103.158 shows the good statistical significance of the model. Similarly, the QSAR model was used to predict pK_a values for the test set (Table S5). A plot of experimental versus predicted pK_a showed an r^2 value of 0.967, which indicates the high predictive accuracy of the results (Figure 4). The predicted and

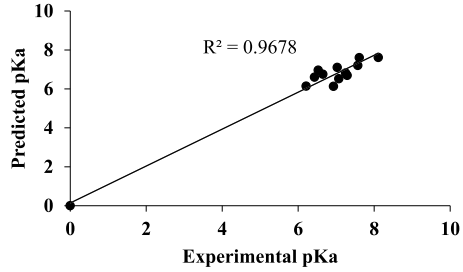

Figure 4. Graph of predicted versus experimental pK_a for the test set.

experimental pK_a values of the test set were comparable to each other, demonstrating the validity of the QSAR model. A plot of residual pK_a versus predicted pK_a values for training as well as test molecules (Figure 5) shows that the prediction error in the model is minimal in the range from −0.46 to +0.79, signifying high accuracy of the model.

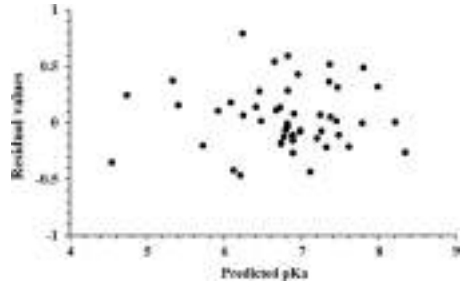

Figure 5. Graph of residual values versus predicted pK_a.

We tried to develop a model with a minimum number of descriptors to be precise, as in the case of small drug molecules, to improve prediction ability, but we observed that reducing the number of descriptors affected parameters like r^2 and q^2 as well as there was poor prediction ability. The present study is with respect to lipid macromolecules having the possibility of multiple interactions and the pK_a having determined in the liposomal system where multiple inter-

https://dx.doi.org/10.1021/acsomega.0c04931
ACS Omega 2020, 5, 32023−32031

actions are envisaged, therefore a large number of terms would be desirable. A larger number of terms would account for multiple interactions and improve the predictivity of the model. Moreover, a large number of terms would be helpful in incorporating structural diversities in the molecule. The QSAR study revealed that all of the 10 contributed descriptors in the model have an impact on determining the pK_a of heterolipids and studying them would be essential for designing new heterolipids with desired pK_a. Descriptors like T_N_O_3, T_N_O_4, and T_N_O_7 reveal that the positional distance between the two heteroatoms oxygen and nitrogen plays an important role in pK_a. T_N_O_3 and T_N_O_4 are negatively contributing descriptors, whereas T_N_O_7 is a positively contributing descriptor. Among the selected descriptors, T_N_O_3 is the most negatively contributing (effect found in molecules 3, 11, 12, 45, 46, and 53). The negative effect of the T_N_O_3 descriptor on pK_a can be easily observed by comparing molecules with and without the T_N_O_3 descriptor. For example, molecules 42 and 53 are not homologous but have some similarity in their structures; molecule 42 (pK_a-7.23) with no T_N_O_3 effect due to the absence of T_N_O_3 has higher pK_a than molecule 53 (pK_a-6.38) with T_N_O_3 and its effect. Similar effects can be observed in molecules 1 and 3, both are homologous, and molecule 1 (pK_a-6.68) with no T_N_O_3 effect has higher pK_a than molecule 3 (pK_a-5.94) with the T_N_O_3 effect. These findings signify the importance of the distance of two heteroatoms in the chemical structure. Similarly, T_N_O_4 also negatively contributes to pK_a (effect found in molecules 6, 9, 15, and 16), whereas T_N_O_7 shows a positive relation (effect found in molecules 17, 18, 33, and 34). While comparing the homologous series of molecules 14−18, the pK_a increases gradually from 4.17 to 7.16 as the positional distance between oxygen and nitrogen increases by changing the length of the spacer (from 1 to 5 number of methylene groups in the spacer). The absence of TNO3 and its negative effect on pK_a in these molecules are the reasons for the observed gradual increase in pK_a. These observations suggest that heterolipids having a bond distance between oxygen and nitrogen atoms beyond 4 forms stable conjugate acids, resulting in higher pK_a.

The hydrogen donor count has a strong positive effect on pK_a and is the strongest positively contributing descriptor. The QSAR study shows that molecules 32, 41, and 48 in the presence of primary and secondary amines in their chemical structure have both predicted as well as experimental pK_a values higher. This could be attributed to their ability to readily share hydrogen bonds to stabilize the conjugate acid. Another important outcome of the study was that pK_a is affected by alkyl substitution on heterolipids. As the number of methylene groups in the alkyl substituents increases, it is expected to increase the basicity and pK_a of molecules due to the (+) inductive effect of methylene groups. Contrary to this expectation, decrease in pK_a was observed with an increase in the number of methylene groups in the case of molecules 37, 38, and 41 and 16, 30, 31, and 47, having 2 and 3° amino groups, respectively. Therefore, it can be concluded that the acidity of heterolipids can be tuned by judiciously selecting alkyl substitutions on amine groups.

As expected, the lack of stereochemically differentiating descriptors in our model leads to the predicted pK_a values of enantiomers 1 and 4 as well as the diastereomeric pairs 6, 7, and 8 and 9, 20, and 21 to be the same, while the experimental pK_a of the enantiomers were found to be the same but the diastereomeric pairs 8 (pK_a-7.29) and 9 (pK_a-6.98) had a specifically profound impact. Other descriptors like T_2_C_0, XlogP, T_N_N_3, T_O_O_2, and rotatable bond count show a negative impact, whereas T_O_O_4 shows a positive impact on pK_a. Though these descriptors play an important role and contribute to pK_a, their contribution is not as significant as that of T_N_O_3 and hydrogen donor count.

3.2. Experimental Validation of the QSAR Model. To effectively design heterolipids with the desired pK_a, it is valuable to understand the impact of the descriptors, the positioning of heteroatoms, the hydrogen bonding, and the number of alkyl groups in the chemical structure of the heterolipid on the pK_a. To validate the present model, numerous heterolipid molecules were constructed computationally, and using the model, their pK_a values were predicted. Among them, eight molecules were selected for synthesis based on criteria such as head group, number of tails, and ease of synthesis and implemented for the validation of our model. Therefore, it can be concluded that the developed QSAR model would be helpful in designing new heterolipids with desired surface pK_a for intracellular delivery of therapeutic molecules such as siRNA.

3.2.1. Synthesis of Heterolipids. Designed heterolipids (Figure 1) were synthesized as per Schemes 1 and 2. Detailed experimental procedure, purification, and characterization data are provided in the Supporting Information. All of the eight heterolipids including four from each series were synthesized in the yield range of 88−96%, indicating the efficiency of the synthetic scheme. Chemical structures were confirmed by Fourier transform infrared (FTIR), ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectral (HRMS) data. All of the associated information related to synthesis and characterization is presented in the Supporting Information.

3.2.2. TNS Assay for Determination of Surface pK_a. Most of the developed LNPs had particle sizes around 100 nm (Figure S28) and they were stable at room temperature for a longer time. The plot of the normalized fluorescence of TNS versus pH allowed us to determine the surface pK_a of synthesized heterolipids in their LNP system (Figure 6). The results of pK_a measurements of all of the heterolipids along with pK_a values predicted by the QSAR model were compiled (Table 3). There is a strong correlation between pK_a values determined experimentally and by the QSAR model with a correlation coefficient of 93.3% (Figure S29). This fact indicates that the QSAR model has a very strong predictive ability with limitations of not being 100% correct and could be considered as a strong and robust alternative to trial and error methods in selecting heterolipids for delivery of nucleic acid therapeutics such as siRNA. Specifically, the model could be employed to design amino lipids for intracellular delivery of molecules to target hepatocytes for hepatic diseases and cancer tissues. Our future plan of work is to investigate and utilize these heterolipids in intracellular siRNA/drug delivery to conclusively establish the approach.

4. CONCLUSIONS

In conclusion, the developed QSAR model shows a good statistical algorithm with a strong correlation of 93.3% between predicted and experimentally determined pK_a values, demonstrating its precision and effectiveness. Furthermore, the developed QSAR model would become a useful tool for designing specific heterolipids with tailored structures, proper-

https://dx.doi.org/10.1021/acsomega.0c04931
ACS Omega 2020, 5, 32023−32031

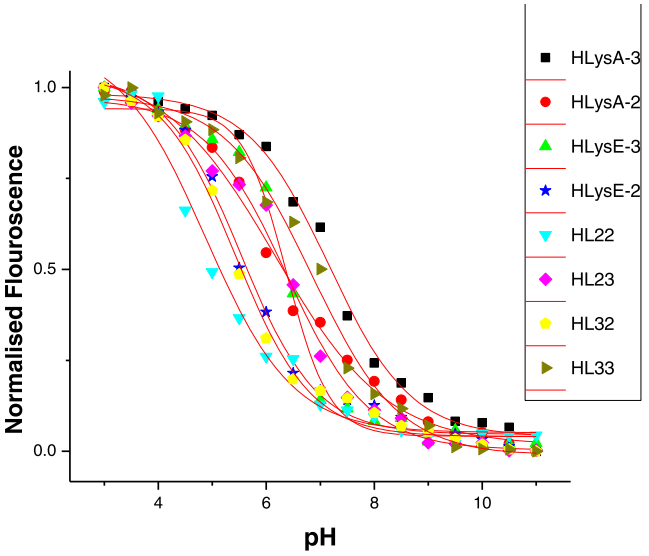

Figure 6. Representative plot for all of the heterolipids showing normalized TNS fluorescence intensity as a function of pH in the presence of liposomes that consist of heterolipids/DSPC/cholesterol/PEG-lipid (40/10/40/10 mol %, respectively). The apparent pK_a value of the amino lipid is the pH at which TNS fluorescence is half of its maximum and it was obtained after fitting the data with a sigmoid function.

Table 3. Comparative Table of Predicted pK_a by the QSAR Model and Experimental pK_a by the TNS Method

heterolipid	predicted pK_a	experimental[a] pK_a (±SD)
HL-22	4.60	5.14 ± 0.02
HL-23	5.96	6.78 ± 0.17
HL-32	5.34	5.54 ± 0.04
HL-33	6.64	7.03 ± 0.04
HLysE-2	5.30	5.84 ± 0.11
HLysE-3	5.97	6.67 ± 0.08
HLysA-2	6.08	6.44 ± 0.05
HLysA-3	6.80	7.30 ± 0.03

[a] n = 3, ±SD = standard deviation.

ties, and pK_a for intracellular delivery of siRNA. The developed QSAR model would be helpful to hold up this innovation by means of providing quick screening of a large number of heterolipid libraries to fasten the process of developing innovative siRNA delivery vehicles.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04931.

Materials and methods which includes the calculation of molecular descriptors; generation of training and test sets; forward stepwise as the variable selection method; partial least-squares (PLS) regression method; model validation and evaluation of the quantitative model; structures of heterolipids with corresponding experimental pK_a values; selected descriptors for 2D-QSAR modeling of the pK_a activity of heterolipids; selected physicochemical and alignment independent descriptors used in the 2D-QSAR model with values; correlation matrix between the physicochemical descriptors and the alignment independent descriptor influencing pKa; comparative data of experimental and predicted pK_a of heterolipids; detailed synthesis protocol of heterolipids with characterization data; particle size of LNPs; and correlation between predicted and experimental pK_a (PDF)

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Notes

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■ ACKNOWLEDGMENTS

D.M.D. is thankful to both the Department of Science and Technology, Govt. of India and Indo-French Centre for the Promotion of Advanced Research (IFCPAR/CEFIPRA) for providing financial assistance under INSPIRE and Raman−Charpak fellowship programs, respectively. The authors gratefully acknowledge VLife Sciences Technologies Pvt. Ltd., Pune, India for providing molecular modeling software VLife MDS (molecular design suite) 4.3.

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https://dx.doi.org/10.1021/acsomega.0c04931
ACS Omega 2020, 5, 32023−32031

Phenytoin Induced Steven Johnson Syndrome: A Case Report

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ABSTRACT

Stevens-Johnson syndrome (SJS) is a disastrous consequence of hypersensitivity reaction precipitated by certain drugs and viral infections. It is an idiosyncratic drug reaction usually associated with drugs like anti-epileptics, non-steroidal anti-inflammatory compounds and antibiotics. The syndrome is characterized by purpuric macules and bullous eruptions involving the mucous membrane which may be followed by systemic manifestations. We report here a case of phenytoin induced SJS, the clinical features of this condition and management of the patient are described in brief. The Naranjo adverse drug reaction causality assessment yielded "probable" causal association between the suspected drug and the adverse drug reaction and severity of the reaction was found to be of "moderately" severe in nature.

Key words: Adverse Drug Reaction, Phenytoin, Steven Johnson Syndrome, Antiepileptics Naranjo, Idiosyncratic Drug Reaction, Toxic Epidermal Necrolysis

INTRODUCTION

Antiepileptic drugs are associated with severe skin reactions such as Stevens-Johnson syndrome (SJS) and Toxic epidermal necrolysis (TEN). Phenytoin is one of the most commonly prescribed antiepileptic agent and is known to cause a plethora of adverse effects.[1] According to WHO, adverse drug reaction is defined as "any response to drug which is noxious or unintended and occurs at a dose normally used in man for prophylaxis, diagnosis or treatment of diseases or for modification of physiological function".[2] The relative risk of SJS/TEN as an important severe cutaneous adverse reactions have been reported with the use of sulfonamide antibiotics, aromatic antiepileptic (phenytoin and carbamazepine), lamotrigine and oxicam NSAIDs. SJS/TEN are rare and severe manifestations of idiosyncratic reaction to certain drugs and are more likely to occur in people infected with human immunodeficiency virus (HIV), with an estimated incidence of 1/1000. They are also rarely associated with vaccination and infections such as mycoplasma, cytomegalovirus and dengue, but are more commonly associated with drugs. SJS and TEN are two entities of the same condition differing only in the percentage of body surface area (BSA) involvement. Usually <10% BSA involvement is seen in SJS, 10-30% BSA in SJS-TEN overlap and >30% BSA detachment is seen in TEN. SJS can present as a nonspecific febrile illness (malaise, headache, cough, rhinorrhea) with polymorphic lesions of skin and mucous membrane characterized by acute blisters and erosions. SJS is associated with a mortality rate of 1-5% which increases to 25-35% in case of TEN.[3,4] Among hospitalized patients approximately about 0.3 to 7% of deaths were reported to be caused by adverse drug reactions (ADR). The spectrum of drug reactions can differ from mild to severe such as SJS which is an uncommon, but with a serious skin-mediated hypersensitivity reaction. Drug induced SJS is one of the

DOI: 10.5530/ijopp.13.4.64

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most common forms of SJS. Antimicrobials (37.27%) have been found to be the most frequent class to cause drug induced SJS, followed by antiepileptic drugs (35.73%) and NSAIDs (15.93%) respectively.[5]

CASE REPORT

A 60 years old male patient was apparently alright 15 days back when he developed lesions over the face which was insidious in onset and gradually progressed to involve lips and mouth. Lesions were also associated with difficulty in swallowing. Patient developed fluid filled lesions over back and bilateral upper and lower extremities, few of which ruptured spontaneously leaving behind erosions [Figure 1-3]. Patient also complaints of reduced sleep and appetite and burning micturition since 2 days. Detailed past history suggested that, the patient had sustained a head injury one and half months back for which he was on *phenytoin* and *nimodipine*. CT scan of brain revealed minimal inter hemispheric bleed, symmetric hypo densities /ischemic changes in frontal white matter bilaterally, extra cranial soft tissues swelling over right frontal region. Patient had also received *amoxyclav* orally for 10 days post the development of lesions. Further, patient took medication (*phenytoin and nimodipine*) for nearly one and half month till the day of admission and it was after that multiple bullae over bilateral thighs, multiple hyper pigmented purperic patches over face, trunk and bilateral upper and lower extremities, diffuse crusting over lips and erosions over bucal mucosa started developing. The patient was immediately admitted in dermatology intensive care unit for further management.

The suspected drug *phenytoin* was stopped. Multiple therapies were prescribed to the patient, On dermatological consultation intravenous fluids of *dextrose normal saline, normal saline* and *ringer lactate* solution was given 8th hourly once a day for 3 days, *mupirocin* ointment, *liquid paraffin* and *1% GV* lotion were applied topically, *triamcinolone* oral paste and *betadine* gargle were used to treat oral ulcer and throat infection, fever was managed by *paracetamol* (650mg) twice a day for 7 days and *cyclosporine (200mg)* was given orally once a day for 7 days to treat skin lesions. *Protein supplement* was started on 3rd day of treatment and anemia was managed with oral iron and vitamin supplements. On the day of hospitalization, the laboratory investigation revealed a marginal elevation of *ESR-60, TC- 21990, eosinophils- 20.4* and decrease in *hemoglobin- 10.2, RBC- 3.83, PCV- 30.9* and *Liver function test* showed decrease *Serum protein- 6.2, serum albumin- 2.8*, increased *SGPT- 101 and SGOT- 65*. Serum electrolyte analysis showed decreased *bicarbonate- 15.7* and Peripheral blood smear report showed *normocytic normochromic* anemia with mild *leucocytosis*. The systemic examination of *BSA reveals 15% involvement* with *SCORTEN severity score of 3*. The immunosuppressant's *(cyclosporine)* doses were tapered appropriately with gradual resolution of the symptoms and the patient was discharged after complete ablation of rashes with proper instructions regarding the possible relapse with the use of aromatic antiepileptic.

DISCUSSION

SJS/TEN are serious adverse cutaneous drug reactions characterized by mucocutaneous tenderness and usually hemorrhagic erosions, erythema and more or less serious epidermal detachment presenting as blisters and areas of denuded skin regions. *Acute generalized exanthematou's pustulosis* (*AGEP*), erythema multiforme (*EM)* major,

Figure 1: Phenytoin induced SJS Hyper pigmented purpuric patches over face, trunk and bilateral upper and lower extremities, diffuse crusting and erosions over lips.

staphylococcal scalded skin syndrome, *pemphigus vulgaris, pemphigus foliaceus*, *severe cutaneous adverse reactions (SCAR) viz; drug hypersensitivity syndrome (DHS)* and *other forms of drug eruption* and acute graft versus host disease are some conditions that might need consideration for differential diagnosis. Some typical drug class have been recognized as the major cause of SJS/TEN in majority of the cases, but *mycoplasma pneumonia* and herpes infections are also well acknowledged.

A large number of drugs are at higher risk of causing SJS/TEN includes: *allopurinol, trimethoprim-sulfamethoxazole* and other *sulfonamide antibiotics, aminopenicillins, cephalosporins, quinolones, carbamazepine, phenytoin, phenobarbital and* oxicam *NSAIDs.* Amongst anti-epileptics, *phenytoin and carbamazepine* have been reported to be the most common cause.[2] SJS/TEN are rare but lethal manifestations of a type IV hypersensitivity reaction with an approximate incidence of 1-2/million/year.[1] In the early stages of the disease progression, the epidermis becomes infiltrated with macrophages and CD8 T-lymphocytes, while the dermis of skin shows CD4 cells in high proportion. It is assumed that the lymphocytes liberate cytokines, which mediate epithelial cells inflammatory response and apoptosis. It should be stressed, however, that the mechanism of hypersensitivity syndrome is believed to engage deficiency or abnormality of the epoxide hydroxylase enzyme that detoxifies the metabolites of aromatic anticonvulsants, associated reactivation of herpes-type viruses and ethnic predisposition with certain human leukocyte antigen subtypes. In the metabolism of aromatic anticonvulsant drugs, the toxic intermediates are capable of accumulating and trigger cell death immediately, or, as prohaptens which bind to T cells evoking immune response.[5,6]

In the acute phase, sepsis is the most common serious risk of SJS/TEN. Organ failure may occur, including pulmonary, hepatic and renal systems.[7] The most common long-term complications of SJS/TEN are ocular (including blindness), cutaneous (pigmentary changes and scarring) and renal. Mucosal involvement with blisters and erosions can lead to strictures and scarring.[8]Patient with SJS/TEN requires multidisciplinary management approach and supportive care that includes; cessation of the suspected causative drug(s), hospitalization (preferably to an intensive care), fluid replacement (crystalloid), nutritional assessment, temperature control, pain relief etc. In the current case, the suspected causative drug *phenytoin* was immediately withdrawn and the patient was managed symptomatically. Cyclosporine (3mg/kg/day) an immunosuppressant drug was given orally once a day for 7 days and tapered appropriately. *Cyclosporine* has encouraging role in the management of uncomplicated cases of SJS, SJS-TEN overlap or TEN.[9] The ideal therapy of SJS/TEN still remains a matter of debate as there are only a limited number of studies of good quality comparing the usefulness of different specific treatments. Though, the expert group recommends prompt withdrawal of the culprit drug, meticulous supportive care and judicious and early (preferably within 72 h) initiation of moderate to high doses of oral or parenteral corticosteroids (prednisolone 1-2 mg/kg/day or equivalent), tapered rapidly within

Table 1: Causality assessment of suspected adverse drug reaction using Naranjo scale.

Question	Yes	No	Don't Know/ NA	Score*
Are there previous conclusive reports on this reaction?	+1	0	0	1
Did the adverse event appear after the suspected drug was administered?	+2	-1	0	2
Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?	+1	0	0	1
Did the adverse event reappear when the drug was re-administered?	+2	1	0	0
Are there alternative causes (other than the drug) that could on their own have caused the reaction?	-1	2	0	2
Did the reaction reappear when a placebo was given?	-1	1	0	0
Was the drug detected in blood (or other fluids) in concentrations known to be toxic?	+1	0	0	0
Was the reaction more severe when the dose was increased or less severe when the dose was decreased?	+1	0	0	0
Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	+1	0	0	0
Was the adverse event confirmed by any objective evidence?	+1	0	0	1
Total				7

**Score: Definite: ≥ 9, Probable: 5-8, Possible: 1-8, Doubtful: 0 Report: The suspected ADR found to be Probable on Naranjo scale assessment.*

7-10 days. Cyclosporine (3-5 mg/kg/day) for 10-14 days may also be used either alone, or in combination with corticosteroids.[7,8]

It is unknown whether systemic corticosteroids are beneficial, but they are often prescribed in high dose for the first three to five days of admission. The management also involves the use of antibiotics for secondary infection but is best avoided prophylactically. Granulocyte colony-stimulating factor (G-CSF) may be of benefit in patients with severe neutropenia. Other drugs reported effective include; *TNF-alpha inhibitors, N-acetylcysteine* and intravenous *immunoglobulins*, however, their role remains controversial.[7,8]

SJS/TEN is potentially very serious with high mortality; however, there has been a trend towards improved mortality in recent years attributed mainly to improvised supportive care against older approaches. People who have survived SJS/TEN must avoid the causative drug or structurally related medicines (*anticonvulsants beta-lactam and NSAIDs and sulfonamides*) for cross-reactivity.[8]

The Naranjo causality assessment for the present case yielded a total score of 7, which indicates that there was a "probability" that the adverse reaction was caused due to the suspected drug (phenytoin) [Table 1] and the Hartwig's severity assessment categorized the observed adverse reaction to level 4 indicating "moderately severe reaction" requiring hospitalization.

CONCLUSION

The suspected ADR was found to have "probable" causal relationship between the suspected drug phenytoin and severity nature of "moderate" category. The report suggests close monitoring of phenytoin usage among population for the occurrence of SJS/TEN type adverse effect and patients should be educated for aromatic anticonvulsants adverse effect and cross reactivity with similar structural molecules.

ACKNOWLEDGEMENT

The authors are thankful to the management of BLDE association, BLDE hospital administration, Dermatology department and staff for supporting the work.

CONFLICT OF INTEREST

The author declares no conflict of interest exists.

ABBREVIATIONS

SJS: Stevens–Johnson syndrome; **TEN:** Toxic epidermal necrosis; **ADR:** Adverse drug reaction; **WHO:** World Health Organization; **HIV:** Human Immunodeficiency Virus; **BSA:** Body Surface Area; **NSAIDs:** Non steroidal anti-inflammatory drugs; **CT:** *computerized* tomography; **ESR:** erythrocyte sedimentation rate; **TC:** Total count,: **RBC:** Red blood cells; **PCV:** Packed cell volume; **SGPT:** Serum Glutamic Pyruvic Transaminase; **SGOT:** Serum glutamic-oxaloacetic transaminase; **SCORTEN:** SCORe of Toxic Epidermal Necrosis; **SCAR:** Severe cutaneous adverse reactions; **DHS:** Drug hypersensitivity syndrome; **AGEP:** Acute generalised exanthematous pustulosis; **EM:** Erythema Multiforme; **CD:** Cluster of differentiation; **G-CSF:** Granulocyte colony-stimulating factor; **TNF:** *Tumor necrosis factor*.

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Drug Induced Hypersensitivity Syndrome Secondary to Phenobarbitone

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ABSTRACT

Phenobarbitone- a barbiturate, non-selective central nervous system depressant drug, primarily used as a sedative hypnotic and anticonvulsant in sub hypnotic doses. It has long term anticonvulsant effect, used in the treatment of generalized tonic-clonic and cortical local seizures. It is also effective in acute convulsive episodes associated with status epileptic's cholera, eclampsia, meningitis, tetanus and toxic reactions to strychnine or local anesthetics. The present case report describes the occurrence of anticonvulsant drug induced hypersensitivity drug reaction followed after to the use of phenobarbitone in a pediatric patient prescribed to treat his seizure disorder. The suspected drug was immediately stopped and the patient was managed symptomatically. The causality assessment of the adverse effect revealed a "probable" causal relationship for the suspected drug and reaction was categorized as "moderately severe" in nature.

Key words: Anticonvulsant, Hypersensitivity, Adverse drug reaction, Phenobarbitone, Barbiturate, Pediatric.

INTRODUCTION

Phenobarbitone belongs to anticonvulsant drug class, it is a long acting barbiturate with a narrow therapeutic index and wide inter individual variability in the rate of metabolism. Barbiturate binds to the β subunits of the υ-aminobutyric acid a (gaba-a) receptors, increasing the duration of opening of the chloride ion channel and potentiating the neuroinhibitory effect of gaba. Anticonvulsant Hypersensitivity Syndrome (AHS) was a term first coined by shear and Spielberg [1, class ii] in 1988 to describe an idiopathic hypersensitivity reaction seen decades earlier in patients exposed to the traditional aromatic anticonvulsants. The traditional aromatic antiepileptic drugs (AEDS); phenytoin, carbamazepine, phenobarbital and primidone, have an aromatic benzene ring in common, the toxic arena oxide metabolite of which was first implicated as a potential trigger of AHS.[1] aromatic anticonvulsant–induced Severe Cutaneous Adverse Drug Reactions (SCARS), such as Stevens–Johnson Syndrome (SJS), Toxic Epidermal Necrosis (TEN) and Drug Rash with Eosinophilia and Systemic Symptoms (DRESS) or drug-induced hypersensitivity syndrome, are rare, but potentially fatal immune-mediated adverse drug reaction (ADR). SCARS typically appear 1–8 wk after receiving anticonvulsant drug. DRESS is characterized by fever, cutaneous rash, lymphadenopathy, eosinophilia and internal organ involvement. The incidence varies from 1:1000 to 1:10,000 exposures. SCARS are frequently associated with aromatic anticonvulsant drugs such as phenobarbital (pb), phenytoin (pht) and carbamazepine (cbz), which are the first-line drugs used in seizure disorder in children.[2-5]

CASE REPORT

A 3 year old male pediatric patient was presented with symptoms of skin lesions over scalp, face, trunk, buttocks and both lower limbs since 25 days [Figure 1& 2].

DOI: 10.5530/ijopp.13.3.48

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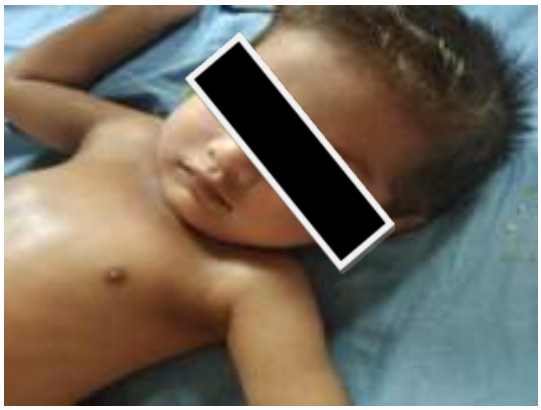

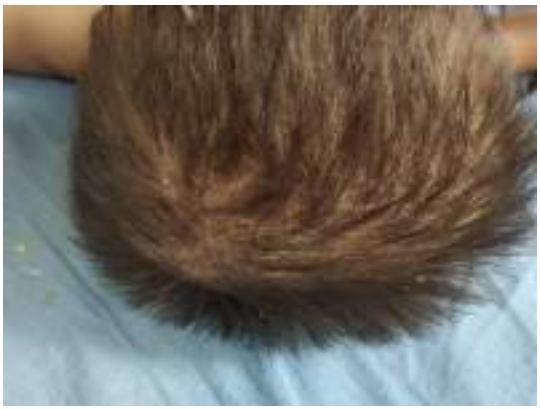

Figure 1 and 2: Phenobarbitone induced cutaneous lesions on trunk and scalp.

Table 1: Causality assessment of suspected ADR using naranjo scale.

Questions	Yes	No	Don't know/NA	Score*
Are there previous *conclusive* reports on this reaction?	+1	0	0	1
Did the adverse event appear after the suspected drug was administered?	+2	-1	0	2
Did the adverse reaction improve when the drug was discontinued or a *specific* antagonist was administered?	+1	0	0	1
did the adverse event reappear when the drug was re-administered?	+2	-1	0	0
Are there alternative causes (other than the drug) that could on their own have caused the reaction?	-1	+2	0	2
Did the reaction reappear when a placebo was given?	-1	+1	0	0
Was the drug detected in blood (or other fluids) in concentrations known to be toxic?	+1	0	0	0
Was the reaction more severe when the dose was increased or less severe when the dose was decreased?	+1	0	0	0
Did the patient have a similar reaction to the same or similar drugs in *any* previous exposure?	+1	0	0	0
Was the adverse event confirmed by any objective evidence?	+1	0	0	1
Total				7

[definite ≥9 or greater, probable for a score of 5-8, possible for 1-4 and doubtful if the score is 0].
Report: the suspected adr found to be probable on naranjo causality assessment

Detailed past history revealed that the patient suffered with epilepsy for which he has been prescribed with phenobarbitone 30mg orally. Patient took the medication since 1½ month and it was after that he started developing red raised skin lesions which was sudden in onset and progressive in nature associated with itching for which oral cefixime was prescribed for 10 days and later was stopped by the pediatrician. There was history of low grade fever of since 20 days associated with cold and cough but not associated with chills and rigors. The skin lesions all over body were severe and the patient was immediately admitted to the teaching hospital and the suspected drug phenobarbitone was stopped. Peripheral smear report shows normocytic normochromic smear with lymphocytic leukocytosis and eosinophilia with reactive thrombocytosis. Multiple therapies were prescribed to the patient *(tab deflazacort and levetiracetam, atarax, prednisolone sodium* in the form of oral solution and *white soft paraffin* as topical moisturizer). The hematological investigation reported; wbc-21400, eosinophils-16.3%, platelet count-5.17, mch-24g/dl and mchc-30g/dl. The glucocorticoid doses were tapered appropriately with gradual resolution of the symptoms and the patient was discharged after complete ablation of lesions with proper instructions regarding the possible relapse.

DISCUSSION

Phenobarbitone was the first efficacious anti-epileptic introduced in 1912. It depresses the central nervous system by increasing the frequency and duration of chloride channels opening by acting on $GABA^{A}$ receptor subunits, thereby allowing a steady flow of these ions into neuronal cells which hyperpolarizes the cell's membrane and increases the threshold for the action potential, thus, effective in the treatment of seizures.[5]

Anticonvulsant Hypersensitivity Syndrome (AHS) as observed in the current case is a delayed adverse drug reaction likely to be associated with the use of aromatic anticonvulsant drug- phenobarbitone. AHS usually present with the classical symptoms that include dermatologic rashes, fever and evidence of systemic organ involvement. The diagnosis is often based on the recognition of these sign and symptoms and clinical judgment. The possible mechanism which thought to play have more or less these three components viz; deficiency or abnormality of the epoxide hydroxylase enzyme that detoxifies the metabolites of aromatic amine anticonvulsants, associated reactivation of herpes-type viruses and ethnic predisposition with certain human leukocyte antigen subtypes. The accumulation and binding of arena oxides, the toxic intermediate metabolite of anticonvulsant drugs to macromolecules, causes cell death and also act as prohaptens that bind to t cells, initiating an immune response and systemic reactions. The management includes withdrawal or discontinuation of associated anticonvulsant drug, use of systemic corticosteroids and related symptomatic treatment. The literature also reports cross sensitivity to other aromatic anticonvulsant drugs in 40-80% of ahs cases, warranting avoidance of aromatic anticonvulsant drug among patient with ahs history. Further, existence of familial association in patients with ahs history has exposed greater risk for the occurrence of ahs in patient's family members if they use aromatic anticonvulsant drugs.[6]

In the current case, the patient was presented with complaints of skin lesions over scalp, face, trunk, buttocks and both lower limbs. The initial assessment reveals use of Phenobarbitone (30mg) by the patient for the past 4 weeks to treat seizures. Suspecting phenobarbitone as a causative agent for the observed skin lesion the drug was immediately stopped and later substituted with levetiracetam to prevent the episodes of seizure. Tab *deflazacort* 12mg was prescribed for first 2 days followed by anti-histamine - *atarax (hydroxyzine hydrochloride)* and *prednisolone* for symptomatic management of the patient. Topical application of white soft paraffin and liquid paraffin were employed for moisturizing and soothing effect. A reduction in the skin lesions ensued after phenobarbitone withdrawal. The patient condition improved gradually and the lesions finally subsided at the time of discharge. Suspecting phenobarbitone as the probable cause for the observed adverse effect, a causality assessment was carried out using naranjo scale [Table 1] to assess the causal relationship. A total score of 7 was obtained, which indicates that there was a probability that the adverse reaction was caused due to the suspected drug itself. Hartwig's scale was used to assess the severity of the observed adverse effect which reported the severity as a moderately severe reaction.[7]

CONCLUSION

The suspected ADR was found to have "probable" causal relationship between the suspected drug phenobarbitone and the observed adverse drug reaction through Naranjo causality assessment. Severity assessment through hart wig's scale put the observed ADR under "moderately" severe reaction category. The report suggests close monitoring of phenobarbitone usage among patient population for the occurrence of anticonvulsant hypersensitivity reaction.

ACKNOWLEDGEMENT

The authors are thankful to the management of BLDE Association and BLDE Hospital and staff for supporting the work.

CONFLICT OF INTEREST

The author declares no conflict of interest exists.

ABBREVIATIONS

CNS: Central nervous system; **GABA-^{A}:** Gama-aminobutyric acid-a; **AHS:** Anticonvulsant hypersensitivity syndrome; **AEDS:** Antiepileptic drugs; **SCARs:** Aromatic anticonvulsant–induced severe cutaneous adverse drug reactions; **SJS:** Stevens–johnson syndrome; **TEN:** Toxic epidermal necrosis; **DRESS:** Drug rash with eosinophilia and systemic symptoms; **ADr:** Adverse drug reaction; **PB:** Phenobarbital; **PHT:** Phenytoin; **CBZ:** Carbamazepine; **MCHC:** Mean corpuscular hemoglobin concentration; **MCH:** Mean corpuscular hemoglobin.

SUMMARY

A pediatric patient on phenobarbitone therapy to manage his seizures was presented to the hospital with the skin lesions all over the body which was later attributed to and caused by phenobarbitone as an anticonvulsant hypersensitivity drug reaction.

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Current Trends in Biotechnology and Pharmacy
Vol. 14 (1) 48-50, January 2020, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2020.1.5

Pemphigus vegetans: a rare case report

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Abstract

Pemphigus vegetans is a rare vesiculo-bullous autoimmune disease which is a rare variant of pemphigus vulgaris characterized by vegetating plaques in the flexures and lesions in the oral cavity. It is a less common disease and involves the mucosa and skin due to disintegration of cellular adherence (acantholysis) resulting in intradermal split. The lesions are usually painful and if untreated it may be fatal. A 68 years old male patient was admitted with complaints of fluid filled skin lesions over trunk since 3 months. The lesions later ruptured spontaneously and healed with crusting. The lesions gradually progressed to involve axilla, scalp and extremities with no itching. Successful implementation of dexamethasone –cyclophosphamide –pulse (DCP) therapy along with other adjuvant drugs has induced disease remission.

Key words: Pemphigus vegetans, Autoimmune disease, Acantholysis.

Introduction

Pemphigus vegetans (Pveg) is a rare vesiculobullous autoimmune disease which is a less common variant of pemphigus vulgaris characterized by heaped up, cauliflower like vegetating plaques in the flexures and rarely in oral cavity. This disorder affects chiefly middle aged adults(1,2).It is caused by auto antibodies directed against desmogleins 1 and 3, which are transmembrane glycoprotein's and results in loss of intercellular adhesion between intact keratinocytes which results in acantholysis. The incidence of the disease is 0.09 to 1.8%. In a study conducted in India majority of pemphigus patients have been diagnosed to have pemphigus vulgaris followed by pemphigus foliaceus, pemphigus erythematosus and pemphigus vegetans. This disease occurs mainly on interiginous areas, scalp and face but may occur anywhere on the skin surface. The oropharynx, oesophagus, stomach, duodenum, anus, nasal vulvovaginal, laryngeal and conjunctival mucosa can also be affected (3). Oral involvement is reported in 60-80% of pemphigus cases and cerebriform tongue, characterized by a pattern of sulci and gyri of the dorsum of tongue has been reported in 50% cases of Neumann type vegetans (4). In Pemphigus vegetans blisters are fragile that usually rupture and leave an area of erosion and have a tendency to develop excessive granulation tissue and crusting in some patients. A positive Nikolsky sign (positive when gentle shearing pressure to pink or normal looking skin results in the formation of an erosion or extension of bulla) often can be elicited. Possible complications of pemphigus include infection of skin, sepsis, rarely death. Pemphigus isn't contagious and there is no way to predict who will get it.

Case report

A 68 years old male patient was admitted in dermatology ward with complaints of fluid filled skin lesions over trunk since 3 months. The lesions later ruptured spontaneously to leave

Current Trends in Biotechnology and Pharmacy
Vol. 14 (1) 48-50, January 2020, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2020.1.5

behind raw areas, which got covered with crusts. The lesions gradually progressed to involve axilla, scalp and extremities. The lesions were painful and not associated with itching.

On Cutaneous examination, multiple crusted plaques well noted over anterior and posterior aspect of trunk and lower extremities, vegetating plaques, vesicles and bullae on bilateral axilla, umbilicus and hyper pigmented patches on scalp and upper extremities were observed (Fig. 1 and Fig. 2). Hyper pigmented ridges of the nails and buccal mucosa was observed with few hyper pigmented patches, some of the erosions were secondarily infected with pus formation.

On investigation complete haemoglobin and liver function tests were within normal limits except for mild hypoproteinemia. Culture from pus produced grants of gram positive cocci and gram negative bacilli.

Diagnosis: A provisional diagnosis of Pemphigus vegetans was made based on the clinical features. A Tzanck smear was prepared from a fresh vesicle and acantholytes cells were noted. Confirmation by skin biopsy is done after observing suprabasal split in histopathological examination and immunoglobulin G deposits within the epidermis in direct immunofluorescene.

Medication: After confirmation of diagnosis, patient was treated with DCP (140 mg of dexamethasone dissolved in 500 ml of 5% dextrose for 3 consecutive days, 500 mg of cyclophosphamide was also given on the second day) After 3 days patient was started on prednisolone 30 mg/day. Patient was also given appropriate antibiotics, calcium supplements, antacids and anti- histamines.

Discussion

Pemphigus vegetans is a rare autoimmune disease and affects people irrespective of gender. This disease occurs mainly on interiginous areas, scalp and face, Similar to that in the present case patient developed fluid filled lesions initially over trunk which later ruptured and got covered with crusts. The lesions gradually progressed to axilla, scalp and extremities. The aetiology in the present case is unknown. As it is auto immune disease in most cases it is unknown what triggers the disease but the mechanism underlying the pathology is IgG auto antibodies directed against desmogleins , a keratinocytes protein which helps in intercellular adhesion and results in acantholysis histologically and bulla formation clinically. Sometimes, pemphigus develops as a side effect of medications, such as antihypertensive drugs and chelating agents, this

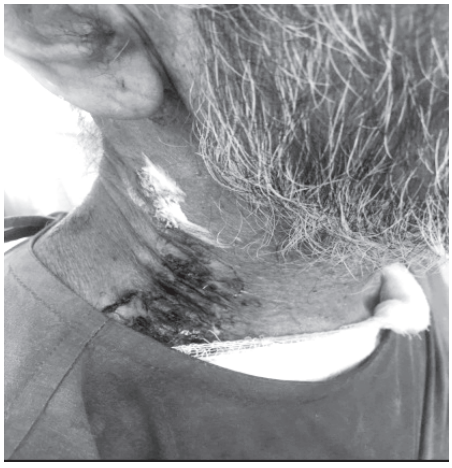

Fig.1: Pemphigus vegetans Lesions on neck

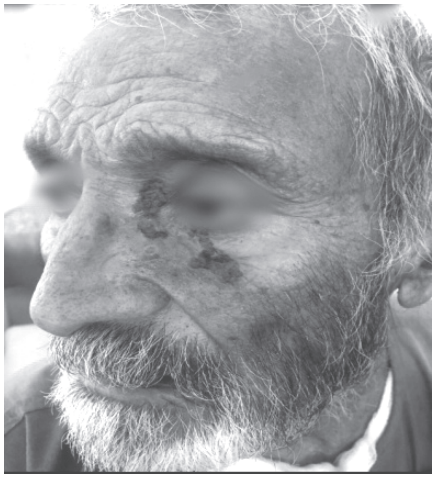

Fig. 2: Ruptured pemphigus vegetans lesions covered with crusts

type of disease disappears when medication is stopped. A preliminary diagnosis of Pemphigus vegetans was established in the present case on the basis of presenting clinical features and a Tzanck smear test, which reveals multiple acantholytic cells (Tzanck cells). The smear test is quite significant in the early diagnosis of Pemphigus vegetans and can be performed with ease and rapidity (5).

The goal of treatment is to induce complete remission while minimizing treatment related adverse effects. The first priority for patient management is to attain rapid disease control. This is typically achieved through the administration of systemic glucocorticoids. Although systemic glucocorticoids therapy is highly effective, the high doses and long treatment periods that are needed to maintain the clinical response may lead to serious or life-threatening side effects. Introduction of dexamethasone-cyclophosphamide pulse (DCP) therapy for pemphigus group of disorders by Pasricha and Gupta in 1984 has revolutionized the therapy of pemphigus. Administration of suprapharmacologic doses of drugs in an intermittent manner is known as “pulse therapy”, which refers to intravenous (IV) infusion of high doses of steroids for one or more days for quicker, better efficacy and to decrease the side effects of long term steroids. The most common side effects of pulse therapy are mood and behavior alteration, hypokalemia, diarrhea, arrhythmias and shock. If administered properly, dexamethasone - cyclophosphamide pulse (DCP) therapy has the potential to affect lifelong recovery from pemphigus (6). In the present case, the patient was managed with dexamethasone - cyclophosphamide pulse (DCP) therapy to control and prevent the development of new lesions. The DCP regimen was followed by oral prednisolone therapy 30mg/day and the patient was managed symptomatically.

Conclusion

The treatment of Pemphigus diseases is a challenge. However the mortality rate of pemphigus vegetans has reduced with the advent of new therapies and treatment modalities. Appropriate treatment and personal care helped patient to improve health condition. Untreated Pemphigus vegetans is often fatal because of many possible complications hence importance should be given for pemphigus vegetans treatment.

Acknowledgement

The authors are thankful to the management of BLDE association for the required support.

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https://doi.org/10.46344/JBINO.2020.v09i05.08

CONTACT DERMATITIS SECONDARY TO PHENYLEPHRINE EYE DROP: A CASE REPORT

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(Received on Date: 16th June 2020 Date of Revision& Acceptance: 14th July 2020 Date of Publish: 1st September 2020)

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ABSTRACT

Phenylephrine, a sympathomimetic drug, is commonly used in eye examinations to dilate the pupil of the eye and to differentiate scleritis from episcleritis. It is extensively used as a mydriatic agent by ophthalmologists and may cause allergic contact reactions even though it is rare. Here is a case of 46 years old female patient with acute-onset edema and erythema of both eyes associated with watering, a burning sensation, and moderate discomfort characterized by contact dermatitis. The patient develops adverse drug reaction to an eye drop containing Tropicamide 0.8%w/v + Phenylephrine 5%w/v used prior to eye fundus examination. Although uncommon, cases of allergic reaction to Phenylephrine cannot be ruled out, hence it is advisable to all the clinicians/Ophthalmologist to encourage and conduct the sensitivity test for Phenylephrine allergy, in order to minimize the possible adverse effects and ruinous consequences.

Keywords: Phenylephrine; Allergic reaction; Contact Dermatitis.

No: of Figures : 01 **No: of References: 05**

INTRODUCTION

Phenylephrine is a sympathomimetic drug commonly used to dilate the pupil of eye and to differentiate scleritis from episcleritis in fundus examination. Tropicamide is a nonselective muscarinic antagonist commonly used for mydriasis due to its fast onset and short duration.[1] Allergic contact reactions with phenylephrine are rare despite its extensive use as a mydriatic agent by ophthalmologists.[2] Its action is due to a direct sympathomimetic effect upon the myoneural junction, resulting in mydriasis.[3] Common adverse effects of phenylephrine include subjective burning, stinging with lacrimation, rebound hyperemia, and liberation of iris pigment into the anterior chamber. Less common, systemic adverse effects include tachycardia and elevation of systemic blood pressure.[5] Even though instances of allergic reactions are rare, phenylephrine has been reported to cause contact dermatitis as in case.

Case:

A 46-year-old Female patient was admitted to a female medicine ward with chief complaints of headache, vomiting (5-6 episodes) and syncopial attacks (loss of consciousness 2 episodes) and a known case of hypertension since two months, not on medications of hypertension. Upon patient history, she has been referred to ophthalmology department for fundoscopy and opine tests to examine hypertensive retinopathy. Administration of tropicamide 0.8%w/v + phenylephrine 5%w/v eye drops while conducting fundoscopy, After 2-3 hours of instillation the patient complained of severe burning In eyes, which further developed adverse reaction of swelling of eyes and redness surrounding both eyes (Fig. 01). Upon Re-reference to ophthalmology department suspected an allergic reaction towards tropicamide 0.8%w/v + phenylephrine 5%w/v eye drops and advice milflox plus (ketorolac 0.4%w/v + moxifloxacin 0.5% w/v) eye drops (QD) to overcome the adverse reaction. Reference of dermatology department reported the case as contact dermatitis secondary to tropicamide 0.8%w/v + phenylephrine 5%w/v eye drops.

Discussion:

Phenylephrine is a-receptor sympathomimetic drug exhibiting vasoconstrictive activity and is frequently used in ophthalmology as a mydriatic agent and nasal decongestant in topical formulations. Local complications like conjunctival irritation, corneal edema, or release of iris pigment into the anterior chamber and systemic cardiovascular symptoms such as hypertension and adverse reactions to tropicamide are rare, although allergic contact dermatitis to phenylephrine has been reported on many occasions from different parts of the world. In the largest documented series Herbst *et al*. performed retrospective analysis of 1641 patients with periorbital dermatitis. Of these, 1053 were diagnosed as allergic periorbital dermatitis and 43 (4.1%) showed positive patch test reaction to phenylephrine. Borch *et al*. observed a higher frequency (15%) of positive reaction to

phenylephrine in their series of 32 patients.[2] In contact dermatitis, specifically sensitized CD4 T helper cells provide the immunologic memory that accounts for the more rapid and intense response that occurs after re-exposure with the allergen.[4]

Fig. 01. Erythema and edema of eyelids with conjunctiva congestion.

Conclusion:

Although uncommon, but the cases of allergic reaction to phenylephrine can occur in some patients. The incidence of allergy caused by the administration of tropicamide 0.8%w/v + phenylephrine 5%w/v eye drop is the case study of a patient presented with a severe contact dermatitis. It is important for all eye care clinicians to realize that adverse effects to diagnostic eye drops are possible and can occur following the most routine of visits. Such reactions can be caused by dilating agents, anesthetics, or preservatives, and these may be allergic or toxic. Clinicians/ophthalmologists should take special care to identify the instigating agent, and if possible to avoid occurrence of such adverse reactions, suggested prior examinations should be performed and use such agents on patients during future exams. Clinicians/Ophthalmologists also should understand how best to manage iatrogenic adverse effects when they encounter them in order to restore a patient's visual function as quickly as possible.

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To Study the Prescribing Pattern of Drugs in Dengue Patients of a Tertiary Care Hospital: A Prospective and Observational Study

S.M. Biradar[1] , K. Maurya[2] , A. Naikal[3] , S. Sarnadgowd[4] , Rajesh M. Honnutagi[5] , Ravi Kattimani[6] , Shivakumar B.[7] , V.T. Kallyanappagol[8]

Section:Research Paper, Product Type: Journal-Paper

Vol.7 , Issue.6 , pp.159-165, Dec-2020

Online published on Dec 31, 2020

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How to Cite this Paper

- **IEEE Citation**
- **MLA Citation**
- **APA Citation**
- **BibTex Citation**
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IEEE Style Citation: S.M. Biradar, K. Maurya, A. Naikal, S. Sarnadgowd, Rajesh M. Honnutagi, Ravi Kattimani, Shivakumar B., V.T. Kallyanappagol, "To Study the Prescribing Pattern of Drugs in Dengue Patients of a Tertiary Care Hospital: A Prospective and Observational Study," *International Journal of Scientific Research in Biological Sciences*, Vol.7, Issue.6, pp.159-165, 2020.

Abstract :

Dengue fever (DF) is an acute febrile illness which is transmitted by mosquito bite. Annually 5 lakhs of patients are hospitalized and more than 20,000 deaths are reported worldwide. Till today there is no specific therapy available for DF, therefore it is very essential to study the prescribing pattern of drugs

among dengue patients in order to make the pharmacotherapy streamline and reduce the mortality rate. A Prospective observational prescription pattern study was conducted for a period of six months in a tertiary care hospital. The sample size of the study was 100 from medicine and pediatric departments and relevant information were extracted from respective files and selected patients. A total of 661 drugs were prescribed among selected dengue patients, in whom 145 were anti-invectives, out of them 143 drugs were antibiotics, Cefixime contributes 35% and followed by Doxycyclin 25.8% and remaining 516 drugs were as supportive therapy. DF was more prevalent in males and prominent in age group of 11-20 years. As concern with the laboratory diagnosis, it was seen that the NS1positive were 97% and IgM positive were 3%, indicates the most of the patients were brought to the hospital in time, because the NS1 is detected early stage of dengue infection. The hematological values (SBP, DBP, PCV, PLT & TC) were in the optimal range indicating the treatment was started abruptly and prescription was most appropriate to the medical condition of patients.
Key-Words / Index Term :
Prescribing pattern; Dengue fever (DF); Anti-infective; Supportive therapy

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GOVERNMENT OF INDIA
Regional Office for Health & Family Welfare
(Directorate General of Health Services, Ministry of Health and FW)
2nd Floor 'F' Wing, Kendriya Sadan
Koramangala, Bangalore – 560 034
Phone Direct :25537310, Office : 25537688
Fax : (080) 25539249
Email: rhobng@nic.in

Senior Regional Director (H & FW)
No.ROH&FW /NVBDCP/2020-21/ Date: 23.10.2020

To

The District Health and Family Welfare officer,
Bijapur

Sir,

Sub: MDA - 2020 – Independent Evaluation Team visit – information reg.
Ref: MDA – 2020 conducted from 27.9.2020 to 05.10.2020, in Bijapur Dt.

With reference to the above, the Dr. Shilaja Patil, Professor, Department of Community medicine, BLDE Medical College will be visiting Bijapur District, during the first week of November, for conducting the Independent Evaluation on Mass Drug Administration -2020 (2 taluks- Sindagi and Muddebihal) in Bijapur District. Kindly provide them the required data of your district pertaining to General information, and MDA programme for carrying out the Independent Evaluation of MDA.

I request that necessary arrangements may kindly be made for their mobility for the field visit and extend local staff assistance.

Yours faithfully,

(Dr.K.Ravi Kumar)
Senior Regional Director (H&FW)

Copy for information to:

1. The Joint Director(NVBDCP), Directorate of Health Services, Ananda Rao Circle, Bangalore.
2. Director / Dean, BLDE Medical college, Bijapur.
3. Dr. Shilaja Patil,Professor and HOD., Department of Community Medicine, BLDE Medical College, Bijapur
4. The District Vector Borne Disease Control Officer, Bijapur- with a request to provide necessary data, assistance of local staff etc.,

BLDE (DEEMED TO BE UNIVERSITY)
[Declared as Deemed-to-be-University u/s 3 of UGC Act, 1956 vide Government of India Notification No.F.9-37/2007-U.3(A)]
The Constituent College
SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

Date: 26.11.2020

Report of "Independent Evaluation of Mass Drug Administration for Elimination of Filariasis"

It is our privilege to inform you that we have successfully completed the Independent Evaluation of Mass Drug Administration Programme for Elimination of Filariasis.

Background:

Mass drug administration was done in the endemic (9 districts) and non-endemic (21 districts) areas from 21.09.2020 to 17.10.2020 in Karnataka. Out of 09 endemic districts 03 districts (Raichur, Vijayapura, Bagalkot) were selected for "Survey of Sentinel and Spot Check Sites in MDA District: Year 2020". Therefore, Professor and HOD Department of Community Medicine, BLDE (DU) Shri B M Patil Medical College, Vijayapura was invited to evaluate 02 taluks of District Vijayapura (Tq. Sindagi and Tq. Muddebihal). Our department Faculty, Staff and PG's conducted the evaluation of the two talukas (4 clusters) from 09.11.2020 to 12.11.2020.

Brief report of District Vijayapura:

The total population of District Vijayapura is 792999, out of which 7,29,559 are eligible population. Among them 6,71,220 people received Mass drug administration, hence the coverage is 92% as per district health authorities .

For the purpose of providing health education regarding MDA 291 banners, 20000 handbills, 2000 posters, 8000 stickers, 3000 DD books were sent to different PHCs from the district office. 20 processions, 25 group meetings and 150 individual meetings with the general population were conducted. (Source: District Malaria Surveillance Office & District performance report by Regional Office for Health and FW, Govt of India, Bangalore).

Methodology:

An online training programme was conducted by Dr Ravikumar, Regional Director and Dr. Kumar, Regional Office for Health and FW, Govt of India, Bangalore and group. Protocols for monitoring and evaluation guidelines were provided to us. We categorized 02 Taluks (Sindagi and Muddebihal) of Vijayapura District in 4 clusters for evaluation purpose depending upon the drug distribution coverage as High Coverage, Medium Coverage and Low Coverage sites, 3 sites in rural and 1 in urban.

Sl. No.	Taluk	Cluster	Coverage type
01	Sindagi	Devarhippargi	Low coverage (rural)
02	Sindagi	Moratgi	High Coverage (rural)
03	Muddebihal	Yelguru Village	Medium Coverage (rural)
04	Muddebihal	Talikote	Low coverage (urban)

We did house to house survey to assess the barriers for coverage and compliance of general public towards MDA.

Results:

Total of 261 households in 04 clusters, covering population of 1264 were included in the process of evaluation. Out of them 64.2% were aged above 15years and 35.8% were aged below 15years. MDA full course was consumed by 89.64% of the total study population. The reasons for non - consumption were old age and <2yr (36.64%), severely sick (21.37%), no information regarding MDA (9.92%) and others. There were no major adverse reactions reported by the study population. ANM and ASHA were the key persons in providing information regarding MDA to the study population and >60% of them had knowledge regarding filariasis, its onset and spread.

Conclusion:

Our survey showed that MDA is not just restricted to the distribution of drugs only but also plays key role in implementation of programme, encouraging people to consume tablets, providing health education, managing side effects, and logistics. But still in some area like Urban Talikote it is essential to address the issues like religious beliefs linked to low compliance to make the program more efficient and achieve the goal of filariasis elimination.

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI.B.M.PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA.

DEPARTMENT OF COMMUNITY MEDICINE

Date: 26.11.2020

Staff involved in the "Independent Evaluation of MDA"

Principal Investigator: Dr. Shailaja S. Patil, Prof & Head, Department of Community Medicine

Team A	**Team B**
Name of the staff	**Name of the staff**
Dr. M R Gudadinni (Associate Professor)	Dr. M C Yadavannavar (Professor)
Dr. Tanuja P Pattankar (Assistant Professor)	Dr. Santosh Patil (Assistant Professor)
Dr Arthy & Dr Rupayan (PG students)	Dr. Priyanga & Dr Vijay (PG students)
Mr. Sanjeev Khanagoudar (MSW)	Mr. Y B Biradar (MSW)

(Signature of the Investigator)

Professor & Head

Department of Community Medicine

2444/20
28/12/20

NATIONAL INSTITUTE OF MENTAL HEALTH & NEUROSCIENCES
(INSTITUTE OF NATIONAL IMPORTANCE) BENGALURU - 560 029

NIMH:A&E:TM:TRG-NA:2020/613 Date: 18.12.2020

The Principal
Shri B.M. Patil Medical College,
Hospital and Research Centre
Vijayapur- 586 103.

Sir/Madam,

Sub: Request for Permission to undergo training at this Institute – reg.
Ref: Your letter dated 07.12.2020.

* * * * *

With reference to the above, I am directed to convey the permission of the Competent Authority for the students of your Institution to undergo training at this Institution as follows:

1	Number of trainees	09
2	Name of the trainees	**Duration**
	Dr. Ayesha Rehman, Dr. Likitha M and Dr. Namratha B M	**02.01.2021 to 31.01.2021**
	Dr. Swathi N R and Dr. Shravya E	**01.02.2021 to 28.02.2021**
	Dr. Lakshmi S Pillai and Dr. Devendra	**01.03.2021 to 31.03.2021**
	Dr. Sai Prasad and Dr. Sankalpa Saha	**01.04.2021 to 30.04.2021**
3	Department at which training permitted	**Neuroanaesthesia & Neurocritical Care**
4	training fee	Rs.10,000/- per month or part thereof per trainee

- The trainees should compulsorily carry their college ID cards while posted at NIMHANS.
- One stamp size photo should be given at the time of joining for issue of temporary ID card. (ID card should be returned at the end of training without fail)
- Trainees should carry a copy of this letter without fail.
- ***The training fee for the whole duration of training has to be paid by SB collect (online) on the day of joining. The training fee once paid will not be refunded.***

I am also directed to inform you that the visiting students/trainees should make their own arrangement for accommodation. However all efforts will be made to provide hostel accommodation, but this will be subject to availability, ***based on Manager, Hostel report (080-26995095)***/**Supervisor, Cauvery Hostel, (080-26995092)** as on the date of joining and on payment of charges as below, **Accommodation will not be provided to the candidates coming earlier than the scheduled date of training.**

1. Hostel Rent: Rs.100/- per day

NOTE: ***In case of any damage of assets/property in the Hostels i.e., movable and immovable property of NIMHANS by the trainees, the college shall be directly responsible for such act of the trainees. The loss incurred has to be borne by the Institution/College deputing the trainees. Further, the attendance certificate for training of such trainees will be withheld.***

On arrival, the trainees must contact the undersigned for further needful.

Yours faithfully,

ADMINISTRATIVE OFFICER (A&E)

Copy to: The HOD of Neuroanaesthesia & Neurocritical Care, NIMHANS
The Manager/Supervisor, NIMHANS Hostels, NIMHANS

Administrative Officer (A & E)
National Institute of Mental Health & Neuro Sciences, Bangalore - 560 029

☎: 08026995015 Email: training@nimhans.ac.in Website : http://www.nimhans.ac.in

HOD (Dept. of Anesthesiology)
Shri B. M. Patil Medical College
Hospital & Research Centre.
VIJAYAPUR-586103

28 DEC 2020

Anesthesiology

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The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

2324/20

07 DEC 2020

To
The Director/Office in Charge,
National Institute of Mental
Health & Neurosciences,
BANGALORE.

Sub: Posting of 2nd year Post-graduate students for speciality training

Sir,

As per the requirement of BLDE (Deemed to be) University, Vijayapur the P.G. students of M.D. Anaesthesiology have to undergo one month training in Neuro Anaesthesia in speciality centres of National and International repute.

Therefore I request you to permit following P.G. students of this Institution to undergo training in Neuro Anaesthesia at your Institute. Please let me know the formalities to be completed in this regard.

Sl.No	Name of the PG Students	
1	Ayesha Rehman	01-01-2021 to 31-01-2021
2	Likitha M	
3	Namratha B M	
4	Swathi N R	01-02-2021 to 28-02-2021
5	Shravya E	
6	Lakshmi S Pillai	01-03-2021 to 31-03-2021
7	Devendra	
8	Sai Prasad	01-04-2021 to 30-04-2021
9	Sankalpa Saha	

Please consider our request and accord the necessary permission.

Thanking you,

Yours sincerely,

07 DEC 2020

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR- 586103

NATIONAL INSTITUTE OF MENTAL HEALTH & NEUROSCIENCES
(INSTITUTE OF NATIONAL IMPORTANCE) BENGALURU - 560 029

NIMH:ACA-B:TRG-NEURO:2021/47 Date: 15.01.2021

The Principal
Shri B.M. Patil Medical College,
Hospital and Research Centre
Smt. Bangaramma Sajjan Campus
B.M. Patil Road, Vijayanagar – 586 103.

Sir/Madam,

Sub: Request for Permission to undergo training at this institute – reg.
Ref: Your letter dated 06.01.2021.

*****

With reference to the above, I am directed to convey the permission of the Competent Authority for the students of your institute to undergo training at this Institution as follows:

1	Number of the trainees	**06**
2	Name of the trainees	**Duration**
	Dr. Gandhi Sani Abhinandan, Dr. Shruti Herimeth, Dr. Swetha N, Dr. Mayur C M, Dr. Pallavi Janardhan Reddy and Dr. Rinto Jose Pratheeksh	**Regretted** *(As all the slots are full in February, 2021)*
	Dr. Chetan R Rajpurohit, Dr. Yash Jhamnani, Dr. Dr. Nethra N, Dr. Deeksha K, Dr. Rutuja Patil and Dr. Vijaya Lakshmi D	**01.03.2021 to 31.03.2021**
3	Department at which training permitted	**Neurology**
4	Training fee	**Rs.10,000/- per month or part thereof per trainee**

- The trainees should compulsorily carry their college ID cards while posted at NIMHANS.
- One stamp size photo should be given at the time of joining for issue of temporary ID card. (ID card should be returned at the end of training without fail)
- Trainees should carry a copy of this letter without fail.
- ***The training fee for the whole duration of training has to be paid by SB collect (online) on the day of joining. The training fee once paid will not be refunded.***

I am also directed to inform you that the visiting students/trainees should make their own arrangement for accommodation. However all efforts will be made to provide hostel accommodation, but this will be subject to availability, ***based on Manager, Hostel report (080-26995095)***/**Supervisor, Cauvery Hostel, (080-26995092)** as on the date of joining and on payment of charges as below, **Accommodation will not be provided to the candidates coming earlier than the scheduled date of training.**

1. Hostel Rent: Rs.100/- per day

NOTE: ***In case of any damage of assets/property in the Hostels i.e., movable and immovable property of NIMHANS by the trainees, the college shall be directly responsible for such act of the trainees. The loss incurred has to be borne by the Institution/College/deputy the trainees. Further the attendance certificate for training of such trainees will be withheld.***

On arrival, trainees must contact the undersigned for further needful.

Yours faithfully

ADMINISTRATIVE OFFICER(A&E)

Copy to: The HOD of Neurology, NIMHANS
The Manager/Supervisor, NIMHANS Hostels

VICE PRINCIPAL (ACADEMIC)
BLDE (Deemed to be university)
Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapura-586103. Karnataka

Administrative Officer (A & E)
National Institute of Mental Health & Neuro Sciences, Bangalore - 560 029

BLDE
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Declared as Deemed to be University u/s 3 of UGC Act, 1956
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

35/21

06 JAN 2021

To

The Administrative Officer (A&E),
NIMHANS,
BANGALORE.

Sub: Posting of 2nd year Post-graduate students for speciality training

Sir,

As per the requirement of BLDE (Deemed to be) University, Vijayapur the P.G. students of M.D. General Medicine have to undergo one month training in Neurology in speciality centres of National and International repute.

Already you have permitted our students for posting from 01-09-2020 to 31-10-2020 due to covid-19 pandemic PG students couldn't attend the posting.

Kindly permit our PG students for one month Neurology posting in two batches as mentioned below:

Sl.No	Name of the PG Students	DEPARTMENT	Period of Posting
1	Dr Gandhi Sani Abhinandan	General Medicine	01-02-2021 to 28-02-2021
2	Dr Shruti Hiremath	General Medicine	
3	Dr Swetha N	General Medicine	
4	Dr Mayur C M	General Medicine	
5	Dr Pallavi Janardhan Reddy	General Medicine	
6	Dr Rinto Jose Pratheeksh	General Medicine	
1	Dr Chetan R Rajpurohit	General Medicine	01-03-2021 to 31-03-2021
2	Dr Yash Jhamnani	General Medicine	
3	Dr Nethra N	General Medicine	
4	Dr Deeksha K	General Medicine	
5	Dr Rutuja Patil	General Medicine	
6	Dr Vijaya Laksmi D	General Medicine	

Please consider our request and accord the necessary permission.
Thanking you,

Yours faithfully,

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR-586103

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU) : Phone: +918352-262770, Fax: +918352-263303 , Website: www.bldeuniversity.ac.in
College : Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

ಶ್ರೀ ಜಯದೇವ ಹೃದ್ರೋಗ ವಿಜ್ಞಾನ ಮತ್ತು ಸಂಶೋಧನಾ ಸಂಸ್ಥೆ
Sri Jayadeva Institute of Cardiovascular Sciences and Research
(Govt. Of Karnataka – Regd. Autonomous Institute)
Bannerghatta Road, 9th Block Jayanagar, Bengaluru – 560069
Ph:+91-80-22977400/600, **Academic Section & Fax:**080-22977281
Website: www.jayadevacardiology.com **Email:** director@jayadevacardiology.com
Academic section email: jayadevacardiology.academic@gmail.com

Ref:
SJICS&R/AS/PG-Training/2020-21

Date:
22/01/2021

The Principal,
BLDE (Deemed to be university)
Shri B.M. Patil Medical College
Hospital & Research Centre,
Vijayapura – 586103

Sir,

Sub: Training Programme for PG students-reg.
Ref : Your letter No. 34/21 dated 06/01/2021

With reference to the above, we write to inform you that, the following PG students of your college are permitted to undergo training in the department of cardiology at this Institute on the dates mentioned against their names on payment of fees of Rs. 12500/- per month per student, through DD drawn in favour of the Director, SJIC&R, Bangalore – 69.

SL No.	Student name	Training Period
1.	Dr. Chetan R Rajpurohit	01/02/2021 to 28/02/2021
2.	Dr. Yash Jhamnani	
3.	Dr. Nethra .N	
4.	Dr. Deeksha K	
5.	Dr. Rutuja Patil	
6.	Dr. Vijaya Lakhsmi .D	
7.	Dr. Gandhi Sani Abhinandan	01/03/2021 to 31/03/2021
8.	Dr. Shruti Hiremath	
9.	Dr. Swetha N	
10.	Dr. Mayur C.M	
11.	Dr. Pallavi Janardhan Reddy	
12.	Dr. Rinto Jose Pratheeksha	

The students have to submit Covid-19 **RT-PCR Negative** report at the time of reporting. Failing which postings will be cancelled.

Thanking you,

Your's faithfully,
K.C.
ACADEMIC SUPERINTENDENT

Note: 1. CET candidate must carry, CET allotment letter (Karnataka Examination Authority – Candidate copy) attested by the respective college Principal/Dean/Director.
2. Please send your request letters for Peripheral Postings atleast 3 months in advance.

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Declared as Deemed to be University u/s 3 of UGC Act, 1956

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

.4/21

06 JAN 2021

To
The Director,
Sri.Jayadeva Institute of Cardiology,
BANGALORE.

Sub: Posting of 2nd year Post-graduate students for speciality training

Sir,

As per the requirement of BLDE (Deemed to be) University, Vijayapur the P.G. students of M.D. General Medicine have to undergo one month training in Cardiology in speciality centres of National and International repute.

Already you have permitted our students for posting from 01-09-2020 to 31-10-2020 due to covid-19 pandemic PG students couldn't attend the posting.

Kindly permit our PG students for one month Cardiology posting in two batches as mentioned below:

Sl.No	Name of the PG Students	DEPARTMENT	Period of Posting
1	Dr Chetan R Rajpurohit	General Medicine	01-02-2021 to 28-02-2021
2	Dr Yash Jhamnani	General Medicine	
3	Dr Nethra N	General Medicine	
4	Dr Deeksha K	General Medicine	
5	Dr Rutuja Patil	General Medicine	
6	Dr Vijaya Laksmi D	General Medicine	
1	Dr Gandhi Sani Abhinandan	General Medicine	01-03-2021 to 31-03-2021
2	Dr Shruti Hiremath	General Medicine	
3	Dr Swetha N	General Medicine	
4	Dr Mayur C M	General Medicine	
5	Dr Pallavi Janardhan Reddy	General Medicine	
6	Dr Rinto Jose Pratheeksh	General Medicine	

Please consider our request and accord the necessary permission.
Thanking you,

Yours sincerely,

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU) : Phone: +918352-262770, Fax: +918352-263303 , Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
College : Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

BLDE (DEEMED TO BE UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 of UGC Act, 1956 vide Government of India Notification No.F.9-37/2007-U.3(A)]

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

23-04-2021

To

The Director,
NIMHANS,
Bengaluru 560029

Sub: Relieving of our six PG students reg.,

Sir/Madam,

With reference to the above cited subject, Due to the increase of COVID-19 Pandemic I request you to relieve following PG students, Dept. of General Medicine of our Institution who are attending training at your institution immediately.

1) Dr Gandhi Sani Abhinanadan
2) Dr Shruti Hiremath
3) Dr Swetha N
4) Dr Mayur C M
5) Dr Pallavi Janardhan Reddy
6) Dr Rinto Jose Prateeksh

Yours sincerely,

PRINCIPAL.

Copy to:

The Medical Superintendent, Shri B M Patil Medical College Hospital & Research Centre, Vijayapura.

The Professor and HOD of General Medicine - for information and needful.

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303 , Website: www.bldeuniversity.ac.in, E-mail:office@bldeuniversity.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldeuniveristy.ac.in

NATIONAL INSTITUTE OF MENTAL HEALTH & NEUROSCIENCES (INSTITUTE OF NATIONAL IMPORTANCE) BENGALURU - 560 029

NIMH:A&E:TM:TRG-NEURO:2021/180 Date: 10.02.2021

The Principal
BLDE (Deemed to be University)
Shri B.M. Patil Medical College, Hospital and Research Centre
Smt. Hangaramma Sajjan Campus
B.M. Patil Road
Vijayapur - 586 103.

Sir/Madam,

Sub: Request for Permission to undergo training at this institute – reg.
Ref: Your letter dated 27.01.2021.

*****

With reference to the above, I am directed to convey the permission of the Competent Authority for the students of your institute to undergo training at this Institution as follows:

1	Number of trainees	06
2	Name of the trainees	**Dr. Gandhi Sani Abhinandan, Dr. Shruti Hiremath, Dr. Swetha N, Dr. Mayur CM, Dr. Pallavi Janardhan Reddy and Dr. Rinto Jose Prateeksh**
3	Department at which training permitted	Neurology
4	Date and Duration	01.04.2021 to 30.04.2021
5	Training fee	Rs.10,000/- per month or part thereof per trainee

Note: ***Permission is subject to written assurance by Director/Dean/Principal/HOD of the above mentioned college/university that all the students who are posted will attend activity/duties of Neurology department everyday as per the timings of the department and will not take any planned leave during the period of posting.***

- The trainees should compulsorily carry their college ID cards while posted at NIMHANS.
- One stamp size photo should be given at the time of joining for issue of temporary ID card. (ID card should be returned in the end of training without fail)
- Trainees should carry a copy of this letter without fail.
- ***The training fee for the whole duration of training has to be paid by Debit/Credit Card on the day of joining. The training fee once paid will not be refunded.***

I am also directed to inform you that the visiting students/trainees should make their own arrangement for accommodation. However all efforts will be made to provide hostel accommodation, but this will be subject to availability, ***based on Manager, Hostel report (080-26995095)***/**Supervisor, Cauvery Hostel, (080-26995092)** as on the date of joining and on payment of charges as below, **Accommodation will not be provided to the candidates coming earlier than the scheduled date of training.**

1. Hostel Rent: Rs.100/- per day

NOTE: ***In case of any damage of assets/property in the Hostels i.e., movable and immovable property of NIMHANS by the trainees, the college shall be directly responsible for such act of the trainees. The loss incurred has to be borne by the Institution/College deputing the trainees. Further, the attendance certificate for training of such trainees will be withheld.***

On arrival, the trainees must contact the undersigned for further needful.

Yours faithfully,

ADMINISTRATIVE OFFICER (A&E)

Copy to: The HOD of Neurology, NIMHANS
The Manager/Supervisor, NIMHANS Hostels, NIMHANS

Administrative Officer (A & E)
National Institute of Mental Health & Neuro Sciences, Bangalore-560 029

ಶ್ರೀ ಜಯದೇವ ಹೃದ್ರೋಗ ವಿಜ್ಞಾನ ಮತ್ತು ಸಂಶೋಧನಾ ಸಂಸ್ಥೆ
Sri Jayadeva Institute of Cardiovascular Sciences and Research
(Govt. Of Karnataka – Regd. Autonomous Institute)
Bannerghatta Road, 9th Block Jayanagar, Bengaluru – 560069
Ph:+91-80-22977400/600, **Academic Section & Fax:**080-22977281
Website: www.jayadevacardiology.com **Email:** director@jayadevacardiology.com
Academic section email: jayadevacardiology.academic@gmail.com

Ref: SJICS&R/AS/PG-Training/2021-22

Date: 01/09/2021

The Principal,
Shri B.M. Patil Medical College,
Hospital & Research Centre,
Vijayapura - 586103

Sir,

Sub: Training Programme for PG students-reg.
Ref : 1|Your letter No. 1741/2021 dated: 27/08/21.
Ref : 2|This office letter No. SJICR/AS/PG-Training/2021-22 dt. 20/07/2021

In partial modification to the above said letter cited under reference (2) the following PG students of your college are permitted to undergo training in the department of cardiology at this Institute on the dates mentioned against their names on payment of fee of Rs. 12500/- per student per month through DD, drawn in favour of the Director, SJIC&R, Bangalore -69.

SL No.	Student name	Training Period
1.	Dr. Sneha Mukerjee	01/09/2021 to 30/09/2021
2.	Dr. Chirag Sajjanar	
3.	Dr. Santhosh B.T	
4.	Dr. K. Swetha Sri	
5.	Dr. Shirish Patil	
6.	Dr. Sai Santhosh Jajimi	
7.	Dr. Panchal Jatin Praveen	
8.	Dr. V. Obul Reddy	
9.	Dr. Sujay .V	
10.	Dr. Sailee R Belvi	
11.	Dr. M. Victoria	01/11/2021 to 30/11/2021
12.	Dr. Patil Bhushan Vijay	
13.	Dr. Gudimetla R. K. Reddy	
14.	Dr. G. Sahith Reddy	
15.	Dr. Priyanka Tomar	
16.	Dr. Y Sethu Reddy	
17.	Dr. Vivan Sanjiv Vyas	
18.	Dr. Prashanth M.R	
19.	Dr. Mayuri M.B	
20.	Dr. Niktha R	
21.	Dr. Sunil R	

The students have to submit Covid-19 **Latest RT-PCR Negative** report at the time of reporting. Failing which postings will be cancelled.

Thanking you,

Yaur's faithfully,

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR- 586103

ACADEMIC SUPERINTENDENT

Note: 1. Please send your request letters for Peripheral Postings atleast 3 months in advance.

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

To 1241/2021
The Director,
Sri.Jayadeva Institute of Cardiology,
BANGALORE.

27 AUG 2021

Sub: Modified Posting of 2nd year Post-graduate students for speciality training

Sir,

In continuation with your permission letter No:SJICS&R/AS/PG-Training/2021-22,Dtd:20/7/2021, in the view of Covid-19 clinical work load is more and new Postgraduate students have not been admitted till date. Therefore we request you to kindly modify the specialty training One month instead of Two Months as per the details given below:-

Sl.No	Name of the PG Students	DEPARTMENT	Period of Posting
1	Dr Sneha Mukerjee	General Medicine	01-09-2021 to 30-09-2021
2	Dr Chirag Sajjanar	General Medicine	
3	Dr Santhosh B T	General Medicine	
4	Dr K Swetha Sri	General Medicine	
5	Dr Shirish Patil	General Medicine	
6	Dr Sai Santosh Jajimi	General Medicine	
7	Dr Panchal Jatin Praveen	General Medicine	
8	Dr V Obul Reddy	General Medicine	
9	Dr Sujay V	General Medicine	
10	Dr Sailee R Belvi	General Medicine	
01	Dr M Victoria	General Medicine	01-11-2021 to 30-11-2021
02	Dr Patil Bhushan Vijay	General Medicine	
03	Dr Gudimetla R K Reddy	General Medicine	
04	Dr G Sahith Reddy	General Medicine	
05	Dr Priyanka Tomar	General Medicine	
06	Dr Y Sethu Reddy	General Medicine	
07	Dr Vivan Sanjiv Vyas	General Medicine	
08	Dr Prashanth M R	General Medicine	
09	Dr.Mayuri M B	General Medicine	
10	Dr Nikitha R	General Medicine	
11	Dr Sunil R	General Medicine	

Please consider our request and accord the necessary permission.
Thanking you

Yours sincerely,

PRINCIPAL
BLDE (Deemed to be University)
SHRI B. M. Patil Medical College Hospital & Research Centre,
VIJAYAPUR- 586103

NATIONAL INSTITUTE OF MENTAL HEALTH & NEUROSCIENCES
(INSTITUTE OF NATIONAL IMPORTANCE) BENGALURU - 560 029

No.NIMH:A&E:TM:TRG-NEURO:2020/756 Date: 16.09.2021

The Principal
Shri B.M. Patil Medical college, Hospital and Research Centre
Smt. Bangaramma Sajjan Campus
B.M. Patil Road (Sholarpur Road)
Vijayapura – 586 103.

Sir,

Sub: Request for reschedule to undergo training at this Institute – reg.
Ref: Your letter request dated 18.08.2021, for reschedule of training period.

* * * * *

With reference to the above and, in partial modification of our letter dated 11.02.2021, I am directed to convey the permission of the Competent Authority for **the following PG students** for reschedule of training in the Department of Neurology for the period as mentioned below:

Name of the trainees	Duration
Dr. Y Sethu Reddy, Dr. V Obul Reddy, Dr. Patil Bhushan Vijay, Dr. Sunil R, Dr. Sailee R Selvi, Dr. Chirag Sajjanar, Dr. Gudimetla R K Reddy, Dr. M Victoria, Dr. Nikitha R and Dr. Sanjay V	**01.10.2021 to 31.10.2021**
Dr. Sneha Mukherjee, Dr. K Swetha Sri, Dr. G Sahith Reddy, Dr. Santosh B T, Dr. Priyanka Tomar, Dr. Prashanth MR, Dr. Panchal Jatin Praveen, Dr. Mayuri, Dr. Sai Santhosh Jajimi, Dr. Vivan Sanjiv Vyas, Dr. Shrirish Patil	**01.12.2021 to 31.12.2021**

All other terms and conditions of our letter-dated 11.02.2021 remain unaltered.

Yours faithfully

ADMINISTRATIVE OFFICER (A&E)

Copy to: The HOD of Neurology, NIMHANS

HOD Neuro

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR-586103.

23 SEP 2021

BLDE

(DEEMED TO BE UNIVERSITY)

Declared as Deemed to be University u/s 3 of UGC Act, 1956

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

1676/2021

18 AUG 2021

To
The Administrative Officer (A&E),
NIMHANS,
BANGALORE.

Sub:Modified Posting of 2nd year Post-graduate students for speciality training

Sir,

In continuation with your permission letter No:NIMH/A&E:TM:TRG-NEURO:2021/181,Dtd:11/02/2021,and as per the Telephonic discussion held with your office, in the view of Covid-19 clinical work load is more and new Postgraduate students have not been admitted till date. Therefore we request you to kindly modify the speciality training One month instead of Two Months as per the details given below:-

Sl.No	Name of the PG Students	DEPARTMENT	Period of Posting
1	Dr Y Sethu Reddy	General Medicine	01-10-2021 to 31-10-2021
2	Dr V Obul Reddy	General Medicine	
3	Dr Patil Bhushan Vijay	General Medicine	
4	Dr Sunil R	General Medicine	
5	Dr Sailee R Selvi	General Medicine	
6	Dr Chirag Sajjanar	General Medicine	
7	Dr Gudimetla R K Reddy	General Medicine	
8	Dr M Victoria	General Medicine	
9	Dr Nikitha R	General Medicine	
10	Dr Sujay V	General Medicine	
01	Dr Sneha Mukherjee	General Medicine	01-12-2021 to 31-12-2021
02	Dr K Swetha Sri	General Medicine	
03	Dr G Sahith Reddy	General Medicine	
04	Dr Santosh B T	General Medicine	
05	Dr Priyanka Tomar	General Medicine	
06	Dr Prashanth M R	General Medicine	
07	Dr Panchal Jatin Praveen	General Medicine	
08	Dr.Mayuri	General Medicine	
09	Dr Sai Santosh Jajimi	General Medicine	
10	Dr Vivan Sanjiv Vyas	General Medicine	
11	Dr Shirish Patil	General Medicine	

Please consider our request and accord the necessary permission.

Thanking you

Yours sincerely

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre
BIJAPUR

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.

BLDE (DU) : Phone: +918352-262770, Fax: +918352-263303 , Website: www.bldeuniversity.ac.in, E-mail:office@bldeuniversity.ac.in

College : Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldeuniveristy.ac.in

ಶ್ರೀ ಜಯದೇವ ಹೃದ್ರೋಗ ವಿಜ್ಞಾನ ಮತ್ತು ಸಂಶೋಧನಾ ಸಂಸ್ಥೆ
Sri Jayadeva Institute of Cardiovascular Sciences and Research
(Govt. Of Karnataka – Regd. Autonomous Institute)
Bannerghatta Road, 9th Block Jayanagar, Bengaluru – 560069
Ph:+91-80-22977400/600, **Academic Section & Fax:**080-22977281
Website: www.jayadevacardiology.com **Email:** director@jayadevacardiology.com
Academic section email: jayadevacardiology.academic@gmail.com

Ref: SJICS&R/AS/PG-Training/2021-22

Date: 27/09/2021

The Principal,
BLDE (Deemed to be University),
Shi B.M. Patil Medical College
Hospital & Research Centre,
Vijayapur - 586103

Sir,

Sub: Training Programme for PG students-reg.
Ref : Your letter No – 1852/21, dated 13/09/2021.

With reference to the above, we write to inform you that, the following PG students of your college are permitted to undergo training in the Department of Paediatric Cardiology at this Institute on the dates mentioned against their names on payment of fee of Rs. 12500/- per month per student, through DD, drawn in favour of the Director, SJIC&R, Bangalore - 69.

SL No.	Student name	Training Period
1	Dr. Lathish Ganapat	01/11/2021 to 30/11/2021
2	Dr. Siri Chandana	01/12/2021 to 31/12/2021
3	Dr. Tanmay Tyagaraj	
4	Dr. P. Mounica	01/01/2022 to 31/01/2022
5	Dr. Ranjima .M	

The students have to submit Covid-19 **Latest RT-PCR Negative** report at the time of reporting. Failing which postings will be cancelled.

Thanking you,

Your's faithfully,
K. C.
ACADEMIC SUPERINTENDENT

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre
Vijayapur-586103
04 OCT 2021

Note:
1. Please send your request letters atleast 3 months in advance for clinical training.
2. The students posted for external postings at this institute shall compulsory bring a copy of this permission order along with them at the time of reporting.

o/c

BLDE

(DEEMED TO BE UNIVERSITY)

Declared as Deemed to be University u/s 3 of UGC Act, 1956
The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

1857/21 13-09-2021

To
The Director,
Sri.Jayadeva Institute of Cardiology,
BANGALORE.

Sub: Posting of 2nd year Post-graduate students for speciality training

Sir,

As per the requirement of BLDE (Deemed to be University), Vijayapur the P.G. students of M.D. Paediatrics have to undergo One month training in Paediatric Cardiology in Speciality Centres of National and International repute.

Therefore I request you to permit following P.G. students of this Institution to undergo training in Paediatric Cardiology at your Institute. Please let me know the formalities to be completed in this regard.

Sl.No	Name of the PG Students	DEPARTMENT	DATE
1	Dr Lathish Ganapat	Paediatrics	01-11-2021
2	Dr Siri Chandana	Paediatrics	01-12-2021
3	Dr Tanmay Tyagaraj	Paediatrics	01-12-2021
4	Dr P.Mounica	Paediatrics	01-01-2022
5	Dr Ranjima M	Paediatrics	01-01-2022

Please consider our request and accord the necessary permission.

Thanking you

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR- 586103

NATIONAL INSTITUTE OF MENTAL HEALTH & NEUROSCIENCES
(INSTITUTE OF NATIONAL IMPORTANCE) BENGALURU - 560 029

NIMH:A&E:TM:TRG-NEURO:2021/1010

Date: 13.12.2021

The Principal
BLDE (deemed to be University)
Shri B.M. Patil Medical College Hospital & Research Centre
Vijayapura – 586 103.

Sir/Madam,

Sub: Request for Permission to undergo training at this institute – reg.
Ref: Your letter dated 06.12.2021.

* * * * *

With reference to the above, I am directed to convey the permission of the Competent Authority for the students of your institute to undergo training at this Institution as follows:

1	Number of trainees	11
2	Name of the trainees	**Duration**
	Dr. Gudimetla R K, Dr. Sahith Reddy, Dr. Vivaan Vyas, Dr. Prashanth M R, Dr. Sailee Belvi, Dr. Chirag M Sajjanar,	**03.01.2022 to 31.01.2022***
	Dr. Victoria M, Dr. K Swetha Sri, Dr. Sai Santosh Jamini, Dr. Obul Reddy and Dr. Nikitha R	**01.2.2022 to 28.02.2022***
3	Department at which training permitted	Neurology
4	Training fee	Rs.10,000/- per month per trainee

6
5

Note: ***Permission is subject to written assurance by Director/Dean/Principal/HOD of the above mentioned college/university that all the students who are posted will attend activity/duties of Neurology department everyday as per the timings of the department and will not take any planned leave during the period of posting.***

* ***based on COVID 19 Pandemic situation and guidelines RTPCR negative report (latest by 72 hours) or COVID Vaccination report to be provide on the day of joining***

- The trainees should compulsorily carry their college ID cards while posted at NIMHANS.
- One stamp size photo should be given at the time of joining for issue of temporary ID card. (ID card should be returned at the end of training without fail)
- Trainees should carry a copy of this letter without fail.
- ***The training fee for the whole duration of training has to be paid by SB collect(online) on the day of joining. Excess payment of training fee will not be refunded.***

I am also directed to inform you that the visiting students/trainees should make their own arrangement for accommodation. However all efforts will be made to provide hostel accommodation, but this will be subject to availability, ***based on Manager, Hostel report (080-26995095)***/Supervisor, Cauvery Hostel, (080-26995092) as on the date of joining and on payment of charges as below, **Accommodation will not be provided to the candidates coming earlier than the scheduled date of training.**

1. Hostel Rent: Rs.100/- per day

NOTE: *In case of any damage of assets/property in the Hostels i.e., movable and immovable property of NIMHANS by the trainees, the college shall be directly responsible for such act of the trainees. The loss incurred has to be borne by the Institution/College deputing the trainees. Further, the attendance certificate for training of such trainees will be withheld.*

On arrival, the trainees must contact the undersigned for further needful.

Yours faithfully

ASSISTANT ADMINISTRATIVE OFFICER (A&E)

Copy to: The HOD of Neurology, NIMHANS
The Manager/Supervisor, NIMHANS Hostels, NIMHANS

BLDE

(DEEMED TO BE UNIVERSITY)

Declared as Deemed to be University u/s 3 of UGC Act, 1956

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

2398/21

06 DEC 2021

To
The Administrative Officer (A&E),
NIMHANS,
BANGALORE.

Sub: Posting of 2nd year Post-graduate students for speciality training

Sir,

As per the requirement of BLDE University, Vijayapur the P.G. students of M.D. General Medicine have to undergo one month straining in Neurology in speciality centres of National and International repute.

Therefore I request you to permit following Six P.G. students (two Batches) of this Institution to undergo training in Neurology at your Institute. Please let me know the formalities to be completed in this regard.

Sl.No	Name of the PG Students	DEPARTMENT	Period of Posting
01	Dr. Gudimetla R K	General Medicine	01.01.2022 to 31.01.2022
02	Dr. Sahith Reddy	General Medicine	
03	Dr. Vivaan Vyas	General Medicine	
04	Dr. Prashanth M R	General Medicine	
05	Dr. Sailee Belvi	General Medicine	
06	Dr. Chirag M Sajjanar	General Medicine	
1	Dr. Victoria M	General Medicine	01.02.2022 to 28.02.2022
2	Dr. K Swetha Sri	General Medicine	
3	Dr. Sai Santosh Jamini	General Medicine	
4	Dr. Obul Reddy	General Medicine	
5	Dr. Nikitha R	General Medicine	

Please consider our request and accord the necessary permission.

Thanking you

Yours sincerely,

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre,
VIJAYAPUR-586103

Lata Mangeshkar Medical Foundation's
DEENANATH MANGESHKAR HOSPITAL & RESEARCH CENTER
Erandawane, Pune 411 004. Tel. : 020 - 40151000 / 49153000 Email : info@dmhospital.org, Website : www.dmhospital.org

PST09S/1

DMH/2021/AMS/GEN/20

24th July 2021

CERTIFICATE

This is to certify that **Dr. Bindu Madhav Yendigeri** has successfully completed his Observer ship in **ICU Department,** at Deenanath Mangeshkar Hospital And Research Centre, Pune, India.

Duration of Observer ship : 19th July 2021 To 21st July 2021

We wish all the best for his future.

Dr. Asmita Bhave
Asst. Med. Superintendent
(Academics)

Dr. Prasad Rajhans
Chief Intensivist
(ICU)

LATA MANGESHKAR MEDICAL FOUNDATION
DEENANATH MANGESHKAR HOSPITAL PUNE-4

Lata Mangeshkar Medical Foundation's
DEENANATH MANGESHKAR HOSPITAL & RESEARCH CENTER
Erandawane, Pune 411 004. Tel. : 020 - 40151000 / 49153000 Email : info@dmhospital.org, Website : www.dmhospital.org

DMH/2021/AMS/GEN/25

24th July 2021

CERTIFICATE

This is to certify that **Dr. Vijay Kumar** has successfully completed his Observer ship in **ICU Department,** at Deenanath Mangeshkar Hospital And Research Centre, Pune, India.

Duration of Observer ship : 22nd July 2021 To 24th July 2021

We wish all the best for his future.

Dr. Asmita Bhave
Asst. Med. Superintendent
(Academics)

LATA MANGESHKAR MEDICAL FOUNDATION
DEENANATH MANGESHKAR HOSPITAL PUNE-4

Dr. Prasad Rajhans
Chief Intensivist
(ICU)