Annual Report

2019-2020

Bharati Vidyapeeth (Deemed to be University)

INTERACTIVE RESEARCH SCHOOL FOR HEALTH AFFAIRS

IRSHA
INTERACTIVE RESEARCH SCHOOL FOR HEALTH AFFAIRS

Bharati Vidyapeeth Deemed University

Interactive Research School for Health Affairs (IRSHA)

Annual Report July 2019- June 2020

Overview of Director

It is my privilege to present the Annual report of Interactive Research School for Health Affairs (IRSHA) for the year 2019-20. The major success of this year is setting up facility of **National Immunogenicity & Biologics Evaluation Center (**NIBEC) and its formal inauguration. All the departments of IRSHA were successful in receiving financial support of Rs.1129.73 Lakhs from national funding agencies for carrying out their research work. This year student fellowships of Rs. 32.36 lakhs were received. In the current year 6 students were awarded PhD degree.

In the year 2019-20 research work at the institute culminated into 22 publications research articles; 2 book chapter and 3 patent applications.

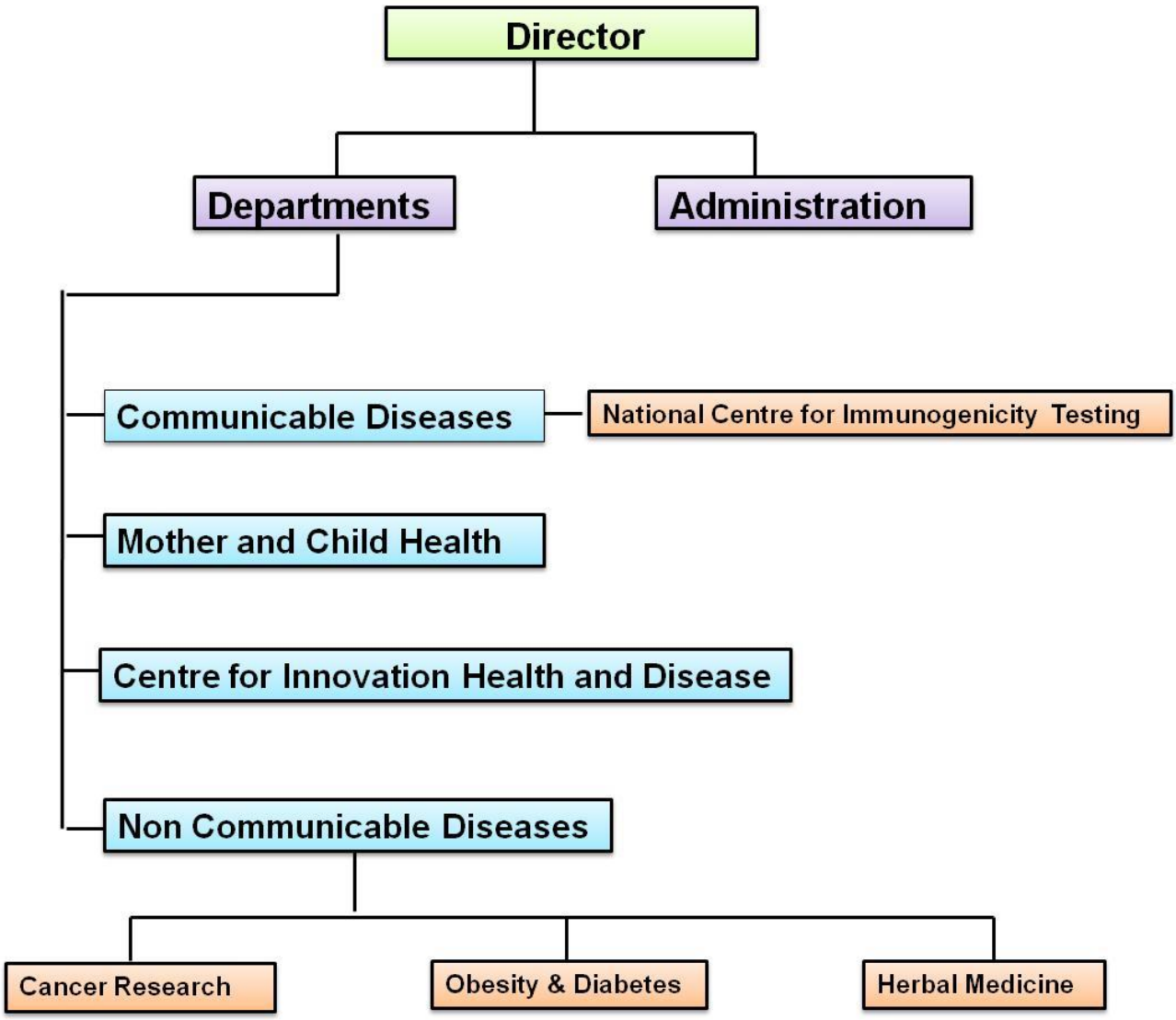
Several activities had been organized at the institute and also the staff and students participated in national and international events. A brief summary of these events, activities and achievements by all the staff members has been presented in the current report.

I appreciate the support and hard work of all the scientists, technical and administrative staff for their commendable performance.

Finally I sincerely thank the management for extending all the support for undertaking our research work.

Dr A C Mishra, M Sc, Ph D, LL B, FASc, FNA
Director

Organogram


Director
Departments
Administration
Communicable Diseases
National Centre for Immunogenicity Testing
Mother and Child Health
Centre for Innovation Health and Disease
Non Communicable Diseases
Cancer Research
Obesity & Diabetes
Herbal Medicine

Name of the Programme: Mother and Child Health

1. **Title:** Investigating Mechanisms Leading to Preeclampsia (**Project ID:** MCH/17/1/E); **Funding:** ICMR, Centre for Advanced Research; **Duration:** March 2017 to March 2022; **Sanctioned Amount**: Rs. 7,55,55,247/- **Investigators: PI** - Dr. Sadhana Joshi; **Co PI**- Dr. Girija Wagh, Dr. Sanjay Lalwani, Dr. Sanjay Gupte; Co-Investigators - Dr. Giriraj Chandak; Dr. Savita Mehendale, Dr. Arun Kinare, Dr. Priscilla Joshi, Dr. Leena Srivastav, Dr. Hemant Mandke, Dr. Anvita Kale, Dr. Deepali Sundrani, Dr. Nisha Wadhwani; **Ph.D. Students**: Aditi Godhamgaonkar; Vaishali Kasture (DST Inspire-SRF); Juhi Nema (CSIR-JRF); Anjali Jadhav (ICMR-SRF); Kinjal Dave (CSIR-JRF); **Human Ethical Approval**: IEC/2015/37, dated 03.10.2015

Background: The current study aims to follow pregnant women from early pregnancy until delivery, to examine changes across gestation in nutritional, biochemical, and molecular measures and identify the underlying mechanisms which influence the pathophysiology of preeclampsia (PE). This will be useful in development/validation of biomarkers for early prediction of PE. The study will also follow up the children's growth during infancy and their neurodevelopment at the age of 2 years.

Work done: All consenting participants were recruited at 11–14 weeks of gestation (visit 1 – V1), assigned a unique participant code, and were subsequently followed across gestation at three time points viz. 18–22 weeks (visit 2 – V2), 26–28 weeks (visit 3 – V3) and at delivery. A total number of 1134 pregnant women have delivered till date from two hospitals (Bharati Hospital and Gupte Hospital) of which 844 are normotensive controls, 156 developed gestational diabetes mellitus (GDM), 102 women with PE, 23 with both PE and GDM, 9 with gestational hypertension. Maternal blood was collected at each time point; cord blood and placenta were collected at delivery. Information on subjects clinical history, medication, SLI, physical activity, 24 hr dietary recall, FFQ, ultrasonography and color Doppler measures are recorded at each time point.

Children Follow Up: Follow up of children for anthropometric measurements at various time points is ongoing. The children are followed up as per the vaccination routine/schedule at 6 wks, 10 wks, 14 wks, 6 months, 9 months, 12 months, 15 months, 18 months and 24 months.
Developmental Scores: The Development Assessment Scale for Indian Infants (DASII) is being administered at 2 years of age by trained Psychologists at both the hospitals.

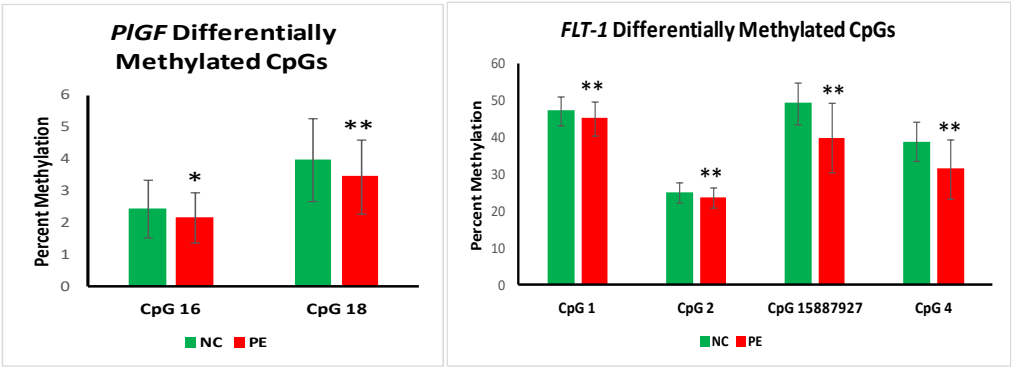
Biochemical Analysis: The biochemical analysis for various parameters like folate, vitamin B_{12}, homocysteine, calcium, magnesium, high-sensitivity C-reactive protein (hsCRP), fatty acids, vascular endothelial growth factor (VEGF), soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), vitamin D, Prostaglandin E2 (PGE2), thromboxane B2 (TXB2), 8 – hydroxy guanidine, malionaldehyde (MDA), sEndoglin (sEng), hypoxia brain derived neurotrophic factor (BDNF), nerve growth factor (NGF) is ongoing.
Epigenetic work: Genomic DNA was isolated from the placental samples and was bisulfite-treated using methylation kits. Bioinformatic was undertaken for selection of regions for DNA methylation.

Preliminary analysis on PE and non-PE groups suggests that:
Women who develop PE have a higher age, BMI and blood pressure right from early pregnancy until delivery as compared to non-PE women. Percent women in the PE group conceiving through IVF and IUI was higher as compared to the non-PE group and delivered by caesarean section

Majority of the women in the PE group were nulliparous
TSH levels were observed to be higher in women with PE in early pregnancy
Fetal measurements such as biparietal diameter, head circumference, abdominal circumference and fetal weight at 32-35 weeks were lower in women with PE as compared to non-PE group
Doppler measure such as mean uterine artery PI was higher in PE as compared to non-PE group
Women with PE had placentae with lower thickness and had a higher percent of bilobed and irregular shaped placentae
Babies born to mothers with PE had a lower birth weight, length and head circumference. The percent of SGA babies was higher in the PE group as compared to non-PE group
Women who had a high BMI i.e. >25kg/m2 in early pregnancy (11-14 weeks of gestation) developed either GDM or PE

Methylation of PlGF and FLT-1
We, for the first-time report that lower placental DNA methylation of both PlGF and FLT-1 (Fig.1), two critical genes involved in angiogenesis are strongly associated with preeclampsia. We also demonstrate lower PlGF but comparable FLT-1 expression between preeclampsia and normotensive controls. The study also reveals gestational age as an important factor influencing placental DNA methylation in term and preterm preeclampsia. Our results suggest a mechanistic basis towards the role of sFLT-1/ PlGF ratio as a biomarker for risk prediction of preeclampsia. Altered methylation of these genes in the placentae may influence angiogenesis, placental growth as well as intrauterine fetal development which may predispose the children to higher risk of cardiometabolic disorders in future. Hence, future studies need to identify maternal nutritional factors driving these consequences in a systematically conducted study which can then be used to alleviate the risk of development of preeclampsia.

Figure 1: DNA methylation at statistically significant differentially methylated CpGs in *PlGF* and *FLT-1* genes



Values are expressed as mean ± SD. NC: normotensive control, PE: Preeclampsia, PlGF: placental growth factor, FLT-1: fms-like tyrosine kinase-1, p, Level of Significance; *p <0.05,

**p<0.01 as compared to NC. ID for CpG 24192328 in *PlGF* and CpG 15887927 in *FLT-1* is taken from details of CpG sites available on 450K Bead chip array from Illumina.

2nd Advisory Committee Meeting:
The second Advisory Meeting was held on the 20th Feb, 2020 which involved clinicians, research scientists, and research experts. The committee members felt that the study has made good progress and appreciated the efforts made by all the investigators at both the hospitals.

2nd Advisory Meeting held at Gupte Hospital

2.Title: Early Interventions to Support Trajectories for Healthy Life in India (EINSTEIN). Healthy Life Trajectories Initiative (HeLTI) (**Project ID:** MCH/17/2/E) Multicentric Project; **Funding:** DBT; **Sanctioned Amount:** Total Sanctioned Rs. 743.44 Lakhs; IRSHA Share: Rs.13.50 Lakhs; **Duration:** Dec 2017 to Nov 2020; **Investigator at IRSHA**: Dr. Sadhana Joshi; **Human Ethical Approval**: IEC/2018/34

Background: The study is a community-based, cluster randomized intervention with three arms (pre-conception, pregnancy and control) set in rural Mysore, South India, with individual villages forming the basis for the cluster. The primary outcome at age 5 years in the children across all HeLTI cohorts is adiposity, measured by fat mass index. Other key outcomes at 5 years include; overweight and obesity, glucose metabolism, blood pressure, and infant/child development.

Work done:
Formative work: Mysore Team commenced the formative work in November 2018 in three villages
Community engagement: Extensive community engagement to explain the study and assess the community's interest and willingness to not only participate, but also contribute to the study design and delivery
Qualitative work: Undertook focus group discussions (FGDs) with village women, husbands, mothers/mothers-in-law, village leaders and officials, and local community health staff.
Quantitative work:
Analyses of fatty acids have been undertaken at IRSHA, Bharati Vidyapeeth, Pune

Plasma fatty acid profile revealed a high n6/n3 PUFA ratio (total n6=33.51 g/100g (SD 4.57); total n3=1.51 g/100g (SD 0.60); n6/n3 ratio=26:1

Intervention development: The core members of the India and Canada teams conceptualised the intervention modules and prepared the outline in February 2019. The intervention will be delivered across four phases. The local team then developed six pre-conceptional modules: General Health; Healthy Eating; Health Lifestyle; Keeping Clean; Positive Thinking; and Preparing for Pregnancy.
Harmonisation and governance : All four HeLTI teams have worked together to harmonise data variables and intervention domains and we have achieved a high degree of harmonisation

3. Title: Pro-neurotrophins /p75NTR Signalling Contributes to Increased Apoptosis in Preterm Placentae (**Project ID**: MCH/17/3/E); **Funding**: DST-SERB; **Sanctioned Amount**: 40.48 Lakhs; **Duration:** June 2017 to June 2020; **Investigators: PI:** Dr. Preeti Chavan Gautam; **Co-Investigators:** Dr. Sadhana Joshi; **Human Ethical Approval**: IEC/2017/11
PROJECT TRANSFERRED TO PUNE UNIVERSITY
Biochemical and molecular analysis is ongoing

4.Title: Influence of Maternal One-carbon (1C) Metabolism in Placental Function, Fetal Growth and Programming **(Project ID:** MCH/18/1/E) Multi-Institutional; **Funding:** DBT; **Sanctioned Amount:** Total Sanctioned Rs. 170.00 lakhs; IRSHA Share: Rs. 68.5; **Duration:** March 2018 to Feb 2021; **Principal Investigator at IRSHA**: Dr. Sadhana Joshi; **Human Ethical Approval**: IEC/2018/44

Background: The primary goal of this proposal is to examine the influence of maternal 1C metabolism on placental structure and function and understand the molecular mechanisms underlying these events.

Work done:
Isolation of Placental Plasma Membranes: The stored placental homogenates were used for the isolation of placental syncytiotrophoblast basal and microvillous plasma membranes. A total of 28 samples have been processed for isolation of these plasma membranes [(basal membrane (BM) and microvillous membrane (MVM)] which are snap frozen and stored in aliquots in -80ºC.
a) Microvillous Membrane (MVM) Alkaline Phosphatase Activity: Alkaline phosphatase activity was 22.93 ± 9.98 fold higher in MVM-vesicles compared to placental homogenates (n=28), and did not significantly differ between the groups.
b) Basal Membrane (BM) VDAC1 Protein Expression: The mean enrichment of VDAC expression in BM compared to placental homogenates was 31.41 ± 20.85 (n=28), and did not significantly differ between the groups.

Expression of Nutrient Transporters in Isolated Membranes: Western blot analysis for vitamin B12 receptor CD320 in the MVM and BM of control and preeclampsia placental samples is standardized and has been completed on all 28 samples. Vitamin B12 estimations

Chemiluminescent microparticle immunoassay (CMIA) technology was used to estimate vitamin B12 (Abbott Diagnostics, Abbott Park, IL, USA) in maternal and cord plasma.

Results and conclusion

Vitamin B_{12} levels:Vitamin B_{12} levels were similar in the maternal plasma between normotensive control women and women with preeclampsia. Similarly, vitamin B_{12} levels were similar in the cord plasma between normotensive control women and women with preeclampsia. In the NC women, vitamin B12 levels were higher in the cord (median [25, 75thC], 225 pg/ml [148,187]) as compared to maternal blood (149 pg/ml [148,319]) ($p<0.05$). Maternal and cord blood B12 levels were similar in women with PE (median 148 pg/ml both, $p>0.05$).

CD320 protein levels: CD320 protein expression was similar in the BM and MVM in normotensive control women (Figure 1[A]). CD320 expression was higher in the BM compared to MVM (+20%, $p=0.057$) in the preeclampsia placentae. Preeclampsia women showed lower CD320 expression in MVM (-60%; $p<0.05$) and BM (-40%; $p=0.081$) compared to NC group (Figure 1[B]). Figure 4 [A] shows a representative western blot image of CD320.

Figure 2[A] CD320 Expression in the MVM and BM across NC and PE placentae

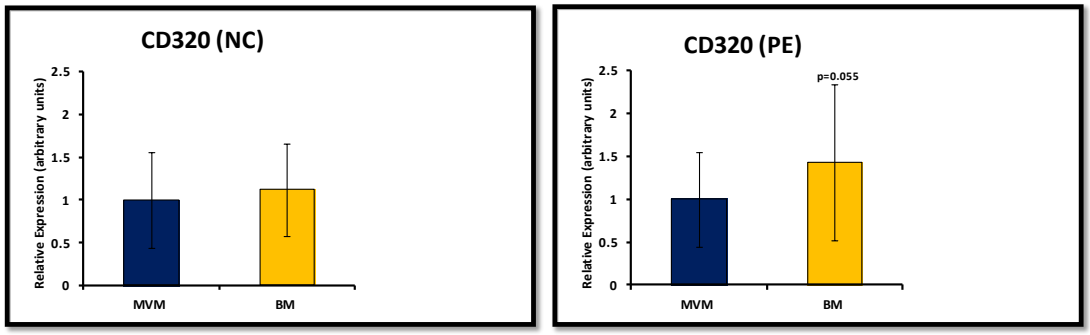
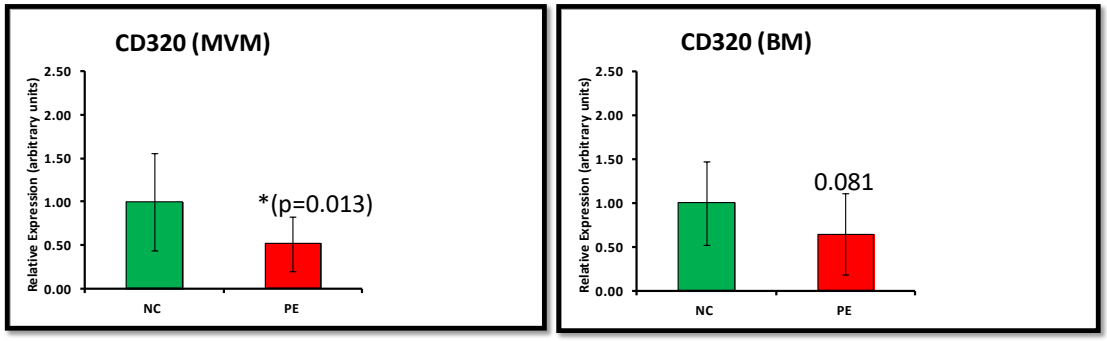


Figure 2[B] CD320 Expression in the MVM and BM between NC and PE placentae



Values represented as Mean ± SE; NC: Normotensive Control; PE: Preeclampsia

*$p<0.05$ as compared to Normotensive Control

Association of vitamin B12 and CD320 expression with birth weight

Maternal B12 levels were positively associated ($r=0.505$; $p<0.05$) while CD320 expression on both MVM and BM were negatively associated with birth weight of the baby ($r= \sim 0.6$, $p<0.05$).

This is the first study to report expression of vitamin B12 receptor CD320 in the syncytiotrophoblast MVM and BM of normotensive and preeclamptic pregnancies. Reduced MVM CD320 expression in placentas from preeclampsia may explain the failure to achieve higher B12 concentrations in fetal circulation in preeclampsia pregnancies. Higher placental CD320 expression in lower birth weight babies suggests higher expression of CD320 transporter to promote fetal growth. This is further supported by the positive association of maternal vitamin B12 with baby weight.

5. Title: OPTIMISE: Optimal preconception nutrition to offset inflammation and non-communicable disease risk in pregnant women and their children in China, India and South Africa; **Funding:** Medical Research Council, United Kingdom; **Duration** : 5 years; Project Sanctioned but not initiated; **Investigators: Principal Investigator** Dr Kalyanaraman Kumaran University of Southampton Human Development and Health; **Co-Investigator** Professor Caroline Fall University of Southampton Human Development and Health Co-Investigator Professor Philip Calder University of Southampton Human Development and Health Co-Investigator Dr Mark Johnson Imperial College London Surgery and Cancer; Co-Investigator Dr Amanda SferruzziPerri University of Cambridge Physiology Development and Neuroscience; Co-Investigator Professor Shane Norris University of the Witwatersrand Faculty of Health Sciences Co-Investigator Professor Stephen Matthews University of Toronto Physiology; Co-Investigator Dr Stephen Lye University of Toronto Physiology; Co-Investigator Dr Ghattu V Krishnaveni CSI Holdsworth Memorial Hospital Research; Co-Investigator Dr Giriraj Chandak CSIR - Centre for Cellular and Molecular; Co-Investigator Dr catherine birken Hospital for Sick Children (SickKids) Paediatrics and Genetics Co-Investigator Professor Cindy-Lee Dennis University of Toronto Unlisted; Co-Investigator Dr William Fraser University of Sherbrooke Faculty of Medicine and Health Sciences Co-Investigator Professor Hefeng Huang Huang Shanghai Jiao Tong University Medical School; Co-Investigator Professor Luigi Bouchard University of Sherbrooke Faculty of Medicine and Health Sciences; Co-Investigator Dr Fengxiu Ouyang Shanghai Jiao Tong University; Co-Investigator Dr Yanting Wu Shanghai Jiao Tong University Medical School; Co-Investigator Dr SADHANA JOSHI Bharati Vidyapeeth University IRSHA, Pune (School for Health Affairs)

Hypothesis: We propose that inflammation is an important modifiable factor underlying an inter-generational cycle of non-communicable disease (NCD) risk in low- and middle-income countries (LMICs). We hypothesise that recent dietary changes in LMICs (causing the 'double burden of malnutrition') set up a chronic inflammatory state which increases the risk of type 2 diabetes (T2DM) and cardiovascular disease (CVD). Among pregnant women, this inflammatory state leads to pregnancy complications (gestational diabetes, hypertensive disorders and pre-term birth) and placental changes that impair fetal growth. These disrupt fetal neurodevelopment and increase fetal adiposity. Optimising maternal diet and nutritional status before and during pregnancy will reduce inflammation, prevent pregnancy complications and improve newborn body composition. Long term benefits, beyond the scope this project, will be reduced NCD risk

in the mother, and improved brain development and reduced cardiometabolic disease in the offspring.

OPTIMISE aims to leverage a unique trio of harmonised randomised controlled trials (RCTs) taking place in China, India and South Africa to:
Determine context-specific nutritional factors influencing inflammatory load among young women and how nutrition interacts with other drivers of inflammation
Elucidate relationships between maternal inflammatory load and common adverse pregnancy outcomes (gestational diabetes, hypertensive disorders, pre-term birth and fetal growth restriction)
Determine if a package of interventions to optimise women's nutrition before and during pregnancy reduces inflammatory load and these adverse pregnancy/birth outcomes
Investigate mechanisms, including altered placental structure, inflammation and nutrient transport capacity, linking inflammatory load with adverse pregnancy outcomes.

6. **Title:** Epigenetic regulation of placental peroxisome proliferator activated receptor (PPAR) in women delivering low birth weight babies (**Project ID:** MC/19/1/E); **Funding:** DBT BioCARe; **Sanctioned Amount:** 35.50 Lakhs; **Duration:** April 2019 to April 2022; **Investigators:PI**- Dr. Deepali P. Sundrani **Co-Investigators**: Dr. Sadhana Joshi; Dr. TusharPanchanadikar

Background: Low birth weight (LBW) babies are associated with fetal and neonatal morbididty and motality and are at increased risk for non-communicable diseases in later life. However, the molecular determinants of LBW are not well understood. The placenta is known to play a key role in 'programming' the fetus for risk of diseases in later life. Peroxisome proliferator-activated receptor (PPAR) is a key transcription factor that regulates placental angiogenesis and its activity regulated by ligands such as long chain polyunsaturated fatty acids. This study aims to understand the molecular mechanisms (DNA methylation and microRNA regulation) underlying the association of maternal fatty acid status and PPAR in the placenta of women delivering LBW babies.

Work Done:
Recruitment and sample collection for 100 NBW(control) and 70LBW subjects is completed.
Subjects history and clinical information and neonatal characteristics have been recorded
Gestational age, placental weight and neonatal characteristics like birth weight, length and chest circumference is lower in the LBW group as compared to NBW group.
Placental dimensions like major axis, minor axis, breadth and trimmed placental weight are lower in LBW group as compared to NBW group.
Standardization of RT-PCR for mRNA expression of PPAR alpha, PPAR gamma and PPAR delta is completed and mRNA expression analysis is completed on 100 NBW and 53 LBW samples.
Reduced expression of key transcription factors PPARalpha and PPARgamma was observed in the placentae of women delivering LBW babies. Considering the critical role of these transcription factors in placental angiogenesis and development, reduced expression of these PPAR may contribute to placental insufficiency in LBW cases.

Standardization for methylation analysis of PPARalpha, PPARdelta and PPARgamma genes is completed.
Estimation of methylation levels on NBW and LBW samples is on-going.

7. Title: Epigenetic regulation of angiogenic factors in assisted reproductive technology (ART) and non-ART derived placentae **(Project ID**: MC/19/2/E); **Funding:** DBT; **Sanctioned Amount**: 59.91 Lakhs; **Duration:**July 2019 to July 2022; **Investigators: PI-** Dr. Deepali P. Sundrani **Co-Investigators:** Dr. Sadhana Joshi; Dr. Sanjay Gupte

Background: In India, the rate of infertility is on the rise thereby increasing the demand for assisted reproductive technology (ART) procedures. ART treatment coincides with several phases of epigenetic programming during gametogenesis and early embryo development. During these stages,*de novo* methylation and chromatin remodeling takes place which influences the placental structure and function by switching on and off various genes. This study aims to examine the placental epigenetic patterns of angiogenic factors in women undergoing ART procedures and also examine their association with maternal one carbon metabolites and fatty acid profile.

Work Done:
Recruitment of patients was carried out atGupte Hospital and till date we have completed recruitment and sample collection of 84 women who conceived naturally (Non-ART group) and 24 women who have underwent ART procedures (ART group).
Subjects history and clinical information and neonatal characteristics have been recorded.
Placenta and maternal blood samples at delivery are collected, processed and stored at -80°C.
Higher maternal age and blood pressure is observed in women of the ART group.
In order to compare the mRNA expression patterns of angiogenic factors, total RNA is isolated from the collected placental tissue samples. This RNA is converted into cDNA for further RT-PCR analysis.
To check the efficiency of the primers for RT-PCR analysis, standardization of VEGF, PlGF, FLT-1, KDR and GAPDH (internal control) primers has been successfully completed.
Placental DNA isolation for methylation analysis of angiogenic factors is initiated.

8. Title: Exploring the Effect of Maternal Omega-3 Fatty Acids and Vitamin E Supplementation on Hepatic Phospholipids in a Rat Model of Preeclampsia (**Project ID:** MC/18/1/I) **Funding:** Intramural; **Duration**: Aug 2018 to March 2021; **Sanctioned Amount**: -; **Investigators: PI-** Dr. Anvita kale, Dr. Sadhana Joshi; ; **Technical Staff**: Rahat Khan; **Animal Ethical Approval:** BVDU/IRSHA/2017-2018/582

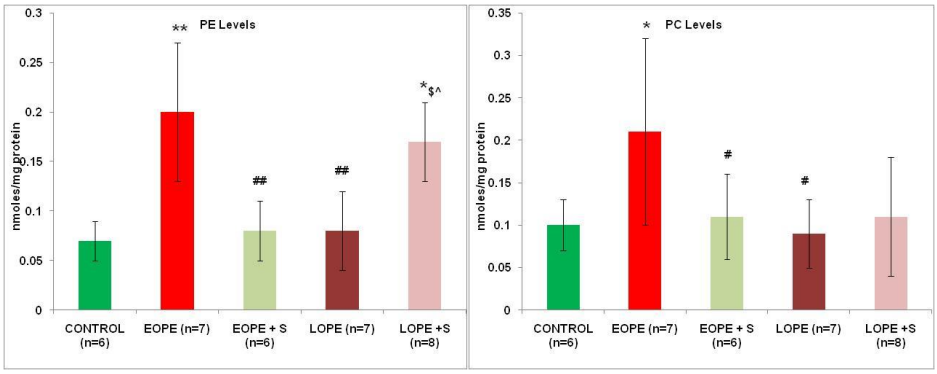
Background: Preeclampsia (both early onset and late onset) leads to increased risk of developing cardiovascular disorders (CVD) in the mother. Dysregulation in the lipid metabolism/ transport is crucial in CVD. We report the effect of supplementation of omega-3 fatty acids and vitamin E on hepatic phospholipids in an animal model of preeclampsia at d20 of gestation.

Work done: Phospholipid Analysis was done using the TLC procedure and PEMT gene expression done using the RT PCR method. Analysis was done on collected tissue wherein

pregnant Wistar rats (n=8 in each group) were assigned to control; early onset preeclampsia (EOPE); late onset preeclampsia (LOPE); EOPE + supplementation (EOPE + O + E) and LOPE + supplementation (LOPE + O + E). Levels of phosphatidyl ethanolamine (PE) ($p<0.01$) and phosphatidyl choline (PC) ($p<0.05$) increased only in EOPE but not in the LOPE group.

Results and conclusion: Key findings of the present study are: 1) Phospholipids PE and PC levels increase only in EOPE and supplementation lowers these levels 2) PC levels were similar to control in LOPE group but supplementation increased the levels of PE. 4) PEMT gene expression was lower only in LOPE group and supplementation increased these levels.

Fig.3: Levels of PE and PC Phospholipids in Dam Liver at D20 of Gestation



Our study demonstrates differential levels of phospholipids (PE and PC) in both subtypes of preeclampsia (EOPE and LOPE). Supplementation of Omega-3 fatty acids and vitamin E maintains the levels of PC in both early and late onset preeclampsia suggesting adequate supply of PC-DHA from the liver to the peripheral tissues. Our study implicates the beneficial effects of a combined supplementation of Omega-3 fatty acids and vitamin E in maintaining the levels of phospholipid PC which may help in preventing the lipid dysregulation in preeclampsia.

9. Title: Placental Endoplasmic Reticulum Stress Markers in Women with Preeclampsia and their Association with Maternal One Carbon Metabolites (**Project ID:** RBMH/FW/2019/1/RA); **Funding:** ICMR; **Sanctioned Amount:** Rs. 14,38,720/; **Duration:** July 2019 to June 2021; **Investigators:** Dr. Akriti Sahay, Dr. Sadhana Joshi, Dr. Girija Wagh; **Human Ethical Approval**: BVDUMC/ IEC/ 66

Background: The current proposal aims to examine the mRNA (by RT-PCR) and protein levels (by ELISA) of endoplasmic stress markers from the placenta of women with preeclampsia and compare them with normotensive control women. The study will also examine the association of placental ER stress with maternal one carbon metabolites and fatty acids.

Work done: mRNA levels of ATF-4, CHOP and PERK and eIF2alpha has been completed on 50 control and 46 preeclampsia samples.

Results
The mRNA levels of ATF-4, CHOP and PERK were similar in women with preeclampsia and normotensive control women.
The mRNA levels of eIF2 alpha were lower in women with preeclampsia as compared to normotensive control women.
ATF-4, CHOP and PERK mRNA levels were similar in control, term preeclampsia and preterm preeclampsia group. However, mRNA levels for eIF2aplha were higher in term preeclampsia group as compared to normotensive control group.
All ER stress markers were higher in women with preeclampsia as compared to normotensive control group in women who underwent vaginal deliveries.

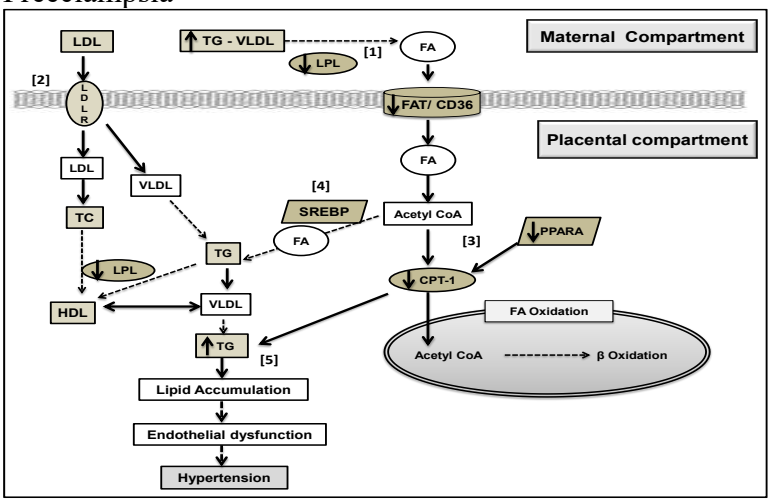
10 Title: Placental Lipid Transport and Fetal Growth in Preeclampsia (**Project ID**:MCH/18/1/RA); **Funding:** Indian Council of Medical Research; **Duration**: Sept 2018- Sep 2021; **Sanctioned Amount:** 14.42 Lakhs; **Investigators:** Dr. Amrita Khaire, Dr. Sadhana Joshi, Dr. Girija Wagh; **Human Ethical Approval**: BVDUMC/ IEC/ 33A

Background: The present study examined the placental and maternal lipid profile and expression of genes involved in placental lipid metabolism in women with preeclampsia.

Work done: Analysis of placental lipid levels (total cholesterol, triglyceride, HDL, LDL) was carrie out from 40 normotensive and 40 women with preeclampsia. The expression of genes involved in placental lipid metabolism (SREBP1; PPARα; LDLR; LPL; CD36; CPT1B,C) was also studied.

Results and conlcusion:
Higher placental total cholesterol and HDL levels in term preeclampsia while triglyceride levels were higher in preterm preeclampsia
Lower placental mRNA expression of PPARα, LPL, CD36 and CPT1 in preterm preeclampsia.
A negative association of mRNA levels of PPARα, CPT1, LPL with systolic and diastolic blood pressure and a positive association of placental triglycerides with diastolic blood pressure.
This is the first study that simultaneously analyzed maternal and placental lipids and placental lipid metabolism in women with preeclampsia. The findings of this study suggest that women with preeclampsia exhibit higher lipid: lipoprotein ratios and altered placental lipid metabolism. These changes are more pronounced in the women with preeclampsia delivering preterm as compared to those delivering at term suggesting a higher cardiovascular risk for these women in later life. The increased placental triglyceride levels in the preterm preeclampsia group are indicative of disturbed lipid metabolism in the placenta.

Fig 4: Mechanism Illustrating the Changes in Placental Lipid Metabolism in Preterm Preeclampsia



The lower LPL expression may lead to lower hydrolysis of triglyceride carrying lipoproteins in the placenta [1]. LDLR may mediate normal cholesterol transport; however reduced LPL activity may negatively affect the conversion of total cholesterol to its lipoprotein carriers (such as HDL), thereby affecting lipid transport [2]. The lower expression of PPARα and CPT1 may contribute to the reduced fatty acid oxidation; thus facilitating the diversion of acetyl CoA towards fatty acid synthesis through SREBP1 [3,4]. These changes may possibly lead to increased triglyceride synthesis and accumulation in the placenta which may further lead to endothelial dysfunction [5].

11. Title: Vitamin D Status and Long Chain Polyunsaturated Fatty Acid Metabolism in Pregnancy (**Project ID**:MCH/15/1/P) **Sanctioned Amount:** 21 Lakhs; **Duration:** April 2015 to April 2020 **Funding:** UGC; **Guide:** Dr. Sadhana Joshi; **PhD Student**: Anindita Nandi (UGC JRF/SRF)

Background: Vitamin D may influence the one carbon cycle by inhibiting the production of homocysteine and decreasing oxidative stress by inducing CBS enzyme. Lower oxidative stress will result in lower COX-2 expression and TXB2 production thereby lowering the ratio of TXB2 to 6-keto PGF1α. We therefore hypothesize that maternal vitamin D status influences LCPUFA metabolism through alterations in the one carbon cycle leading to an adverse pregnancy outcome.

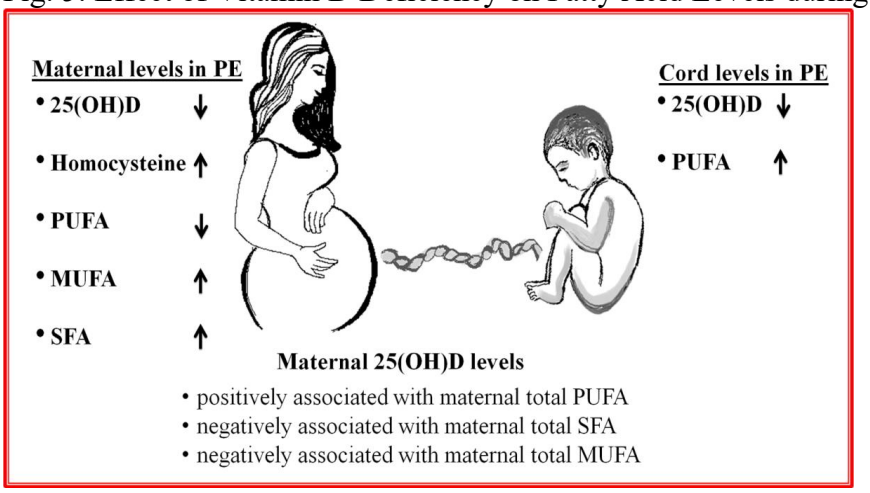
Work done: The present cross-sectional study recruited 69 normotensive control (NC) and 50 women with preeclampsia at delivery. maternal and cord serum levels of 25(OH)D, maternal,cord and placental LCPUFA levels and maternal LCPUFA metabolites (TXB2, 6-keto PGF 1α) and the placental levels of VDR, RXR, PLA2, COX-2 in the above women We have analyzed

Results and conclusion:
Maternal 25(OH)D levels were negatively associated with maternal systolic and diastolic BP ($p<0.01$ for both).

Maternal and cord serum 25-hydroxyvitamin D [25(OH)D] levels were lower ($p<0.01$ for both) in women with PE compared to NC women.
Maternal plasma total polyunsaturated fatty acids (PUFA) levels were lower ($p<0.05$) while levels of total saturated fatty acids (SFA) and total monounsaturated fatty acids (MUFA) were higher ($p<0.05$ for both) in women with PE.
Cord erythrocyte PUFA levels were higher ($p<0.01$) in PE women. Maternal 25(OH)D levels were positively associated with maternal total PUFA ($p<0.01$) and negatively associated with maternal total SFA ($p<0.05$), total MUFA ($p<0.01$).
Women with preeclampsia had lower placental protein and mRNA levels of CBS enzyme, higher plasma MDA levels and higher placental levels of AA and omega-6 fatty acids. Women with preeclampsia also demonstrated higher placental mRNA levels of COX-2 as compared to NC.
Maternal 25(OH)D levels were negatively associated with maternal plasma MDA levels. Placental VDR levels were positively associated with CBS while maternal MDA levels were positively associated with serum levels of TXB2 levels.

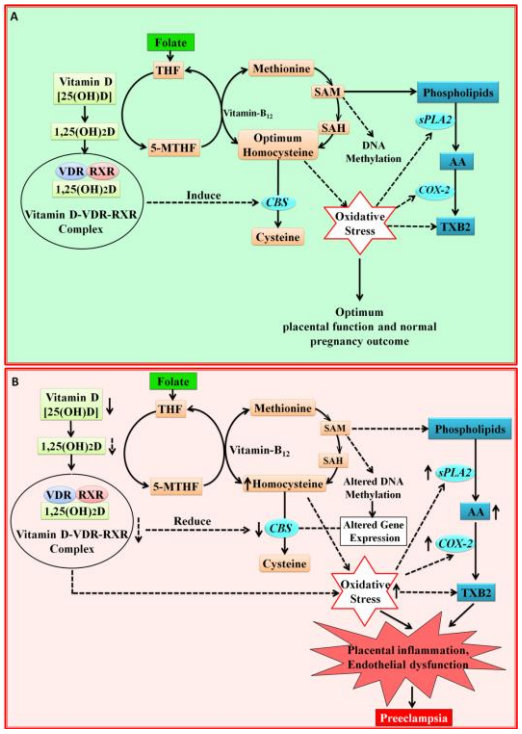
Maternal vitamin D deficiency exists in women with preeclampsia. It may increase oxidative stress by increasing the production of homocysteine in the one carbon cycle. Increase in oxidative stress further influences the inflammatory pathway of LCPUFA and the production of vasoconstrictor thromboxanes which may lead to inflammation and endothelial dysfunction in preeclampsia.

Fig. 5: Effect of Vitamin D Deficiency on Fatty Acid Levels during Pregnancy



PE: preeclampsia; 25(OH)D: 25-hydroxyvitamin D; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids

Fig. 6: Possible Mechanism through which Maternal Vitamin D Influences Arachidonic Acid Metabolism through the One Carbon Cycle (A) Normal Pregnancy (B) Preeclampsia



25(OH)D: 25-hydroxyvitamin D; 1,25(OH)2D: 1,25-dihydroxyvitamin D; 5-MTHF: 5-methyltetrahydrofolate, AA: arachidonic acid, CBS: cystathionine-β-synthase, COX-2: cyclooxygenase-2, sPLA2: secreted phospholipase A2, ROS: reactive oxygen species, RXR: retinoid X receptors, SAM: S-adenosylmethionine ; SAH: S-adenosylhomocysteine; THF: tetrahydrofolate, TXB2: thromboxane B2, VDR: vitamin D receptor

12.Title: One carbon cycle metabolites and apoptosis in preeclampsia (**Project ID**:MCH/15/2/P) **Funding:** Indian Council of Medical Research; **Duration:** Sept 2015- Sep 2020 Sanctioned Amount: 24, 42 lakhs. PhD: Vaishali Kasture Guide: Dr. Sadhana Joshi; **Animal Ethical Approval:** IAEC/ CPCSEA/ BVDUMC/2670/2017/002/016) on 24/03/2017; Human **Ethical Approval:** BVDU/MC/E 53

Background: The present study aims to examine maternal one carbon components and LCPUFA and their influence on placental apoptosis in preeclampsia. This is the first study which will examine the effect of omega-3 fatty acid and vitamin E supplementation on placental apoptotic markers .

Work Done :
Animal Study: Examined the expression of placental protein and mRNA levels of apoptotic markers (Bax, Bcl2, caspases 8 and 3) in early onset and late onset Preeclampsia.Examined the expression of placental protein and mRNA levels of angiogenic markes (VEGF, FLT-1, PPAR-g and HIF-1 alpha) in early onset and late onset Preeclampsia.

Results and Conclusion:
Placental protein levels and mRNA levels of VEGF were lower in both EOP and LOP groups, whereas supplementation of omega-3 fatty acids and vitamin E was beneficial only in case of the LOP group.

Supplementation was beneficial in normalizing the HIF-1 α mRNA levels. Protein levels of BAX and Caspase-3 were higher at d20 of gestation in the EOP.
Placental protein levels of caspase-8 were also higher in the EOP group as compared to LOP and control at d20 of gestation. Bcl-2 protein levels were lower in both the subtypes of preeclampsia
Apoptotic index was higher in both EOP and LOP groups as compared to control. However, supplementation was beneficial in reducing the apoptotic index only in the LOP group.

13. Title: Maternal Vitamin D and its Association with Angiogenesis in Preeclampsia. (**Project ID:**MCH/17/1/P); **Funding:** CSIR-SRF, Duration: 2017-2022, **Sanctioned Amount**: 22.94 lakhs, **Guide:** Dr. Sadhana Joshi**; PhD** Student: Juhi Nema (CSIR JRF/SRF) **Ethical Approval**: IEC/2015/37, dated 03.10.2015

Background: The current study explores the association of maternal vitamin D levels with angiogenic growth factors in preeclampsia.It also focuses on the potential mechanisms through which maternal vitamin D may regulate angiogenesis in preeclampsia.
.
Work done:
Human study: Vitamin D was estimated on 66 normotensive control women and 31 women with preeclampsia. Maternal serum vitamin D (25(OH)D) levels were estimated at four different time points across gestation that is 11-13 weeks, 18-22 weeks, 26-28 weeks and at delivery. Cord blood serum vitamin D (25(OH)D) were also estimated.
Animal study: The effect of preconception vitamin D deficiency or supplementation on blood pressure and pregnancy outcome has been examined. We have also analyzed the effect of vitamin D deficiency or supplementation on placental angiogenic growth factors. The mRNA and protein levels of angiogenic growth factors such as: Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), Flt-1 (fms-like tyrosine kinase receptor) and hypoxia inducible factor 1 alpha (Hif1α) has been analyzed.
Results and Conclusion:
More than 50 % of women are found to be vitamin D deficient at all four time points across gestation. Mean serum vitamin D levels were found to be lower in women with preeclampsia as compared to control women.
Our animal study for the first time demonstrates the effect of maternal vitamin D status (deficiency and supplementation) starting from pre-pregnancy and continuing during gestation on pregnancy outcome and angiogenesis in a rat model of preeclampsia. Our study demonstrates that Vitamin D deficiency increased both systolic as well as diastolic blood pressure.
In contrast, vitamin D supplementation reduced the blood pressure. Vitamin D deficiency also lowered the protein levels of pro-angiogenic proteins VEGF and Flt-1 while supplementation improved both these levels.

14. Title Investigating the role of enzymes regulating the one carbon metabolism in preeclampsia (**Project ID:** RBMH/FW/18/1/P); **Funding:** Indian Council of Medical Research (Senior Research Fellowship to Anjali Jadhav); **Duration:** 10th October 2018-9th October 2021; **Sanctioned Amount:**16.22 Lakhs; **PhD student:** Anjali Jadhav **Guide:** Sadhana Joshi; **Human Ethical Approval**: Yes ((Institutional Ethics no. : IEC/ 2018/ 44)

Background: Our earlier studies in women with preeclampsia have reported an altered one carbon cycle, reduced omega-3 fatty acids and increased homocysteine and oxidative stress. It is likely that these changes in the maternal micronutrients and long chain polyunsaturated fatty acids (LCPUFA) could influence the regulation of enzymes involved in the one carbon metabolism which may further affect the methylation pattern. The current study examines the levels of enzymes regulating the one carbon cycle in the placenta of women with preeclampsia and compare them with normotensive women.

Work done: Recruitment of patients, Collection of maternal blood and placenta collection of included patients. Total RNA from placental samples was isolated using Trizol method and quantified by the Eppendorf BioPhotometer plus and cDNA was prepared. Gene Expression Levels of One Carbon Cycle Enzymes in the Placenta (MTHFR, MTR and MAT)

Results and conslusion:
Placental gene expression of *MAT2A* and MS genes were significantly lower in preeclampsia women as compared to control group. The levels of placental MTHFR gene was also lower but did not reach statistical significance (p=0.29)
A negative association of placental gene expression of MAT and MS with systolic blood pressure
A positive association of placental gene expression of all the genes (MTHFR,MAT,MS) with head circumference of the baby

This study for the first time evaluated the effects of maternal micronutrients and omega-3 fatty acid given individually or in combination on the enzyme gene expression of the one carbon cycle. This has implications for epigenetic programming of the developing fetus. The changes observed in the present study may have influence on the epigenetic programming of the developing fetus. This study may thus provide some vital information that will help to explain the mechanisms involved in the role of altered one carbon cycle in the pathophysiology of preeclampsia.

15. **Title:** Influence of maternal one carbon metabolites on placental epigenetic patterns (**Project ID:** RBMH/FW/18/2/P)**Funding**:CSIR; **Sanctioned Amount:** 22.94 lakhs ; **Duration:** August 2018 – August 2023; **Guide**: Dr. Sadhana Joshi; **PhD Student**: Kinjal Dave (CSIR JRF/SRF) **Ethical Approval:** BVDU/MC/51

Background: Alterations in the one carbon metabolism which supplies methyl group for all biological methylation reactions can result in changes in the DNA methylation patterns.
The current study therefore aims to examine the placental CpG methylation and mRNA expression levels of angiogenic factors *PEMT* and *FADS* in women with preeclampsia and compare it with normotensive women. We also aim to examine the association of the CpG methylation patterns with maternal blood pressure and fetal outcome.

Work done: A total of 200 placental tissues (100 normotensive controls, 100 preeclampsia) were collected from central maternal region and stored at -80°C. Genomic DNA was isolated from placental samples using the DNeasy Blood and Tissue kit
Methylation studies are completed and analysis is ongoing

Name of the Program: Cancer Research

Title: Evaluating the anticancer activity of homeopathic potencies of *Terminalia chebula* (TC) in breast cancer cell lines and analyzing the best potency for activity in breast cancer mouse model. (Project ID: **CR/16 (16-19)/1/E**);**Funding:** EMR, AYUSH CCRH; **Duration:** 2016-2019; **Sanctioned Amount:** 42.98 lakhs; **Investigators: PI-** Dr. Ruchika Kaul-Ghanekar; **Co-PI**- Dr. Nilesh Shah (Homeopathy College, BVHC); **Co-Investigators- NA; Ph.D. Students:** Apoorva Parimoo; **Animal Ethical Approval:** Yes

Background: In the initial year of present project, we conducted a detailed study for anticancer activity of various TC potencies i.e. MT, 3X, 3C, 6C, 30C, 200C, 1M, 10M, 50M and CM on breast cancer cell lines (MCF-7 and MDA-MB-231) and noncancerous breast epithelial cell line (MCF10A). Out of these, 6C and 50M showed significant anti-cancer activity. In the following year we conducted the acute toxicity study, where both 6C and 50M were found to be safe. 6C was taken further for dose range finding (DRF) study and was found to be safe in Swiss albino mice. Simultaneously, we initiated the mechanistic studies to find the mode of action.

Work done: In the present study, we determined the phytochemical composition of homeopathic potency 6C and MT of TC. Further, we evaluated the effect of 6C potency on lactate release from MDAMB231 cells.

Results:
LCMS showed presence of phytocompounds in MT and 6C.
6C caused intracellular acidosis that lead to apoptosis and, hence, lactate release from the breast cancer cells.

Conclusion: 6C potency functions as an anti-cancer agent by causing apoptosis of MDAMB231 cells by intracellular acidosis. The anticancer activity of homeopathic preparation of TC could be attributed to the cumulative effect of phytocompounds with anticancer potential.

Title: Evaluating the anticancer activity and mechanism of action of Unani formulation Habbe Musaffi Khoon (HMK) against cervical cancer (**Project ID**: Z 28015/61/2018-HPC (EMR)-AYUSH-C); **Funding:** EMR, AYUSH CCRM; **Duration:** 2018-2021; **Sanctioned Amount:** 57,56,500/-; **Investigators: ; PI-** Dr. Ruchika Kaul-Ghanekar; **Co-PI**-Dr Gazalla Mulla, Dr Prerna Raina ; **Co-Investigators-** Nil; **Ph.D. Students:** Ms Nidhi Sharma

Background: In the first year (2018-2019) of the project, the effect of HMKaq was evaluated on the viability of cervical cancer cell lines, SiHa and HeLa. HMKaq decreased the viability of cervical cancer cells in a dose dependent manner, indicating the potential anticancer activity. Phytochemical analysis of the extract showed presence of different phytocompounds having anticancer activity.

Work done: The mechanism of action of Habbe Musaffi Khoon was studied in terms of regulating apoptosis in cervical cancer cell lines.

Results:
In HeLa, HMKaq induced cell cycle arrest.
In SiHa, HMKaq induced apoptosis through mitochondrial depolarization and intracellular Ca^{2+} influx. In SiHa, at 160 µg/ml dose, HMKaq increased the intracellular Ca^{2+} levels by 4.1 folds (Figure 1.A) with simultaneous decrease in mitochondrial membrane potential by 4.9 folds (Figure 1.B).

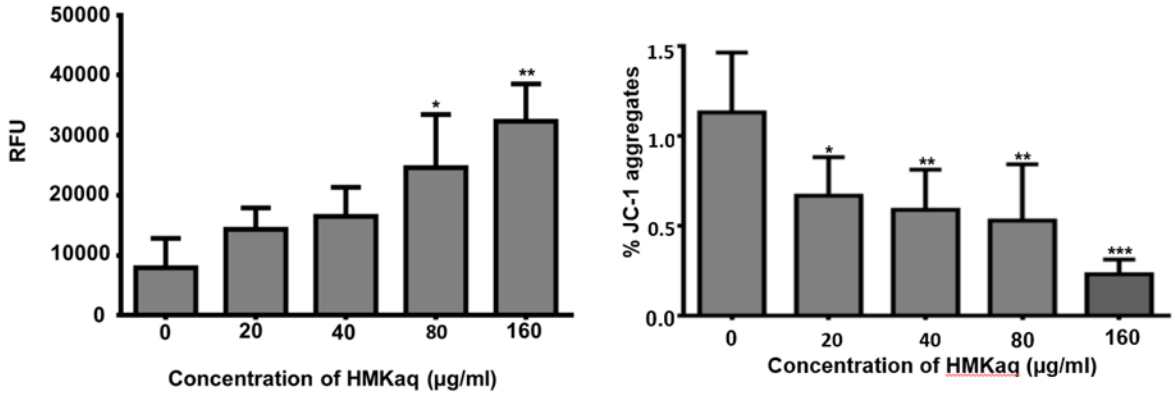


Figure1. Effect of HMKaq on apoptosis in SiHa. HMKaq increased (A) intracellular calcium levels and decreased (B) mitochondrial membrane potential. HMKaq All data has been presented as means ± SD of three independent experiments. *p<0.05, **p<0.01, ***p<0.001 indicate statistically significant differences compared to the control untreated cells

Conclusion: Habbe Musaffe Khoon (aqueous extract) shows potential anticancer activity against cervical cancer cells.

Title: Evaluating the effect of Alpha Linolenic acid, an omega-3 fatty acid on the modulation of epigenetic markers in Cervical cancer cells **(Project ID:** EMR/2017/001208**); Funding:** DST SERB; **Duration:** 2018-2021; **Sanctioned Amount:** 33,16,800/-; **Investigators:** NA; **PI-** Dr. Ruchika Kaul-Ghanekar; **Co-PI-** Dr Preeti Gautam-Chavan; **Co-Investigators-** NA; **Ph.D. Students**: Ms Amrita Ulhe

Background: Cervical cancer is caused majorly by Human Papilloma virus infection, which acts by integration of viral DNA into host genome. A number of epigenetic alterations occur during all the stages of cervical carcinogenesis in the host genome, which include global DNA hypomethylation, hypermethylation of key tumor suppressor genes and histone modifications. Nutrition has been shown to play an important role in the regulation of various cancers. Earlier in the first year of the project, we have reported the anticancer activity of ALA along with epigenetic studies. ALA induced significant inhibition of HDAC1 and DNMT1 expression in cervical cancer cell lines indicating epigenetic modulation. Hence, in the following year we have further studied the effect of ALA on global DNA methylation and DNMT3b in cervical cancer cell lines.

Work done: The effect of ALA on global DNA methylation and DNMT3B expression was studied by ELISA.

Results:

ALA increased 5-mc content in HeLa, SiHa and C33A compared to control untreated cells.

Compared to control cells, ALA decreased the expression of DNMT3b in HeLa, Siha and C33A.

Conclusion: ALA increased global DNA methylation by reducing 5-mc content and reduced DNMT3B expression, indicating epigenetic modulation in cervical cancer cells.

Title: Phytochemical standardization and evaluation of anti-cancer and immunomodulatory activity of Unani formulation, Itrifal Ghudadi.(**Project ID** : F.No.3-64/2019-CCRUM/Tech.); **Funding :** AYUSH-EOI Unani; **Duration :** 2020-2023; **Sanctioned amount:** Rs.43,57,750/-; **PI :** Dr. Ruchika Kaul Ghanekar; **Co-PI :** NA; **Project JRF :** Ms. Samradni Pingale

Background : Itrifal ghudadi is an Unani formulation mainly used to treat all kinds of glandular swellings in the human body. The reported anti-cancer activities of various components present in Itrifal Ghudadi prompted us to investigate the antiproliferative nature of the formulation. Phytochemical standardization of Itrifal ghudadi is required to determine the major chemical constituents of the formulation, which may be responsible for the anticancer activity.

Work done: The authentic plant material resources were identified and plant material was obtained. The preparation of traditional Unani formulation Itrifal Ghudadi has been optimized under the guidance of Hakim. Extensive review of literature has been done.

Title: Evaluating the anticancer activity of homeopathic preparation of *Linum usitatissimum* in breast cancer cell lines (**Project ID:** z.28015/02/2018-HPC (EMR)AYUSH-D); **Funding:** EMR, AYUSH CCRH; **Duration:** 2018-2021; **Sanctioned Amount:** 42,02,500/-; **Investigators:** NA; **PI-** Dr. Prerna Raina; **Co-PI**-. Dr Swati Shinde; **Co-Investigators-** NA; **Ph.D. Students:** Ms. Rupika Pawar

Background: Adjunct therapies are frequently explored in cancer patients. Among these, Complementary and Alternative Medicines (CAM) are widely explored, which also include Homeopathic medicines. In the present study, anticancer potential of homeopathic preparation of *Linum usitatissimum* (LU), commonly known as Linseed or Flaxseed, is being evaluated against breast cancer. In the first year of the project, we have reported that different potencies (6C, 12C, 30C, 200C and 1M) of LU significantly reduced the viability of breast cancer cell lines (MCF-7, MDA-MB-231). In the second year of the project we studied the effect of potentized distilled alcohol (DA) of all the tested potencies on the vitality of breast cancer cell lines and non-cancerous mammary epithelial (MCF-10A) cell lines.

Work done: The effect of potentized D.A (6P, 12CP, 30CP, 200CP and 1MP) of *Linum usitatissimum* (LU) on the viability of breast cancer cell lines MCF-7, MDA-MB-231 and non-cancerous mammary epithelial cell line MCF-10A was evaluated and compared with the respective potencies of LU. The LU potency with potent anticancer activity and safe on non-cancer cell line was determined. Further, mechanism of action for anticancer activity of best potencies was determined by caspase assay.

Results:
The potentized D.A (6P, 12CP, 30CP, 200CP and 1MP) of LU reduced the viability of breast cancer cells MCF-7, MDA-MB-231 significantly
6C and 30C were found to have potent anticancer activity with breast cancer cells MCf-7 and MDA while non-significant alteration in viability of non-cancer cell line MCF-10A at higher dilutions.

The effect of 6C and 6CP on generic caspase expression in MDA-MB-231 cells at non killing dilution of 1:500 was studied. At molecular level compared to 6P, 6C significantly increased (3.1-folds) the expression of generic caspases. These preliminary results suggest that although the 6C potency of LU and its DA showed similar activity (in terms of reduction in viability of breast cancer cells, there is a difference in their activity at molecular level.

Conclusion: The potentized D.A (6P, 12CP, 30CP, 200CP and 1MP) of *Linum usitatissimum* significantly reduced the viability of breast cancer cell lines, MDA-MB-231 and MCF-7 but was found to be safe in the non-cancerous cells MCF10A. The 6C potency of LU at non-killing dilution (1:500), compared to potentized DA, significantly increased the expression of caspases, an indicator of apoptosis.

Title: Comparing vaginal microflora diversity between healthy and cervical cancer women for identifying isolates having probiotic and anticancer potential **(Project ID:** EMR/2017/001208**); Funding:** DST WOS-A; **Duration:** 2018-2021; **Sanctioned Amount:** 32,06,000/-; **Investigators:** NA; **PI-** Dr. Ashwini Kamble**; Co-PI-** NA; **Co-Investigators-** NA; **Ph.D. Students**: NA; **Human Ethical Approval: Yes**

Background: Vaginal probiotics are gaining significance as anticancer agents. In the present study, we evaluated the diversity of vaginal microflora among healthy volunteers and cervical cancer patients to isolate probiotics with anticancer activity. In the first year o (2018-2019), we got ethics committee approvals and collected vaginal swabs from healthy and cervical cancer women from Bharati Medical Hospital and Sassoon General Hospital, Pune. Further, isolation of different microflora from the collected swabs was done. In the second year of the project, we have evaluated the probiotic characteristics (hemolytic activity, acid-bile tolerance study and antibacterial activity) of the isolated microflora.

Work done
1. Vaginal microflora was isolated and purified on different growth media.
2. The isolated strains were evaluated for their probiotic characteristics such as hemolytic activity, acid-bile tolerance study and antibacterial activity.

Results:
Total 86 vaginal swabs (45 from healthy and 41 from cervical precancerous/cancerous subjects) were collected.
251 cultivable microflora (111 microflora from samples of healthy and 140 from samples of pre/cancerous subject) were isolated.

The isolates were subjected for characterization of probiotic potential (non-pathogenicity, acid-bile tolerance and antibacterial activity against isolated vaginal pathogens).

Conclusion: The vaginal microflora isolated from swabs of healthy subjects mostly found to be non-pathogenic in nature with probiotic potential than the microflora isolated from precancerous/cancerous subjects.

Title: Role of Selected Phytochemicals in Regulation of Aberrant Lipid Metabolism in Prostate Cancer**. (Project ID:** CA/16 (17-18)/5/E**); Funding:** Nil; **Duration:** 2017-2022; **Sanctioned Amount:** Nil; **Investigators:** NA; **PI-** Dr. Ruchika Kaul-Ghanekar; **Co-PI-** Nil; **Co-Investigators-** NA; **Ph.D. Students:** Minal G. Mahajan

Background: Aberrant Lipid Metabolism in Prostate Cancer (PC) may lead to Castration Resistant Prostate Cancer (CRPC). Phytochemicals have been reported to target various markers in lipid pathway in different cancers. Matairesinol (MR) was previously reported to exhibit anticancer activity against breast cancer, an adenocarcinoma. Since prostate cancer is an adenocarcinoma, MR was tested for its activity against it. MR reduced the viability and growth kinetics of PC3 (androgen- independent). It also decreased mitochondrial membrane potential of PC-3. In the present year, MA was tested for its activity on LNCaP (androgen- dependent) cell line. Further, MA was studied for its effect in regulation of genes involved in lipid metabolism in both the PCa cell lines.

Work done: Evaluated the effect of matairesinol (MR) on growth kinetics and mitochondrial membrane potential in prostate cancer cell line (LNCaP). Further, the role of MR in regulating expression of de novo fatty acid synthesis genes was determined in prostate cancer cells.

Results:
MR altered the growth kinetics of LNCaP cells in a dose-dependent manner. Interestingly, at 80 µg/ml concentration, MR decreased mitochondrial membrane potential.
MR decreased the expression of SREBP-1c and FASN in LNCaP and PC-3. Also, expression of FASN.

Conclusion: Matairesinol has a potential to regulate aberrant de novo fatty acid metabolism in prostate cancer cell lines.

Title: To study the effect of phytochemicals on Repolarization of macrophages in THP-1 derived macrophage model (**Project ID**: CR/18 (18-22)/6/P) (DBT JRF); **Funding:** DBT : 4,91,280.00; **Duration:** 2019-2021; **Sanctioned Amount:** 25,25,440.00; **Investigators:** NA; **PI-** Dr. Ruchika Kaul-Ghanekar; **Co-PI**- NA; **Co-Investigators-** NA; **Ph.D. Students:** Amol Rajendra Chaudhary (DBT-JRF); **Animal Ethical Approval:** BVDUMC/2666/2017/002/012

Background: In cancer patients and *in vivo* preclinical experimental models, high-grade tumor-associated macrophages (TAMs) correlate with poor prognosis and reduced overall survival.

Chemokines and growth factors produced by stromal and tumor cells in tumor microenvironment recruit macrophages and may influence the phenomenon of macrophage activation into pro (M2) or anti-inflammatory (M1) subtypes. One of the strategies to target TAMs is macrophage reprogramming (from M2 to M1). Various chemotherapeutic drugs such as doxycycline, metformin and paclitaxel have been shown to induce macrophage polarization. Various natural compounds such as zoledronic acid, emodin, gingerol have been shown to repolarize M2 macrophages to M1. Thus, the present study aims at evaluating the potential of phytocompounds in repolarization of macrophages polarization in breast cancer.

Work done: Protocols optimized for identification and generation of M1 and M2 from THP-1 cell line. Further *in vitro* safety of phytocompound MA001 on THP -1 (TDM0), M1(TDM1) and M2 (TDM2) was evaluated by viability assay.

Results:

M0, M1 and M2 pool of macrophages were successfully isolated by differentiation of THP1 cells.

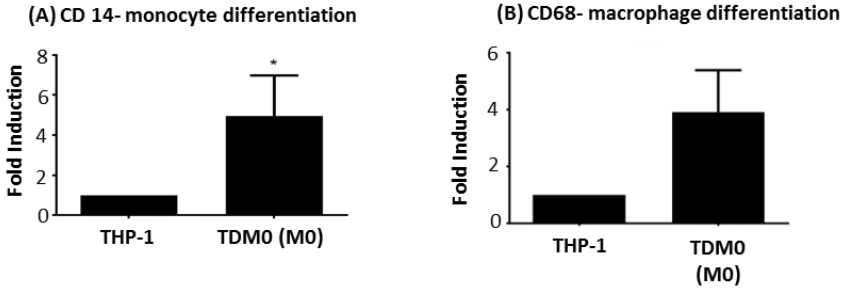


Figure 1: Effect of phorbol 12-myristate 13-acetate (PMA) on THP-1. Expression of CD14 (A) and CD68 (B) increased in TDM (M0) cells after exposure of THP-1 cells to PMA for 24h. The results were analyzed by unpaired t-test. Data is represented as mean± SD, *- $p < 0.05$

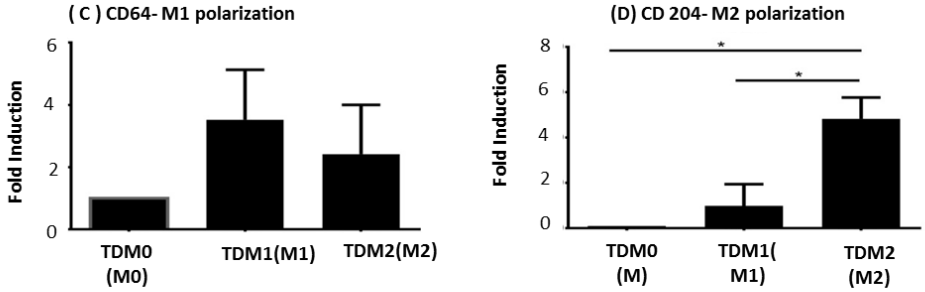


Figure 2: Expression of CD64 (C) and CD204 (D) increased to more extent in TDM1 and TDM2 respectively when compared with TDM0 cells. Data are expressed as mean ± SD. Differences between groups were analyzed using one-way annova. (*P<0.05).

MA001A phytocompound reduced viability of TDM0 in a dose dependent manner without significantly affecting the viability of M1 and M2

Conclusion: The phyto-compound MA001A is not affecting the viability of M1 and M2 hence is safe for macrophage polarization

Title: Evaluation of anti-cancer potential of selected phytochemicals against breast cancer stem cells (**Project ID**:) (DBT-JRF); **Funding:** DBT : 4,60,000.00; **Duration:** 2019-2022;

Sanctioned Amount: 25,25,440.00; **Investigators:** NA ; **PI-** Dr. Ruchika Kaul-Ghanekar; **Co-PI**-Nil ; **Co-Investigators-** NA; **Ph.D. Students:** Ms. Akanksha Mahajan

Background: Cancer stem cells (CSCs) play an important role in breast cancer recurrence in patients. Majority of cells in tumors are non- tumorigenic and are marked by limited self-renewal ability, however only a small subpopulation of cancer cells has the ability to self- renew and initiate tumors. These cells are referred to as cancer stem cells (CSCs) or tumor- initiating cells. Breast cancer stem cells (BCSCs) are shown to exhibit unique growth abilities including self-renewal, differentiation potential, and resistance to most anti-cancer agents including chemo- and/or radiotherapy. In the present study, we would screen the phytocompounds that target the cancer stem cells and study the underlying mechanism.

Work done: The model in triple negative breast cancer cell line (MDAMB231) has been standardized.

Title: Evaluating the effect of selected bioactives on cytokine and chemokine regulation in prostate cancer (**Project ID**:) (DST Inspire); **Funding:** DST Inspire 4,51,520.00; **Duration:** 2019-2022; **Sanctioned Amount:** 22,57,600.00; **Investigators:** NA; **PI-** Dr. Ruchika Kaul-Ghanekar; **Co-PI**- Nil; **Co-Investigators-** NA; **Ph.D. Students:** Ms. Rama A. Rajadnya

Background: Current treatment for prostate cancer (PCa) involves androgen deprivation. However, this therapy may lead to castration resistant PCa. Pro-inflammatory cytokines and chemokines play major role in androgen receptor (AR) activation in prostate cancer development. We are interested in studying the role of bioactives on the cytokine and chemokine regulation in prostate cancer. The extensive literature search supported our hypothesis, where use for phytocompounds to prevent and inhibit the progression of PC has been reported. Earlier studies in our lab demonstrated the potential anticancer activity of some of the bioactives. Hence in the present year we analyzed the in silico activity of bioactives for drugability, pharmacokinetics and toxicity. Based on this analysis we selected Matairesinol and 6 Gingerol for anticancer activity on prostate cancer.

Work done: Screening of few phytocompounds by in silico approach was done and the bioactives with good pharmacokinetic profile and minimum toxicity was chosen for further in vitro studies.

Results:

- Based on in silico studies, Matairesinol (MA) and 6 Gingerol (6G) phytocompounds exhibited excellent pharmacokinetic profile and minimum toxicity and they were taken for further studies.

- Phytocompounds, MA and 6G decreased the growth of prostate cancer cell line PC3. MA showed better results and thus was taken forward for further studies.
- MA decreased the number of colonies in dose dependent manner with more decrease at 80 ug/ml dose.

Conclusion: MA decreased the viability more significantly than 6G and thus would be taken for further studies.

Name of the Programme: Obesity-Diabetes

1. **Title:** Effect of Yoga intervention on skeletal muscle linked glucose homeostasis in pre-diabetic individuals (Project ID:) Funding:DST (SATYAM); Duration: March 2019-March 2022; Sanctioned Amount:Rs.46, 74,200/-; Investigator: PI:Dr. Supriya Bhalerao; Co-Investigators; Dr. Jayshree Kharache (16.03.2019- 31.08.2019);Dr. Pranita Ashok (01.09.2019- till date); Co-Investigator :Mrs. Anita Patil; Project Staff: Dr. Tanuja Sawant (SRF) ; Dr. Shubhangi Harke (JRF); Ethics Approval:IEC/2019/05 (04.03.2019);IEC/2019/35 (06.07.2019 amended) IEC/2019/05

Background: Currently prevalence of diabetes is rising in India. India ranks second in case of pre-diabetes, increasingly recognized as an important asymptomatic metabolic state. The role of Yoga has been well documented in pre-diabetes, though its mechanism of action has not been explored so far. In the present study, pre-diabetic individuals are randomly allocated to follow either Yoga or exercise for a period of 4 months. The effect of these interventions is being assessed on functional capacity of skeletal muscles as they form the major site for glucose uptake and their strengthening may enhance proper glucose disposal. It is expected that the project will enable to bridge the gap in existing knowledge about Yoga and its effect on skeletal muscle linked glucose homeostasis.

The project work started in April 2019 with the following objectives.

1. To evaluate the effect of Yoga interventions on muscle mass, strength, endurance and Flexibility which are direct or indirect indicators of fat deposition in skeletal muscles.
2. To study the association between changes in muscle quality/ functionality and glycemic control.

Work done:

During the first 6 months, the preparations for initiating the study were completed, which included:

- **Ethics approval and CTRI registration:**
  The Institutional Ethics Committee (IEC) approval was obtained on 4th March 2019 (IEC/2019/05). As per the suggestions of the Advisory Committee (detailed below), the protocol was amended and the IEC approval for the amended protocol was sought on 6thJuly 2019 (IEC/2019/35). The trial was registered prospectively in Clinical Trial Registry of India (CTRI/2019/05/019149).
- **Procurement of instruments and development of SOPs:**
  For muscle assessment, various equipment viz. Hand grip dynamometer, Leg strength dynamometer, Flexibility box, TRX band (for Pull ups), Dumbbells, were procured along with Stadiometer and Karada Scan (required for anthropometry). Standard Operating

Procedures (SOPs) were drafted for each equipment. These SOPs were verified by the subject experts.

- **Promotional activities:**
  As community screening is the major channel for identification of pre-diabetics in this project, promotional literature such as pamphlets, banners, audio-visuals etc. were developed for outreach and circulated in nearby Institutes/organizations/residential societies. Promotional and awareness lectures, more than 20 in number, were organized in various institutes and housing societies in a span of 4.5 months since the first lecture on 16th July 2019. 'Walk for diabetes' campaign was conducted in the month of January, 2020.
- **Development of data documentation measures:**
  A Case Record Form (CRF) was developed taking into consideration the nuances of the study protocol. The data captured using CRF is further entered in Epi Info™. This software is available in public domain, which has been customized according to the data points in our study.

  This project is an example of Interdisciplinary research such as epidemiology (for community screening), clinical research, Yoga and physical training. This not only robust planning but also ongoing quality check. To ensure the quality, following measures have been adopted.

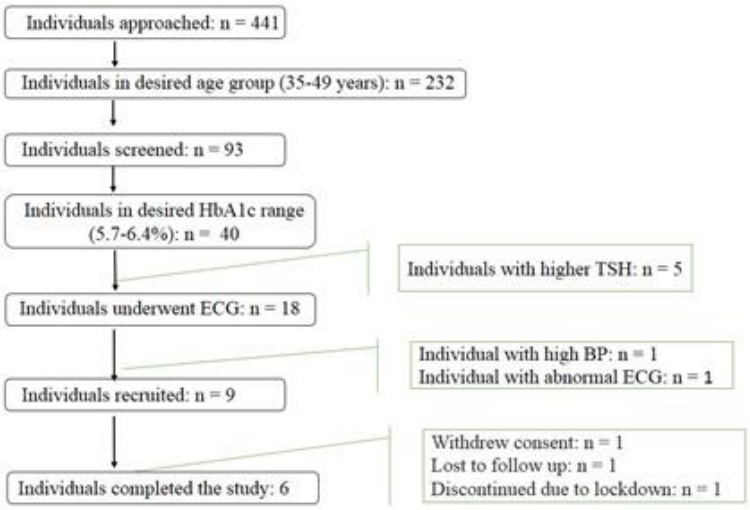
- **Constitution of Advisory cum Data Monitoring Board**
  This board comprises of 3 members; Dr. Vijaya Pandit (Professor and Head, Department of Pharmacology, Bharati Vidyapeeth Medical College), Dr. Girish Tillu (Principal Investigator, AYUSH Center of Excellence, Center for Complementary and Integrative Health, Interdisciplinary School of Health Sciences, Savitribai Phule Pune University) and Dr. Vaishali Deshmukh (Consulting Endocrinologist, Pune). We have also invited Yoga and exercise experts (as additional members) for these meetings.The board meetings have been conducted every 6 months; 18thApril, 2019 followed by 24thJanuary, 2019. The 3rdmeeting was held telephonically on 14thJune 2020 to discuss the conduct of the project in the 'new normal environment' due to COVID 19 pandemic.
- **Study monitoring by an Independent monitor**
  An independent study monitor has been appointed to review all the study related documents. The first monitoring was done on 7thMarch, 2020 following which all necessary changes have been made in the study documents for compliance as per the monitoring report.

Results:

Recruitment status:

The actual recruitment of participants began in October 19. We approached around 450 individuals for screening. Of these, 232 were found to be in desired age group as per the eligibility criteria. Out of them, 93 individuals voluntarily agreed to undergo screening, of which 40 individuals were in desirable range of Glycosylated haemoglobin, indicative of their pre- diabetic status. Only 18 individuals from the 40 shortlisted individuals responded for further testing of ECG, as per the screening process. Further, due to reasons like elevated levels of TSH, BP and abnormal report of ECG, 9 individuals could be recruited, of which 6 individuals completed the study (3 in each group) Figure 1.

Fig.1 Consort flow chart:



Since March 20, due to COVID 19 pandemic the participant screening and recruitment procedures are halted.

2. **Title:** Evaluation of the effect of Triphala on Obesity associated Cognitive impairments **(Project ID:** OB/15 (15-18)/8/P**);** Funding: Generated funds & Sakal India Foundation 2019; Duration:2016-2020; Sanctioned Amount:Rs.88, 000/-; Investigators: PI- :Dr. Supriya Bhalerao; Co-PI- ; Ph.D. Students: Shital A Giramkar; Human Ethical Approval: BVDUMC/IEC/80; Animal Ethical Approval: BVDUMC/1891/2018/002/020

   Background: Obesity associated cognitive impairment is a relatively unexplored area. The growing epidemic of obesity however necessitates the need to understand this association and also to explore treatment options that can prove safe and effective. The

present study is planned to evaluate the effect of Triphala in obesity associated cognitive impairment with the following objectives:

1. To study the association of obesity and its pathophysiology with cognition in young age adults
2. To standardize the study formulation, Triphala
3. To evaluate the effect of Triphala on free fatty acid induced neuronal Lipotoxicity
4. To study the effect of Triphala in rat model of cognitive impairment associated with high fat diet induced obesity

Work done:

Objective 1:

With Institutional Ethics Committee permission, healthy, young age (18–35 y) adults of normal weight (NW: BMI 18.5–24.9 kg/m2) and obese category (OB: BMI ≥ 30.0 kg/m2) are being recruited. Their demographic details, clinical history, anthropometric measurements, body composition (bio-impedance method) are recorded. Following this, their cognitive capacities are assessed using Addenbrooke's Cognitive Examination – ACE-III, (2012). Of the approved sample size (50 obese + 50 non-obese), 5 obese and 25 non-obese participants have been recruited so far.

Objective 2:

During earlier years of the project, the formulation has been standardized. The corresponding manuscript has been communicated to the Indian Journal of Pharmaceutical Sciences

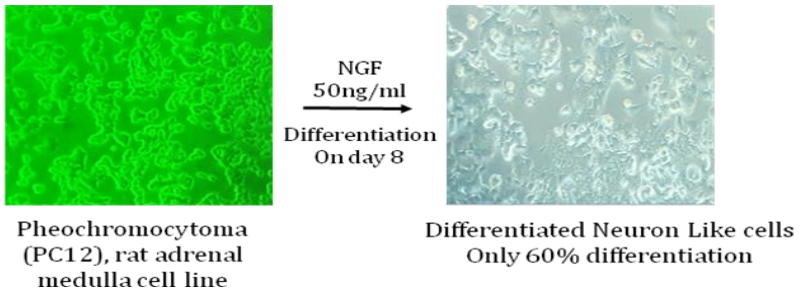
Objective 3:

Results

For the work related to Objective 3, Pheochromocytoma cell line (PC12), a rat adrenal cell line, was procured from NCCS. The cells are being maintained, freeze stored & sub cultured in respective media.

After passaging, seeding density of cells for 24 well plate (2 x 10^3 cells/cm^2 12 well plate) has been standardized. For differentiation of PC12 to neuron like cells, concentration of Nerve Growth Factor (NGF) has been optimized as 50ng/ml. Since we have observed only 60-70% of cell differentiation patterns, we have shifted the work on other neuronal cells namely SHSY5Y (Neuroblastoma cell line).

Fig 2: Differentiated cells



Objective 4:

The study to achieve objective 4 has been planned in 60 male Wistar rats of 6-7 weeks old (100-110gm) divided in following groups:

Table 1: Different study groups

No	Name	Treatment
I	Normal Control (NC)	Normal pellet diet for 120 days
II	Disease Control (DC)	HFD (35% Kcal) for 120 days
III	Positive Control 1 (PC 1)	HFD for 90 days + Metformin (200 mg/kg bw/day) for 30 days
IV	Positive Control 1 (PC 2)	HFD for 90 days + Rivastigmine (10 mg/kg bw/day) for 30 days
V	Treatment Group I	HFD for 90 days + Aqueous extract of Triphala (50 mg/kg bw/day) for 30 days
V	Treatment Group II	HFD for 90 days + Aqueous extract of Triphala (100 mg/kg bw/day) for 30 days
VI	Treatment Group III	HFD for 90 days + Aqueous extract of Triphala (150 mg/kg bw/day) for 30 days
VII	Reversible Group	HFD for 90 days then continued on normal diet for 30 days

The following Parameters are planned for assessment in above grouped animals:

- Body Weight
- Food intake
- Behavioral parameters on 90^{th} and 120^{th} day:
    - Water maze

- Activity monitoring

The animals will be humanely sacrificed after completion of the experiment (i.e. Day 90). Blood samples and tissues like liver, brain and adipose tissues will be collected and stored for histopathology, biochemical analysis.

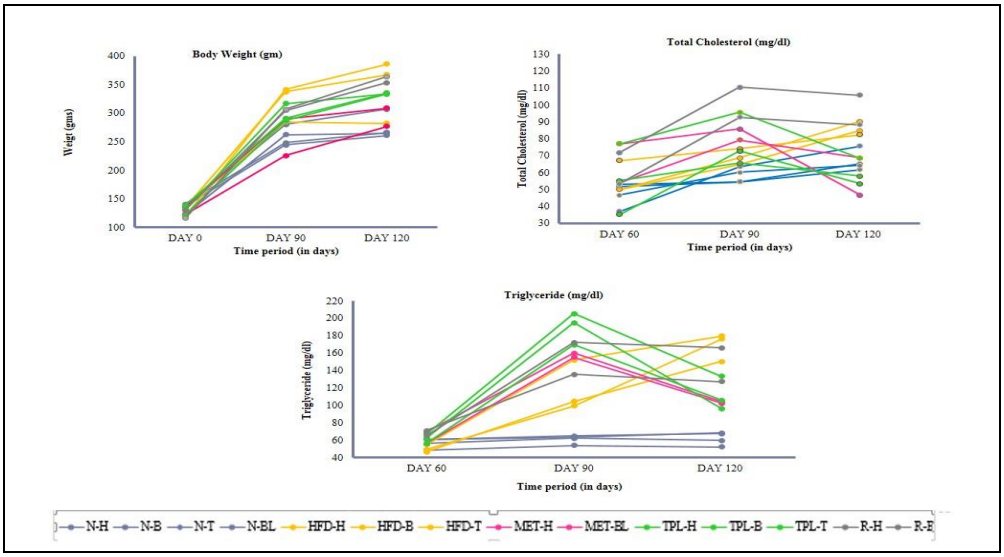
- Lipid Profile: Triglyceride, Cholesterol, HDl-cholesterol, LDL
- Oxidative stress markers (Serum/ brain): Superoxide Dismutase and Malondialdehyde on 120th day
- Acetyl choline esterase (Serum) on 120th day
- Fatty acid composition of serum/ brain homogenate on 120th day
- Gene expression studies from liver, brain and adipose tissues (PPAR-g, SREBP, TNFα, NFκβ, glut 4, BDNF, NGF etc.) on 120th day
- Brain histology (Atrophy, inflammation, vascular damage etc.), Liver and adipose tissue histology after completion of the experiment on 120th day

After Institutional Animal Ethics Committee Permission, the experiment was initiated. So far, we have completed study on 14 animals [NC = 4, DC = 3, PC1 = 2, Treatment Group III: 3, Reversible Group:2] and recorded weekly body weight, food intake, behavioral analysis by water maze and performed lipid profile, and oxidative stress marker (MDA) estimations.

Result

In the last year, we have completed a batch with 14 animals and above-mentioned parameters were assessed.

Fig 3: Anthropometry and biochemical observations



Data obtained from study suggests that HFD resulted in increased body weight and BMI. This increase was accompanied with changes in lipid profile viz. increased levels of cholesterol, triglycerides, LDL and decrease in HDL levels. Triphala showed improvement in body weight, BMI and lipid profile. The behavioral changes in the animals were studied using Morris Water Maze. In this test, we observed that the Normal Control rats took less time to explore the platform than the HFD treated animals (DC).

3. **Title:** Evaluation of Triphala and Trimad for their effects on adipocyte biology and lipid metabolism (**Project ID**: OB/16 (16-19)/1/E); **Funding:** Ministry of AYUSH; **Duration**:Feb 2016 – Jan 2019 **Sanctioned Amount**: Rs. 28, 32, 000/-; **Investigator: PI**: Dr. Supriya Bhalerao; **Co-Investigator** 1:Dr. Arul Mozhi S.; Co-Investigator 2: Dr. Ruchika Kaul-Ghanekar; **Project Staff** :Ms. Abhilasha Dolle (From 1$^{s\,t}$ April 19- January 20); Mr. Suresh Khadke (From 1st February 20- March 20**); Ethics Approval:** BVDUMC/3293/2016/007/002

Background: The project work was started in year 2017 with the following objectives:
To investigate the effect of aqueous extracts of Triphala & Trimad by studying

1. Extent of adipocytes differentiation, cellular triglyceride levels and expression of genes involved in differentiation process in 3T3 fibroblast cell line model
2. Changes in lipid and glucose, inflammatory markers, oxidative stress along with expression of genes regulating lipid metabolism in rat model of HFD induced obesity

In the first 2 years of the project, Triphala has been studied in cell line model while both formulations have been evaluated in an animal model of obesity. The results of these experiments have been presented in the earlier reports**.**

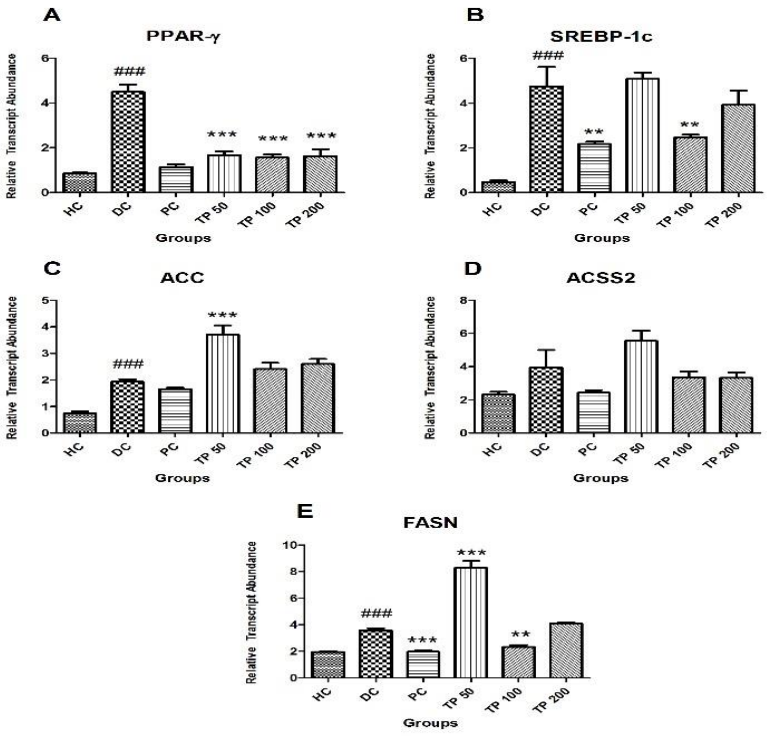
Work Done:
In the last year, we studied the expression profile of transcription factors and regulatory genes involved in fatty acid metabolism from the liver tissue of the animals fed with HFD as well as HFD with study drugs. The following genes were considered for this study:

- Peroxisome proliferator-activated receptor gamma (PPAR-)
- Sterol regulatory-element binding proteins (SREBP)
- Fatty acid synthetase (FASN)
- Acetyl-CoA carboxylase: (ACC)
- Acyl-CoA Synthetase Short Chain Family Member 2: (ACSS2)

Results:
All the fatty acids metabolizing gene expression was up-regulated in Disease Control (DC) group on obesity induction as compared to Hgroup. (Fig.2A-E). Triphala treatment showed significant

downregulation of FASN, SREBP-1c and PPAR-γ gene, while it was not significant in ACC and ACSS2 gene when compared with DC group. The lower dose of Triphala (50mg/kg) did not exhibit this effect.

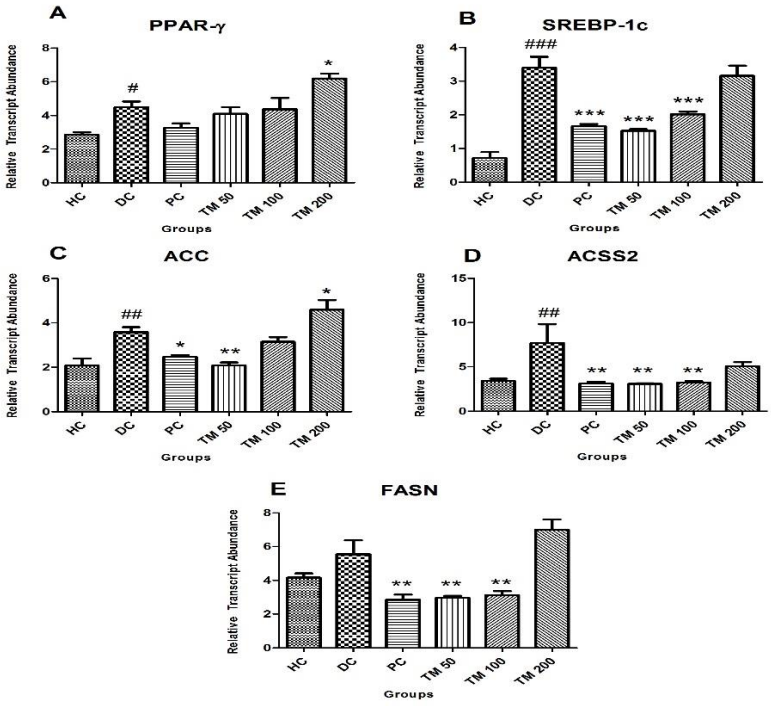
Fig.4: Effect of Triphala on modulation of genes involved in fatty acids metabolism



A-E shows expression profiles of the PPAR-γ, SREBP, ACC, ACSS2, FASN. HC: Healthy control, DC: Disease control, PC: Positive control (treated with Atorvastatin 1.2mg/kg), TP 50- Triphala 50mg/kg, TP 100- Triphala 100mg/kg, TP 200- Triphala 200mg/kg.
Data expressed as Mean ± SE (n=3 animals per groups). #p≤0.05; ##p≤0.01; ###p≤0.001 as compared to HC, *p≤0.05; **p≤0.01; ***p≤0.001 as compared to DC; using One-way ANOVA followed by Dunnett's Multiple Comparisons Test

Expression of PPAR-γ, SREBP-1c, ACC, ACSS2 and FASN gene were significantly up regulated in the DC group as compared to HC group. Except PPAR-γ, Trimad treatment showed significant down regulation of other genes.

Fig.5: Effect of Trimad on modulation of genes involved in fatty acids metabolism



A-E shows expression profiles of the PPAR-γ, SREBP, ACC, ACSS2, FASN. HC: Healthy control, DC: Disease control, PC: Positive control (treated with Atorvastatin 1.2mg/kg), TM 50- Trimad 50mg/kg, TM 100- Trimad 100mg/kg, TM 200- Trimad 200mg/kg.
Data expressed as Mean ± SE (n=3 animals per groups). #p≤0.05; ##p≤0.01; ###p≤0.001 as compared to HC, *p≤0.05; **p≤0.01; ***p≤0.001 as compared to DC; using One-way ANOVA followed by Dunnett's Multiple Comparisons Test

Conclusion: In the 3T3-L1 cell line model, Triphala significantly decreased adipogenesis by reducing lipid accumulation and inhibiting the expression of adipogenic genes in concentration dependent ways. In a rat model, administration of HFD demonstrated significant increase in weight along with food intake of Disease Control (DC) rats as compared to Normal Control. A significant increase was observed in Triglyceride, Cholesterol & fasting blood glucose with decrease in HDL-Cholesterol. The inflammatory markers (IL-6, CRP, leptin), serum and liver oxidative stress marker (MDA) were found raised, while the protective markers such as adiponectin and anti-oxidant enzyme like SOD were found decreased. Brain monoamine levels were found to be altered and reflected increased hunger and reduced satiety levels in obese rats. These changes were supported by histological changes in organs viz. fatty changes in liver and hypertrophy of adipocytes in DC rats as compared to NC. Hepatic gene expression for genes involved in fatty acid metabolism like PPAR-γ, SREBP, FASN, ACC& ACSS2) was found to be upregulated in HFD fed rats.

After 21 days of treatment with aqueous extracts of Triphala and Trimad in 3 doses showed reversal of all the above-mentioned changes though in varying degree. We did not observe any dose dependent response as there was no statistically or biologically significant difference seen among its 3 doses used in the study.

4. Title: Association between Prakriti (Ayurvedic concept of constitution) and presence of Metabolic Syndrome **(Project ID:** OB/17/2/I/P**); Funding**: Intramural-Generated funds; **Sanctioned Amount** : Rs.18, 000/-; **Investigator:PI**:Dr. Supriya Bhalerao;**Co-Investigator**: Dr. Jayshree Gothankar, Dept. of Community Medicine; BV Medical College; **Project staff**:Dr. Poonam Gupte; **Duration**: 2017-2019; **Ethics Approval** : BVDUMC/IEC/95A

Background: Prakriti (Constitution) of an individual is defined by the relative proportions of the three doshas (functional entities in the Ayurvedic paradigm) Vata, Pitta and Kapha. These three entities represent distinct characteristics and therefore body functions. The dominant functional entity is identified based on appearance, temperament and habits of an individual and this phenotype is described to be responsible for susceptibility of the person to specific disease. With this background, we planned the present study to explore the association of Prakriti with presence of Metabolic Syndrome (MS). MS is a cluster of at least 3 of 5 medical conditions viz. large waist size (for males≥ 102 cm; for females ≥ 88cm), elevated blood pressure (≥ 130/85 mm Hg), fasting blood glucose (≥100 mg/dl) & triglycerides (≥ 150 mg/dl) and low levels of high-density cholesterol (for males < 40mg/dl; for females < 50mg/dl).

Objectives

To study association between Prakriti types and presence of metabolic syndrome (MS).

Results:

During last year, 82 individuals were recruited. Screening and recruitment of the participants was continued in this year to complete target sample size of 100 individuals. The project has been completed with recruitment of 84 individuals, of which, 34 are males and 50 are females. As shown in Fig 2, the study participants showed dominance of Pitta Prakriti (65/84).

Fig 6: Distribution of Prakriti in study participants

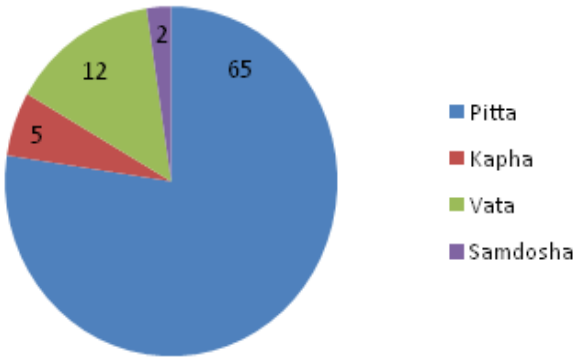


Table 2: Association of Prakriti and Metabolic Syndrome

	Case	Control
Pitta Prakriti	16	49
Non-Pitta Prakriti	2	17

Conclusion: Pitta Prakriti as a constitutional trait of individuals (Odds ratio: 2.78; 95% CI 0.58-13.34) can be considered as independent risk of metabolic syndrome. A study with large population needs to be carried out for confirming these results. Manuscript for the generated data is under preparation.

5.Title: Predictors of Diabetes in first degree relatives of type II DM individuals: Visceral Fat and Visceral Adiposity Index (Project ID: OB/17/1/I**); Funding:** Intramural-Generated funds; **Sanctioned Amount**:Rs. 25000/-;**Duration:** Sep 2017- 2019; **Investigator: PI :** Dr. Vijay Mate, Dept. of Pharmacology, BV Medical College; **Co-Investigator**: Dr. Supriya Bhalerao; **Project Staff** : Dr. Sarika Mane; **Ethics Approval**:BVDU/MC/54

Background: The present study was planned to investigate the association of visceral fat percentage and visceral adiposity Index (VAI) with insulin resistance (marker for risk of diabetes) in the First Degree Relatives (FDRs) of diabetic patients. The participant recruitment was started in August 2018. In the last year, we have recruited 59 volunteers.

Objective

To assess whether visceral fat and visceral adiposity index predict the risk of diabetes in FDR of type II DM

Work done: A total of 85 First degree relatives of T2DM individual in the age group of 18-60 years were recruited in the study. Out of them, data from only 80 participants (33 Male and 47 Female) was analyzable. The rest 5 participants were found to be diabetic in the screening process. During last year, analysis of the data was carried out. The mean age (± SD) of the study participants was 35.65 (±12.38) years. The results of anthropometric parameters, body composition and blood investigations are presented in Table 4.

Table 4: Anthropometry, body composition and biochemical investigations for of FDRs

Parameters	Mean ± SD
Anthropometry	
Body weight (kg)	67.64 ± 14.15
Body Fat %	31.34 ± 6.83
Waist circumference (WC) (cm)	94..91 ± 9.36
Hip circumference (cm)	103.68 ± 8.63
W/H ratio	0.92 ± 0.05
Body composition	
Body mass index (BMI) kg/m^2	25.26 ± 4.38
Visceral fat (%)	8.5 (0.5-21)
Visceral Adiposity Index	1.27(0.63-4.91)
Subcutaneous Fat (%)	25.81 ± 9.15
Skeletal Muscle Mass (%)	26.70 ± 4.63
Biochemical investigations	
Fasting sugar (mg/dL)	82.48 ± 11.29
Fasting insulin (mIU/L)	5.16 (<1-19.15)
HOMA-IR	1.13 (0.20- 4.82)
Haemoglobin	13.15 ± 1.87
Total Cholesterol (mg/dL)	152.44± 34.43
Triglycerides (mg/dL)	78 (48-361)
HDL (mg/dL)	47.89 ±2.37
SGPT (IU/L)	28.64±5.55
SGOT (IU/L)	26.88 ± 4.24
Alkaline Phosphatase (IU/L)	60.64 ± 16.81
A/G Ratio	1.22 ± 0.27

All the studied blood parameters including fasting sugar and insulin were found to be in normal range.

Out of 80 recruited individuals, 41 of them had higher Insulin resistance (HOMA-IR 1- ≥2) while only nine individuals were found to have a higher percentage of visceral fat (≥ 15). Visceral Adiposity index (VAI) is a recently developed indicator of visceral adiposity for determining high risk of obesity. More than 50 % (46 FDRs) of the study participants were in the range of 1.0-2.0. Nineteen individuals were in low risk range of VAI and 15 individuals were in high risk range (>2). (Table 5)

Table 5. Distribution of FDRs on different range of calculative indices of insulin resistance and fat distribution

Parameters	**Range**	**No. of participants**
HOMA-IR	0.2- <1.0	39
	1.0-2.0	32
	>2	09
Visceral Fat (%)	≤ 9	44
	9.1-14	27
	≥ 15	09
Visceral Adiposity Index (VAI)	0.2- <1.0	19
	1.0-2.0	46
	>2	15

All recruited FDRs were categorized according to age group criteria of Indian Diabetes Risk Score (IDRS). This score includes four risk factors viz. age, Family history, waist circumference and physical activity. Since we observed values of all parameters within normal range, we categorized the participants according to the IDRS specified age groups. It was observed that the participants in the age group 35-49 years had maximum BMI, Insulin, HOMA-IR and visceral fat % levels as compared to participants from other 2 age groups. In this age group, adiponectin levels were lower as compared to other two groups. The participants above 50 years of age showed higher levels of sugar, TG/HDL ratio as well as VAI (Table 6).

Table 6: Age wise distribution for risk parameters Median (Range) values

Parameters	Age(years)		
	18-35 (n=40)	35-49 (n=30)	≥50 (n=10)
Waist circumference (WC) (cm)	96(71-119)	92(68-113)	98(77.5-118)

Body Mass Index (BMI)	24.7 (16-34.9)	25.62(21.5-32.2)	23.05(15.9-31.5)
Insulin (mIU/L)	4.99 (<1- 19.15)	5.6 (1.02-16.81)	4.57 (2.68-9.63)
Sugar (mg/dL)	80.5(63-104)	83.5 (61-103)	90.5 (71-120)
HOMA-IR	0.97(0.28-4.82)	1.17(0.20-3.11)	1.08 (0.48-2.09)
TG/HDL	1.61 (1.03-4.93)	3.79 (3.46-4.57)	3.92 (3.65-4.23)
Visceral Fat (%)	8.25 (0.5-17)	9 (0.5-20)	7.25(1.5-17.5)
Visceral Adiposity Index	1.22(0.620-4.33)	1.28 (0.70-4.90)	1.41 (0.90-3.11)
Adiponectin	71.85(12.9-209)	67.65(17.4-176.7)	81.3(27.3-169.3)

Conclusion: The present study highlights the importance of age in impairment of glucose homeostasis in the FDRs. While increased visceral fat was found to be associated with high insulin levels (and therefore high HOMA-IR values) in the participants of age group 35-49 years, Visceral Adiposity Index was accompanied with high sugar levels in the participants above the age of 50 years. The specific association of these 2 parameters with age and glycemic indices needs to be explored in large sample size. Manuscript for the above data is under preparation.

Name of the program/group: Herbal Medicine

1.Title: Studies on 'Vidanga'- traditionally used plants with respect to their Pharmacological Activities **(Project ID: HM/18/1/P); Funding Agency:** NA; **Duration:** Registered (2018); **PhD Student**: Kartikey T Jagtap; **Name of the Guide:** Dr. Suresh D. Jagtap

Background: Vidanga has several medicinal properties. Different species are correlated with local name Vidangathroughout India and investigated for their medicinal properties. Vidanga is one of the herbs commonly used in Ayruveda. It is considered to support the intestine and keep the digestive system healthy. The genus *Embelia* belongs to family Myrsinaceae. Species of *Embelia* like *E. ribes, E. basaal, E.* ***drupacea*** and *Maesaindica* are known for their medicinal use since thousands of years.Vidanga has strong traditional as well as experimental base for its use: in skin ailments like acne and pimple; in constipation; digestive track in piles; as a brain tonic. Therefore parameters related to these conditions will be studied to find out potent *Embelia* species as Vidanga.

The market samples of Vidanga are available in mixtures of aliedVidanga species, so we can observe ambiguity in the market drugs

Objectives:
To identify and collect all Vidanga species and development of simple field identification key
To study the ambiguity in identification of authentic Vidanga drugs from market samples using In-vitro comparative antioxidant study
To study more effective species of Vidanga using In-vitro comparative antioxidant study on self-collected samples
To carry out pharmacogonistic studies on fruits of selected Vidanga species using In-vivo anti-inflammatory studies
To confirm the most potent species of Vidanga by pharmacogonistic studies using In-vivo immunomodulatory potential

2.Title: Chemometric analysis & Development of Methodology for quality standardization of 'Vidanga'. (Project ID: HM/18/2/P); **Funding Agency**: CSIR HRDG JRF; **Duration**: 5 Years; **PhD Student**: Manoj Khavate; **Name of the Guide**: Dr. Suresh D. Jagtap

Background: Therapeutic properties of the genus *Embelia* are attributed by Embelin or embolic acid present in berries of plants, The concentration of active principle in plants may vary on account of environmental conditions or ecosystem of the plant, maturity at the time of collection, substitutability on the basis of perceived efficacy or generic name and adulteration are obvious. These factors cause ambiguity for authentication of Embelin in the traded herbal raw materials; therefore there is a need to standardize a method for rapid, accurate and non-destructive method to detect the authentic species.

Objectives:

- Development of standardization parameters by co-relating biological activity and chemometric parameters
- Generation of biological activity based chemical profile of Vidanga
- The Chemometric standardization of Vidanga will throw light on rapid, non destructive detection method for authentic species.
- The study can set a model to solve problem related to ambiguity among the traditionally used plants.

3.Title: Development and evaluation of new synbiotic formulation against candidal inflammation of intestine. **(Project ID: HM/18/3/P); Funding Agency**: Self funded; **Duration:** 24 months; **Ph.D. Student**: Mr. Mayur. A. Aswani; **Name of the Guide**: Dr. Suresh. D. Jagtap

Background: Prebiotics have been discussed with respect to the systemic effects they exert on the host's health, metabolism and immune system. The ability to regulate the composition of the gut microbiota by prebiotic dietary substance and probiotic microorganisms is an interesting approach in the control and treatment of some major diseases. Prebiotics are emerging as promising nutraceuticals in various medical conditions, including IBD. Since prebiotics are easy to administer, inexpensive, and lack significant toxic side effects they may become an attractive alternative or adjunct to standard therapeutics in inflammation conditions.

Objectives:

To purify and characterize prebiotics from selected plant parts with anti-candida and and anti-inflammatory properties

To formulate and screen synbiotics for anti-inflammatory activity

To characterize the activity of selected synbiotic against inflammation

To characterize the activity of selected synbiotic for immunomodulatory activity

Name of the program/group: Osteoarthritis
Projects (Intramural/Extramural/Industrial/Ph.D.)

Title of the Project:Investigating modulation of matrix metalloproteinases in synoviocytes pertinent to osteoarthritis as well as through treatment with ayurvedic formulations. (Project ID: OA/13/1/P);
Funding Agency: Funded through INNO INDIGO
Duration: 4.5 years
Ph.D. Student: DhanashreeIngale
Name of the Guide: Dr Suresh Jagtap andDr. Abhay A. Harsulkar
Background:KneeSynovial fluid (SF) accumulates a plethora of biochemical factors that are synthesized and released by several adjoining cells under stress. These factors are in turn prove essential for worsening disease condition. This thesis work was designed to treat the SW-982 a human synovial fibroblast cells with the SF obtained from OA patients and the release of NO measured as an inflammatory response of the cells. Using the MMPs and TIMPs as representative markers of catabolic and anabolic processes, it was attempted to study the delicate balance in knee synovial fluid and synovial tissue. Accordingly, the synovial samples from patients undergoing total knee replacement were analyzed for expression of MMPs and TIMPs and Synovial fluid to estimate the activity levels of MMPs and TIMPs
Objectives:
Studying MMPs and TIMPs in Synovial Fluid (SF) and synovium from OA patients
Studying MMPs and TIMPs in synovium from OA patients
A cell-based assay for modulation of MMPs and TIMP using formulations, on synoviocytes challenged by SF from OA patients
Chromogenic Assays of formulations for modulation of MMPs and TIMPs
Anti-inflammatory and Antioxidant potential of selected herbal formulations for modulation of MMPs and TIMP

Title of the Project:"Decoding the synovial immunefunction at cellular and molecular level in knee osteoarthritis."
Funding Agency:Self funded
Duration:24 months
Ph.D. Student:MsVanshika Shrivastava
Name of the Guide:Dr Abhay Harsulkar
Background:Osteoarthritis (OA) is the most common form of degenerative disease. OA poses an enormousburden on individual family and society owing to its debilitating feature; affecting mainly theworking class at the peak of their career. Low-grade chronic inflammation due to immunologicaldysfunction along with the axis of “synovium-synovial fluid-cartilage” is the key feature of OApathophysiology, which unfortunately, none of the current therapy addresses. Omega 3 fatty acids and many natural products are indicated to potentially address this pathophysiology. In the present module of work, it is planned to investigate the immune dysfunction in synovial joint by studying differentiationin effector immune cells in presence of synovial fluid collected from OA patients. This is a novelapproach to mimic the in vivo disease conditions. Immunomodulatory properties of omega 3 fatty acids and a select natural products will be deciphered using these cell assays.
Objectives:

To study the transition of monocyte into microphase and hematopoietic stem cells into mast cells with reference to knee osteoarthritis

To study expression of various transcription factors during this cell differentiation sequence

To study the transition links between the innate nonspecific immune mechanism to acquired and targeted immunity during the progression of osteoarthritis

To use this cell assay system to screen different compounds such as omega 3 fatty acids and a select natural products that can potentially arrest the immune cell differentiation.

Title of the Project: Elucidating the molecular mechanism of a novel synbiotic formulation in Non-Alcoholic Fatty Liver Disease (NAFLD)

Funding Agency: Self-funded

Duration: 24 months

Ph.D. Student: Akib Nisar

Name of the Guide: Dr. Abhay A. Harsulkar

Background: Prebiotics have been discussed concerning the systemic effects they exert on the host's health, metabolism and immune system. The ability to regulate the composition of the gut microbiota by prebiotic dietary substance and probiotic microorganisms is an interesting approach in the control and treatment of some major diseases. The non-alcoholic fatty liver disease (NAFLD) is the metabolic disorder affecting more than 25% population globally. Gut-liver axis is one of the important components which has been proven to be linked to NAFLD. Recent studies have shown improvement in NAFLD through gut microbiota by incorporating potential probiotics and synbiotics. This study is focused to target NAFLD by using novel synbiotic formulation and naturally isolated plant prebiotics. The special emphasis has been given to molecular mechanisms to fill the research gap.

Objectives:

Screening of potential prebiotics and probiotics.

To prepare synbiotic formulations and its anti-inflammatory activity.

To evaluate the efficacy of selected synbiotic against NAFLD and mechanism of action- *in vivo* studies.

To collect the blood from the hepatic portal vein and peripheral blood from the synbiotic treated animal model and its assessment.

Name of the Programme: Communicable Diseases

1.Title: Establishment of a novel Electronic Surveillance System for dengue in Pune: an initiative for Smart Cities Mission **(Project ID**: CD/17/1/E); **Funding**: ICMR; **Duration:** March 2017 – February 2020; **Sanctioned Amount:** 400.1 Lakh; **Investigators: PI** - Dr A. C. Mishra; **Co-Investigators** - Dr Vidya Arankalle (IRSHA), Dr Sanjay Lalwani, Dr Arundhati G Diwan, Dr. M Modak (Bharati Vidyapeeth Medical College), Anand P Kulkarni, Dr Varsha Vaidya; Ph.D. Students: None; **Human Ethical Approval:** IEC/2017/04, renewed IEC/2018/11, IEC/2019/06

Background: This study was initiated in March 2017. Through this study, we propose to develop a smart web based system for data collections from different clinics/hospitals and appropriate programs for processing of data to produce real time streaming of information on epidemiological and clinical parameters of dengue. During the previous year, we customized the CDC Epi info-software program for recording clinical and epidemiologic information on dengue cases in Pune and monitor the data in real time. Clinical and virologic surveillance of dengue was undertaken throughput the year.

Objectives:
Development of web based electronic system to collect, store, retrieve and process data on dengue
Clinical and virological surveillance of dengue in Pune city
Attempt to develop an early warning system for dengue cases to facilitate advance public health action.

Work done:
1. Development of web-based electronic system to collect, store, retrieve and process data
CDC Epi info-software program customized as per our requirement was used for recording the dengue surveillance data during 2019. Demographic and clinical information of suspected dengue patients was collected in the field directly on tablet / mobile and transmitted real-time to central server both online as well as by connecting the mobile with computer. Laboratory results were then entered into the system online. A dashboard was created to get automatic results on daily basis to facilitate real time monitoring of data and sharing with stakeholders like clinicians. Mapping of positive/negative cases, seasonal trend, age/sex/ward-wise distribution of cases was automatically updated on the dashboard after new entries (Fig.1,2).

Fig.1. Schematic representation of web-based electronic disease surveillance system

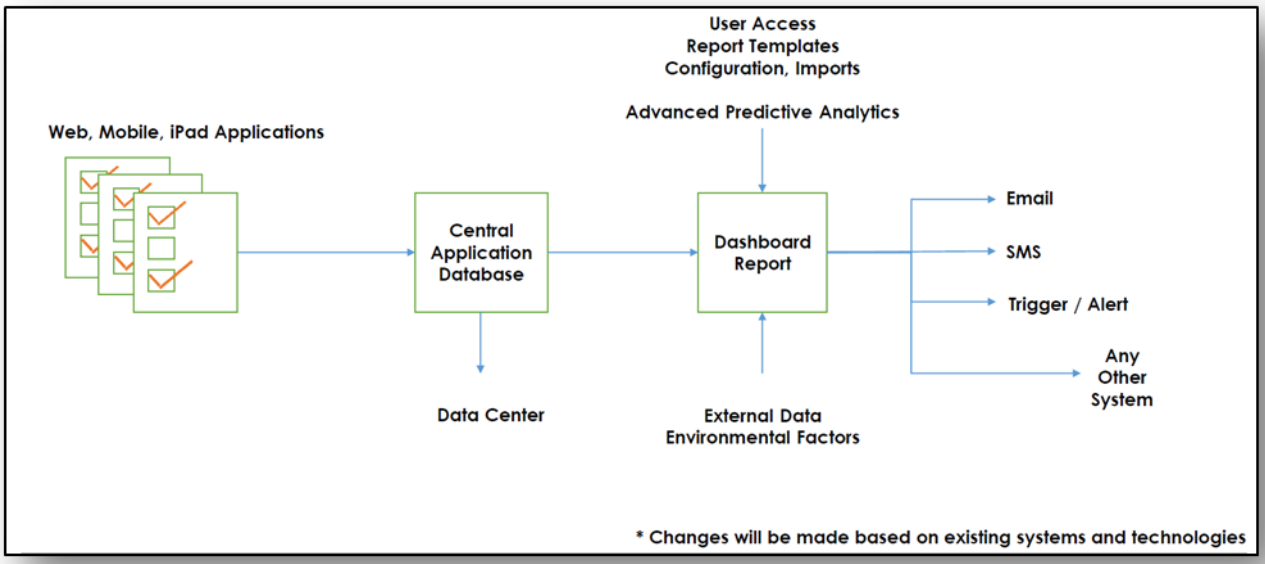
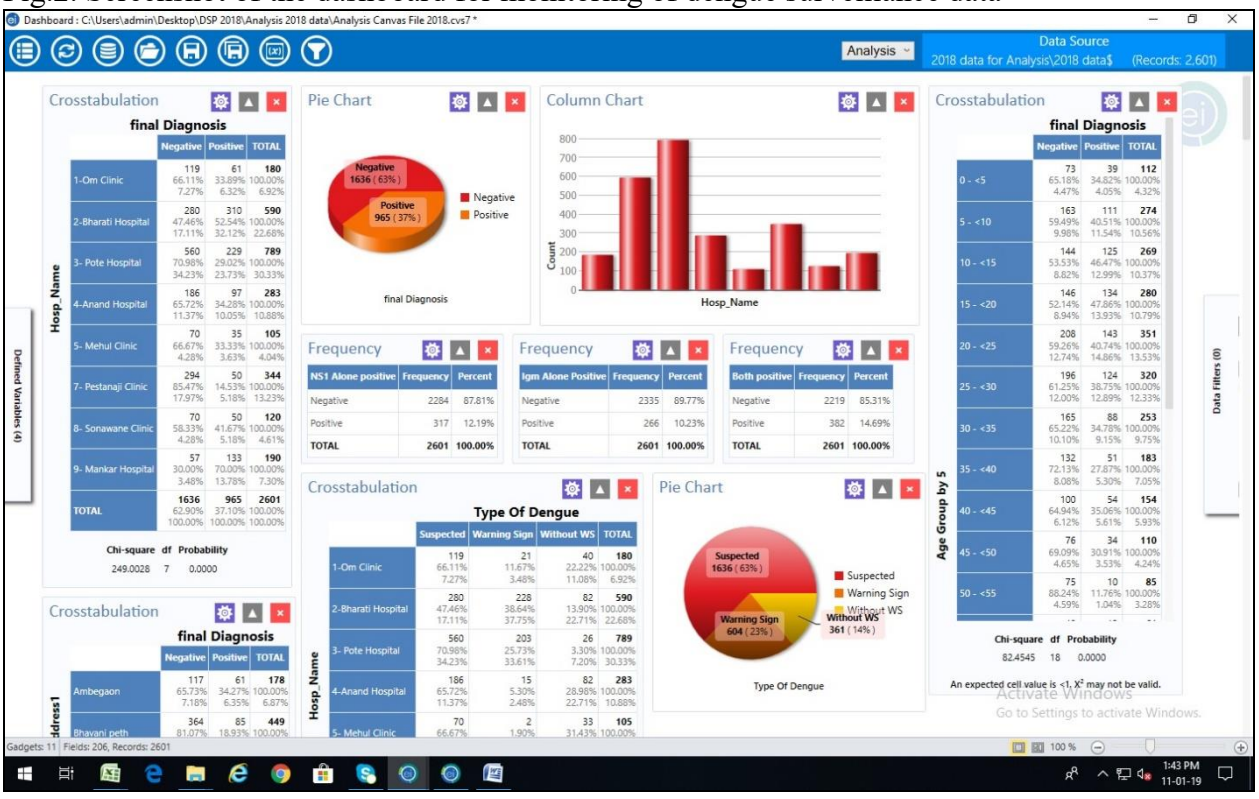
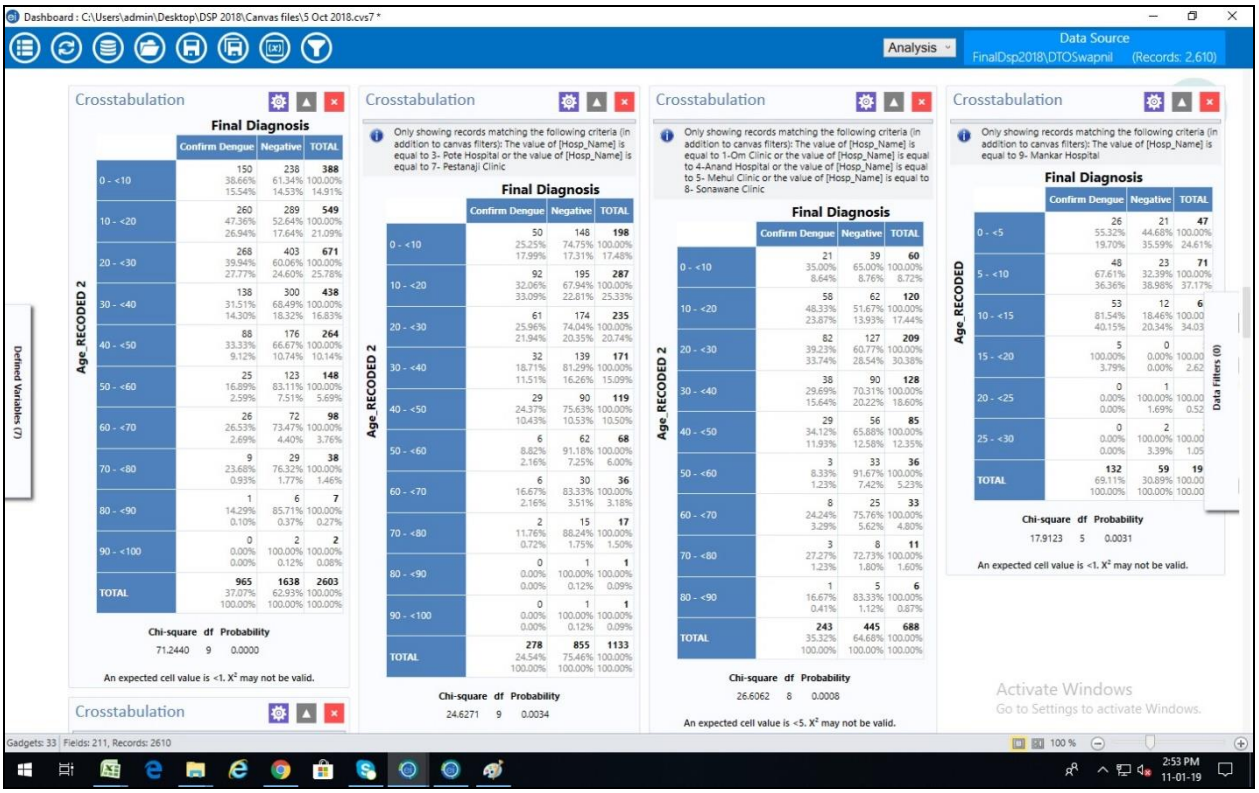


Fig.2. Screenshot of the dashboard for monitoring of dengue surveillance data



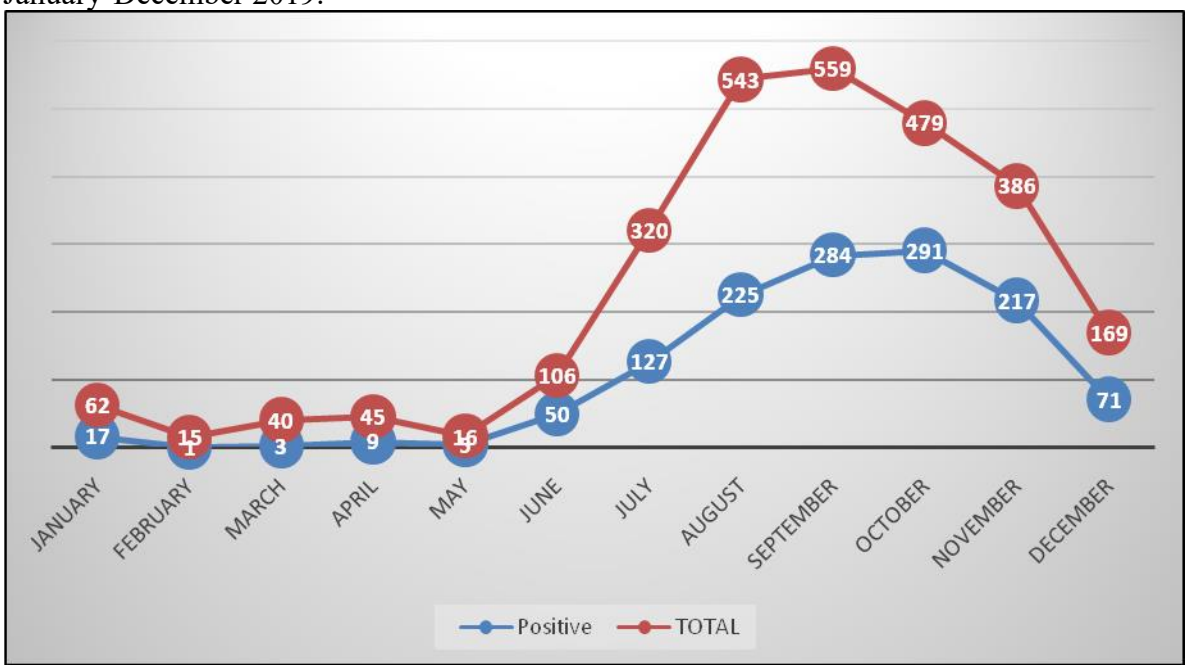


2. Clinical and virological surveillance of dengue during 2019 season

During 2019, dengue surveillance was continued at 4 sites in Dhankawadi ward - one corporation hospital Pote hospital), tertiary care hospital (Bharati hospital) and two clinics of general practitioners (GP), and one pediatric hospital (Mankar Hospital) from an adjacent, different ward (Sinhgad Road). A total of 2740 samples were collected from dengue suspected patients during January – December 2019. All samples were tested for NS1 antigen and anti-DENV IgM antibody using J Mitra NS1 ELISA and Panbio Dengue IgM Capture ELISA respectively. 1300 (47.4%) of the tested samples were positive for NS1 and/or IgM-anti-DENV antibodies and classified as confirmed dengue positive. Of these, 889 were tested for anti-DENV IgG using Panbio IgG Capture ELISA and 322 (36.2%) were identified as secondary dengue infections.

Figure 3 depicts month-wise distribution of suspected and serologically confirmed dengue cases during 2019. The months of September and October witnessed maximum number of dengue cases.

Fig.3. Month-wise distribution of suspected (total) and confirmed (positive) dengue cases during January-December 2019.



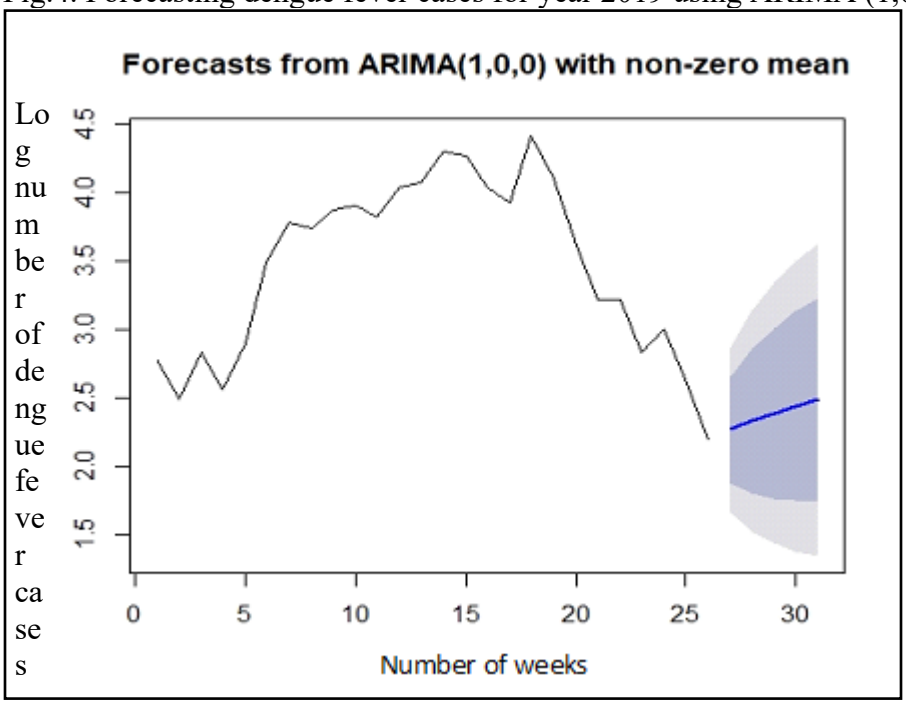
To determine the circulating serotypes, a total of 382 NS1 positive serum samples were subjected to NS1 RT-PCR or real-time PCR based serotyping. DENV serotype could be determined in 216 (56%) of the samples and the distribution was as follows - DENV-1: 142 (65.7%), DENV-2: 52 (24%) and DENV-3: 17 (7.8%). Thus, DENV-1 serotype was found to be predominant during 2019 dengue season. Additionally, co-infection was detected in 5 patients (2.3%) - 2 with serotype DENV-1/-2 and 3 with serotype DENV-1/-3.

74 NS1 positive serum samples were used to isolate DENV by infecting Vero cells. 51 (68.9%) DENV isolates were obtained of which 49 were serotyped as DENV-1 – 29, DENV-2 – 15 and DENV-3 – 05.

3. Development of early warning system for dengue cases by using time series data

Effective monitoring and forecasting of dengue fever outbreaks is essential in preventing the spread of dengue fever. We investigated predictive modelling using the Auto Regressive Integrated Moving Average - ARIMA (1,0,0) time-series model for forecasting dengue fever using our data from Pune region for the best forecast for the upcoming dengue season. ARIMA method, which is derived from three basic time-series methods: autoregressive (AR), moving average (MA) and autoregressive moving average (ARMA), was chosen to be the best fit method for our data using the R Software. We built time series model based on the data from 2016-2018 and used it for forecasting dengue fever cases for 2019 (Fig.4.).

Fig.4. Forecasting dengue fever cases for year 2019 using ARIMA (1,0,0) time series model



Conclusion:

A web-base electronic system was developed for real-time monitoring of dengue surveillance in Pune. The electronic system with minimal or no manual intervention could be used for providing customized laboratory reports to clinicians.

During January-December 2019, 47.4% dengue positivity was noted among suspected dengue cases. DENV-1 was the predominant serotype.

Preliminary studies using ARIMA (1,0,0) time series model suggest strong possibility that predictive model system can be developed to give good predictions for dengue fever outbreaks.

2.Title: Immune response of Indian preterm infants to pentavalent vaccine (**Project ID**: CD/17/3/E); **Funding**: DHR-ICMR; **Duration:** July 2017 – December 2019; **Sanctioned Amount:** Rs. 41.08 Lakh; **Investigators: PI** - Dr Vidya Arankalle; **Co-Investigators** - Dr A. C. Mishra (IRSHA), Dr. Nandini Malshe, Dr Sonali Palkar, Dr Sanjay Lalwani (Bharati Vidyapeeth Medical College); Ph.D. Students: None; **Human Ethical Approval**: IEC/2017/31

Background: The project was concluded this year after 6 months extension. The primary aim of our study was to assess if the current national recommendations for pentavalent immunization of preterm infants are appropriate and ensure adequate immune response. Earlier work revealed that as compared to infants born full term [FT, gestational age (GA) > 36weeks), preterm infants grouped according to GA in PT1 (28-32weeks) and PT2 (32-36weeks) developed adequate antibody response against tetanus and diphtheria, lower titers against HiB and HBsAg while suboptimal anti-pertussis response was independent of GA. To understand the basis for such differential responses, we determined (1) relationship of GA with proportion of circulating

immune cells and cytokines at pre- and post-vaccination and (2) recall response of PBMCs from antibody positive and negative infants, post-stimulation with different immunogens.

Objectives:

To assess the antibody response of pre-term babies to pentavalent vaccine in comparison to term babies

To compare circulating immune cells / cytokine profiles in preterm and term infants

To compare response of *in-vitro* antigen-stimulated PBMCs in preterm and term babies.

Work done:

Proportion of Immune cell frequencies in the infant groups examined:

Table 1 describes circulating immune cell frequencies in the infant groups. To identify parameters influencing immune response to the individual components of pentavalent vaccine, multiple regression analysis was done. In univariate analysis, a significant association of GA with antibody response was found for all the antigens except HiB. Importantly, GA was identified as a significant independent variable influencing response to tetanus ($p=0.0005$) and to a lesser degree for pertussis ($p=0.054$) and diphtheria ($p=0.06$). Though the response to tetanus was universal, GA did influence titers, increasing with GA. Frequency of pDCs emerged to be the single independent variable positively impacting humoral response to diphtheria.

Correlation analyses:

Table 2 provides relationship of antibody titers against vaccine component antigens with variables such as gestational age and several immune cells / cytokines present in PT1/PT2/FT infants at the time of vaccination. Of the immune cell parameters evaluated, univariate analysis revealed significant association of only few with antibody response. Gestation age was identified as a significant variable influencing antibody response to tetanus, diphtheria, pertussis and HBsAg, but not to HiB. For HiB and HBsAg, only single parameter was significant and hence multivariate analysis was not possible. Anti-tetanus response was significantly associated CD4, CD8, monocyte and pDC 276 frequencies, but, multivariate analysis identified gestation age as the only predictor for antibody titres. In case of diphtheria toxoid, univariate analysis showed significant associations of humoral response with CD4%, unswitched memory B cells, dendritic cells (mDC & pDC) and monocytes, however, multivariate analysis revealed a strong association with pDC only. For pertussis with compromised seroprotective titres in all the study groups, a significant association with gestation age, monocytes, and plasma levels of IL-6, IL-10 and Il-2 was found in univariate analysis, but plasma IL6 levels emerged to be the significant parameter in multivariate analysis.

Recall immune responses to constituent immunogens of pentavalent vaccine:

To understand the comparative response of term and preterm infants to pathogen exposure, infant PBMCs from immunized infants were stimulated with component antigens of pentavalent vaccine. Memory profile and functionality of B cells, T cells and dendritic cells was determined (Table 3). None of the infants from all the study groups exhibited any modulation when the PBMCs were stimulated with tetanus or diphtheria showing high titers. For the remaining three immunogens, birth status-specific alterations were noted. As against significant rise (> 1.5fold) of all the indicated markers in the FT group, the response of both PT1 and PT2 was much lower. PT1 documented an increase of 2.06fold (Bordetella: central memory CD8 T cells, CD300a MFI) and 2.35fold (HiB: TNF-α & IFN-γ bifunctional central memory CD8 T

cells). In the PT2 infants, central memory CD8 T cells (CD300a MFI) increased 2.2fold with pertussis and 1.02-fold, increased, central memory CD8 T cells (TNF-α MFI) and BCMA+ plasma cells raised 1.5fold with HBsAg while central memory CD8 T cells (CD300a MFI) increased 2.33fold for HiB. Overall, there was a prompt proliferation of central memory T cell compartment with Th1 cytokine secretion in term infants as compared to preterm infants. Immune cells of term infants showed highest capability to respond to the component immunogens, particularly inactivated whole cell of Bordetella, HBsAg and PRP moiety of Hemophilus influenzae B, suggestive of robust immunological memory development.

Conclusion:

- Comparative antibody response of Indian infants classified according to gestational age, to the component immunogens of the pentavalent vaccine is provided for the first time.
- It is satisfying to note that irrespective of gestational age, all the infants developed adequate antibody response against tetanus, diphtheria and, protective but lower antibody levels for HiB and hepatitis B.
- Suboptimal response to pertussis in all the infant groups emerged as a major concern.
- In addition to generating data on the relationship of circulating immune cells and cytokines with GA, the results revealed that both humoral and cellular immune responses of preterm infants were dependent on the type of the immunogen.
- Preterm infants born before 32 weeks of gestation may need an extra dose of pentavalent vaccine for long lived robust immune response.
- A further follow up till the receipt of booster dose is necessary to identify the window of susceptibility to these pathogens.

Table 1: Immune cell frequencies in all study groups at enrolment and after pentavalent vaccination.

	Enrolment			Post pentavalent vaccination		
Type of Immune cell	PT-1 (N=33)	PT-2 (N=80)	FT (N=80)	PT-1 (N=24)	PT-2 (N=55)	FT (N=54)
CD4% (Median & IQR)	10.65±5.97 (5.76-13.68)	12.94±7.88 (5.94-17.93)	15.54±7.88 (10.2-19.2)	12.19±5.558 (8.54-15.85)	16.89±7.7 (10.5-20.93)	18.99±6.86 (12.9-25.43)
CD8% (Median & IQR)	17.42±10.27 (7.30-27.5)	10.86±8.33 (4.84-13.6)	12.11±7.14 (7.31-15.1)	19.98±8.19 (16.95-24.38)	12.57±6.07 (8.22-17.35)	15.49±6.33 (11.65-18.35)
B cell % (Median & IQR)	17.5±9.93 (8.6-25.55)	16.73±8.70 (8.95-23.5)	18.94±10.23 (10.6-24.3)	18.23±7.90 (11.15-25.95)	20.87±8.83 (14.85-28.03)	22.63±8.61 (15.83-27.45)
Class Switched B cells % (Median & IQR)	25.15±14.53 (14.63-30.93)	25.73±13.91 (14.8-32.9)	19.92±11.95 (8.75-29.8)	28.48±9.501 (20.7-35.1)	32.8±15.38 (25.3-39.03)	29.82±8.32 (24.73-34.38)
Unswitched B cell% (Median & IQR)	73.07±16.15 (63.35-82.95)	73.75±14.37 (66.4-85.2)	79.86±11.97 (70.2-91.25)	69.15±9.824 (60.48-77.7)	65.88±15.19 (60.5-73.23)	70.18±8.32 (65.63-75.27)
Memory B cell% (Median & IQR)	11.27±15.17 (3.10-9.28)	9.86±13.83 (3.63-8.63)	10.07±10.33 (3.9-10.6)	13.98±12.66 (6.063-15.08)	14.68±16.57 (5.03-15.98)	8.03±3.61 (5.17-10.3)
Class Switched Memory B cell % (Median & IQR)	34.26±22.91 (16.6-55.7)	32.42±20.58 (14.6-44.9)	28.79±17.97 (12.25-42.95)	38.37±22.23 (18.05-52.7)	46.09±22.26 (32.08-64.35)	49.79±15.24 (38.68-61.2)
Unswitched memory B cells % (Median & IQR)	64.17±22.34 (43.75-79.98)	66.98±20.37 (54.8-83.8)	71.21±17.97 (57.05-87.75)	59.95±20.82 (47.3-73.3)	53.01±21.55 (35.65-67.58)	50.52±14.74 (38.8-61.33)
Myeloid Dendritic cells (mDC)% (Median & IQR)	0.20±0.12 (0.09-0.27)	0.35±0.24 (0.19-0.47)	0.33±0.22 (0.16-0.44)	0.30±0.19 (0.17-0.47)	0.32 ± 0.19 (0.185-0.44)	0.32±0.21 (0.18-0.44)
Plasmacytoid Dendritic cells (pDC)% (Median & IQR)	0.11 ±0.09 (0.05-0.17)	0.2±0.15 (0.09-0.26)	0.2±0.14 (0.1-0.26)	0.11±0.06 (0.05-0.17)	0.19±0.19 (0.09-0.24)	0.15±0.09 (0.08-0.19)
Monocytes % (Median & IQR)	5.5±3.82 (2.5-6.79)	7.75±4.0 (4.77-10.4)	8.34±4.24 (5.06-11.1)	7.4±3.8 (5.033-10.63)	7.0±3.4 (4.58-8.57)	6.26±3.09 (3.58-9.28)

Table 2: Multivariate analysis to identify independent variables influencing antibody response to the pentavalent vaccine components*

Immunogen	Variable	Univariate (p value)	Multivariate (p value)
Tetanus toxoid	Birth status	<0.001	0.0005
	CD4%	0.048	0.2
	CD8%	0.045	0.2
	pDC%	0.028	0.2
	Monocyte%	0.025	0.15
Diphtheria toxoid	Birth status	0.046	0.06
	CD4%	0.049	0.09
	USB%	0.042	0.16
	mDC%	0.002	0.2
	pDC%	0.007	0.03
	Monocyte%	0.04	0.6
B.pertussis (Whole cell)	Birth status	0.049	0.054
	Monocyte%	0.01	0.055
	IL-2 (pg/ml)	0.02	0.055
	IL-6 (pg/ml)	<0.001	0.001
	IL-10 (pg/ml)	0.04	0.8
HiB(PRP)	mDC%	0.025	NA
HBsAg	Birth status	0.034	NA

*****Parameters with significant influence on antibody titers in univariate analysis were included for multivariate analysis

Table 3: Fold changes in functional parameters of immune cells following antigenic stimulation of cultured PBMCs

Type of immune cell	Functional parameter	*Bordetella* (whole cell) stimulation PT-1 (Fold rise)	PT-2 (Fold rise)	FT (Fold rise)
Central memoryCD4 T cells	IFN-γ MFI	1.11	0.47	11.21
	TNF-α (%)	0.00	0.00	1.84
Central memory CD8 T cells	TNF-α (%)	0.00	0.81	3.16
	TNF-α MFI	0.12	0.50	2.56
	CD107a & IL-2 (%)	1.10	0.60	1.78
	TNF-α & IFN-γ (Bifunctional) %	0.00	0.69	2.25
	CD300a MFI	2.06	2.20	1.74
Polyfunctional Central memory CD8 T cells	CD107a MFI	1.00	0.74	1.54

Type of immune cell	Functional parameter	HBsAg stimulation PT-1 (Fold rise)	PT-2 (Fold rise)	FT (Fold rise)
Polyfunctional Central memory CD4 T cells	% cells	0.5	0.3	1.5
Effector Memory CD4 Tcells	IFN-γ MFI	1.0	0.9	2.0
Central memory CD8 T cells	TNF-α MFI	0.8	1.5	15.6
	TNF-α & IFN-γ (Bifunctional) %	0.0	1.0	2.0
	CD300A MFI	0.0	0.8	2.7
	TNF-α & IL-2 (Bifunctional) %	0.0	0.7	2.0
	Il-2 MFI	0.0	0.9	1.6
	TNF-α %	0.0	1.3	6.0
Polyfunctional Central memory CD8 T cells	CD107A MFI	1.2	1.0	1.6
Effector Memory CD8 Tcells	CD300A MFI	1.2	0.4	2.2

Plasma cells	BCMA +(%)	1.0	1.5	1.7
	BCMA MFI	1.0	1.1	1.7
		Hib PRP stimulation		
Type of immune cell	Functional parameter	PT-1 (Fold rise)	PT-2 (Fold rise)	FT (Fold rise)
Central memory CD4 T cells	CD40L MFI	1.13	1.16	1.70
	TNF-α %	0.00	0.00	1.89
Central memory CD8 T cells	TNF MFI of bifunctional (TNF-α & IL-2)	1.00	1.02	2.96
	Bifunctional (TNF-α & IFN-γ)	0.94	0.64	2.06
	CD300a MFI	1.35	0.69	3.14
	Bifunctional (TNF-α & IL-2)	0.00	0.51	4.40
	Il-2 MFI of Bifunctional (TNF-α & IL-2)	0.00	1.21	4.44
	Bifunctional (TNF &IFN-g)	2.35	0.83	2.76
	IFN-γ MFI of Bifunctional (TNF-α & IFN- γ)	1.25	1.02	1.66
	CD300a MFI	0.80	2.33	1.93
Effector memory CD8 T cell	CD300a MFI	0.00	0.00	1.79
Myeloid DC	CD86 MFI	1.01	0.96	1.71

3.Title: Norovirus surveillance among children with non-rotavirus associated gastroenteritis in Pune, India (**Project ID**: CD/18/1/E); **Funding:** Centers for Disease Control and Prevention (CDC), USA and intramural support from Bharati Vidyapeeth; **Duration:** January 2018 - December 2019; **Sanctioned Amount:** CDC, USA (Reagents for rotavirus and norovirus testing) and Bharati Vidyapeeth (Rs. 5 lakhs); **Investigators: PI**: Dr. Ruta Kulkarni; **Co-Investigators:** Dr. V. Kalrao, Dr. S. Mankar, Dr. M. Sangamnerkar; Ph.D. Students: None; **Human Ethical Approval:** IEC/2017/32 renewed as IEC/2018/47, IEC/2019/53

Background: This study was initiated in January 2018 with an aim to study the rate and trends of norovirus infections among children with acute gastroenteritis. During the year 2018, norovirus surveillance was undertaken among children hospitalized for acute gastroenteritis in Pune.

Objectives:
To determine the rate of norovirus infection among children with non-rotavirus associated gastroenteritis in Pune, India.
To identify the norovirus genotypes circulating among children in Pune, India.

Work done: Norovirus surveillance was continued during January to December 2019. A total of 82 stool samples were collected during this period from sporadic cases of acute gastroenteritis among children (≤5 years of age) getting treatment at three hospitals in Pune (Bharati hospital, Mankar hospital and Chinmay hospital). All these samples were tested for rotavirus by ELISA. The samples found to be negative for rotavirus were tested by real-time RT-PCR for detection of norovirus. Genotyping of the norovirus-positive samples is in progress.

Results:
Of the 82 stool samples collected during 2019, 38 (46.3%) were positive for rotavirus. On testing of the remaining 44 rotavirus-negative samples for norovirus, 13 (29.5%) were found to be positive for Genogroup II norovirus; none of the samples showed presence of Genogroup I norovirus.
Overall, during the project duration (2018-2019), norovirus prevalence among children with non-rotavirus associated gastroenteritis in Pune was found to be 21.3% (32/150).

4.Title: Evaluation of different adjuvants for development of potent chikungunya vaccine (**Project ID**: CD/18/2/E); **Funding:** DST-SERB; **Duration:** May 2018 – May 2021; **Sanctioned Amount:** 32.2 Lakhs; **Investigators: PI:** Dr. Harshad Padmanabh Patil; Ph.D. Students: Ms. Mrunal Gosavi; **Animal Ethical Approval:** BVDUMC/1890/2018/002/019

Background: This study was initiated in May 2018 with an aim to evaluate inactivated CHIKV or VLP together with various adjuvants by systemic immunization routes for induction of immune response. During the previous year, CHIKV was propagated in Vero cell line and inactivated by treatment with β-propiolactone. Immunization of mice using the inactivated CHIKV, and investigation of the generated antibody response was undertaken.

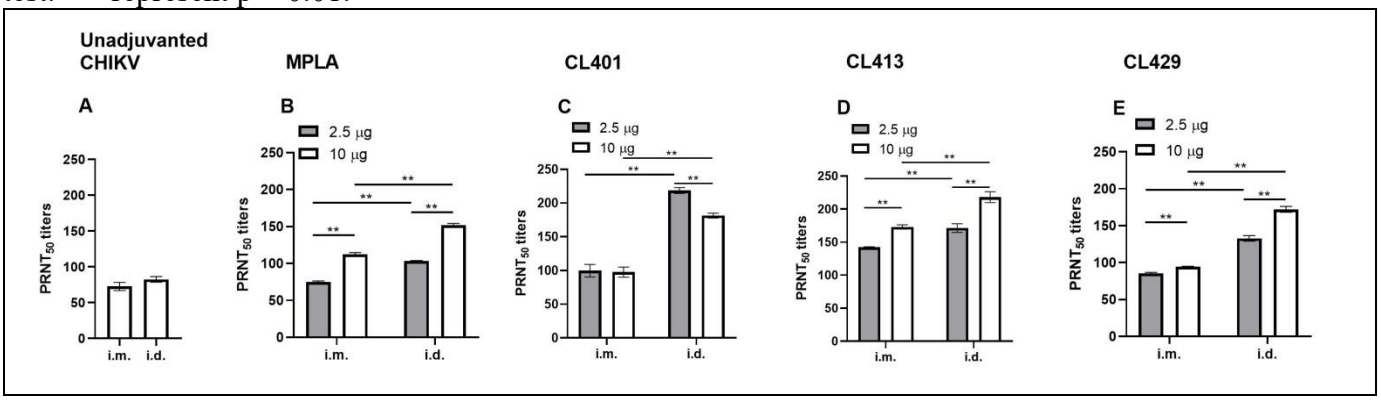
Objectives:
Isolation of CHIKV virus from serum samples and production of inactivated CHIKV for immunization in mice.
Analysis of CHIKV specific humoral immune responses after administration of adjuvanted CHIKV vaccine.
Analysis of CHIKV specific cellular immune responses after administration of adjuvanted CHIKV vaccine.
Analysis of T and B cells for immunological memory and migration towards skin after immunization against CHIKV.

Work done:
Work on objectives 2 and 3 was executed in the year.

Neutralizing antibody titers levels after immunization

An increase in PRNT50 titers was observed after immunization with adjuvanted inactivated CHIKV for both i.m. or i.d. route (p=0.014 to <0.0001, Fig. 5A-E). All the mice administered with adjuvanted formulations by i.m. route, expect for MPLA or CL429 (2.5 µg), induced higher titers (p=0.014 to 0.001). In case of i.d. delivery, adjuvanted formulations induced higher titers as compared to unadjuvanted CHIKV administered by same route (p<0.0001). I.D. delivery was found to be better than i.m. delivery for induction of neutralizing antibodies for all adjuvants. Highest $PRNT_{50}$ titers were induced by 2.5 µg CL401 and 10 µg CL429 after i.d delivery.

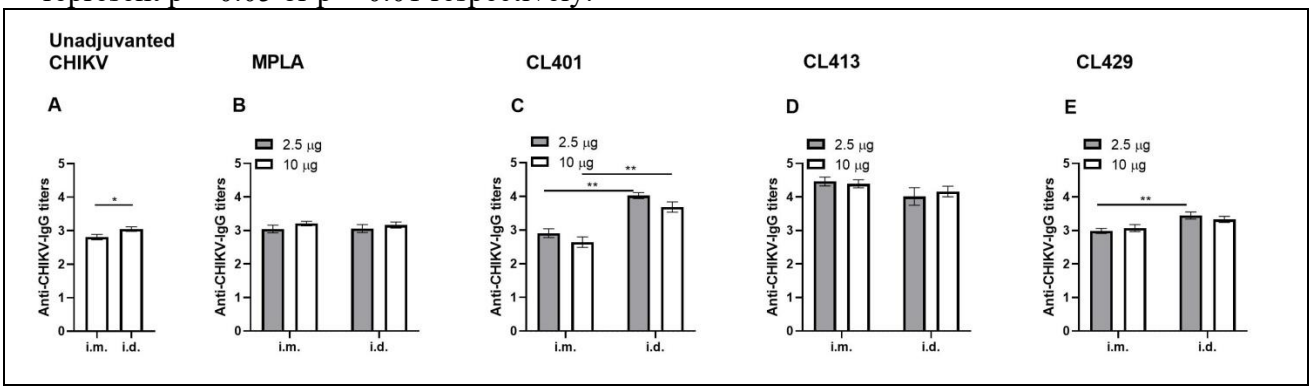
Fig 5. Neutralizing antibody response after immunization. Mice (n=6) were immunized twice on day 0 and 21 with 2.5µg inactivated CHIKV with or without 2.5 or 10 µg adjuvants either by i.m. or i.d. route. Blood was collected on day 51. PRNT50 assay using 600 pfu/ml CHIKV was used to execute the assay. P values were determined with unpaired, two-sided Mann–Whitney U-test. ** represent p < 0.01.



IgG response after immunization

Except for CL413 and MPLA (10 µg), no other adjuvant induced IgG titers higher than unadjuvanted CHIKV after i.m. delivery. On the contrary, after i.d. delivery, all adjuvants expect MPLA elicited greater anti-CHIKV-IgG titers (p=0.027 to <0.0001). No difference in antibody titers was observed when 2.5 or 10 µg adjuvant was used.

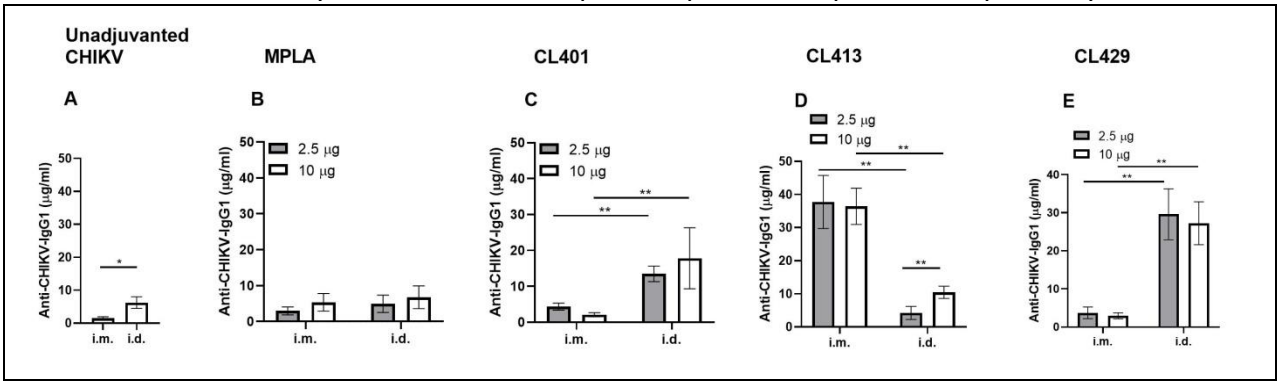
Fig 6. Anti-CHIKV-IgG response after immunization. Serum collected from mice (n=6) one month (i.e on day 51) after second dose was used for anti-CHIKV-IgG titer determination using indirect ELISA. P values were determined with unpaired, two-sided Mann–Whitney U-test. * or ** represent $p < 0.05$ or $p < 0.01$ respectively.



IgG1 response after immunization

I.d. delivery of unadjuvanted CHIKV induced significantly higher IgG1 as compared to i.m. delivery (Fig.7A). CL413 induced significantly higher IgG1 titers (Fig.7D) in comparison to other adjuvants (0.002 to 0.0043). After i.d. delivery, CL401 (p=0.041, Fig.7C) and CL429 (p=0.015 to 0.0087, Fig.7E) induced higher IgG1 as compared to unadjuvanted inactivated. Highest IgG1 was induced by CL413 after i.m. delivery and CL429 after i.d. delivery.

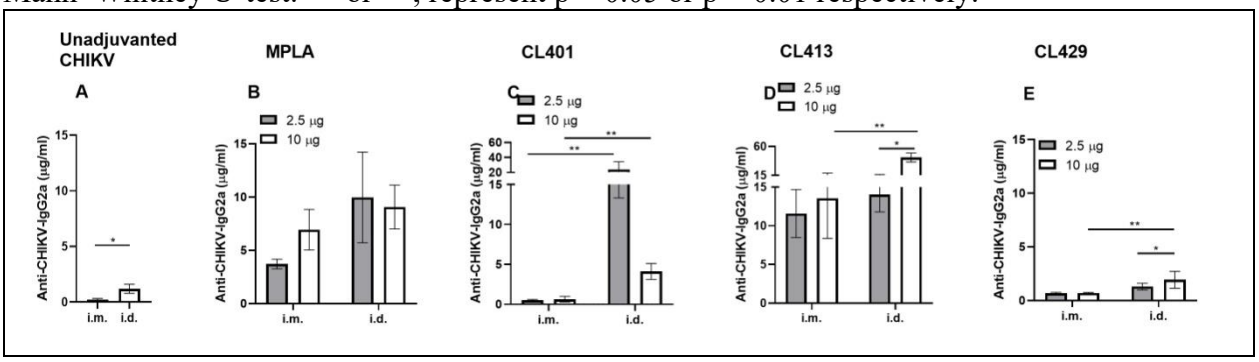
Fig 7. Anti-CHIKV-IgG1 response after immunization. ELISA was performed to determine CHIKV specific IgG1 level after immunization. Serum collected from mice (n=6) one month after second dose was used (i.e on day 51.) P values were determined with unpaired, two-sided Mann–Whitney U-test. * or **, represent $p < 0.05$ or $p < 0.01$ respectively.



IgG2a response after immunization

Similar to IgG1, i.d. delivery of unadjuvanted CHIKV resulted in higher IgG2a titers as compared to i.m. delivery (Fig 8A). In case of i.m. delivery, except for CL401, all adjuvanted elicited higher titers than unadjuvanted CHIKV (p=0.015 to 0.0043). CL429 induced lowest IgG2a (Fig. 8E) after i.d. delivery while highest was induced by CL413 (Fig. 8D). Expect, MPLA (10 µg, Fig. 8B) and CL429 (Fig. 8E), other adjuvants induced higher levels of IgG2a after i.d. delivery (p=0.015 to 0.0043).

Fig.8. Anti-CHIKV-IgG2a response after immunization. ELISA was performed to determine CHIKV specific IgG2a levels after immunization. Serum collected from mice (n=6) one month post-second dose was used (i.e. on day 51). P values were determined with unpaired, two-sided Mann–Whitney U-test. * or **, represent $p < 0.05$ or $p < 0.01$ respectively.



Conclusion:

Intradermal delivery of adjuvanted inactivated CHIKV induced higher neutralizing antibodies than unadjuvanted CHIKV.

Among all adjuvants, CL413 induced highest antibody titers after delivery by both i.m. and i.d. routes while MPLA induced lowest.

5.Title: Development of potent adjuvanted respiratory syncytial virus vaccine for mucosal delivery (**Project ID**: CD/19/1/E); **Funding:** Wellcome-DBT India Alliance; **Duration:** January 2019 - December 2023; **Sanctioned Amount:** 1.69 crore; **PI:** Dr. Harshad Padmanabh Patil; **Co-Investigator:** Dr. Vidya Arankalle; **Animal Ethical Approval:** BVDUMC/1881/2018/002/010

Background: This study was initiated during January 2019. Plan of the current study is to evaluate RSV-virus-like-particle vaccine together with chimeric adjuvants that are recognized by two PRR ligands for immunogenicity after sublingual or pulmonary delivery in mice and using system immunology. During the previous year, RSV virus A2 strain obtained from American Type Culture Collection (ATCC) was subjected to whole genome sequencing, and primers were designed and procured for cloning of M, G, F and structural protein sequence of RSV in plasmids of interest to obtain respective proteins or RSV-VLP.

Objectives:

Production of candidate RSV vaccines, consisting of VLPs plus different combinations of adjuvants

Determination of the immunological and protective properties of these vaccine candidates in mice

Evaluation of the effects of these vaccine candidates on human PBMC or PBMC-derived cells

Work done: Most of the work of first objective out of three was carried out during the year

RNA extraction and cDNA preparation

RNA was isolated from RSV-A2 strain (ATCC) propagated on Hep2 cells. cDNA specific to M, G and F was prepared using a moloney murine leukemia virus (rMoMuLV) RT kit and gene

specific forward primer with and without a 6x-HIS tag. The reverse transcription reaction consisted of 2 µl of 10x RT buffer, 0.8 µl 25X dNTP Mix (100 mM), 1 µl each of RNase Inhibitor and reverse transcriptase, 2 µl of specific primer (10µM), 3.2 µl of nuclease free water and 10 µl of RSV-RNA. The thermal cycler conditions used were: 25°C for 10 mins, 37°C for 120 mins and 85°C for 5 mins. The sequences of the primers used are as follows in the table below:

Table 4: Forward primer sequences

primer	sequence (5' – 3')
RSV-GFwd	cgc***GGATCC***ATGCAAACATGTCCAAAAACAAG
RSV-GHIS-Fwd	cgc*GGATCC*ATGCACCATCACCATCACCATCAAACATGTCCAAAAACAAG
RSV-FFwd	cgc**GGATCC**ATGGAGTTGCTAATCCTCAAAG
RSV-FHIS-Fwd	cgc*GGATCC*ATGCACCATCACCATCACCATGAGTTGCTAATCCTCAAAG
RSV-MFwd	cgc***GGATCC***ATGGAAACATACGTGAACAAGCTTC
RSV-MHIS-Fwd	cgc*GGATCC*ATGCACCATCACCATCACCACGAAACATACGTGAACAAGCTTC

*bold letters indicate insertion of 6x HIS tag, bold and underlined indicate site for restriction enzyme *BamHI*

Amplification of RSV M, G and F genes

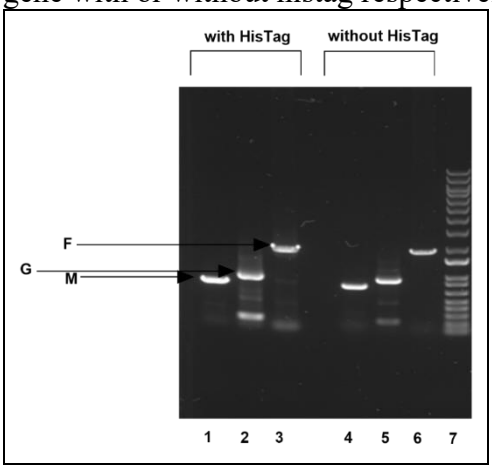
cDNA was amplified by PCR using forward primers with or without HisTag listed in Table 1 and reverse primers mentioned in Table 2. The PCR reaction consisted of 10µl of 5X SuperFi ™ Green Buffer, 1 µl of 10 mM dNTP mix, 2.5 µl of forward and reverse primer (10µM), 1 µl of Platinum SuperFi DNA Polymerase (2 U/ µL), 5 µl of cDNA template and 28 µl of nuclease free water. The thermal cycler conditions used were: initial denaturation of 98°C for 30 sec, followed by 98°C for 10 sec, 60°C for 10 sec and 72°C for 90 sec for 35 cycles and a final extension of 72°C for 5 mins. Amplified products were confirmed (Fig. 9)

Table 5: Reverse primer sequences

primer	sequence (5' – 3')
RSV-GRev	ccg***CTCGAG***AGTAACTACTGGCGTGGTGTG
RSV-FRev	ccg***CTCGAG***TATAACTATAAACTAGGAATCTAC
RSV-MRev	ccg***CTCGAG***TAATCTTCCATGGGTTTG

*bold and underlined letters indicate site for restriction enzyme *XhoI*

Fig.9. Amplification of RSV- M, G and F specific sequences. RSV M, G and F genes were amplified using cDNA by PCR. RSV M (770 bp), G (908 bp) and F (1883 bp) gene (with and without 6x HIS tag). In above figure, lane 1 and 4 has amplified M gene with or without histag respectively, lane 2 and 5 has G gene with or without histag respectively, Lane 3 and 6 has F gene with or without histag respectively. Lane 7 has 1kb ladder**.**

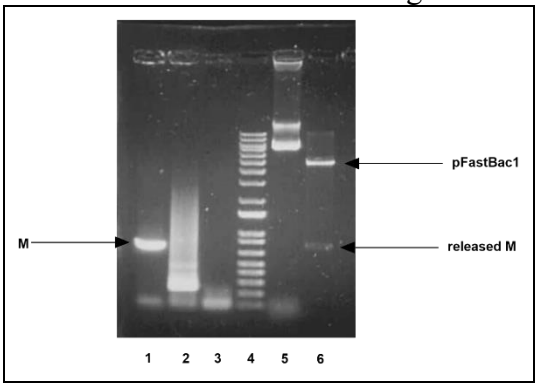


The amplified M, G and F gene products corresponding to 770 bp, 908 bp and 1883 bp respectively were gel purified.

Cloning of RSV M, G and F genes into pFastBac1 vector

Purified PCR products and vector pFastBac1 were digested by using *BamHI* and *XhoI* restriction enzymes at 37°C for 60 min. After restriction digestion, the PCR products and pFastBac1 were ligated using T4 DNA ligase at vector-insert ratio of 1:3. Chemically competent DH5α *E. coli* Max Efficiency were used for transformation. The recombinant clones were selected on Luria Bertani agar supplemented with 100 µg/ml ampicillin. Positive recombinant clones were screened by performing colony PCR using forward and reverse primer pairs for RSV M, G and F genes. After screening, positive clones were grown in LB broth supplemented with 100 µg/ml ampicillin and grown at 37°C for ~ 24 h. Plasmid was extracted and confirmed for gene insertion by sequencing and restriction digestion with BamHI and XhoI (Fig. 10).

Fig. 10. Cloning of RSV-M in pFastBac1. In above figure, **lane** 1 consists of purified M gene, **lane** 4 has 1kb marker, **lane** 5 consist of undigested pFastBac1 while **lane** 6 consist of M /pFastBac1 vector digested with BamHI and XhoI. M gene released from M /pFastBac1 is observed in **lane** 7. Similar images were obtained for RSV-G and F.



Generation of recombinant Bacmid

M, G and F specific bacmids were obtained by transformation of recombinant pFastBac1 vectors into DH10Bac *E. coli*. For transformation, 100 µL of DH10Bac *E. coli* Max Efficiency competent cells were transformed with 100 ng of M, G or F/pFastBac1 plasmid, grown with 900 µL of SOC media and incubated at 37°C for ~ 4 h. Incubated cells were plated onto selection LB agar plates (50 µg/mL kanamycin, 7 µg/mL gentamycin, 10 µg/mL tetracycline, 40 µg/mL IPTG, and 200 µg/mL X-gal) and grown at 37°C for 48 h. Isolated large white colonies were picked and restreaked onto fresh selection plates and grown at 37°C for 48 h. Positive recombinant transformants were screened by colony PCR using M13-F and M13R primers followed by gene specific primer pairs mentioned above. Positive clones were obtained for RSV M,G or F without histag (Fig 11).

Producing recombinant Baculoviruses

PCR confirmed recombinant bacmids were used for transfection of Sf9 and ExpiSf9 cells for producing recombinant baculoviruses expressing the RSV M, G and F proteins. Cellfectin II and ExpiFectamine reagents were used for transfection of Sf9 and ExpiSf9 cells respectively. P0 virus was harvested 96hrs after transfection and used to infect Sf9 or ExpiSf9 cells to generate P1 virus stock. Cytopathic effect was observed in the transfected cells (Fig. 12).

Fig. 11. Generation of recombinant bacmids. Lanes 1, 2 and 3 show confirmed recombinant pFastBac1 vector with M, G and F. Lanes 6,7 and 8 show confirmed recombinant M, G and F bacmids by amplification using gene specific primers. Lanes 11, 12 and 13 show undigested recombinant M, G and F bacmids. Lanes 4, 7 and 5, 10 shows resolved 100bp and 2kb DNA markers respectively.

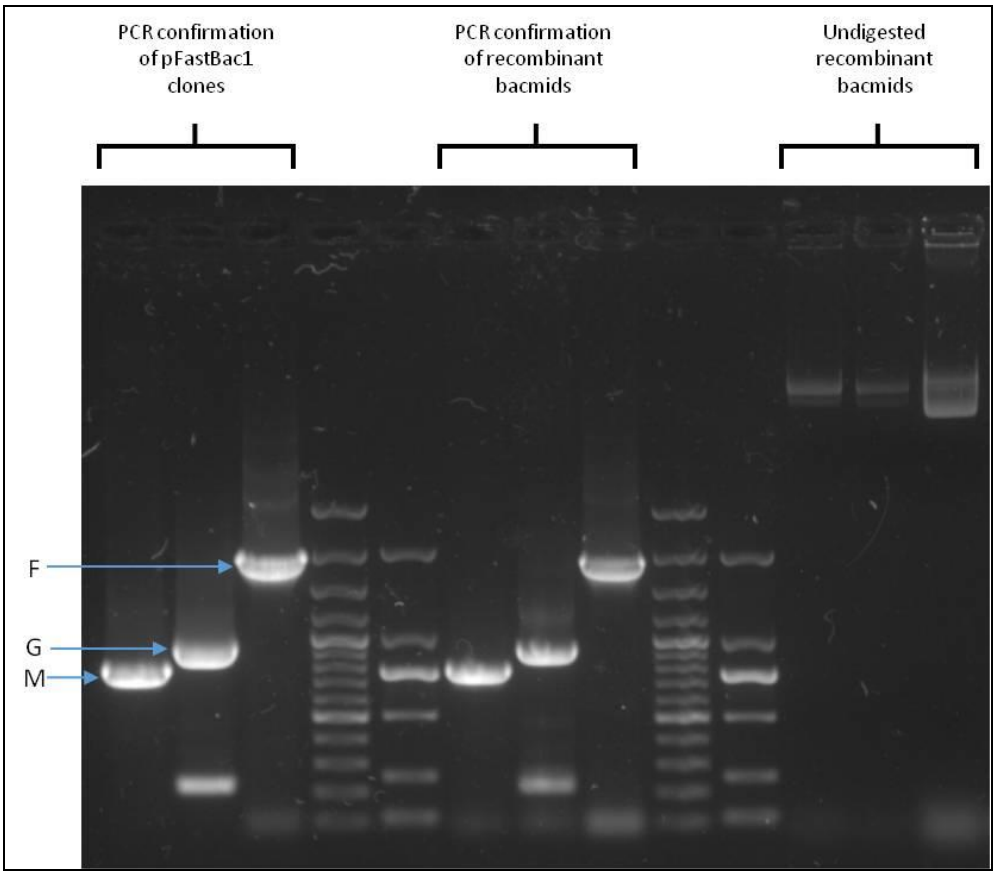
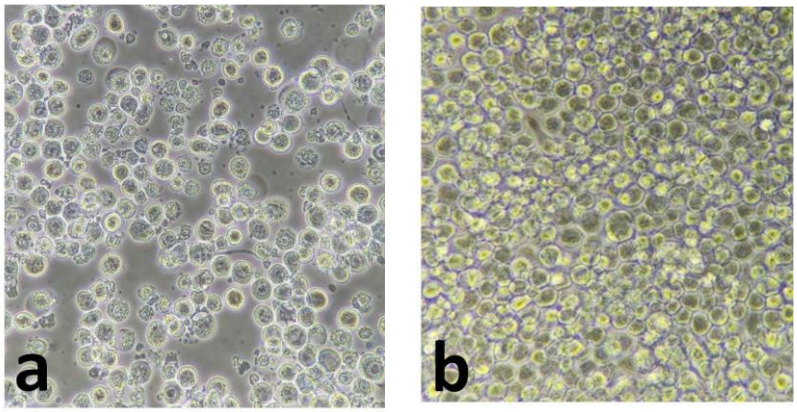


Fig. 12. Transfection of Sf9 cells. (a) Sf9 cells showing infection with RSV-M baculovirus. (b) Mock transfection control.



Conclusion: RSV M, G and F specific bacmids were successfully produced using baculovirus expression system. Transfection of SF9 and ExpiSF9 cells was done to generate recombinant baculoviruses with expression of the three proteins.

6.Title: Platelet derived exosomes and their role in endothelial dysfunction in dengue infection (**Project ID**:CD/19/2/E); **Funding:** DBT-BioCARe; **Duration:** March 2019 – March 2022; **Sanctioned Amount:** Rs. 46.4 Lakh; **Investigators: PI** - Dr Shubham Shrivastava; **Co-**

Investigators – Dr Deepak G Bhosle (Bharati Vidyapeeth Medical College); **Ph.D. Students:** Ms. Sayali Vedpathak; **Human Ethical Approval:** IEC/2019/15 and IEC/2020/46

Background: This study was aimed at evaluation of the role of platelet derived exosomes in endothelial dysfunction during dengue infection. During the previous year, after obtaining necessary approvals, the study was initiated and Human Umbilical Vein Endothelial Cells **(**HUVEC) were procured from American Type Culture Collection (ATCC).

Objectives:

To investigate the microRNA expression profiles of platelet- derived exosomes in longitudinal samples of dengue patients with different clinical presentations.
To determine the role of platelet-derived exosomes and exosome associated microRNAs in endothelial dysfunction.

Work done:

Sample Collection, platelet Isolation and purity

During dengue season 2019, blood samples from healthy controls (n=6) as well as patients (mild dengue category, n=23) were collected in acid citrate dextrose buffer (ACD) tube. After isolating Platelet rich plasma (PRP), purity of platelets was determined by using flow cytometry CytoFLEX (LX, Beckman Coulter). Platelets were stained with (1) CD45 (to check the depletion of leukocytes) (2) CD41 and CD61 (to check the presence of platelets) and (3) CD62P (to check the activation status of platelets) as shown in Fig. 13. Data was analysed by CytExpert and Graphpad Prism softwares. Fig. 14 depicts (A) the depletion of CD45 marker in platelet fraction and (B) percentage of CD62P in platelets of healthy controls and dengue patients. The purity of platelets was > 99%.

Fig.13. Gating strategy of platelet staining

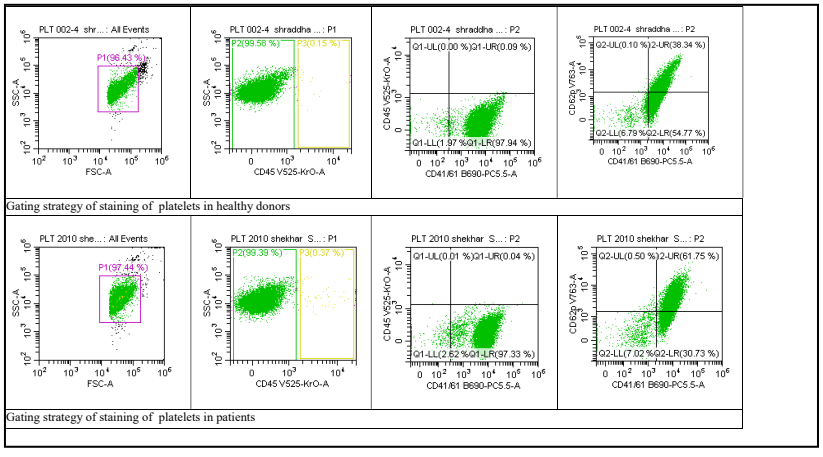
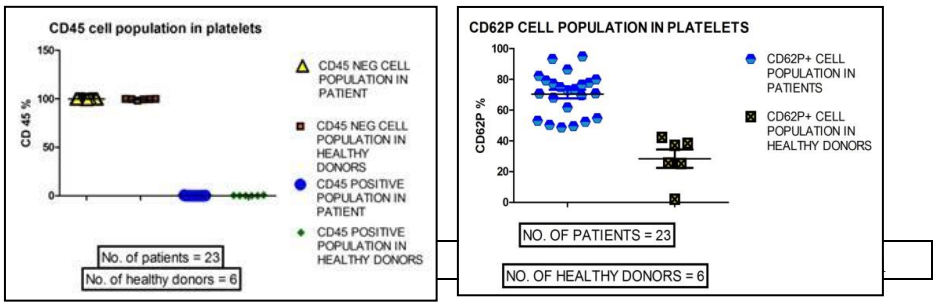


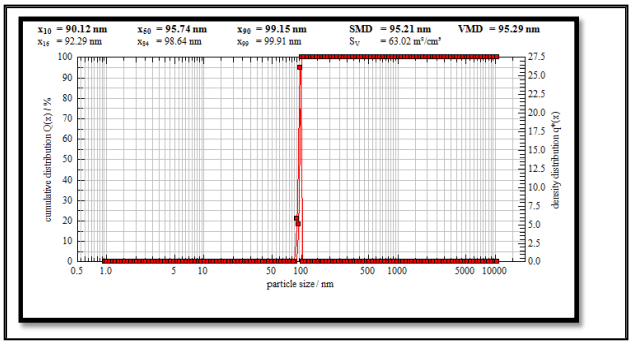
Fig. 14. (A) Depletion of CD45 marker in platelet fraction and (B) Percentage of CD62p in platelets of healthy controls and dengue patients.



Characterization of Platelet derived exosomes

Size of exosomes was examined by Dynamic Light Scattering (DLS) method. Our results suggest that size of exosomes varied from 95 to 100nm (Fig. 15).

Fig.15. Representative image of size distribution analysis of purified exosomes.



Conclusion:

Dengue patients exhibited higher levels of platelet activation in comparison with healthy subjects. Sample collection will be continued in second year. On selected number of samples, microRNAs profiling from platelet derived exosomes will be carried out.

7.Title: Development of NS1 reduction neutralization test (NRNT) for detection and quantitation of neutralizing antibodies against dengue virus (**Project ID:** CD/20/1/I); **Funding:** Intramural; **Duration:** January 2020 – December 2020; **Sanctioned Amount:** NA; **Investigators: PI**: Dr. Ruta Kulkarni; **Co-Investigators:** Dr. Shubham Shrivastava, Dr. AC Mishra, Dr. Vidya Arankalle; **Ph.D. Students**: None; **Human Ethical Approval:** IEC/2016/43

Background: Immunogenicity assessment in dengue vaccine clinical trials relies on evaluation of neutralizing antibody response elicited by vaccination. The plaque reduction neutralization test (PRNT) is considered as the "gold standard" test for detection and quantitation of anti-DENV neutralizing antibodies. In view of the practical difficulties issues associated with the use of PRNT for large scale testing, it is necessary to explore alternative tests for assessment of anti-DENV neutralizing antibody response. The NS1 protein of dengue virus (DENV) is secreted during viral replication in cell cultures, and can be detected by ELISA in the infected cell culture

supernatant. The ability to use NS1 as a marker for viral infectivity in cell culture suggests the possibility of developing an NS1 ELISA-based neutralization test as an alternative to PRNT.

Objectives:

To standardize NRNT for detection and quantitation of neutralizing antibodies against dengue virus

To assess the performance of NRNT in comparison to the gold standard PRNT

Work done:

The NRNT procedure was standardized using our in-house propagated, well-characterized DENV1-4 isolates. The basic design of NRNT involves infection of Vero cells in a 96-well plate with DENV pretreated with different dilutions of test serum (suspected to contain anti-DENV antibodies). The effect of virus-antibody interaction on virus infectivity in Vero cells is evaluated using NS1 antigen capture ELISA. The $NRNT_{50}$ titer is determined as the serum dilution which gives 50% reduction in NS1 ELISA absorbance with respect to virus control (DENV untreated with antibody). For standardization, different concentrations of input virus and incubation period were compared. Infection with 100 TCID virus and incubation for 48 hours post Vero cell infection was found to give optimum results. The optimized assay was then used to test 9 DENV IgG-negative sera to determine the specificity of the assay; none showed reactivity at 1:10 dilution in NRNT.

The performance of NRNT was then compared with PRNT by testing 42 DENV IgG-positive serum samples with known $PRNT_{50}$ titer, against all 4 DENV serotypes. Among these 42 samples, excellent agreement was noted between the titers determined by both methods (Spearman correlation co-efficient, r=0.92). For the individual serotypes, correlation between the $PRNT_{50}$ and $NRNT_{50}$ titers was as follows.

DENV-1 r=0.91
DENV-2 r=0.87
DENV-3 r=0.95
DENV-4 r=0.90

Conclusion:

NRNT appears to be an attractive alternative to PRNT for detection and quantitation of neutralizing antibodies against DENV. Due to the use of 96-well format and ELISA as the detector system, the NRNT is amenable to high throughput and automation, and will be more suitable for use in large scale studies such as vaccine clinical trials.

8.Title: Circulating adaptive immune cell profiles in dengue patients with or without warning signs. (**Project ID**: CD/19/1/I); **Funding:** ICMR (as part of Dengue Surveillance Project CD/17/1/E); **Duration**: July 2019 – Jan 2020; **Sanctioned Amount:** NA; **Investigators: PI** – Dr. Archana Kulkarni-Munje; **Co-Investigators** – Dr. Vidya Arankalle, Dr. A. C. Mishra; **PhD students:** None; **Human Ethical Approval**: IEC/2017/04, renewed IEC/2018/11, IEC/2019/06

Backgound: Dengue virus (DENV) is an arthropod-borne single stranded RNA virus of genus *Flavivirus*. It is comprised of 4 closely related but antigenically distinct serotypes, DENV-1, -2, -3, and -4. The virus is endemic in more than 100 tropical and subtropical countries of the world. Ailments caused by DENV infection include undifferentiated fever, dengue fever (DF),

dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). According to WHO's 1997 documentation, DF is clinically defined as an acute febrile illness with two or more manifestations of headache, retroorbital pain, myalgia, arthralgia, rash, and so on. Symptoms of DF can last 2–7 days. Besides nutrition, age, and sex, the following 3 factors, viral strain virulence, host genetics, and host immune status, are major contributors to DHF. The adaptive immune response to DENV infection contributes to resolution of infection and plays the key role in protection from reinfection. Conversely, it may also play a critical role in the enhancement of disease severity in most patients with DHF/DSS. In general, immune responses are essential for the resolution of DENV infection; however, as DHF is associated with secondary infections and symptoms of DHF emerge at the time when viremia is declined, DHF/DSS is thought to be consequences of immunopathology. While the evidence suggesting a pathogenic role for T cells is sparse, recent data abound to indicate protective roles for serotype-specific and cross-reactive T cells against DENV infection. Characterization of adaptive immune responses that occur during natural infection with varied clinical presentation is important to understand the DENV pathogenesis.

Objectives:

- To understand differences in the profiles of circulating adaptive immune cells in dengue suspected patients diagnosed as dengue or non-dengue
- To assess if patients with or without warning signs differ with respect to immune profiling
- To assess if patients with primary or secondary dengue differ with respect to immune profiling

Work done:

For this study, blood samples collected from suspected dengue patients were collected at the time of first visit to the physician / hospital. Dengue diagnosis was provided as part of the ICMR-Dengue project. We analyzed 392 dengue patients and 152 patients with other febrile conditions for T cell and B cell immune profile by polychromatic flow cytometry. The study comprised 247 adult and 145 paediatric cases. that were categorised as patients with warning signs and without warning signs as per WHO criteria. The patients were also classified as primary and secondary dengue based on the presence of IgG-anti-DENV antibodies at >22IU/ml (Panbio, IgG-capture ELISA). The flow cytometry analysis included CD4, CD8 T cells (Central & Effector Memory T cells, CCR10 expression) follicular T helper cells (ICOS & IL-21 expression), B cells (Memory B cells, class switched and unswitched memory B cells, plasmablast cells,) and its subsets.

Results:

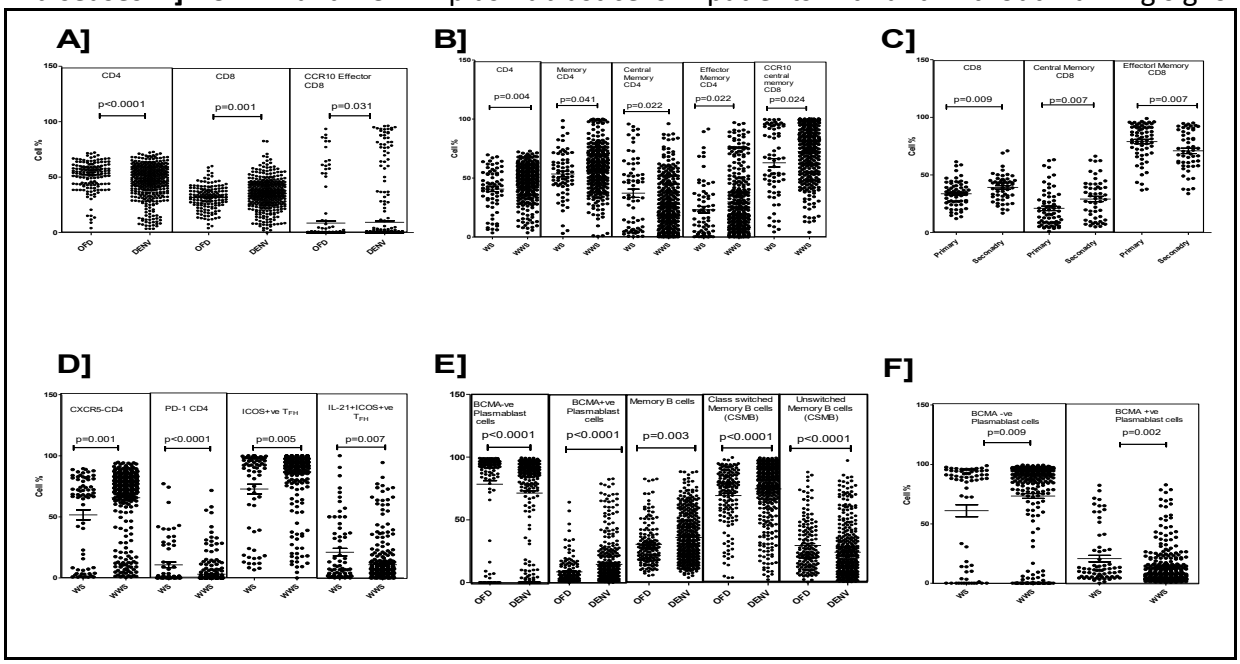
The results are summarized as follows.

- An expansion of CD8 T cell compartment was evident in DENV infection and skin homing CCR10 marker was also predominantly expressed on CD8 T cells. Whereas DENV infection exhibited a lower CD4 T cell number as compared to other febrile conditions. **(Fig.16A)**
- The patients with warning signs showed lower CD4 T cell pool comprising lower effector memory CD4 T cells and lesser umber of CCR10 +CD8 T cells. But the patients with warning sign presented higher central memory CD4 T cells. **(Fig.16B)**
- Primary DENV infection characterized by lower CD8 T cells and central memory CD8 T cells. But primary DENV exhibited a higher frequency of effector CD8 T cells. **(Fig.16C)**

DENV infection showed activation of peripheral follicular T cell helper cell compartment and increased expression of PD-1 on CD4 T cells increasing further in patients showing warning signs. Warning sign patients showed higher frequency of IL-21+ ICOS+ T_{FH} cells in periphery **(Fig.16D)**

DENV infection showed rise in B cell maturation antigen expression on plasmablast cells, higher memory B cells and class switched memory B cells (**Fig.16E**). Patients with warning sign showed highest number of BCMA positive plasmablast cells. (**Fig.16F**)

Fig. 16. Adaptive Immune response to DENV infection. Vertical scatter plots shows frequency of **A]** CD4, CD8 and CCR10+ Effector CD8 T cells in DENV infection and other febrile diseases. **B]** CD4 T cells and memory CD4 T cell subsets and CCR10+central memory CD4 T cells in patients with and without warning sign. **C]** CD8 T cells and memory CD8 T cell subsets in primary and secondary DENV infection. **D]** T_{FH} subsets in patients with and without warning signs. **E]** Plasmablast cells and memory B cell subsets in DENV infection and other febrile diseases. **F]** BCMA+ and BCMA- plasmablast cells in patients with and without warning signs.



Conclusion:

DENV infection manifested marked activation of CD8 T cells and follicular T helper cells.

Highest T_{FH} activation was apparent in DENV infection with warning signs (WS).

Primary DENV infection exhibited expansion of effector CD8 T cells as against central memory CD8 T cells evident in secondary infection.

DENV infection induced long-lasting plasma cells evident from higher number of BCMA+ plasmablast cells with highest frequency in patients with WS. The antibodies produced by these cells may either protect from exposure to homotypic DENV infection or may lead to severe pathologic sequel in secondary infection by a heterotypic DENV strain.

9.Title: Age dependent evaluation of immunoglobulin G response after chikungunya virus infection (**Project ID**: CD/19/2/I); **Funding:** Intramural; **Duration:** July 2018 - December 2019; Sanctioned Amount: NA; **Investigators: PI:** Dr. Harshad P. Patil; **Co-PI/ Co-Investigators:** Dr. Vidya A. Arankalle, Dr. A.C. Mishra; **Ph.D. Students:** NA; **Human Ethical Approval:** IEC/2017/04, renewed IEC/2018/11, IEC/2019/06

Background: Antibodies play a critical role in neutralization of the virus. Virus exposure and immunological factors such as cytokines, chemokines or molecular factors elicited after CHIKV infection modulate IgG response qualitatively and quantitively. Moreover, degree of antibody modulation is shown to be age dependent. Age dependent IgG response is not available from all age groups that are presumably recovered from CHIKV infection. There is a possibility of difference in IgG or its subtype induction after CHIKV infection. In absence of such information, rational vaccine design is difficult to achieve against CHIKV. Therefore, in this study serum samples from various age groups collected during dengue serosurvey study that were analyzed for anti-CHIKV-IgG were used for IgG and its subtypes titer determination. Total of 796 anti-CHIKV-IgG positive samples were distributed age wise in 170 pools were evaluated. Obtained titers were correlated with the CHIKV neutralization titers. Anti-CHIKV-IgM (n=15) positive samples were also analyzed for IgG and its subtype to check the CHIKV specific antibody pattern difference.

Objectives:

To quantify antibody (IgG and IgG-subtype) responses to chikungunya virus healthy individuals.

To correlate binding antibody titers with neutralizing antibody titers

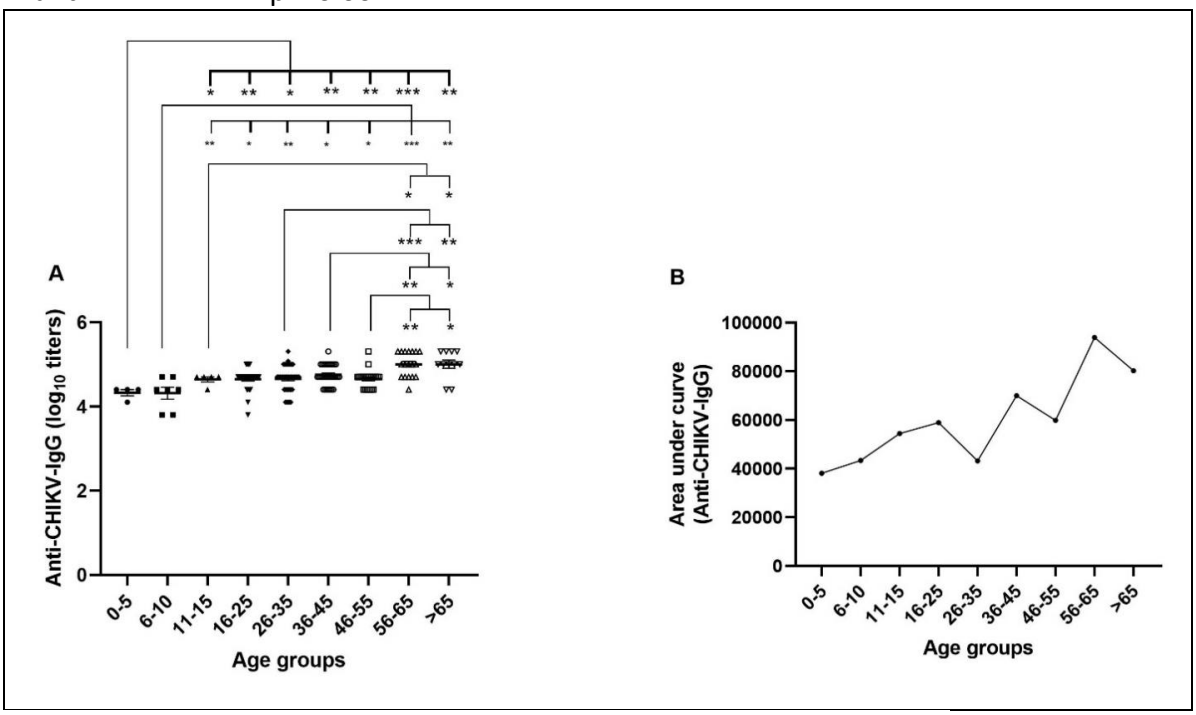
Work done:

Comparison of ELISA and PRNT for determining anti-CHIKV antibody titers

Earlier, we had used PRNT for the evaluation of our ELISA positivity and found excellent correlation (Patil et al. 2020). For quantitation, PRNT detecting neutralizing antibodies is most appropriate. However, for testing of large number of samples, especially in serosurveys, PRNT is of limited utility because of higher cost and longer time. Therefore, we first compared titers of 76 pools tested earlier by PRNT (neutralizing antibodies) with ELISA (binding antibodies) titers. The mean titers determined by ELISA (72084, log10: 4.72) and PRNT (1567)., ELISA values being 46 fold higher than PRNT. We then determined anti-CHIKV-IgG titers in ELISA for 170 pools representing different age groups (Fig. 17A). A trend towards increase in IgG titers with age was observed. Children in the age groups 0-5 and 6-10 (mean IgG titer: 25600, $\log_{10}$: 4.33) had significantly lower IgG titers than the other age groups. On the other hand, age group 56-65 or >65 (IgG titer: 124469, $\log_{10}$: 5.01) has significantly high antibody titers than all the age groups except 16-25 (IgG titer: 50994,

log_{10}: 4.65). Area under the curve (AUC, Fig. 17B) for various age groups was calculated between dilution 1:1600 till 1: 102400 to understand affinity of IgG towards CHIKV. An increase in AUC was observed with increase in age. Age group 0-5 had lowest while age group 56-65 had highest AUC.

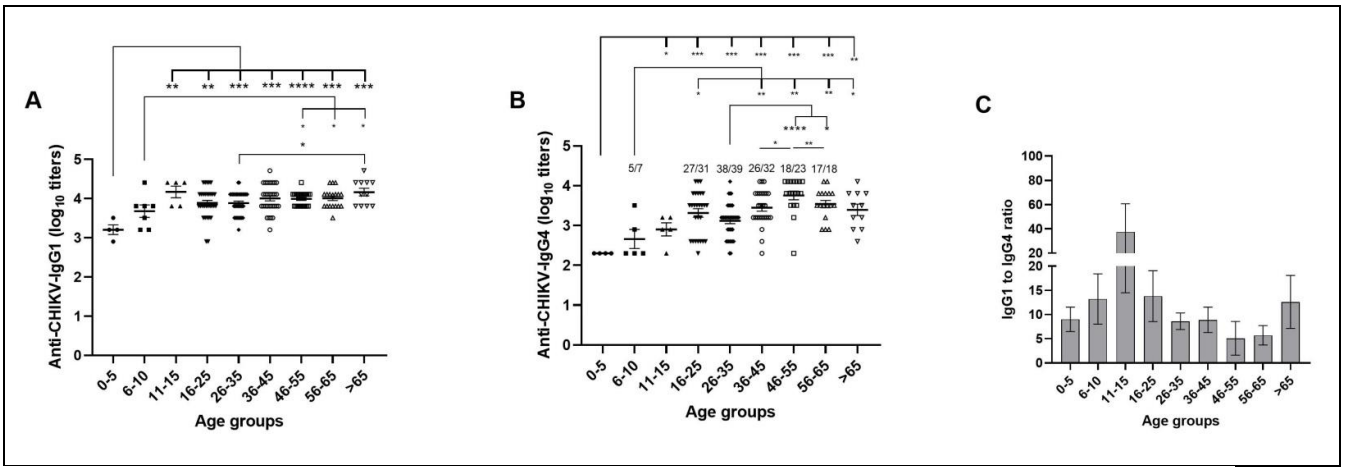
Fig.17. Antibody response after CHIKV infection. Serum pools (n=170) of anti-CHIKV-IgG positives samples from various age groups were evaluated for (A) anti-CHIKV-IgG titers. Data are presented as average ± standard error of mean. (B) Area under curve of serum dilutions for each age group. Levels of significance are presented as * $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.



Phenotype of persisting anti-CHIKV-IgG antibodies after CHIKV infection.

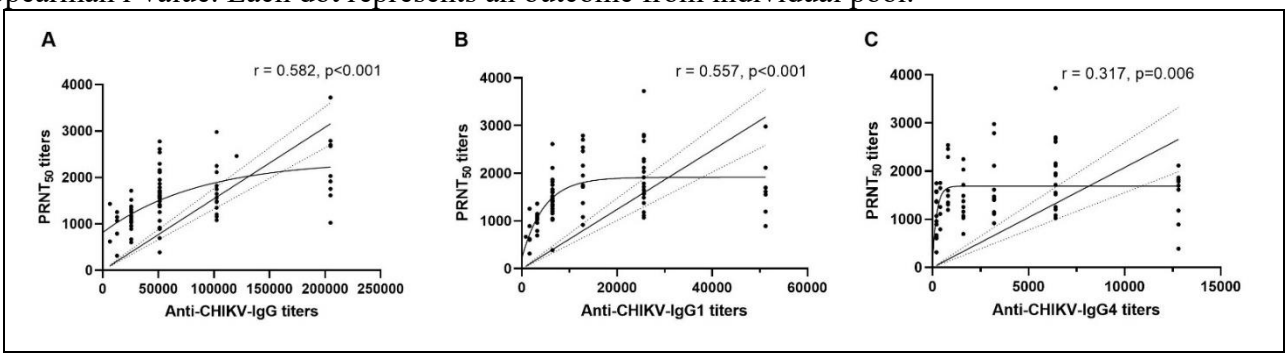
Next, we carried out IgG subtype analysis using ELISA (Fig.18A-C). None of the 170 pools representing 796 serum samples from different age groups were reactive for IgG2. IgG3 antibodies were detected in 5 pools (two pool from age group 56-65 or >65 and one from age group 26-35). IgG1 was present in all the pools and IgG4 in 151/170 (88.82 %) pools. Age group 11-15 exhibited highest IgG1/IgG4 ratio while 46-55 had the lowest ratio. The pattern of IgG and IgG1 titers (Fig. 18A) was similar with titers gradually increasing till age 11-15 years and remaining comparable in the subsequent age groups (p=0.02-0.95).

Fig.18. Phenotype of antibody response after CHIKV infection. Pooled serum (N=170) positive for anti-CHIKV-IgG was used to quantify (A) anti-CIHKV-IgG1 and (B) anti-CHIKV-IgG4. Obtained titers were used to calculate (C) age wise CHIKV specific IgG/IgG4 ratio. Data are presented as average ± standard error of mean. Levels of significance are presented as * $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ and **** = $p < 0.0001$



Correlation between neutralizing and binding antibody titers: Correlation analyses with PRNT (Fig. 19A-C) revealed moderate, positive correlation with anti-CHIKV-IgG ELISA titers (Spearman r= 0.582, p=<0.001, 95% CI of slope: 0.013 to 0.017). Similarly, a positive correlation of $PRNT_{50}$ titers was obtained with anti-CHIKV-IgG1 titers (r=0.557, p<0.001, 95% CI of slope: 0.050 to 0.073). However, anti-CHIKV-IgG4 titers showed poor correlation of (r=0.317, p=0.0.06, 95% CI of slope: 0.155 to 0.259).

Fig.19. Correlation between PRNT50 and ELISA titers. Neutralizing antibody titers obtained by PRNT50 assay and binding antibody titers obtained by ELISA were plotted to understand role of (A) IgG (B) IgG1 or (C) IgG4 in CHIKV neutralization. The correlation is demonstrated as Spearman r value. Each dot represents an outcome from individual pool.

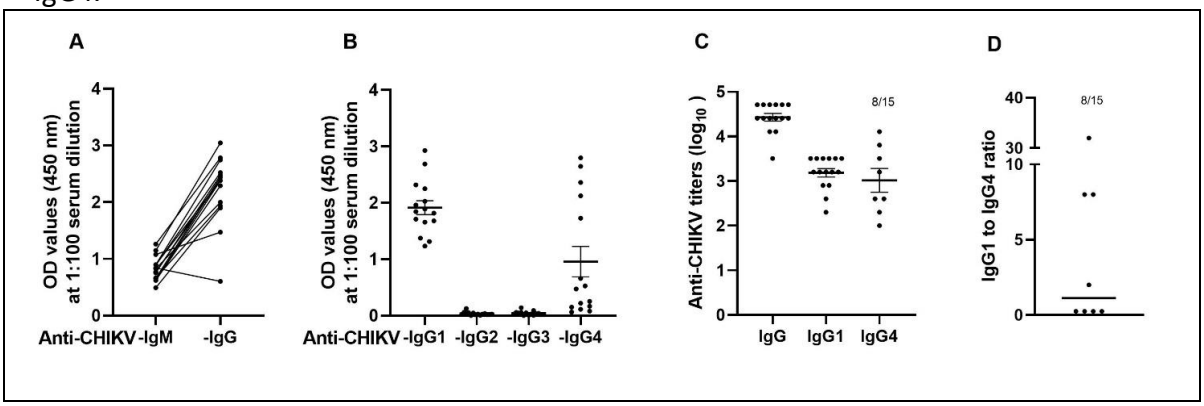


Antibody response among chikungunya patients in acute phase

Failure to detect IgG2 and IgG3 antibodies, as reported by Nayak et al or Kam et .al respectively, prompted us to analyze serum samples (n=15) that were positive for both anti-CHIKV-IgM and -IgG for IgG-subtypes (Fig.20A). As samples collected during serosurvey represent prior exposure to CHIKV before variable period, we determined status during the acute phase. For this, 15 acute-phase samples were used. IgG antibody subtype ELISA revealed induction of IgG1 in all the samples while 8/15 (53.33%) were positive for IgG4 (Fig.20B). IgG2 and IgG3 antibodies were undetectable. The mean log_{10} titers of anti-CHIKV-IgG1 and IgG4 were 3.1 and 3.0 respectively (Fig.20C). To our surprise, 4/8 IgG4 positive samples had higher titer than corresponding IgG1 titers. The IgG1/IgG4 ratio for these 4

samples was 0.25 (Fig.20D). For the other four samples with predominant IgG1 the ratio varied between 2 to 32.

Fig.20. Antibody response in acute sample. N=15 samples were evaluated for presence of both (A) anti-CHIKV-IgM and anti-CHIKV-IgG antibody and (B) IgG subtypes raised against CHIKV. The data as presented as OD values at 450 nm at serum dilution 1:100. These samples were used to determine (C) anti-CHIKV-IgG, -IgG1 and -IgG4 titers. (D) IgG1/IgG4 titer ratio was calculated for serum samples that were positive for both anti-CHIKV-IgG1 or -IgG4.



Conclusions:
Children in the age groups 0-5 and 6-10 had significantly lower IgG titers than the other age groups. On the other hand, age group 56-65 or >65.
High levels of IgG1 were obtained in all individuals after CHIKV infection with strong correlation with neutralization titers.

10. Title: Evaluation of circulatory biomarkers for disease severity in hepatitis E (**Project ID**: CD/20/1/E); **Funding:** ICMR; **Duration:** Jan 2020 – Dec 2021; **Sanctioned Amount:** Rs. 81.95 Lakh; **Investigators: PI** - Dr Shubham Shrivastava; **Co-Investigators** –Dr. Vidya A Arankalle (IRSHA), Dr Deepak G Bhosle (Bharati Vidyapeeth Medical College and Hospital), Dr. J Shashtri, Dr. C Pawar (Kasturba Hospital, Mumbai) and Dr. A L Kakrani (D Y Patil Medical College and Hospital); **Ph.D. Students:** Ms. Shweta Chelluboina; **Human Ethical Approval:** IEC/2020/13

Background: Hepatitis E virus (HEV) infection is the major cause of acute viral hepatitis (AVH) in India. HEV infection is usually self-limiting however, chronic infection can occur in immune compromised individuals. About 0.5-4% of individuals infected with HEV develops fulminant hepatic failure (FHF) and high mortality of ~30% is documented in HEV infected pregnant women particularly in third trimester of pregnancy. Identification of novel biomarkers to predict disease outcome in AVH patients who will recover or progress to FHF after HEV infection is largely an unexplored area of research. miRNAs have been described as biomarkers in differentiating normal vs disease conditions by their presence or absence in tissues or in circulation.miR-122 facilitates HEV genotype 1 replication by direct interaction with its target

site in RdRp of HEV genome. Research studies suggested that elevated levels of miR-122 may precede the development of severe liver disease. Secretory S100 proteins have been detected in body fluids and considered as biomarkers in diseases such as atherosclerosis and stroke. Increased expression of S100 proteins and alpha-defensins was observed in pregnant HEV patients with subclinical infection.

Objectives:

- To assess miR-122-5p, miR-134-5p, S100 proteins and alpha defensins as potential circulatory biomarkers for hepatitis E disease severity
- To determine if coinfection with other hepatitis viruses or chronic liver disease impacts serum levels of the above biomarkers

Work done:

Approval was obtained from the Institutional Biosafety Committee and Institutional Human Ethics Committee. Due to the emergence of pandemic, collection of samples from hepatitis patients from Pune and Mumbai could not be done.

11.Title: Establishment of National Centre for Immunogenicity Testing, NCIT to evaluate vaccines in clinical trials (**Project ID:** CD/19/3/E); **Funding:** DBT-BIRAC (Under National Biopharma Mission); **Duration:** March 2019 – March 2023; **Sanctioned Amount:** Rs. 16 crore; **Investigators: PI** - Dr A C Mishra; **Co-Investigators** – Dr. Vidya Arankalle, Dr. Shubham Shrivastava, Dr. Harshad Patil, Dr. Ruta Kulkarni, Dr. Rashmi Virkar, Dr. Archana Kulkarni-Munje, Dr. Suhas Mhaske, Dr. Sudha Ramkumar; **Ph.D. Students:** None; **Human Ethical Approval**: IEC/2019/33

Background: This project was initiated in March 2019 with an aim to establish a GCLP compliant laboratory to perform immunogenicity testing for the evaluation of vaccines in clinical trials in India. During the previous year, after obtaining necessary approvals, construction of BSL-2 and BSL-3 laboratories was initiated at IRSHA. With due approval from the authorities, the name of the facility was changed to "National Immunogenicity and Biologics Evaluation Center (NIBEC) and the same is used here.

Objectives:

- Establishment of GCLP laboratories for immunogenicity testing of vaccines
- Setting up of dedicated Biosafety 2 and 3 laboratories compliant to both Biosafety and GCLP requirements.
- Acquisition, standardization, validation and finally accreditation of the tests required for immunogenicity testing of vaccines
- Creation of self-sustainable business model, capable of absorbing new technologies and maintain pace with newer developments in the field

Work done:

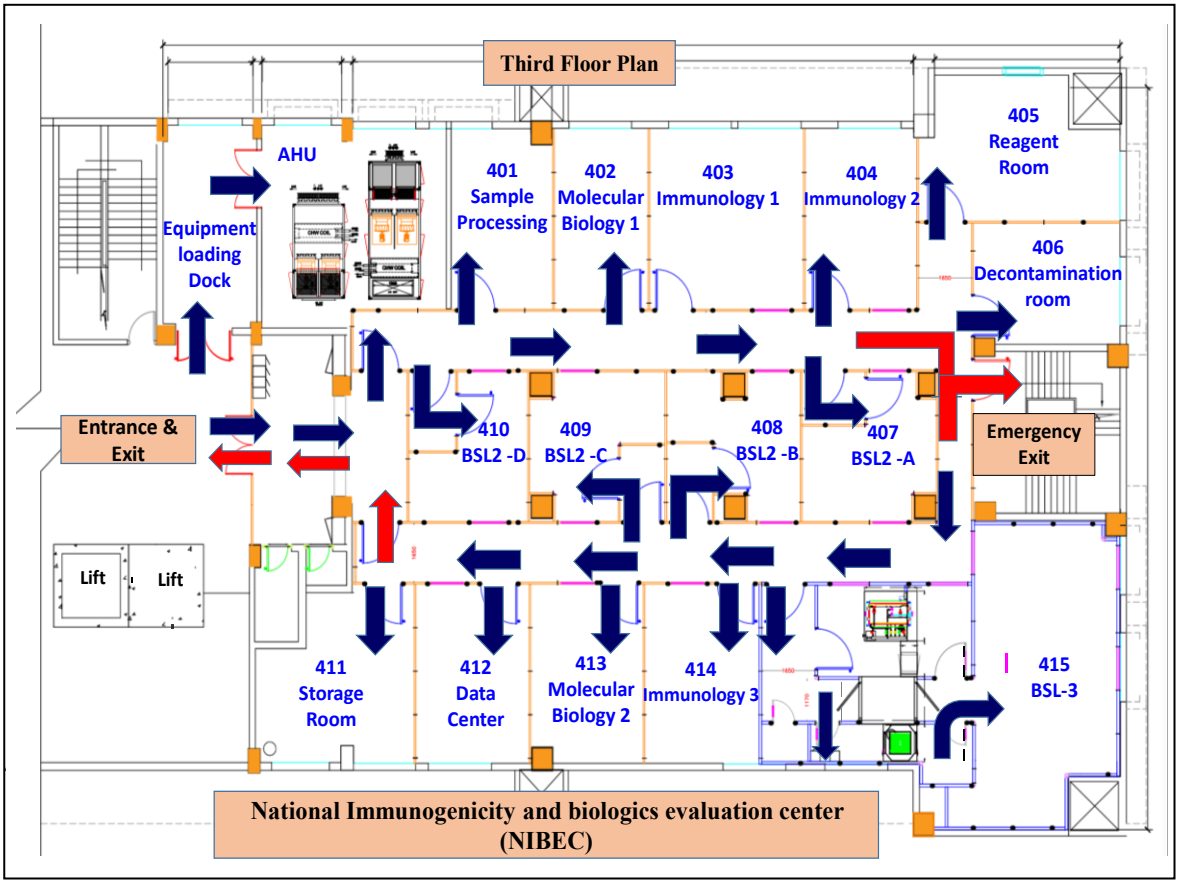
Two of the four main objectives of the project were completed during the year-

- Establishment of GCLP laboratories for immunogenicity testing of vaccines
- Setting up of dedicated Biosafety 2 and 3 laboratories compliant to both Biosafety and GCLP requirements

We established a state of art facility namely, National Immunogenicity and Biologics Evaluation Centre (NIBEC), IRSHA, BVDU, that provides comprehensive immunogenicity testing services to our vaccine manufacturers. The facility comprises of 15 laboratories; 8 Biosafety level-1, 4 Biosafety level-2 and one Biosafety level-3 laboratory. It includes separate sample management room, data center, reagent room, decontamination area and a special lab. BSL-2 and BSL-3 laboratories constitute containment facility to mitigate the risk of exposure of infectious agents handled in these laboratories to laboratory personnel, outside environment and to community. Figure 21 indicates the layout of NIBEC. The laboratories have impervious panels for walls and roof, epoxy flooring, semi open able windows and individual cassette air conditioners. The laboratory access is restricted to authorized personnel, through use of access card only. The facility meets all the necessary biosecurity requirements with CCTV camera and Building Management System (BMS). Liquid waste of the laboratory is decontaminated in the Effluent Treatment Plant (ETP), located in the basement of building and disposal of solid waste is done by autoclaving and handed over to PASSCO.

The facility is equipped with CFR compliant equipment like Real-Time PCR system, Flow cytometer, ELISPOT cum Plaque counter and ELISA reader. All the three types of laboratories are equipped with Biosafety cabinets, CO2 incubators, centrifuge, refrigerators, freezers, autoclave and small equipment. BSL 1 labs are used to conduct work which does not involve handling of live viruses or category 2 viruses involving low volume and low risk or the work that is classified as non-hazardous in nature. BSL 2 labs are used to work with microorganisms of risk category 2 and for risk group 3 organism involving low risk. BSL 3 laboratory is dedicated for the work related to BSL 3 risk group agents and BSL 2 agents grown in larger quantity and the work that produces aerosol. Laboratory plans to implement web-based Laboratory Information Management System (LIMS) and Document Management System (DMS) to ensure data storage, archival and data security.

Fig.21. Layout of NIBEC



The laboratory follows GCLP guidelines and has a team of qualified and experienced scientists, supervisors, testers, biosafety and EHS officer, maintenance and housekeeping staff. The laboratory provides services for immunogenicity evaluation in vaccine and antiviral studies (Table 6,7,8).

Future plan: Acquisition, standardization, validation and accreditation of the tests required for immunogenicity testing of vaccines. We also look forward to the creation of self-sustainable business model, capable of absorbing new technologies and maintain pace with newer developments in the field.

Activities at NIBEC:

NABL Accreditation of Assays:

Tables 6 and 7 provide details of the tests available at IRSHA for immunogenicity assessment and antiviral testing respectively. This year, the primary test required for the assessment of influenza vaccine, haemagglutination inhibition (HI) assay was standardized, validated using ICH guidelines and NABL accredited. For this, H1N1, H3N2 and 2 strains of influenza B viruses and, standard antibodies obtained from NIBSC, UK were employed. The other required assays were standardized and validated. Application for NABL accreditation is under process.

Testing activities during the current year (Table 8):

Immunogenicity evaluation:

We continued supporting Serum Institute of India, Pvt. Ltd. (SIIPL), by quantitating serotype-specific DENV neutralizing antibodies in the monoclonal antibody preparations.

For the influenza vaccine clinical trial performed by SIIPL, we tested 790 serum samples for quantitation of HI antibodies against four different strains of influenza virus.

Antiviral Testing:

For the evaluation of anti-SARS-CoV-2 activity, 12 samples (7 drugs, 4 disinfectants and 1 antimicrobial coated non-porous surface) were screened in an in-vitro system employing internationally approved protocols.

Table 6: Assays available for vaccine studies

Project status	No of assay	Name of assay
NABL accredited	03	HI for influenza viruses
		PRNT for CHIKV antibodies
		PRNT for serotype-specific DENV antibodies
Standardized and validated	05	ELISA for CHIKV-IgG detection and quantitation (inactivated virus-based)
		ELISA for IgG-anti-SARS-CoV-2 (inactivated virus-based)
		PRNT for SARS-CoV-2 antibodies
		Plaque assay based quantitation of dengue viremia
		NSET

		Serotyping qRT-PCR

Table 7: Assays available for testing of antiviral activity

SN	Assay Developed/Standardized	Guidelines used
1	Antiviral Assessment of Drugs against SARS-CoV2 Virus	Published research article
2	Evaluation of Virucidal activity of chemical disinfectants /antiseptics against SARS-CoV-2	As per mutually agreed guideline: BS EN 14476:2013+A2:2019
3	Measurement of antiviral activity on plastics / non-porous surfaces against SARS-CoV-2	As per mutually agreed guideline: ISO:21702
4	Determination of antiviral activity of textile products against SARS-CoV-2	As per mutually agreed guideline: ISO:18184

Table 8: Immunogenicity and antiviral testing services provided during July 2019 – June 2020

Sr. No.	Project Title	Client	Mode
1.	Determination of neutralization potential of monoclonal antibodies against all the 4 DENV serotypes, using PRNT	Serum Institute of India, Pvt. Ltd.	Service
2.	Testing of serum samples from Influenza vaccine clinical trial for haemagglutination inhibition (HI) antibodies against type A and B influenza viruses	Serum Institute of India, Pvt. Ltd.	Service
3.	Antiviral Assessment of Drugs against SARS-CoV2 Virus	Bhami Research Laboratory, Mangalore, India	Service
4.	In vitro assessment of antiviral effect of MOLECUSAN ™ 9 CORONASH ™, a Phytopharmaceutical formulation against SARS-CoV-2 virus.	Vedicinals India PVt Limited	Service
5..	*In vitro* assessment of anti-microbial coating solution porous (fabric) and non-porous (metal-Stainless steel) surfaces against SARS-CoV-2 virus.	Harind Chemicals & Pharmaceuticals Pvt. Ltd, India	Service
6.	In vitro assessment of Naga parpam, (traditionally formulated Siddha drug) against SARS-CoV-2	Dr. M. Hari Hara Mahadevan Research Officer (Siddha) i/c, Siddha	Collaborative

	virus.	Clinical Research Unit (SCRU) Sri Jayachamarajendra Institute of Indian Medicine	

Studies on COVID-19 / SARS-CoV-2:

With the emergence of the current pandemic and detection of COVID-19 cases in travelers from the affected countries to India, IRSHA facility was evaluated and found to be ready to take up the challenge of SARS-CoV-2. After detection of the first COVID-19 case in Pune, various activities were planned, approved by relevant committees and undertaken. Bharati Medical college and hospital were the clinical partners for all the studies. We first isolated the SARS-CoV-2 from patients from Pune, developed appropriate antibody tests and attempted to understand if differential immune response of the patients was responsible for variable outcomes of the viral infection.

12.Title: Isolation and genetic characterization of SARS-CoV-2 from Indian patients (**Project ID**: CD/20/2/I); **Funding:** DBT-BIRAC (as part of NIBEC Project CD/19/3/E); **Duration:** April 2020 – 2021; **Sanctioned Amount**: NA; **Investigators**: **PI:** Dr. Shubham Shrivastava ; **Co-PI/ Co-Investigators**: Dr. Harshad P. Patil, Dr. Suhas Mhaske, Dr. A.C. Mishra, Dr. Vidya A. Arankalle; **Ph.D. Students:** NA; **Human Ethical Approval:** IEC/2020/25

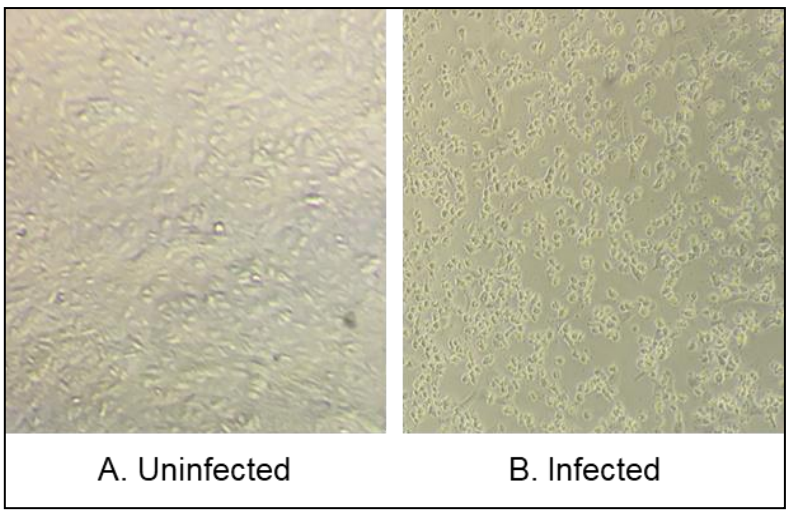
Background:SARS-CoV-2 is highly contagious respiratory virus. It is a member of the Coronaviridae family and an etiological agent of COVID-19. Isolation and characterization of the virus was needed to standardize and validate various assays required for vaccine immunogenicity evaluation.

Work done:

Isolation of SARS-CoV-2 from clinical samples

Nasopharyngeal swab from 16 suspected patients were tested for the detection of RdRp gene of SARS-CoV-2 and added into Vero cells for virus isolation (Table 9). CPE initiation was observed as early as 2 days after inoculation and complete CPE (more than 90% cells detached) was observed on day 3 (Fig. 22).

Fig.22. Phase-contrast microscopic image of Vero-CCL81 cells. (A) Uninfected control cells (B) Cells infected with nasopharyngeal swab of SARS-CoV-2 infected patient exhibiting cytopathic effect (CPE), 3 days after inoculation



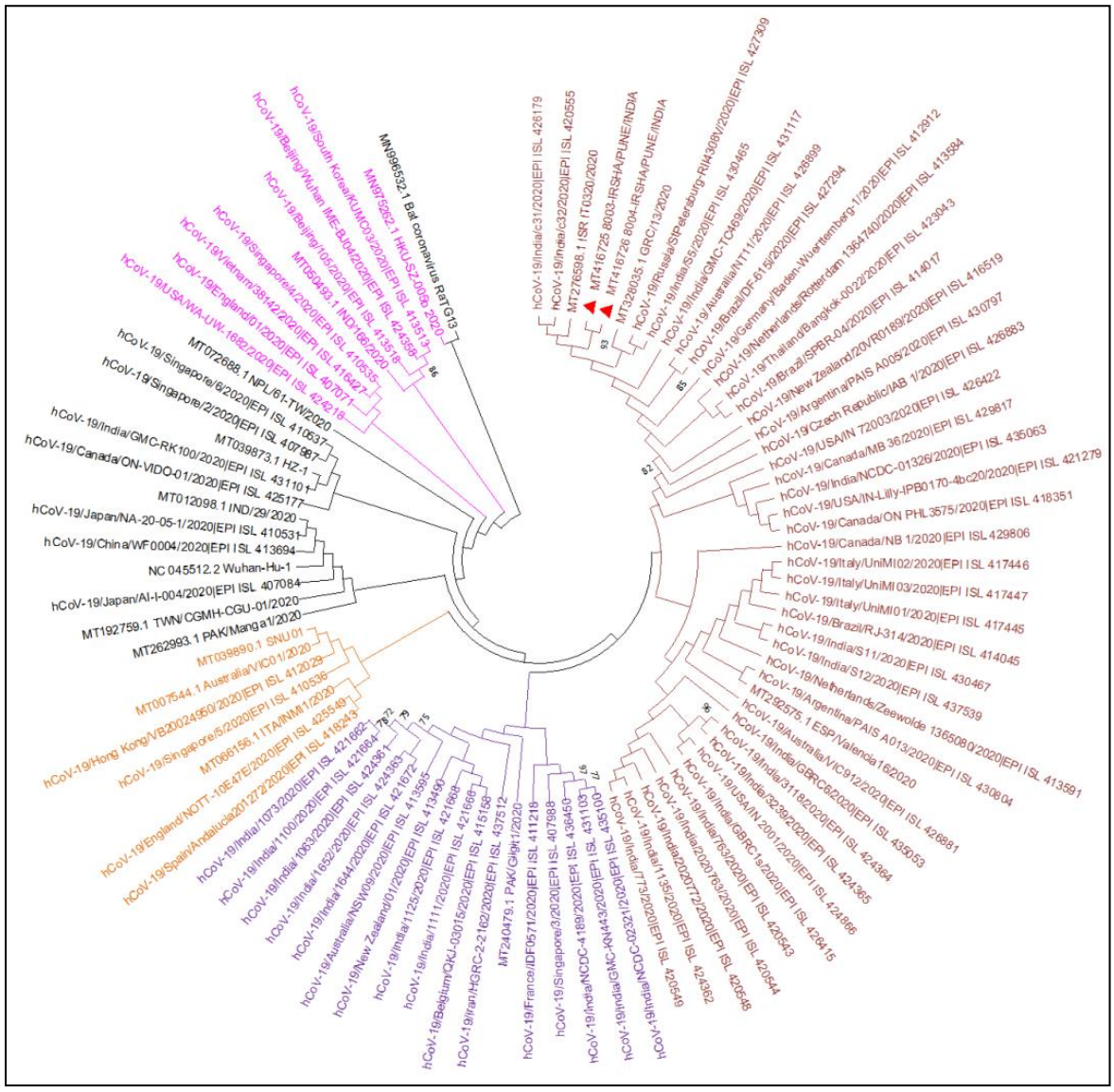
Genomic characterization of virus isolates

Phylogenetic tree revealed that the two IRSHA isolates (CD/20/8003 and CD/20/8004) were closely related to Indian strains (EPI ISL 420555, EPI ISL 426179) isolated from close contacts of infected patients with travel history to Italy. Other closely related isolates from India included strains from West Bengal (EPI ISL 430465). IRSHA isolates grouped together with strains isolated from Israel, Greece, Russia, Australia, Germany, Netherlands, Thailand, Brazil, New Zealand, Argentina, Czech Republic, Italy, Spain, USA, Canada, and few other Indian isolates (Fig.23). The nucleotide divergence within these sequences was found to be 0.02%. The nucleotide similarity of IRSHA isolates were 99.97 ± 0.01% with cluster of sequences with travel history to Italy.

Table 9: Clinical features of SARS-CoV-2 cases used for virus isolation

Sr. No.	Sample ID	Clinical specimens	Post onset days of symptoms	Ct value of RdRp gene	Virus isolation status	Days (CPE appearance)	Virus titer at 3 dpi ($TCID_{50}$/ml)	Viral load (copies/ml)
	CD/20/8001	Throat swab	9	16.64	No	No	No	5.3×10^{9}
	CD/20/8002	Nasopharyngeal swab	Not known	UD	No	No	No	--
	CD/20/8003	Nasopharyngeal swab	5	13.60	Yes	2	$10^{4.34}$	4.6×10^{10}
	CD/20/8004	Nasopharyngeal swab	1	14.89	Yes	2	$10^{5.6}$	1.8×10^{10}
	CD/20/8005	Nasopharyngeal swab	3	21.23	No	No	No	2.1×10^{8}
	CD/20/8006	Nasopharyngeal swab	8	23.24	No	No	No	5.0×10^{7}
	CD/20/8007	Nasopharyngeal swab	6	UD	No	No	No	--
	CD/20/8121-3	Nasopharyngeal swab	12	28.8	No	No	No	--
	CD/20/8137	Nasopharyngeal swab	5	24.6	No	No	No	
	CD/20/8137-2	Nasopharyngeal swab	8	20.99	Yes	2	$10^{5.34}$	--
	CD/20/8137-3	Nasopharyngeal swab	11	21.13	Yes	2	$10^{3.53}$	--
	CD/20/8138	Nasopharyngeal swab	4	19.0	Yes	2	$10^{5.69}$	--
	CD/20/8139	Nasopharyngeal swab	Asymptomatic	29.92	No	No	No	--
	CD/20/8144	Nasopharyngeal swab	Asymptomatic	14.63	Yes	2	$10^{5.94}$	--
	CD/20/8145	Nasopharyngeal swab	Asymptomatic	24.32	Yes	2	$10^{2.98}$	--
	CD/20/8146	Nasopharyngeal swab	Asymptomatic	29.91	No	No	No	--

Fig.23. Phylogenetic tree indicating complete genome sequences of SARS-CoV-2 isolates (n=2) from Pune, India. Each strain is indicated by country, year of isolation and Genbank accession number or GISAID number. The sequences obtained in this study are marked in filled color triangles.



Conclusion:

Successful isolation of SARS-CoV-2 from several patients

Full genome sequence analysis revealed that two IRSHA isolates were closely related to Indian strains isolated from cases with travel history to Italy.

13.Title: Standardization of SARS-CoV-2 antibody ELISAs (**Project ID:** CD/20/3/I); **Funding:** DBT-BIRAC (as part of NIBEC Project CD/19/3/E); **Duration:** April 2020 – 2021; **Sanctioned Amount:** NA; **Investigators: PI**: Dr. Vidya Arankalle; **Co-Investigators:** Dr. Ruta Kulkarni, Dr. Harshad Patil, Dr. Suhas Mhaske, Dr. AC Mishra (IRSHA), Dr. Sonali Palkar, Dr. Sanjay

Lalwani (Bharati Vidyapeeth Medical College); **Ph.D. Students:** None; **Human Ethical Approval:** IEC/2020/25

Background: Coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome – coronavirus-2 (SARS-CoV-2) is a devastating pandemic affecting 216 countries globally. Timely and accurate diagnosis is the mainstay of COVID-19 management. Currently, viral RNA detection in respiratory tract samples by RT-PCR is the method of choice for COVID-19 diagnosis. In view of the limitations of RT-PCR, there is a definite need for sensitive and specific antibody detection tests as a supplement to molecular diagnosis. Moreover, antibody detection assays are needed for estimation of disease prevalence, extent of exposure and immunity to the virus in a particular population, which is important for planning and implementation of disease control measures.

Antibody detection ELISAs can be developed using inactivated whole virus. This approach offers use the advantage of simultaneous detection of antibodies generated against majority of the surface epitopes. Further, recombinant viral proteins such as spike (S) and its domains – S1, S2, receptor binding domain (RBD), nucleoprotein (NP) can be exploited for ELISA development.

Objectives:

To standardize inactivated virus-based ELISAs for SARS-CoV-2 IgG and IgM detection

To clone and express SARS-CoV-2 RBD, S1, S2, full length S and NP proteins using bacterial and baculovirus expression systems

To investigate utility of different SARS-CoV-2 recombinant proteins for IgG and IgM detection ELISAs

Work done:

Inactivated virus-based ELISA

Our in-house propagated and well-characterized SARS-CoV-2 isolate (8004/IND/2020, Genbank Accession No:MT416726) was subjected to β-propiolactone (BPL) treatment for virus inactivation. Complete inactivation of SARS-CoV-2 was achieved after treatment with 0.0125%-0.2% BPL. The BPL-inactivated virus was used for standardization of indirect IgG ELISA.

After comparison of different harvesting times and BPL concentrations, SARS-CoV-2 harvested at 72 hours post infection and inactivated with 0.1% BPL was chosen as the coating antigen. for indirect IgG ELISA. Coating antigen at 30,000 PFU/well, serum dilution of 1:100 and anti-human IgG-HRP conjugate diluted at 1:20000 were found to be optimum for the IgG ELISA. The optimized assay was then used to screen 200 healthy donor sera (collected during 2017-2019, prior to COVID-19 emergence) to determine the specificity of the assay; 6 tested positive for SARS-CoV-2 IgG, thus specificity of the assay was 97%.

The BPL-inactivated virus was also investigated for IgM detection ELISA; however, was not found useful in the indirect ELISA format.

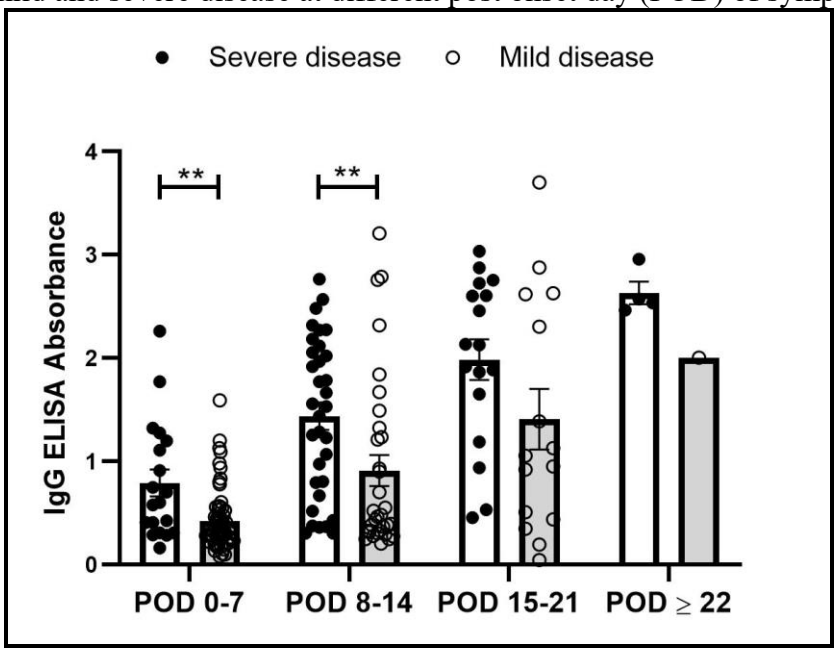
We then used screened 242 samples collected at different intervals from confirmed COVID-19 patients using our in-house IgG ELISA (Table 10). IgG was detected in >50% of the patients within 7 days post onset of symptoms (POD 0-7), and percent IgG detection increased with increase in POD – 80% at POD 8-14, 93.8% at POD 15-21, 100% at POD 22-30.

Table 10: IgG seroprevalence at different post onset day (POD) of symptoms among COVID-19 patients

POD	Total no. of samples tested	No. of samples positive for IgG	Percent IgG positivity
0-3	30	16	53.3
4-7	53	27	50.9
8-14	65	52	80.0
15-21	32	30	93.8
22-30	4	4	100.0
>30	1	1	100.0

Further, we compared the IgG response among different categories of SARS-CoV-2 infected individuals – asymptomatic, mild disease (MD) and severe disease (SD). The ELISA absorbance values among the asymptomatic subjects were comparable to that of the MD patients (P=0.94), however, were lower than the SD patients (P<0.0001). Within the symptomatic group, absorbance values were significantly higher in SD patients during the first 2 weeks of illness (Fig.24).

Fig.24. Comparison of IgG ELISA absorbance values for samples collected from patients with mild and severe disease at different post onset day (POD) of symptoms



Cloning and expression of recombinant SARS-CoV-2 proteins

Spike-RBD (331-524 aa) of our in-house isolated and propagated SARS-CoV-2 strain (CD/20/8003) was cloned in pET45b+ vector and expressed using *E.coli* BL21(DE3)*pLysS* system. The expressed protein was purified using Ni-NTA column; purity and specificity of the protein was confirmed by SDS-PAGE and immunoblotting. The utility of the spike-RBD was investigated for SARS-CoV-2 IgG and IgM detection ELISAs; however, the performance was not satisfactory. For IgG detection, the *E.coli* expressed RBD was not able to

differentiate between COVID-19 patients and negative controls, while for IgM, the detection rate was poor (<30% among confirmed COVID-19 patients).

Conclusion:

BPL-inactivated SARS-CoV-2 based ELISA was successfully developed. This ELISA can efficiently detect IgG antibodies in a significant proportion of COVID-19 patients. Early detection of IgG suggests potential utility of this ELISA as a supplement to RT-PCR in patient diagnosis and contact screening algorithms.

The *E.coli* expressed RBD was not useful for ELISA. Spike-RBD expression in Sf9 cells using baculovirus system is in progress, and this protein will be investigated for ELISA development.

14.Title: Antibody (IgA, IgG and IgG-subtype) responses to SARS-CoV-2 in severe and non-severe COVID-19 patients (**Project ID**: CD/20/4 /I); **Funding:** DBT-BIRAC (as part of NIBEC Project CD/19/3/E); **Duration:** April 2020 – 2021; **Sanctioned Amount:** NA; **Investigators: PI:** Dr. Harshad P. Patil; **Co-PI/ Co-Investigators:** Dr. Vidya A. Arankalle, Dr. A.C. Mishra (IRSHA), Dr. Sonali Palkar, Dr. Sanjay Lalwani (Bharati Vidyapeeth Medical College); **Ph.D. Students:** NA; **Human Ethical Approval:** IEC/2020/25

Background: Several vaccines for SARS-CoV-2 are in different phases of clinical trials in different populations. Understanding of immune response following natural infection and recovery is essential for the evaluation of vaccine-induced response. In view of the importance of IgA / IgG and IgG-subtypes in protection and probably in pathogenesis, we quantitated these antibodies in COVID19 patients presenting with no symptoms or mild/severe disease at different time intervals after the onset of clinical symptoms, by ELISA. Additionally, correlation analyses of antibody titers in ELISA and plaque reduction neutralization test (PRNT) were carried out.

Objectives:

To quantify antibody (IgA, IgG and IgG-subtype) responses to SARS-CoV-2 in severe and non-severe COVID-19 patients

To correlated IgA and IgG antibody in severe and non-severe COVID-19 patients

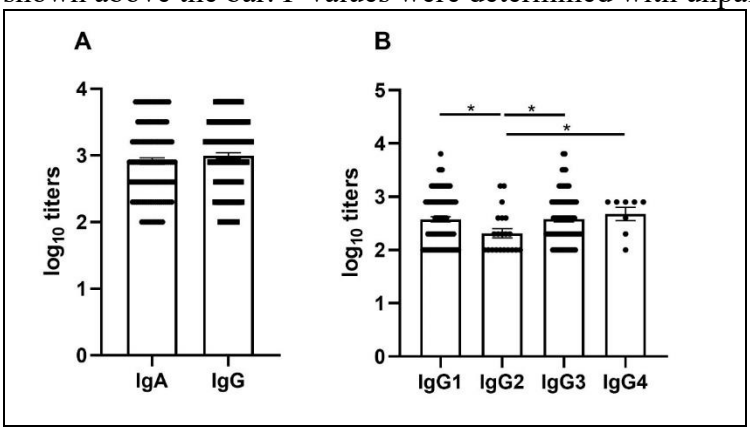
To assess correlation of binding antibody titers with neutralizing antibody titers

Work done:

Detection and quantification of IgG-SARS-CoV subtypes:

The ELISA titers for IgA and IgG were determined in 146 samples positive and were found comparable ($p = 0.190$, Fig.25). Among IgG subtypes (Fig.25B), IgG1 was the predominant subtype detected in 94/135 (69.6%) followed by IgG3 80/135 (59.3%), both suggestive of Th1 response. IgG2 and IgG4 that are result of Th2 response were detected in 19/135 (14%) and 8/135 (5.9%) samples respectively. Both IgG1 and IgG3 were present in 74 samples; IgG1 alone was present in 14 while IgG3 alone was present in 6 samples. IgG2 alone was present in 7 samples and IgG4 alone were present in 1 sample. IgG2 and IgG3 were detected in 1 sample. All the four subtypes were detected in 1 sample while IgG1, IgG2 and IgG3 were present in 6 samples from 5 patients. Overall, positivity of Th1 induced subtypes (100/135, 74%) was higher ($p<0.0001$) than Th2 directed (27/135, 20%).

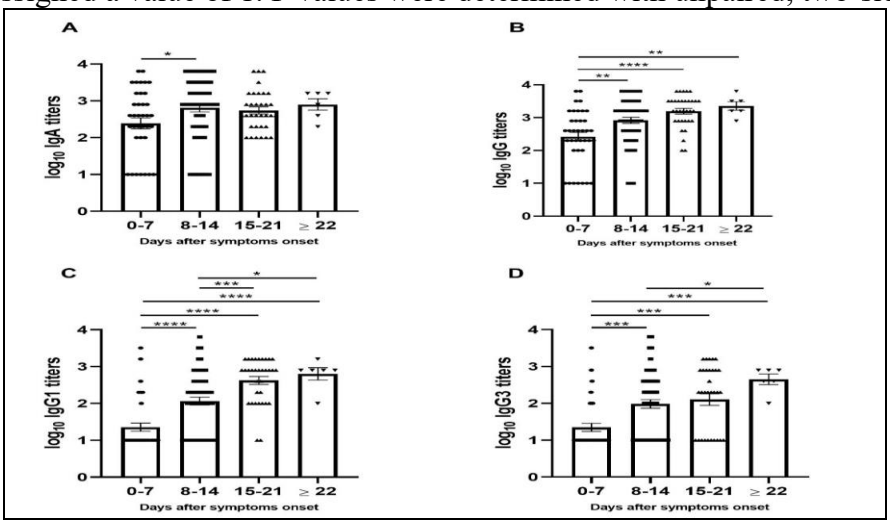
Fig.25. **SARS-CoV-2 antibody responses in COVID19 patients.** (A) Serological response of IgA and IgG in serum or plasma samples. (B) IgG subtype i.e IgG1, IgG2, IgG3, IgG4 response in same samples. Number of samples that were positive for antibodies out of 163 samples are shown above the bar. P values were determined with unpaired, two-sided Mann–Whitney U-test.



Dynamics of IgA and IgG antibodies / subtypes:

IgA and IgG titers were comparable during the first week and showed ~5 fold increase during the second week post-disease onset. A further rise in 3rd week was seen for IgG in the third week (Fig. 26A-B). Antibody dynamics was similar for IgG1 and IgG3 antibodies POD (Fig. 26C-D). In addition, IgG1 titres showed higher correlation with IgG titers (r=0.755) than with IgG3 (r=0.579).

Fig.26. SARS-CoV-2 specific antibody titers days after onset of symptoms from n=146 samples. (A) IgA (B) IgG (C) IgG1 and (D) IgG3 antibody titers. Patients with titer <100 were assigned a value of 1. P values were determined with unpaired, two-sided Mann–Whitney U-test.

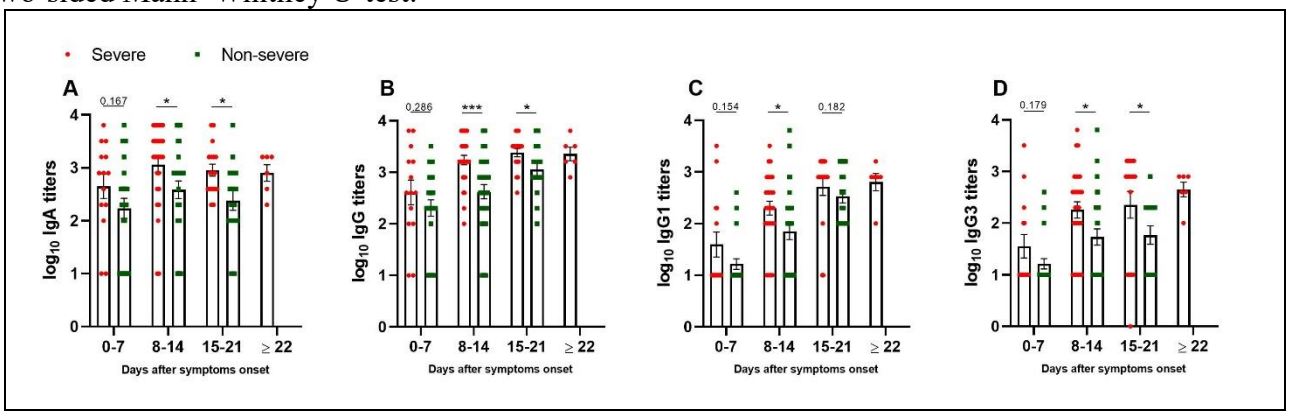


Antibody titers in relation to disease severity:

Comparisons of different antibody levels were compared with respect to disease duration (Fig.27). For IgA, IgG and IgG3, significantly higher titers were recorded in severe disease patients, during 2nd and 3rd weeks. For IgG1, the difference was only during the second week. In both severe and non-sever patients, comparable levels of IgA and IgG were

observed in 1st and 2nd week. However, by 3rd week, IgG levels were higher than IgA in sever (p=0.006) and non-sever (p=0.017).

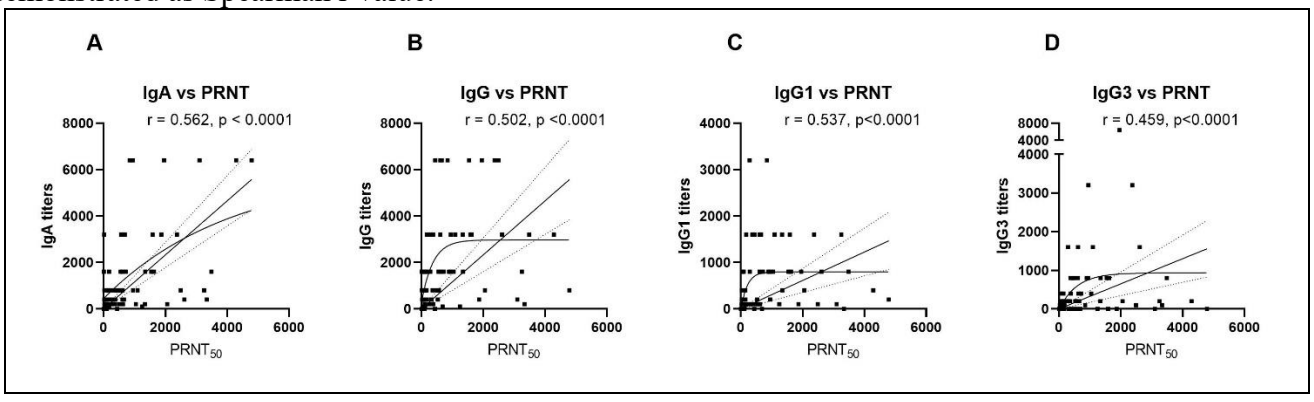
Fig .27. SARS-CoV-2 specific antibody titers days after onset of symptoms in severe and non-severe COVID19 patients. (A) IgA (B) IgG (C) IgG1 and (D) IgG3 antibody titers. Patients with titer <100 were assigned a value of 1. P values were determined with unpaired, two-sided Mann–Whitney U-test.



Correlation analysis among binding and neutralizing antibody titers

To understand the relationship of binding antibodies (ELISA) and neutralizing antibodies (PRNT), a separate analysis was undertaken. Both IgA (r=0.562, p<0.0001) and IgG (r=0.502, p<0.0001) correlated with neutralizing antibodies (Fig. 28). Similar results were obtained when mild and severe patients were compared. Titers correlated with better with IgG1 (r= 0.537, p<0.0001) than IgG3 (r=0.459, p<0.0001).

Fig.28. Correlation of SARS-CoV-2 specific antibody titers obtained by ELISA and neutralizing antibody titers obtained by PRNT50 assay from n=81 samples. Each dot represents a correlation of PRNT50 titer with (A) IgA (B) IgG, (C) IgG1 or (D) IgG3 from an individual and is demonstrated as Spearman r value.



Conclusions:

IgG1 was the predominant subtype detected in followed by IgG3, both suggestive of Th1 response. IgG2 and IgG4 that are result of Th2 response were detected in minimally detected.

IgA and IgG titers did not differ during the first week, severe disease exhibiting raised titers thereafter.

IgA and IgG1 antibodies correlated better with neutralizing antibody titers.

15.Title: Development of neutralization assay for SARS-CoV-2 viruses (**Project ID**:CD/20/5/I); **Funding:** DBT-BIRAC (as part of NIBEC Project CD/19/3/E); **Duration:** April 2020 – 2021; **Sanctioned Amount:** NA; **Investigators: PI:** Dr. Vidya A. Arankalle; **Co-PI/ Co-Investigators:** Dr. Shubham Shrivastava, Dr. A.C. Mishra (IRSHA), Dr. Sonali Palkar, Dr. Sanjay Lalwani (Bharati Vidyapeeth Medical College); **Ph.D. Students:** NA; **Human Ethical Approval:** IEC/2020/25

Background: Multiple candidate vaccines for SARS-CoV-2 are in different phases of clinical trials. Immunogenicity assessment of vaccine candidates in pre-clinical studies and in clinical trials relies on the neutralizing antibody response elicited by vaccination. Thus, there is a definite need for standardization of plaque reduction neutralization test (PRNT) and microneutralization assays for detection and quantitation of neutralizing antibodies against SARS-CoV-2.

Neutralizing antibody response in SARS-CoV-2 infected or vaccinated individuals is conventionally linked with recovery and protection from the disease. To understand the role of neutralizing antibodies in SARS-CoV-2 infection, PRNT test was carried out in asymptomatic individuals and in COVID-19 patients with mild / severe disease.

Objectives:

To standardize plaque reduction neutralization test (PRNT) and colorimetric microneutralization assays (CMNT) for detection and quantitation of neutralizing antibodies against SARS-CoV-2.

To determine the neutralizing antibody titers in SARS-CoV-2 infected individuals

Work done:

Development and standardization of PRNT and CMNT:

For this, the virus isolated at IRSHA was used. The test was standardized by optimization of the reagents and other variables. Samples with $PRNT_{50}$ titer ≥ 20 was considered seropositive. To assess the specificity of the standardized PRNT assay, plasma/serum samples collected from 61 blood donors in 2017-19 were tested for the presence of neutralizing antibodies. All the samples were found to be negative at starting dilution of 1:10 to SARS-CoV-2.

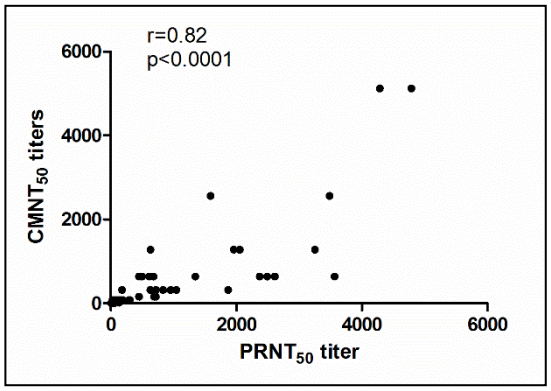
In view of the advantage of use of 96well format over 24well format in PRNT, we standardized microneutralization assay that depends on the inhibition of virus-induced cytopathic effects by the antibodies. To avoid the visual inspection of the inhibition of cytopathic effect (CPE) at each serum dilution under inverted microscope, colorimetric based MNT (CMNT) assay was developed.

Comparison of neutralizing antibody titers by PRNT and CMNT methods:

Performance of CMNT was compared with the gold standard test, PRNT. For this, 63 plasma samples from 45 COVID-19 patients including 14 severe (31 samples) and 31 mild (32 samples) cases were used. Neutralizing antibody (nAb) Seropositivity was 77.8% (49/63) by PRNT and 76.2% (48/63). The sample missed by CMNT had a titre of – in PRNT. PRNT titers varied from 5 to 4788 (mean, 752±147) and CMNT titers varied from 5 to 5120 (mean, 450±128). Overall,

nAb titers obtained by both the methods were comparable (p=0.13) with an excellent correlation (r=0.82, p<0.0001, Fig.29).

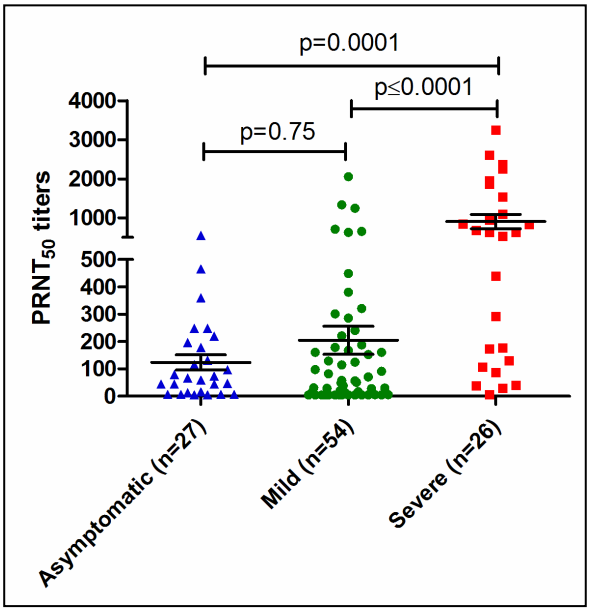
Fig.29. Comparison of neutralizing antibody titers by PRNT and CMNT methods



Neutralizing antibody titers in COVID-19 patients

The nAb seropositivity was comparable among asymptomatic individuals (19/27, 70.4%) and patients with mild disease (MD, 36/54, 66.7%, p=0.93). In contrast, a significantly higher nAb positivity was seen in the patients with severe disease (SD, 25/26, 96.1%, p = 0.03). The nAb titers ranged from 5 to 552 (mean 124±28) in asymptomatic individuals, from 5.0 to 2055 in MD group (mean 205±52) and from 6.5 to 3250 (mean 907±183) in SD groups. The titers were similar in asymptomatic individuals and MD patients. Importantly, SD patients exhibited significantly higher antibody titers when compared to asymptomatic, p=0.0001 and mild, p≤0.0001 categories (Fig.30.).

Fig.30. Comparison of $PRNT_{50}$ titers among samples collected from SARS-CoV-2 positive asymptomatic subjects and COVID-19 patients with mild and severe disease



Conclusion:

Virus neutralization assays, PRNT and CMNT employing live SARS-CoV-2 virus were successfully developed.

Our preliminary data suggests an association between neutralizing antibody titers and disease severity among Indian COVID-19 patients.

16.Title: Assessment of role of circulating immune cells in SARS-CoV-2 pathogenesis (**Project ID:** CD/20/6/I); **Funding:** DBT-BIRAC (as part of NIBEC Project CD/19/3/E); **Duration**: April 2020 – 2021; **Sanctioned Amount:** NA; **Investigators: PI** – Dr. Archana Kulkarni-Munje; **Co-Investigators** –Dr. Vidya Arankalle, Dr. A. C. Mishra (IRSHA), Dr. Sonali Palkar, Dr. Sanjay Lalwani (Bharati Vidyapeeth Medical College); **Human Ethical Approval:** IEC/2020/47

Background: The ongoing pandemic of SARS-CoV-2 has turned out to be an unprecedented threat to global public health and economy. Irrespective of the degree of industrialization or availability of medical infrastructure, all populations have been (and are being) affected. The major focus of the scientific community has been to unravel immunopathogenesis of the disease requiring clear understanding of the immune response in both mild and severe disease forms. In India, the first COVID case was reported on 30th January 2020. Older age and existing comorbidities remain the high-risk factors. In view of the need to understand immunology of the disease in general and among Indian population in particular, we attempted to explore the functional profile of innate immune cells (Monocytes, Dendritic cells & NK cells), and adaptive immune cells (B cells, Follicular T helper cells, CD4T & CD8 T cells) in SARS CoV-2 infected individuals presenting with asymptomatic, mild or severe disease. Further, dynamics of these immune cells and, relation to neutralizing antibody titers was analysed.

Objective:

To understand modulation of circulating immune cells in relation to disease duration and severity

To identify prognostic marker(s) for severe infection

Work done:

Sixty COVID-19 patients and 10 apparently healthy individuals negative for IgG-anti-SARS-CoV2 antibodies were included in this study. Patients admitted to intensive care units for oxygen / ventilator support were designated as suffering from severe disease (SD, n=25) while those with mild (n=20) or no (n=15, asymptomatic) symptoms were classified as MD. Among SD patients, 4 succumbed to the infection while 21 were discharged. Follow up blood samples collected from SD (n=15) and MD (n=5) patients were also included. Median age of mild cases was 38 years whereas in severe cases it was 51 years. Comorbidities such as Diabetes, hypertension, cardiovascular diseases and obesity were present in 20% mild cases as against 64% in severe cases. COVID-19 cases showed a marked lymphopenia as evidenced by lower lymphocyte percentage (median-28.83%) as compared to the control subjects (median-61.06%) ($p<0.0001$). To understand the contribution of major immune cell subsets in the pathogenesis of SARS CoV-2 infection, we evaluated the frequencies of antigen presenting cells (Dendritic cells & Monocytes), Natural killer cells, T cells (CD4 T cells, CD8 T cells and T_{FH}) and B cell subsets (memory B cells and plasmablast cells). Cytokine storm involving a major role of IL-6 is already documented and hence we analysed Th1 (IFN-γ, TNF-α & IL-2) & Th2 (IL-4, IL-6 & IL-10)

cytokine levels in plasma of these patients to delineate the association with these cells, if any. $PRNT_{50}$ data of these samples was also analysed for any association with their immune profile.

Results:
Initially, we compared mild symptomatic and asymptomatic individuals with respect to all the parameters examined. The only difference was seen in the levels of circulating TNF-α levels. Therefore, the two groups were combined (MD) while comparing with the patients with severe disease (SD).

Dynamics of parameters examined in MD and SD patients:

Table 10 shows proportion of immune cells and cytokine concentrations in MD and SD patients at different time points after onset of clinical symptoms. The observed patterns were: (1) Lowering of activated mDCs (CD80+ and CD86+) and increase in TFH cells in the SD patients that continued till 3rd week post-onset. (2) Lower pDCs and marginal reduction in B cells during the 2nd week (p=0.061) and higher IL2+CD4 cells during the first two weeks, in the SD patients (3) Difference only in the first week; increase in HLA DR & CD38+ CD8 and memory B cells and decrease in BCMA+ plasmablast cells in the SD patients (4) modulation during 2nd week, decrease in CD16+ Monocytes and reduction in total NK cells in the SD patients.
Overall, we observed association of early modulation of mature dendritic cells, activated CD8 T cells, follicular T helper cells and IL-2 + CD4 T cells with disease severity. Lower IL-21 receptor expression on memory B cells indicated imbalance in IL-21/IL-21 R ratio. Lower BCMA positive plasmablast cells in severe cases did suggest probable absence of long-term humoral immunity.

Neutralizing antibody titers in relation to the parameters investigated:

Earlier, we observed that severe disease was characterized by higher neutralizing antibody titers. Therefore, the proportions and effector functions of immune cells and cytokines were compared in MD and SD patients with respect to neutralizing antibody response. In univariate analysis, CD86+ pDC (p=0.017), PD1CD4 (0.0051) and memory B cells (p=0.00982) correlated with PRNT titers. However, in multivariate analysis, PD1+CD4 emerged as the single variable influencing PRNT50 titers (p=0.003, R2 = 0.421). Importantly, PD-1 expression on CD4 T cells was higher in severe disease.

Conclusion: Our study revealed that Indian patients exhibited a different set of immunological modulation in SARS CoV-2 infection and identified additional prospective severity prognostic markers such as dendritic cells, activated CD8 T cells, IL-2+ve CD4 T cells and follicular T helper cells.

Table 10: Analysis of immune cell frequencies / cytokines in relation to disease severity and duration									
Name of Immune cell subset	Type of illness	Week 1	Mann Whitney U	Week 2	Mann Whitney U	Week 3	Mann Whitney U	Week 4	Mann Whitney U
mDC (CD80+ & CD86 +) %	Mild	87.6	0.007	87.8	0.001	87.7	0.013	87.8	1
	Severe	31.77		33.33		56.02		83.35	
Plasmacytoid DC %	Mild	0.17	0.012	0.11	0.032	0.15	0.276	0.13	0.121
	Severe	0.06		0.03		0.01		0.06	
CD4 %	Mild	56.8	0.19	59.3	0.753	57.08	0.355	58.12	0.121
	Severe	71.08		63.7		60.64		10.735	
T_{FH} %	Mild	0.15	0.001	0.15	0.012	0.17	0.045	0.16	0.121
	Severe	1.29		1.07		1.04		5.77	
Il-21 MFI (T_{FH})	Mild	18836	0.28	14852	0.038	16471	0.165	15160	0.121
	Severe	21875		21751		17500		15178	
CD16+ Monocytes %	Mild	30.37	0.165	10.16	0.025	10.16	1	10.16	1
	Severe	13.22		17.86		12.39		18.3	
Total NK %	Mild	7.6	0.316	6.2	0.042	6.38	0.355	6.29	1
	Severe	2.88		2.65		3.03		8.866	
HLA DR + CD38 + CD8 %	Mild	0.495	0.041	0.16	0.414	0.29	0.122	0.18	1
	Severe	3.69		2		3.03		0.405	
IL-2 + CD4 %	Mild	0.13	0.005	0.03	0.016	0.06	0.06	0.035	1
	Severe	0.49		0.47		0.3		0.015	
B Cells %	Mild	13.44	0.033	10	0.061	13.01	0.64	11.5	0.439
	Severe	4.63		6.93		7.47		13.66	

Memory B cells %	Mild	14.3	0.001	25.76	0.116	31.26	0.355	28.73	0.121
	Severe	41		42.86		34.78		42.84	
Switched memory B cells %	Mild	81.15	0.589	89.31	0.586	88.22	0.537	88.76	0.439
	Severe	85.23		86.53		90.02		81.73	
Unswitchedmemory B cell%	Mild	18.27	0.643	10.27	0.66	10.97	0.436	10.28	0.425
	Severe	14.03		12.61		10		16.58	
BCMA + Plasmablast cell %	Mild	23.7	0.02	21.32	0.136	23.56	0.563	25.32	0.935
	Severe	13.23		10.25		12.34		21.25	
BCMA MFI	Mild	25326	0.82	34215	0.996	27896	0.036	36547	0.865
	Severe	21457		26359		21789		39875	
IL-6 pg/ml	Mild	6.14	0.939	5.12	0.368	9.68	0.105	5.83	1
	Severe	5.82		27.36		49.13		21.1	

17.Title: Establishment of *In silico* screening platform to screen Dengue NS3 protease inhibitors at National Immunogenicity and Biologics evaluation Centre (NIBEC) to evaluate drugs and other products (**Project ID**: CD/19/3/I); **Funding:** DBT-BIRAC (as part of NIBEC Project CD/19/3/E); **Duration:** December 2019 – March 2020; **Sanctioned Amount:** NA; **Investigators: PI** - Dr. Sudha Ramkumar; **Ethical Approval**: Not applicable; **PhD students:** None; **MSc. Dissertation Students:** Mr. Swarnendu Gosh and Ms. Aabha Thite; **Ethical Approval**: Not applicable

Background: The global occurrence of Dengue poses a significant threat to human health. The lack of long-term protective efficacy of dengue vaccines against each of the 4 dengue virus serotypes urges us to have drugs directed at the viral targets viz., NS2b/NS3 protease that can be used safely as prophylaxis or treatment to effectively ameliorate disease or reduce disease severity and fatalities.

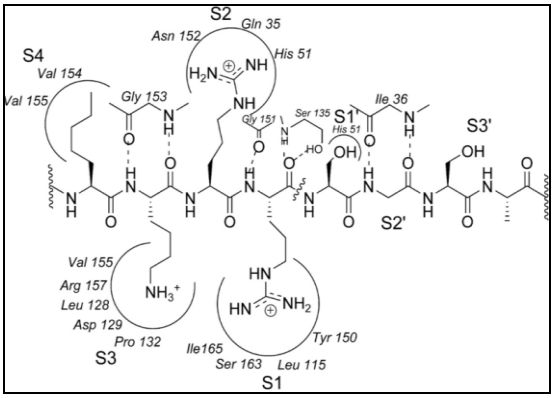
Objective:

1. In-silico molecular modelling and screening of plant triterpenoids against NS3 protease
2. MNTD and CC_{50} estimation for the drugs (Maximum non-toxic dose)
3. Viral load reduction assay (NS1 based)

Work done: Identification of the NS3 protease subsites and residues involved in binding of the NS3 protease inhibitor

Fig. 31. A: Schematic representation of NS3 protease subsites involved in binding the protease inhibitors. B: Allosteric pocket of the dengue virus (serotype 2) NS2B/NS3

A **B**



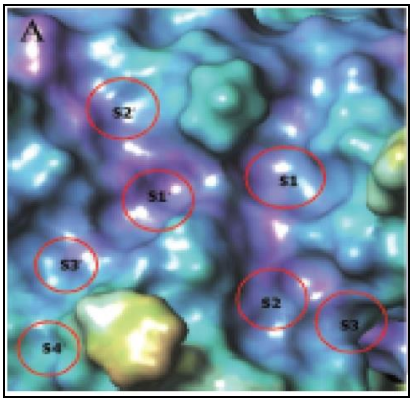


Table 11: Binding energy and interactions of plant terpenoids with amino acids present in NS3 protease active site

Ligand (L)	NAME	Minimum Binding Energy (kCal/mol)	Residues	Binding Site
Positive Control	Cardamonin	-5.93	PRO132, LEU128, TYR161	S3
	Baicalein	-6.47	TYR161, LEU128, GLY153, PRO132, SER135	S1`, S3, S4
	Pinocembrin	-7.18	PRO132, LEU128, TYR161	S3
	Andrographolide	-7.25	PRO132, LEU128, TYR161	S3

L1	Deacetylsalanin	-8.02	ARG54	-
L2	Azadaridione	-8.31	MET59, MET46, LEU47	-
L3	Azadarictin A	-6.91	TYR161, LEU128, GLY153, PRO132, SER135	S1`, S3, S4
L4	Azadiractin B	-7.26	LYS28, LEU47, MET46, ASP50	-
L5	Azadirone	-8.32	ASN105, PRO72, LEU74, ILE86; MET59, LEU47, MET46, GLU52	-
L6	Epoxyazadiradione	-8.01	TYR161, LEU128, PRO132, GLY153; LEU47, MET59	S3, S4
L7	Euphol	-8.85	LEU128, PRO132, TYR161	S3
L8	Gedunin	-8.05	TYR161, LEU128, PRO132	S3
L9	Nimbin	-7.55	GLY153, TYR161, LEU128, PRO132	S3, S4
L10	Salanin	-6.88	PRO132, LEU128, TYR161, HIS51, ARG54	S1`, S2, S3
L11	Salanone Acetate	-7.75	MET46, LYS28, ILE30, LEU47	-

Conclusions: The minimal binding of the plant triterpenoids ranges from -6.91 to -8.85 kcal/mol. The subsite S3 alone and along with S1`, S4 is involved in the binding of the positive controls with known protease inhibitor are the identified sites for screening of NS3 protease inhibitors. Based on the *in silico* data Gedunin, Azadiractin A, Euphol, Nimbin, Salanin and Epoxyazadiradione can be taken up for in vitro cell cytotoxicity and NS3 protease inhibitory assay.

18.Title: Standardization of the Cell viability assay to determine the CC_{50}/MNTD/EC_{50} of the test products at National Immunogenicity and Biologics evaluation Centre (NIBEC)(**Project ID**:CD/20/7/I); **Funding:** DBT-BIRAC (as part of NIBEC Project CD/19/3/E); **Duration:** March 2020 – June 2020; **Sanctioned Amount:** NA; **Investigators PI** - Dr. Sudha Ramkumar, Dr. Rashmi Virkar; **PhD students:** None; **Ethical Approval**: Not applicable

Background: Due to the current COVID 19 pandemic, the research focusing development of antivirals ended up in several enquires at NIBEC for screening of test products viz., drugs/sanitizers/ disinfectants/Hand wash/ antimicrobial coated surface. So antiviral assessment work against SARS-CoV2 has been initiated. Measurement of Cell viability is the primary assays involved in this work. Therefore, standardization of cell viability determination using MTS and MTT reagents has been planned

Objective:

To standardize the MTT assay on Vero cell line

To standardize the MTS assay on Vero cell line

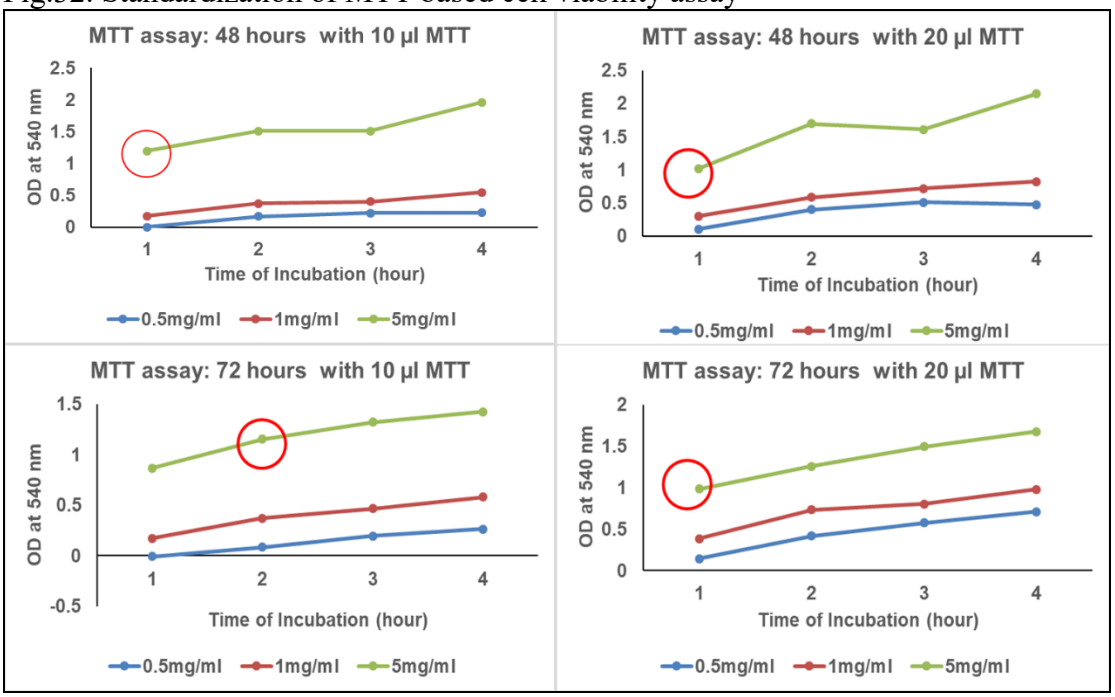
Work done:

MTT Assay: For standardization for cell viability assay with MTT reagent, different parameters were considered (Fig.32).

Time of incubation post seeding

Time of incubation after addition of the MTT reagent

Fig.32. Standardization of MTT based cell viability assay



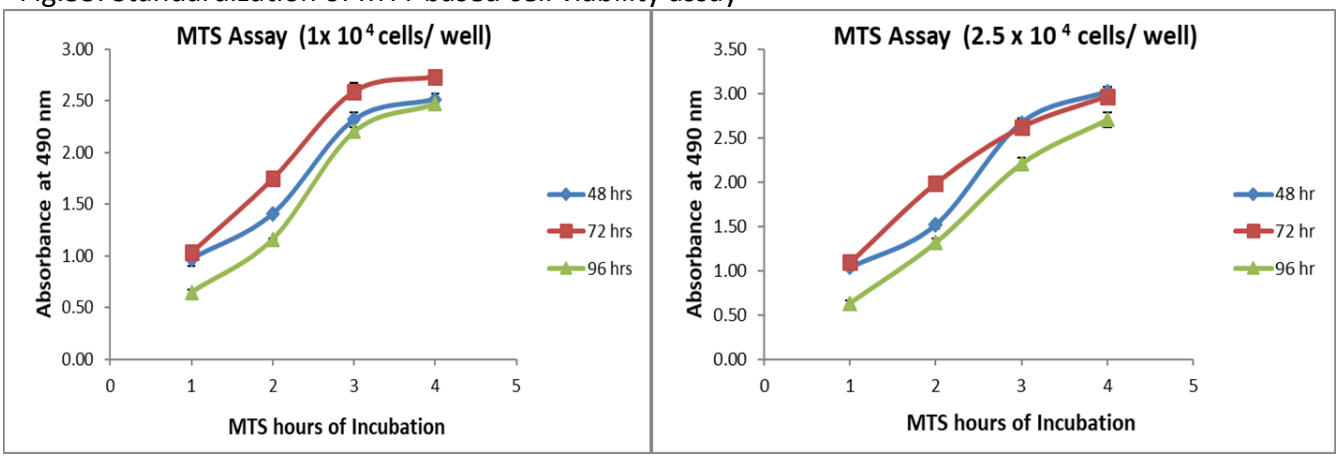
MTS Assay: For standardization for cell viability assay with MTS reagent, different parameters were considered (Fig.33).

Cell count

Time of incubation post seeding

Time of incubation after addition of the reagent

Fig.33. Standardization of MTT based cell viability assay



Conclusion:

- MTT assay should be performed with 20 µl of 5mg/ml MTT reagent for 1 hour. The time of development post addition of test product is 72 hours for 2.5 x 10^4 cells per well
- MTS assay should be performed with 20 µl MTS reagent for 1 hour. The time of development post addition of test product is 72 hours for 2.5 x 10^4 cells per well

Future prospects:

After standardization of cell viability assay, following tasks have been planned.

Standardization of assays for determination of anti-SARS-CoV2 virus antiviral/ virucidal activity of

- Drugs
- Determination of antiviral activity of textile products referring to ISO 18184:2019(en) (Textiles:)
- Measurement of antiviral activity on plastics and other non-porous surfaces referring to ISO 21702:2019(en).
- Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of virucidal activity in the medical area referring to BS EN 14476:2013+A2:2019

Centre for Innovation in Nutrition Health Disease (CINHD)
July 2019- June 20

1.Title: ICAR -AICRP- Linseed Value Addition Centre (**Project ID:** INHD/15 (15-18)/1/E); **Funding:** ICAR, New Delhi; **Duration:** April 2015 onwards; **Scientist in-charge:** Dr. Anand A. Zanwar; **Amount received:** Total 85.41 Lakh (2015-20), 11.97 Lakh (2019-20)

Background: Broad objective is linseed value addition. The objectives for 2019-20 were planned and approved during Annual Linseed Group meeting held at CSAUA & T, Kanpur, UP during 04 – 06 September, 2019. Last year blending of linseed oil with edible oil study, initial 8 month's stability study was performed and this study was continued from 9 to 15 months to assess the long term stability during this year. In omega-3 enriched energy bar study, composition of the formulation was re-designed based on the results obtained in last year's composition. Whey protein was found to improve protein content without much alterations in other physicochemical properties and oxidative stability, as observed in last year's results. Hence this year whey protein group was dropped and studies were performed only with respect to omega-3 fatty acid for incorporation in energy bar. Under development of lignan rich linseed hull powder study, it was concluded that, removal of fat, mucilage and separating hull from whole seed resulted in significant improvement in lignan content for developing lignan rich powder.

Work done (objective-wise):

Effect of ageing on linseed quality, when stored under ambient conditions

Objective: To assess the effect of ageing on oil content, fatty acid profile and SDG lignan content of linseed

Materials and methods:

The seed variety PKV.NL 260 was collected in May 2017. These seeds have been stored at ambient temperature. Seeds are being evaluated for 36 months at the interval of every 3 months.

Results and discussion:

Effect of ageing on linseed quality, when stored under ambient conditions was studied for the period of three year (May - 2017 to 2020). Linseed is mainly exploited for value addition as it is richest source of omega-3 fatty acid and SDG lignan content. Both these components have several beneficial effects for human health. Hence for the present study, these parameters along with oil content were studied for the period of three years to understand how long stored linseed can be used for value addition. Data of three years is compiled in table 1 (May-2017-2020). Significant reduction in oil content has been observed from May-2019 ($p<0.05$) and further more significant reduction ($p<0.001$) in oil content was also observed during third year. Non-significant changes in PUFA content was observed till May-2020. In case of SDG lignan content, non-significant change in content was observed till May-2020.

Conclusion:

It is concluded that, linseed can be stored at cool and dry place at room temperature and away from direct light/heat/moisture. Under these conditions, oil content stable for the period of two years and omega-3 fatty acid and lignan content are stable for three years.

Table 1: Effect of ageing on oil and lignan content and fatty acid profile of linseed

Parameters	May-2017	May-2018	May-2019	May-2020
Oil content (%)	34.53±0.53	33.55±0.35	32.43±0.66	31.61±0.50
SFA (%)	11.94±0.06	11.54±0.51	11.28±0.08	10.59±0.05
MUFA (%)	19.51±0.11	18.40±0.57	19.85±0.50	19.55±0.07
PUFA (%)	68.36±0.16	70.03±1.07	68.84±0.57	69.84±0.02
Lignan (mg/g)	06.07±0.22	04.60±0.29	05.67±0.62	05.88±0.71

A. Blending of linseed oil with edible oil

Objective: To study oxidative stability of blended linseed oil with added antioxidants

Materials and methods:

Following edible oils and their blends with linseed oil were prepared during 2018-19 and evaluated for stability study for the period of 8 months. Palm olein, coconut oil, rice bran oil, mustard oil, palm olein + linseed oil (80:20), coconut oil + linseed oil (80:20), rice bran oil + linseed oil (80:20), mustard oil + linseed oil (80:20). The ratio was 80:20 for all blends as permitted by FSSAI, India regulations. All the oils were purchased from local market. Antioxidants namely Ascorbyl Palmitate (AP - 500 mg/kg) and Tertiary butyl hydroquinone (TBHQ - 120 mg/kg) were added as per Codex standards 210-1999. These oils and their respective blends were kept at room temperature and evaluated for oxidative stability over the period of time. Stability study was continued from 9th month onwards during 2019-20.

Results and conclusion:

At the end of 15th months storage stability study, the efficacy of AP was very significant in controlling peroxidation in case of palm olein as compared to plain palm olein and its blend with linseed oil. In case of coconut oil both AP and TBHQ were comparable. Further AP showed fairly good performance in mustard and rice bran oil in controlling peroxidation as compared to TBHQ. With respect to acid value, all oils and blends were within acceptable limits. In this experiment, comparative data of storage stability study from 2017-2020 wherein we studied 4 antioxidants i.e. α-tocopherol, tri-vitamin E, AP and TBHQ. So, the efficiency of different antioxidants in various blends is observed as given below: AP>TBHQ>Tri-vitamin E> α-tocopherol.

Conclusion: Asorbyl palmitate (500 mg/kg) is preferred over other antioxidants in controlling the peroxidation in blended oils for long term storage stability of experimental edible oil blended with linseed oil.

B. Development of omega-3 enriched energy bar

Objectives: To develop omega-3 enriched energy bar and to carry out storage stability study of omega-3 enriched energy bar

Results and discussion:

In the present set of experiments, four different formulations were tried for linseed value addition in energy bar. Comparative study of emulsion v/s linseed oil with equivalent concentration of omega-3 fatty acid in the form of raw linseed oil and emulsion in the formulation of energy bar was studied. Emulsion two variations i.e. emulsion containing 30% and 60% linseed oil, and raw linseed oil two variations i.e. - 5 gram and 8 gram, groups were studied along with control group which is devoid of linseed/linseed oil/emulsion to finalize the composition of best formulation which can incorporate optimum amount of omega-3 fatty acid without much alterations in physicochemical properties and oxidative stability of energy bar.

The nutritional analysis of energy bar is given in table 2. The protein content was close 12% in all groups including in control group. Carbohydrate % is ranging from 59.10 to 64.10 %. Important micronutrients such as phosphorus, calcium and ash content ranged from 70 – 90 mg/100g, 80.49 – 174.52 mg/100g, 1.58 - 1.79 respectively. Dietary fiber ranged 15.02 to 20.01 %. Unhealthy cholesterol was not detected/below detectable limit in all groups. Absolute fatty acid deliverable by different energy bar in g/100 g of energy bar is also shown in Table 7.3.2. Maximum ALA incorporation of ALA can be done using 60% Emulsion and 8 g linseed oil group in keeping balanced omega-6:3 ratio i.e. closer to 1:1.

Texture profile analysis presented in table 3. Breaking strength using compression test (load taken to break the sample at 1st compression) revealed there was non-significant alteration in group B and slight alterations were noted in C and E as compared to control (group A), however significant reduction was noted in group D as compared to group A. Deflection test (deflection to break the sample) revealed, slight alterations in C group and non-significant alteration in remaining all experimental group (B, D and E group) as compared to control.

As per CIELAB classification, L*value (lightness) was ranged from 27.34 – 30.75, a* (redness) between 3.60 to 5.51, b* (yellowness) 4.02 – 6.91 and dE*ab was ranging between 67.27 – 70.39. These parameters indicates all the bars showed slight or very little alterations w. r. t. to various colour co-ordinates indicating uniformity and homogeneity of experimental formulations as compared to control group formulations (Table 3).

Table no. 3 represents fatty acid profile of energy bar. SFA content was maximum in control energy bar and due to addition of linseed oil/linseed emulsion there was significant reduction in SFA content in all experimental groups as compared control group bars ($p<0.001$). There was non-significant alteration in MUFA content. As control group does not contains omega-3 fatty acid, and because of addition of linseed/linseed oil/linseed emulsion there was very significant alteration in ALA content and thereby PUFA content in all experimental groups as compared control group bars ($p<0.001$). Further omega-6 to 3 ratio was ideal and healthy i.e. 1.72:1 to 1:1. The ideal ratio was recorded in two groups i.e. Linseed emulsion (LO-60 %) and Linseed oil (LO-8g).

As part of stability study of energy bar, oxidative stability parameters (peroxide value, acid values and free fatty acid content) were recorded initially and at the end of 3 and 6 months. Oxidative stability data of 6 months is presented in table no. 4. There was gradual increase in peroxide value, acid value and free fatty acid content in initial values of experimental groups as compared respective values in 3rd and 6th months. Further these parameters were significantly altered in initial values of experimental groups as compared respective values in 3rd and 6th months, however values remain within well acceptable limits as per codex guidelines, indicating stability of linseed oil in raw/emulsified form in energy bar (Table 4).

Conclusion:

Based on nutritional, and colour analysis, it can be concluded that, energy bars prepared using linseed emulsion (30% and 60%) and bars prepared using linseed oil (5g and 8g) were fairly good as compared to control. Even in oxidative stability study, all the primary oxidation parameters were within acceptable limits and fatty acid profile was also unaltered during stability period, indicating the stability of omega-3 fatty acid. With respect to fatty acid profile and omega-6 to 3 ratio, all experimental group performed fairly well as expected. In texture analysis, linseed emulsion (30% and 60%), linseed oil 5 g were fairly comparable, however linseed oil 8 g showed very significant reduction in compression test and showed poor performance in texture analysis which indicated higher amount of linseed oil may not be incorporated in raw form in energy bar formulation.

Table 7.3.2: Nutritional analysis of omega-3 energy bar

Parameters	Unit	Control	Linseed emulsion (LO-30%)	Linseed emulsion (LO-60%)	Linseed oil (LO-8g)	Linseed oil (LO-4g)
Total protein	g/100g	12.38	12.07	12.45	12.5	12.27
Total fat	g/100g	17.06	15.39	22.37	21.31	14.85
Moisture	g/100g	6.22	6.39	3.51	5.05	6.53
Cholesterol	mg/100g	<0.5	<0.5	<0.5	<0.5	<0.5
Carbohydrate	g/100g	62.01	64.10	59.21	59.10	64.00
Phosphorous	g/100g	0.08	0.07	0.09	0.07	0.08
Calcium	mg/100g	171.39	148.86	80.49	80.51	174.52
Dietary fiber	g/100g	19.58	19.86	20.1	16.48	15.02
Ash content	g/100g	1.79	1.58	1.76	1.67	1.67
Total sugar	g/100g	16.49	18.86	20.18	15.14	17.37
Potassium	mg/100g	481.99	488.12	563.15	495.43	576.63
ALA	g/100 g	00	0.91	2.11	2.54	0.97
LA	g/100 g	1.58	1.55	2.12	2.09	1.16
SFA	g/100 g	10.31	8.05	11.41	9.94	8.25
MUFA	g/100 g	1.74	1.78	2.22	2.44	1.48
n-6:n-3	g/100 g	-	1.72	1.00	0.82	1.21

Table 7.3.3: Texture, colour and % fatty acid composition of omega-3 energy bar

Parameters	Control (A)	Linseed emulsion (LO-30%) (B)	Linseed emulsion (LO-60%) (C)	Linseed oil (LO-8g) (D)	Linseed oil (LO-5g) (E)
Texture analysis					
Breaking strength	15.85±0.55	12.46±2.50	7.87±1.61	3.16±1.39	8.65±1.72
Deflection test	1.66±0.10	2.38±0.54	3.43±0.77	3.20±0.89	3.22±0.93
Colour analysis					
L*	27.60±1.73	30.75±3.05	30.33±0.36	28.82±1.97	27.34±2.18

a*	5.2±0.87	5.51±0.32	5.19±0.51	4.78±0.71	3.60±0.43
b*	6.12±1.57	8.11±1.54	6.57±0.37	6.915±1.73	4.02±0.72
dE*ab	70.16±1.77	67.27±2.83	67.47±0.39	69.0025±1.73	70.395±2.22
% Fatty acid composition					
LA (%)	11.59±0.18	12.65±0.49	11.89±0.20	12.28±0.97	9.83±0.50
ALA (%)	0.0±0.0	7.37±0.44	11.80±0.57	14.95±0.21	8.17±1.10
SFA (%)	75.58±0.86	65.42±3.18	63.80±0.70	58.35±1.74	69.46±1.10
MUFA (%)	12.77±0.67	14.50±3.13	12.45±1.08	14.36±0.98	12.49±0.49
PUFA (%)	11.59±0.18	20.02±0.04	23.69±0.37	27.23±0.75	18.00±0.60
O6/O3 ratio	0.0±0.0	1.72±0.17	1.00±0.06	0.82±0.07	1.21±0.22

Table 4: Oxidative stability of omega-3 energy bar

Parameters	Control			Linseed emulsion (LO-30%)			Linseed emulsion (LO-60%)			Linseed oil (LO-8g)			Linseed oil (LO-4g)		
	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months
Peroxide value	0.80±0.00	1.03±0.05	1.23±0.05	1.36±0.05	1.96±0.05	3.6±0.15	1.70±0.00	2.26±0.05	3.6 ±0.1	1.8±0.05	2.36±0.05	3.5±0.1	1.43±0.05	2.60±0.00	3.46±0.15
Acid value	2.18±0.02	3.12±0.04	5.40±0.02	1.74±0.00	2.12±0.02	3.70±0.02	1.80±0.02	1.97±0.01	2.97±0.05	1.88±0.00	2.16±0.00	3.37±0.02	1.88±0.00	2.11±0.00	3.91±0.05
Free Fatty Acid	1.16±0.01	1.60±0.00	2.71±0.01	0.93±0.01	1.16±0.01	1.81±0.02	0.95±0.01	0.97±0.01	1.58±0.005	0.94±0.00	1.08±0.01	1.59±0.005	0.98±0.01	1.03±0.01	1.63±0.005

2. Title: Evaluation of efficacy of novel stabilized omega-3-fatty acid and antioxidants formulation for the prevention and treatment of metabolic syndrome (**Project ID:** INHD/17/1/E); **Funding:** DST-SERB ; **Sanctioned amount:** 40.36 Lakh (2017-20); **PI:** Dr. Anand Zanwar; **Duration:** 29th June 2017 to 28th Sept 2020; **Ethics Committee approval**: BVDUMC/3017/2019/001/006

Background: Last year formulation containing omega-3 fatty acid along with vitamins and micronutrients was subjected for characterization, stability evaluation (oxidative and fatty acid stability, mechanical stability, physical instabilities, optical microscopy, particle size analysis, PDI and zeta potential analysis) and *in vivo* acute toxicity study was carried out.

Objective-wise work done:

To evaluate effect of omega-3-antioxidant formulation in animal model of metabolic syndrome

Male Wistar rats (8-10 weeks old), were housed in a temperature-controlled room, in a 12-h light/dark cycle environment with *ad libitum* access to water and food. Various trials for finalizing the diet compositions were carried out considering the energy values of diet. High fat diet induced animal model of metabolic syndrome initiated with following groups: control diet, High Fat High Carbohydrate (HFHC), HFHC+Omega-3 antioxidants formulations (2-5 g/serve). The first group is having free access to a control diet, remaining all groups are receiving, HFHC diet with an additional 25 % of fructose in water. All the groups having free access to water. Blood samples collected for biochemical analysis. There was significant increase in serum cardiac markers in HFHC group and compared to control group. The key biochemical parameters such as creatinine kinase-MB, lactate dehydrogenase, aspartate aminotransferase, uric acid, triglycerides were significantly reduced due to formulation (4, 5 g/serve) and non-significant reduction in case of other parameters. RBC fatty acid is an important and proven biomarkers for metabolic syndrome. Higher omega-3 levels in RBC is directly related to greater longevity and inversely associated with mortality and incidences of cardiovascular and associated disorders (Harris et al., 2018). In the present study, RBC fatty acid analysis recorded significant improvement in omega-3 fatty acid content ($p<0.001$) in formulation treated groups as compared to HFHC group and inflammatory omega-6 fatty acid was significantly reduced ($p<0.001$), suggesting omega-3 fatty acid supplementation in formulation responsible for protective effect in the experimental model of metabolic syndrome (Figure 1).

Histopathology of heart tissue using Masson's-trichrome staining in animal model of metabolic syndrome:

Cardiac fibrosis is key marker associated metabolic syndrome, especially cardiovascular and associated comorbidity including diabetes and obesity (Cowling et al., 2019, Petta et al., 2017, Cavalera et al., 2014). In the present study, histopathology of heart tissue using Masson’s trichrome stain, showed severe perivascular collagen deposition in high fat high carbohydrate (HFHC) group as compared to control group rats (Figure 2), indicating the HFHC diet responsible for induction of metabolic syndrome in experimental animal model. These changes were significantly reversed/neutralized in formulation treated groups (4 and 5 g/serve), indicating the protective effect of formulation in controlling the diet induced metabolic syndrome in animal model. However formulation 2 g/serve failed to show in controlling these histopathological alterations.

To carry out single dose bioavailability study of emulsified omega-3 antioxidant formulation as compared to fish oil

The pharmacokinetics of omega-3 fatty acid is well established (Lin and Salem, 2007). It is well known that, conversion of α-linolenic acid (ALA) to long chain fatty acid such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is poor. Hence in order to evaluate the bioavailability of omega-3 fatty acid in formulation, the preliminary study was conducted by single dose oral administration of formulation and compared with flax oil and fish oil i*n vivo* model. Figure 3 shows ALA, EPA and DHA content in blood after 2, 4, 6, 8, 12 and 24 hr of oral administration of formulation, flax oil and fish oil. In the formulation group, ALA peak reaches in 2 hr whereas in case of flax oil group peak reaches after 4 hr. This possibly suggests better absorption and improved bioavailability in formulation group. Fish oil was devoid of ALA, therefore, ALA level remained at baseline only. Further it is also seen that, EPA appears earlier within 2 hr in formulation group whereas it takes 4 hr for the flax oil group (Figure 3). This possibly suggests better conversion of ALA to EPA possibly because of the co-factors present in the formulation. In case of DHA, formulation group showed better conversion as compared to flax oil and comparable to the fish oil group, indicating improved bioavailability of omega-3 fatty acid in the formulation group, indicating presence of micronutrients/co-factors such as zinc, magnesium, vitamin B3 and B6 responsible for improved ALA to EPA and DHA conversion.

Figure 1: Levels of different fatty acid in rat RBC in HFHC animal model

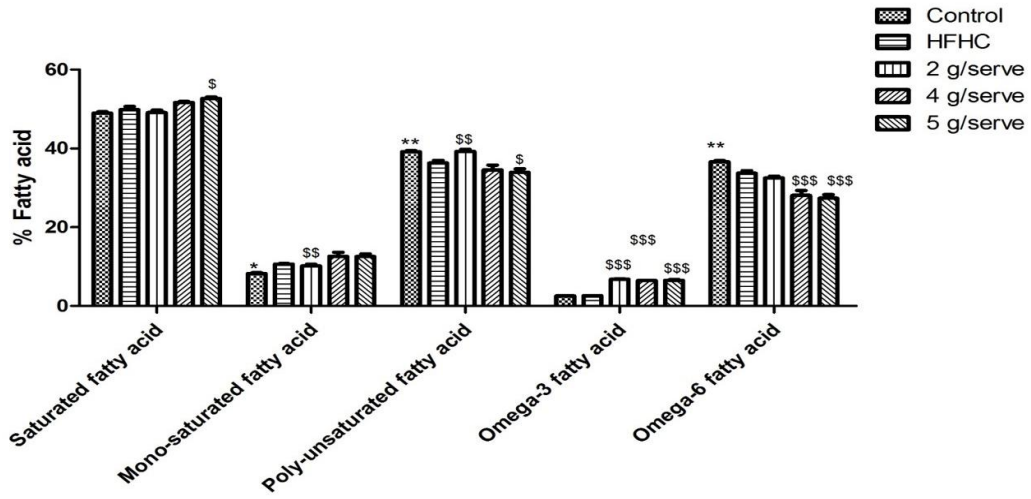


Figure 2: Histopathology of Heart tissues using Masson Trichome stain

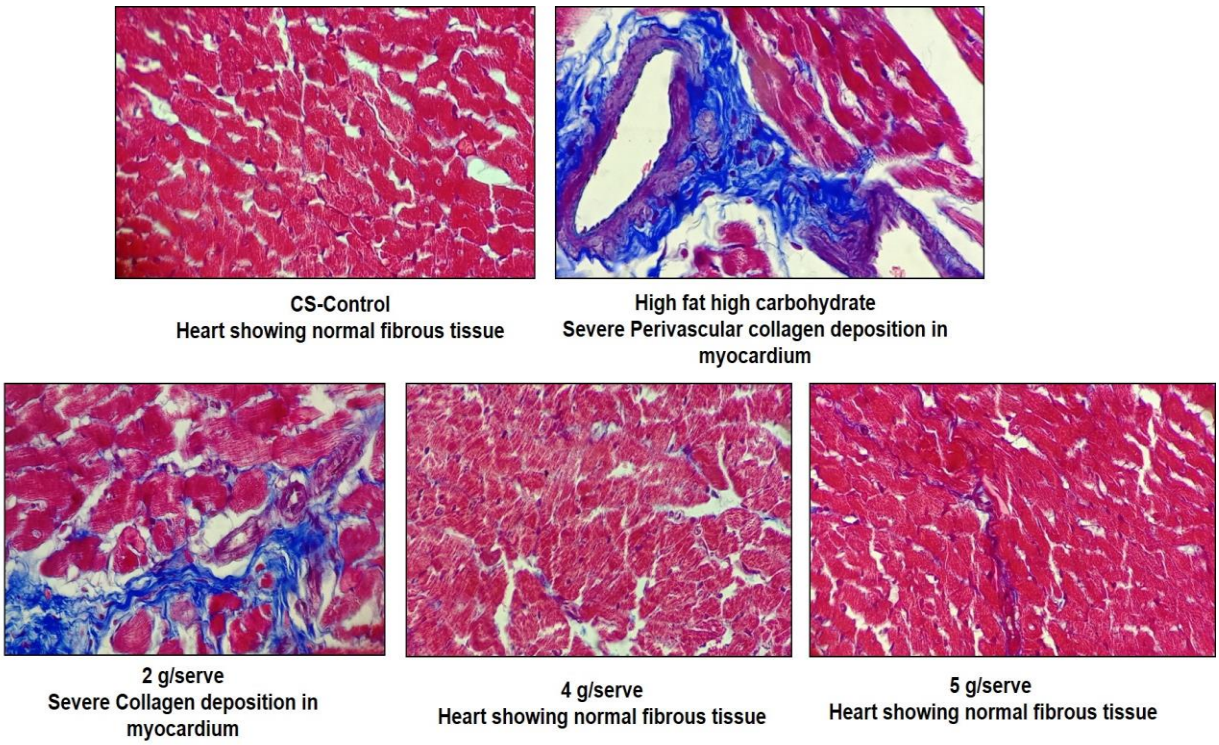
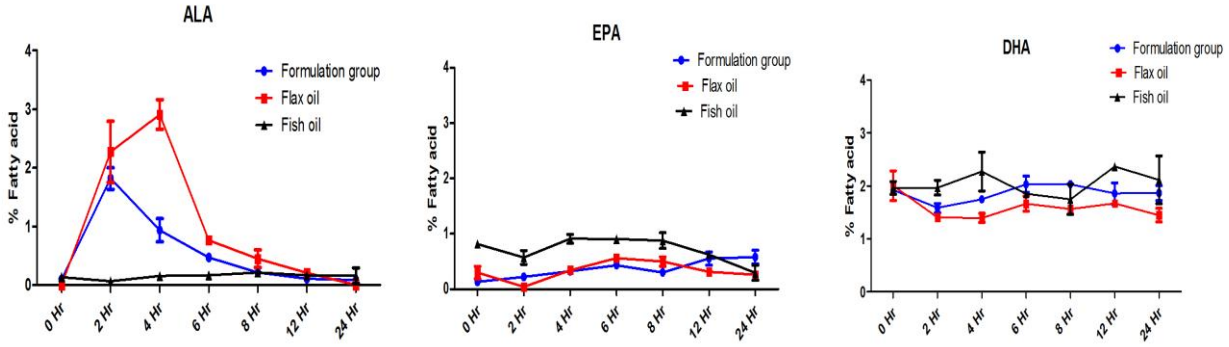


Figure 3: Single dose bioavailability study of formulation as compared to flax and fish oil



Conclusion:

Protective effect of omega-3 antioxidant formulation in metabolic syndrome based on biochemical and histopathology analysis.

Single dose in bioavailability study confirmed better bioavailability of the metabolites of ω-3 fatty acid i.e. EPA and DHA in the formulation treated group as compared to flax oil and comparable bioavailability to that of fish oil.

The conversion of ALA to DHA was better than flax oil and comparable to that of fish oil.

3.Title: Extraction of bioactive lignan and development of value added products from flaxseed (**Project ID:** - INHD/19/1/E (New project started in 2019); **Funding:** SERB and Industry (RWNLF, Pune); **Sanctioned amount:** 51.30 Lakh; **PI:** Dr. Anand Zanwar; **Co-investigator:** M. L. Panse; **Collaborator:** Real World Nutritional Laboratory Foundation, Pune; **Duration:** 27th November 2019 to 26th Nov 2022; **Ethics Committee approval**: BVDUMC/3020/2019/001/009

Background: Flaxseed cake is a by-product as a part of omega-3 oil extraction process at Real World Nutrition Laboratory Foundation (RWNLF), Pune. RWNLF, Pune has developed and successfully commercialized various omega 3 enriched products resourced from flaxseed oil. Leftover cake contains 8% fat, 20% protein, 28% total dietary fiber, carbohydrate less than 5% and essential micronutrients. Extraction of lignan from flax seed and omega-3 nutritious products in some commercially viable ways, is present requirement of RWNLF, Pune. Further stability of developed products is required for certification of the products. Considering health importance and demand, these products will have potential for commercialization. As on today lignan concentrate and lignan based products are not available in India, hence this is a profitable venture for pharmaceutical and neutraceutical, herbal drug industries.

The specific aim of the project is development of simple, efficient, economical lignan extraction method from flaxseed and development of value added food products using flax lignan alone and in combination with omega-3 FA for improved health benefits. Once products are ready, then up-scaling of technology will be done at RWNLF, Pune. Further initially test marketing will be undertaken by RWNLF and based on the consumer and market response up-scaling of product will be carried out.

Objectives:

To extract lignan concentrate by solvent-solvent extraction method

To characterize and nutritional evaluation of lignan concentrate

To study toxicity of lignan formulation

To develop formulation of flaxseed lignan and omega-3 fatty acid

Work done:

Institutional animal ethics committee approval was obtained (Approval no. BVDUMC/3020/2019/001/009 dated 22-10-2019).

Appointment of project staff and training.

Equipment processed by e-tender and BV approval was obtained.

Lab scale trials of extraction of lignan from flaxseed was initiated.

4.Title: Development of infection-resistant urinary Foley catheter (**Project ID:** INHD/16/2/I); **Funding**: BVDU, Pune; **Investigators/ Co-Is / Co-PIs**: Dr. Arnab K. Ghosh; **Duration of the project**: January 2016 – June 2020

Background: We have aimed to develop a novel antimicrobial composition and method of coating to produce antimicrobial coated urinary catheter, which would provide a prolonged and broad spectrum antimicrobial efficacy. Our previous *in vitro* studies demonstrated that newly developed antimicrobial coated urinary catheter (Anti-Bac) was found to be more effective in preventing adherence of uropathogens like *E. coli*, *K. pneumoniae*, *P. aeruginosa*, P. *mirabilis* and *C. albicans* on catheter surface, when compared to uncoated and commercially available "Bactiguard^{TM}" infection resistant urinary catheters. Here, we have evaluated the mechanical properties of the coated Anti-Bac catheter post soaking in artificial urine, compared to uncoated catheter. We have also determined whether coated Anti-Bac catheter has retained antimicrobial activity against the uropathogens, after 6 months of storage at room temperature.

Work done:

1. Evaluation of mechanical properties of catheters post soaking in artificial urine: Uncoated and antimicrobial coated Anti-Bac catheter segments (2 inch long) were placed in

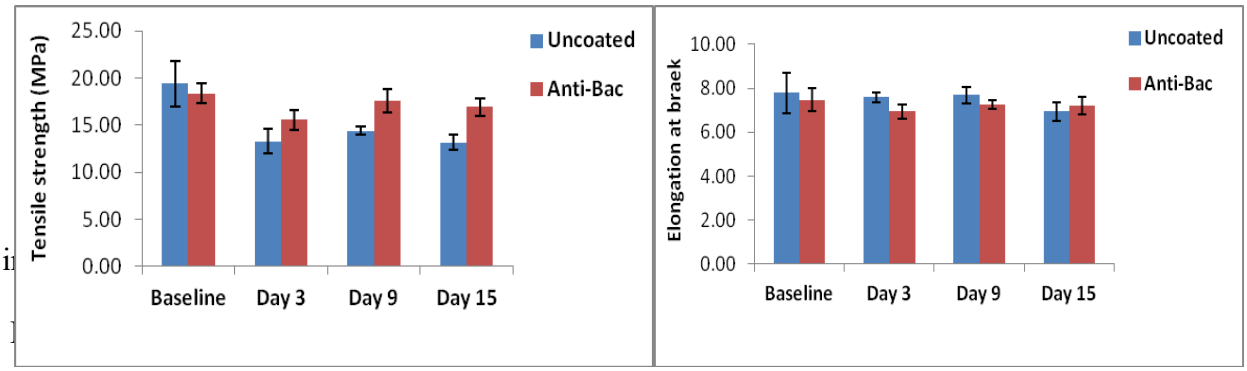
separate tubes containing artificial urine and incubated at 37°C for 3, 9 and 15 days. Triplicate catheter segments from each group were left unsoaked. Physico-mechanical properties (tensile strength, Young's modulus and elongation at break) of the uncoated and antimicrobial coated catheters were assessed using a computerized universal testing machine (Max capacity 1 KN).

2. Testing of antimicrobial activity of the coated catheter during storage: In order to determine if antimicrobial catheters would remain active, uncoated and coated catheters were stored at room temperature (25°C) for 6 months and then tested the antimicrobial activity by immersing the catheter segments (0.5 cm) in sterile artificial urine and incubating at 37°C. Catheter segments were removed for testing from the artificial urine at 1, 5, 10 and 15 days. Antimicrobial activity of the catheter segments against uropathogens at different intervals was evaluated by zone of inhibition test and compared with the baseline zones of inhibitions determined before immersion in urine.

Results:

1. Physico-mechanical properties of the catheter after soaking in artificial urine: Physico-mechanical properties of uncoated and coated Anti-Bac catheters were compared, before and after soaking in artificial urine. There were no statistically significant differences in tensile strength ($p = 0.1522$) [Fig. 4], elongation at break ($p = 0.2375$) [Fig. 5] and Young's modulus ($p = 0.0669$) [Fig. 6] of uncoated and coated Anti-Bac catheters, when compared in their unsoaked state (baseline) or soaked in artificial urine for 3, 9 and 15 days.

Fig. 4: Tensile strength of catheters post-soaking



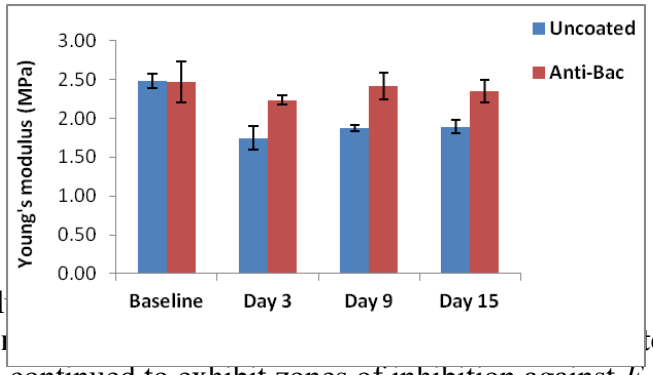


Fig. 6. Young's modul

Retention of antimici **torage**: Coated Anti-Bac catheter segments continued to exhibit zones of inhibition against *E. coli* CFT073, ESBL *K. pneumonia* 185, *E. faecalis* (ATCC 29212) and *C. albicans* 167 throughout 15 days of suspension in artificial urine. However, there were no detectable zones of inhibition against *P. aeruginosa* (ATCC 15442) around the Anti-Bac catheter segment that had been suspended in artificial urine for 10 days or more (Fig. 7). Uncoated catheters did not exhibit zones of inhibition against the tested organisms.

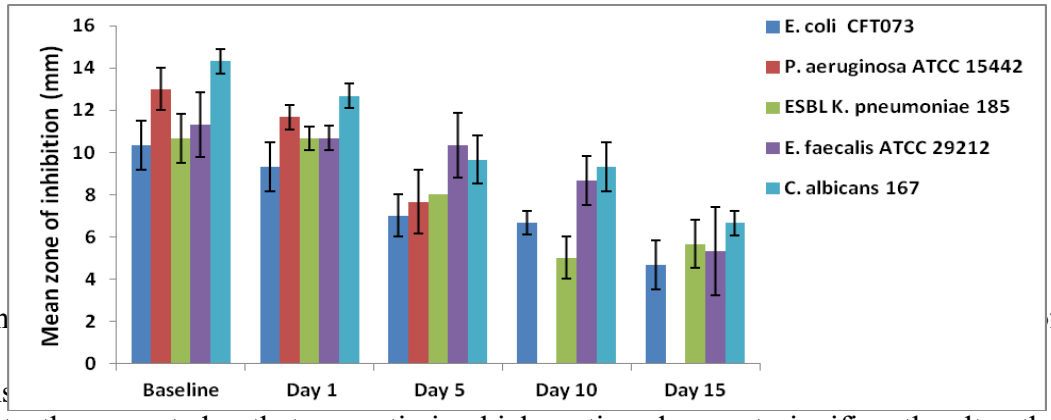


Fig. 7: Zon f storage

Conclusions

Compared to the uncoated catheters, antimicrobial coating does not significantly alter the physico-mechanical properties of the coated Anti-Bac catheters, before or after soaking in artificial urine.

After 6 months of storage at room temperature, coated Anti-Bac catheters have retained antimicrobial activity against the tested bacterial and fungal isolates.

5.Title: Development of a novel antimicrobial hand sanitizer (**Project ID:** INHD/20/1/I (New project initiated in 2020); **Funding:** BVDU, Pune; **Duration:** June 2020-22; **Investigators: PI-** Dr. Arnab K. Ghosh

Background: Hygienic hand antisepsis is one of the most important measures to prevent infections in healthcare settings and outbreak-associated viral infection. World Health Organization (WHO) has proposed two alcohol based hand rubs, commonly referred to as hand sanitizers, in order to reduce the transmission of pathogens by hands. However, there is a continuing desire for a novel antimicrobial composition that is non-irritating, safe, rapidly and persistently effective against broad spectrum of pathogens, including pandemic SARS-CoV2 virus (COVID 19) in various professional and non-professional settings.

Work done:

1. Development of a novel antimicrobial formulation: A novel antimicrobial hand sanitizer, "Germ-free hand sanitizer" has been developed, where ethyl alcohol and benzalkonium chloride were used as active ingredients. Additionally, low concentrations of orange oil, aloe vera extract, 1,2 octanediol and polyquaternium 10 were added in the formulation.

2. Evaluation of rapid antimicrobial efficacy of hand sanitizers in suspension: *In vitro* rapid (15 seconds contact time kill) and broad spectrum antimicrobial efficacy of newly developed hand sanitizer i.e., Germ-free sanitizer was tested and compared to WHO sanitizer and commercially available Dettol sanitizer, against various microorganisms according to the standard ASTM E 2783-11 method. For control, PBS was used instead of sanitizer.

3. Evaluation of persistent activity of hand sanitizers using porcine skin model, simulating standard ASTM E1882-10 method: Our newly developed "Germ-free hand sanitizer" had been evaluated for persistent antimicrobial activity by agar patch test, at the laboratory of Dr. Shanta Modak (our collaborator), Columbia University, USA. Briefly, the test hand sanitizer was applied on a pair of sterilized circular porcine skins. Then, the skins were rubbed together with each other for 30 seconds and allowed to keep at room temperature for 20 min and 40 min., PBS was used as control. The pigskins, previously applied with either test product or PBS, were pressed (using metal cylinders of same weight) for 10 minutes over the trypticase soy agar (TSA) plates inoculated with *S. aureus* culture. The cylinder was then removed and TSA plates were incubated for overnight at 37°C.

Results:

1. Evaluation of rapid antimicrobial efficacy of hand sanitizers:

Rapid antimicrobial efficacy of the hand sanitizers (*viz*. Germ-free sanitizer, WHO sanitizer and Dettol sanitizer) is presented in Table 5. According to ASTM E2783-11 method, all the tested hand sanitizers showed rapid and broad spectrum antimicrobial activity in suspension.

Table 5: Rapid and broad spectrum antimicrobial activity of hand sanitizers:

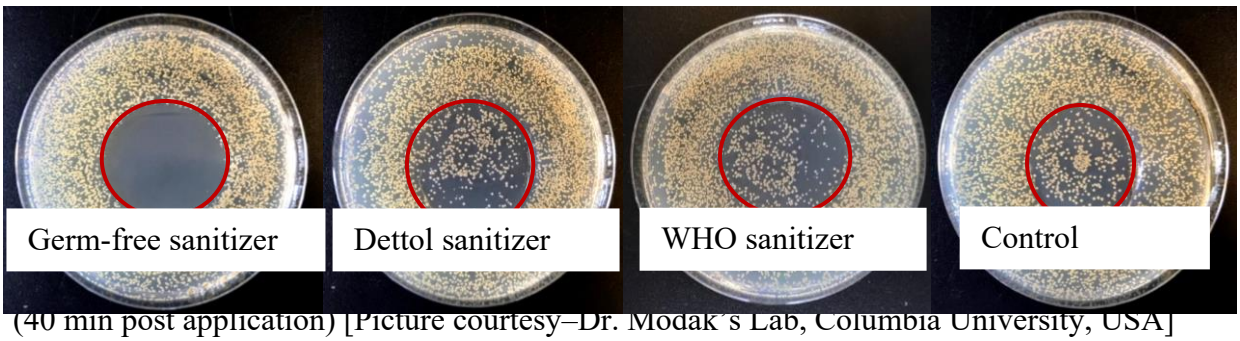
Microorganism	Rapid (15 seconds contact time) antimicrobial efficacy of sanitizers [Log_{10} reduction]		
	Germ-free sanitizer	WHO sanitizer	Dettol sanitizer
S. aureus (ATCC 6538)	5.60 ± 0.18	5.38 ± 0.24	5.15 ± 0.18
E. coli (ATCC 25922)	5.3 ± 0.37	5.3 ± 0.36	5.3 ± 0.27
P. aeruginosa (ATCC 15442)	4.92 ± 0.31	5.25 ± 0.47	5.0 ± 1.00
K. pneumoniae (ATCC 10031)	5.0 ± 0.65	4.8 ± 0.53	5.35 ± 0.65
C. albicans (ATCC 10231)	4.78 ± 0.47	4.35 ± 0.31	4.55 ± 0.56

Values are represented as mean ± SD of three independent experiments (n=3)

Control counts ranged from $5X10^5$ to $1X10^6$ cfu/ml

2. Evaluation of persistent **activity** of antimicrobial hand sanitizers:

Persistent antimicrobial activity of the hand sanitizers were evaluated and compared against *S.aureus* by agar patch test (Fig. 8). Germ-free hand sanitizer showed significantly higher persistent antimicrobial activity compared to WHO and Dettol sanitizers, 20 minutes and 40 minutes after application ($P < 0.05$). On the other hand, WHO sanitizer and Dettol sanitizers showed negligible persistent activity.



(40 min post application) [Picture courtesy–Dr. Modak's Lab, Columbia University, USA]

*Red circles denote the area on inoculated agar plates, where direct contact between S. aureus and test hand sanitizer took place. No viable S. aureus colony had been found, when Germ-free hand sanitizer was interacted with the inoculated agar plate.

Conclusions:

Germ-free hand sanitizer has shown rapid and broad spectrum antimicrobial activity.

Germ-free hand sanitizer kills more than 99.99% of common microbes in suspension.

Germ-free hand sanitizer has shown persistent antimicrobial activity (20 minutes and 40 minutes post application) against *S. aureus*, whereas, WHO and Dettol sanitizers have shown negligible persistent activity.

6.Title: Development of Chromatographic Method for Quantification of Glycolytic Intermediates and Its Correlation with Enzyme Kinetics in Type – II Diabetes (**Project ID**: INHD/16/4/I/P); **Funding:** Institutional; **Duration:** September 2013 to August 2020; **Ph.D. Students:** Ms. Sunita Shivaji Bhise; **Guide-** Prof. Dr. Janhavi R. Rao and Prof. M. V. Hegde; **Human Ethical Approval**: DCGI Reg. No. ECR 518

Background: In our earlier studies, we have demonstrated the enzyme activities of the rate limiting steps of glycolysis that were altered in RBCs from type 2 diabetic patients. In particular, two irreversible ATP utilizing activity enzymes of glycolysis viz. Hexokinase and phosphofructokinase showed significant changes in their activity with marked alterations in kinetic properties. The kinetic parameters of the terminal enzyme lactate dehydrogenase also changed significantly. In view of these observed changes it was of significance to know, if and how the flux of intermediates of the glycolytic pathway is also affected. With this objective, a simple, rapid and sensitive liquid chromatography tandem mass spectroscopy (LC-MS/MS) method has been developed for quantification of five important glycolytic intermediates of the rate limiting steps (glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, 3-phosphoglyceric acid and pyruvate) in human RBCs.

Work done:

- The LC-MS/MS method has been successfully developed and standardized for quantification of five important glycolytic intermediates (glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, 3-phosphoglyceric acid and pyruvate) using standard mixture.
- The developed method is successfully applied to quantify above stated glycolytic intermediates in RBCs from control and type 2 diabetic patients.
- The LC-MS/MS data was successfully analysed and interpreted and sent for publication.

Result and Discussion:

The aim of the present study was to determine the intra-RBC concentrations of sugar phosphate metabolites of glycolysis pathway as influenced by the diabetic state since this has direct bearing on ATP synthesis. Hence in the present studies, a method was developed especially for rapid quantification of targeted five sugar phosphates metabolites of glycolysis pathway of RBCs from Type 2 diabetic subjects as against normal controls, by employing LC-ESI-MS/MS technique. It must be noted that the reported literature values of glycolytic intermediates are absolute quantities and not the intracellular molar concentration that we are presenting here. The targeted sugar phosphates i.e. G-6-P, F-6-P, FBP, 3-PGA and pyruvate is the metabolites i.e. substrates or end-products at the rate limiting steps of glycolysis pathway. The average contents of G-6-P, F-6-P, FBP, 3-PGA and pyruvate from the Control and Diabetic groups are shown in Table. 6

Table. 6 - The concentrations of major Glycolysis Intermediate s in RBCs from Control and Type 2 Diabetic Subjects

Sr. no.	Metabolite	Concentration in RBCs, µM	
		Controls (12)	Diabetics (40)
1	G-6-P	250 ± 67.74	903 ± 82.8*
2	F-6-P	137 ± 2.25	243 ± 0.39*
3	FBP	216 ± 25.74	128 ± 19.9**
4	3-PGA	190 ± 4.28	310 ± 1.60*
5	Pyruvate	205 ± 15.96	148 ± 14.69**

The results are given as mean ± SEM of number of observations indicated in parentheses.
*, $p < 0.0003$, **, $p < 0.05$.
It is apparent that the concentration G-6-P was about 3.5fold higher in the diabetic group. Similarly, the concentration of F-6-P was about 77% higher. Further, the concentration of FBP and pyruvate decreased by 40% and 28 % respectively, whereas that of 3-PGA increased by 63%. Taken together, the results suggest that the flux of glycolysis is altered significantly in the diabetics and that eventually this leads to lactic acidosis. In our earlier studies we found that hexokinase activity is higher in the diabetic RBCs and therefore the 3.5-fold increase in G-6 P is consistent with our earlier observation. We have reported earlier that the phosphofructokinase activity is decreased which is consistent with the lower levels of FBP observed here. It seems that despite higher input of glucose in the glycolytic cycle in diabetic RBC, there seems to be decrease in the ATP production. This needs to be confirmed.
It can be provisionally concluded that from our study of glycolysis in diabetic RBC, there is increased glycosylation of hemoglobin because of higher levels of G6P and also there is alteration in the ATP production, which may be responsible for the loss of deformability of RBC and other diabetic complications
We have reported earlier that the RBC membrane structure is abnormally different in the diabetic group in that the membrane proteins almost double without significant changes in the lipid profiles. As is evident, there is loss of RBC deformability in diabetes. The RBCs are not able to squeeze to pass through nor reform their shape after passing through capillaries. In the process the RBCs breaks and undergoes fragmentation. Fragments of RBCs are already noted in kidney glomeruli in nephropathy. In the RBCs ATP has several important functions. These include binding of oxygen to hemoglobin, restoration of glutathione level, maintenance of Na^+-K^+ pump and restoration of RBC reformability. Thus, it has been reported that binding of ATP to spectrin plays an important role in reformability of the RBCs. Possibly this would suggest that the spectrin confirmation is an energy-dependent process. One therefore wonders as too whether in the diabetic RBCs the binding of ATP results in futile cycles leading to enhance turnover i.e. hydrolysis of ATP after binding to spectrin; increased flux of glycolysis restores ATP concentration that to normality. The results are thus suggestive of increase turnover of ATP and futile cycle. This possibility, however, needs to be carefully evaluated.

Conclusion:

In conclusion our results show that while the flux of glycolysis is enhanced in diabetic RBCs, structural and functional abnormalities remain a matter of concern. Loss of deformability ultimately leads to fragmentation of RBCs; which results in occlusion of capillaries. This intern is the cause for initiation of micro-angiopathy ultimately leading to diabetic complications. As mentioned above, fragments of RBCs in kidney glomeruli has been already been reported. It is not surprising then that a diabetic nephropathy represents the first and foremost example of diabetic complications.

7.Title: Developing Omega-3 Edible Oil Blends and Evaluating Their Effects and Safety in Pre-Clinical Studies (**Project ID:** INHD/16/5/I/P); **Funding:** Institutional and AICRP Centre; **Duration:** Registered in 2018; **Ph.D. Students**: Ms. Asavari Joshi; **Guide:** Dr. Anand A. Zanwar; **Co-Guide:** Prof. M. V. Hegde

Background: During 2018-2019, Palm olein blends (PO) with Flax seed oil (FSO) were prepared and analyzed for nutritive quality, oxidative stability, thermal stability, cell viability and anti-inflammatory effect. Similar studies have been done for Groundnut oil (GNO) and FSO blends.

Work done:

- GNO blends containing 20,10 and 5% FSO have been prepared and indicated as G20, G10 and G5 respectively

Physico-chemical characterization of GNO blends (Nutritive quality, oxidative stability and thermal stability) has been analyzed

- GNO blends have been evaluated for their effect on cell viability in THP-1 and $HepG_2$ cell lines
- Effect of the treatment of individual and PO blended oils on THP-1 cells in terms of cellular fatty acid analysis and lipid accumulation has been evaluated

Results: Physico-chemical characterization of GNO blends

Table 7. Fatty acid composition of GNO blends by Gas Chromatography (GC)

Oil	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Alpha Linolenic acid	ω6: ω3
FSO	6.45±0.142 $^{\#}$	6.46±0.12$^{\#}$	21.66±0.07$^{\#}$	15.00±0.84 $^{\#}$	50.42±0.64$^{\#}$	0.30
GNO	12.13±0.26	3.96±0.28	59.86±0.18	24.05±0.22	0.00±0.00	-
G5	11.60±0.14 $^{\$}$	3.73±0.40$^{\$}$	56.73±0.97$^{*\$}$	23.63±0.36 $^{\$}$	3.49±0.68$^{*\$}$	6.76
G10	11.47±0.21 $^{\$}$	3.77±0.51$^{\$}$	54.84±1.07$^{*\$}$ $^{\pi}$	22.95±0.57 $^{\$}$	6.16±0.26$^{*\$\beta}$	3.72
G20	10.93±0.13 $^{\$}$	3.48±0.56$^{\$}$	48.90±1.72$^{*\$}$ $^{\beta}$	23.27±0.56 $^{\$}$	13.42±1.36$^{*\$\beta}$	1.73

Table 7. Fatty acid (FA) analysis was done by preparing fatty acid methyl esters (FAME) of the individual or blended oils followed by GC. Two-way ANOVA and Bonferroni posttests were applied to determine statistical significance. When compared with GNO, all the blends showed statistically significant change in Oleic acid (OA) and Alpha Linolenic acid (ALA) (*, $p < 0.001$). When compared with GNO, FSO showed statistically significant difference in all the FAs (#, $p< 0.001$). When compared with FSO, all the blends showed statistically significant difference in all the FAs ($, $p< 0.001$). When compared with G5, all the blends showed statistically significant difference in OA and ALA (β, $p< 0.001$) except in case of G10-OA (π , $p< 0.01$). Similar was the case with G10.

Table 8: Various parameters assessed for evaluating stability of blends

Oil	Parameters (immediately after blend preparation)			
	Acid Value (mg KOH/ g oil)	%Free Fatty Acid (as oleic acid)	Peroxide Value (milliequivalent O_2/ kg oil)	Smoke Point (˚C)
FSO	0.48±0.09	0.2±0.01	0.4±0.03	103±1.41
GNO	1.74±0.00*	0.63±0.03$^{@}$	4.05±0.07$^{\%}$	202.5±2.50$^{\#}$
G5	1.74±0.08*	0.66±0.04$^{@}$	4.05±0.21$^{\%}$	191±1.00$^{\#, \omega}$
G10	1.85±0.16*	0.67±0.00$^{@}$	4.05±0.07$^{\%}$	189±1.00$^{\#, \omega\omega}$
G20	1.82±0.04*	0.69±0.02$^{@}$	4.25±0.07$^{\%}$	191.5±0.50$^{\#, \omega}$

Table 8. All the parameters were determined by following standard guidelines of AOAC. One ay ANOVA and Tukey's Multiple Comparison Test was applied to determine statistical significance. In case of Acid Value, when compared with FSO, GNO, G5, G10 and G20 showed statistically significant difference (*, $p<0.05$). No statistically significant difference among GNO and the blends were observed. In case of %Free Fatty Acid (FFA), there was no statistically significant difference between GNO and any blend or among the blends. When compared with FSO, GNO and all blends have statistically significantly high FFA values (@, $p<0.05$). In case of Peroide Value, when compared with FSO, GNO, G5, G10 and G20 showed statistically significant difference (%, $p<0.05$). No statistically significant difference among GNO and the blends were observed. In case of Smoke point, when compared with FSO, GNO and all the three blends have statistically significantly high Smoke points (#, $p<$ 0.01). When compared with GNO, all the three blends have statistically significantly low Smoke points (ω, $p < 0.01$). Smoke points of the blends were statistically not different.

Evaluation effect of GNO blends on cell viability in THP-1 and $HepG_2$ cell lines

Figure 9: Effect of GNO blends on viability of THP-1 and HepG2 cells

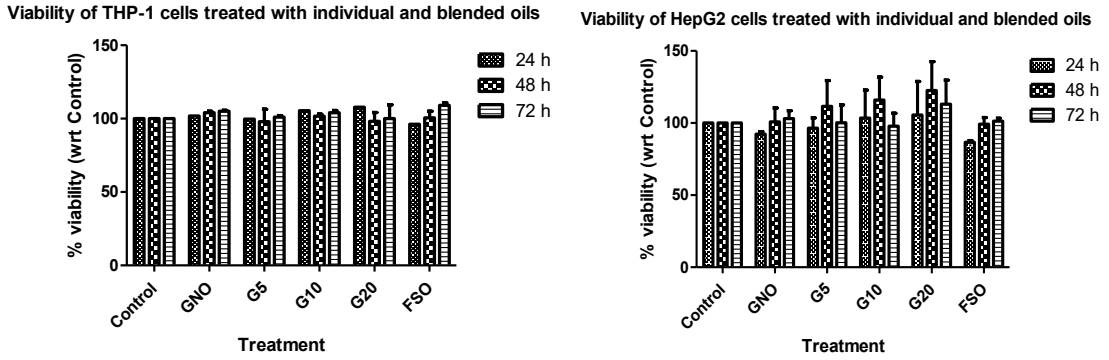


Figure 9. Effect of individual or blended oils on viability of THP-1 and HepG2 was evaluated by MTT assay. Two-way ANOVA and Bonferroni posttests were applied to determine statistical significance. No statistically significant differences in the viability were seen when compared with control for the respective time points in both the cell lines when treated with 250 µg/ml individual oils or blends for mentioned time points. Even the blends did not show statistically significant differences when compared with each other.

Determination of effect of the treatment of individual and PO blended oils on THP-1 cells

Table 9: Effect of Palm olein blends on fatty acid composition of THP-1 cells

FA	C	FSO	PO	PO20	PO10	PO5
Palmitic Acid	19.90±4.76	16.14±3.74	18.90±0.37	17.18±0.85	16.72±2.74	16.63±2.61
Stearic Acid	10.50±2.86	13.62±7.37	8.92±1.43	9.68±0.48	9.77±1.47	8.72±0.07
Oleic Acid	5.91±0.40	9.01±1.41	15.18±3.06 ***	14.91±0.03 ***	10.39±2.70	11.61±5.13 **
Linoleic Acid (LA)	1.76±1.21	3.68±1.25	3.30±1.82	4.04±0.72	2.42±0.68	3.00±0.24
Alpha Linolenic Acid (ALA)	-	7.02±.38***	-	1.33±0.45	0.62±0.02	0.30±0.01
Arachidonic Acid (AA)	4.42±1.51	2.61±1.94	3.81±1.19	3.98±0.24	4.16±0.97	3.67±0.24

Docosahexanoic Acid (DHA)	1.66±0.09	1.31±0.43	1.59±0.47	2.73±1.46	1.87±0.06	1.66±0.04
Others	55.86±7.60	46.60±8.51	48.32±2.16	46.15±2.79	54.05±7.28	54.41±8.17
LA/ALA	-	0.52±0.15	-	3.32±1.67#	3.95±1.23#	10.03±1.28
(LA+AA)/ (ALA+DHA)	3.73±0.02	0.74±0.31	4.74±1.80	2.25±1.18	2.65±0.03	3.41±0.19

Table 9. THP-1 cells were treated with the oils and blends for 48 h. FAME were prepared after total lipid extraction from the cells and were subjected to FA analysis by GC-FID. Data is represented as Mean±SD of %FA of the total extracted lipids. Two-way ANOVA and Bonferroni posttests were applied to determine statistical significance. ***: $p<0.001$vs C, **: $p<0.01$vs C and #: $p<0.01$vs PO5

Figure 10: Effect of the blends on lipid accumulation in THP-1 derived macrophages

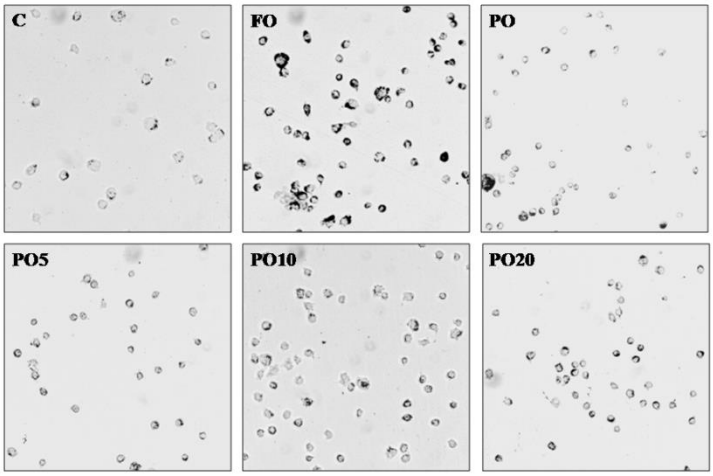


Figure 10. Lipid accumulation in the THP-1 derived macrophages was detected by Oil Red O staining. Figure shows the presence of very few lipid droplets in both the control cells (C) and cells treated with PO. In case of FSO treated cells, intense colored lipid droplets were observed in almost all cells. Similarly, treatment with the blends caused an increase in the intensity of lipid droplets and the number of the cells possessing such droplets.

Conclusion:

Physico-chemical characterization of GNO blends indicates that blending with FSO has not adversely affected the studied parameters. The cell viability data indicates that at 250 µg/ml, upto 72 h, these blends are not affecting viability of the THP-1 cells and HepG2 cells. Palm olein blends resulted in dose dependent increase in ALA percentage with simultaneous lowering of LA/ALA ratio indicating incorporation of ALA from the blends in the cells. Lipid droplet formation in the cells also indicates that fatty acids are utilized in the form of triglycerides and fatty acids from FSO trigger lipid droplet accumulation.

Other Information

Budget

Extramural Grants (newly sanctioned and ongoing) Total Projects:28

Sr no	Funding Agency	Title of the project	Total grant sanctioned (In Lakhs)	Amount Received (INR) In Lakhs	Expenditure (INR) In Lakhs
1	DBT-BIRAC	Establishment of National Centre for Immunogenicity Testing, NCIT to evaluate vaccines in clinical trials	Rs.16 crore	No funds received in financial year 2019-2020.(Funds received in March 2019)	627.10
2	Indian Council of Medical Research, Government of India	Investigating Mechanisms Leading to Preeclampsia	Rs. 681.53	Rs.135.24	Rs.141.93
3	ICMR and Serum Institute of India	Establishment of a novel Electronic Surveillance System for dengue in Pune: an initiative for Smart Cities Mission	Rs.400.10	Rs 67.00	Rs.52.13
3	ICMR Dengue Surveillance	Establishment of a novel Electronic Surveillance System for dengue in Pune: an initiative for Smart Cities Mission	Rs.400.10	0	Rs.45.86
4	DBT Wellcome India Alliance – Ashwini Hinge		Rs 343.84	Rs 77.16	Rs 61.57
4	Department of Biotechnology, Government of India	Early Interventions to Support Trajectories for Healthy Life in India (EINSTEIN). Healthy Life Trajectories Initiative (HeLTI	Total Amount Awarded: Rs. 743.44 IRSHA SHARE: 13.50	-	0
5	Department of Biotechnology, Government of India	Influence of Maternal One-carbon (1C) Metabolism in Placental Function, Fetal Growth and Programming	Total Amount Awarded: 161.98 IRSHA SHARE: Rs. 68.5	Rs 14.04	Rs 18.23

6	Wellcome-DBT Indian Alliance	Development of potent adjuvanted respiratory syncytial virus vaccine for mucosal delivery	168.93	Rs 23.11	Rs 31.07
7	ICMR	Evaluation of circulatory biomarkers for disease severity in hepatitis E	81.95	Rs 21.81	Rs 5.54
8	DBT-Reproductive Biology	Epigenetic regulation of angiogenic factors in assisted reproductive technology (ART) and non-ART derived placentae	Rs. 59.91	Rs 19.99	Rs 14.13
9	Effect of Yoga intervention on skeletal muscle linked glucose homeostasis in pre-diabetic individuals	DST-SATYAM	Rs 46.74	No funds received in financial year 2019-2020.(Funds received in March 2019)	Rs 13.30
10	DBT-BioCARe	Platelet derived exosomes and their role in endothelial dysfunction in dengue infection	Rs 46.39	Rs 16.01	Rs 15.08
11	Department of Science and Technology	Pro-neurotrophins /p75NTR Signalling Contributes to Increased Apoptosis in Preterm Placentae	Rs. 40.48	0	0
12	Department of Biotechnology, Government of India	Epigenetic regulation of placental peroxisome proliferator activated receptor (PPAR) in women delivering low birth weight babies	Rs. 35.50	Rs 12.39	Rs 12.01
13	ICMR-DHR	Immune response of Indian preterm infants to pentavalent vaccine	Rs 34.22	Rs 15.11	Rs 20.89
14	DST-SERB	Evaluation of different adjuvants for development of potent chikungunya vaccine	Rs 32.22	Rs 4.50	Rs 7.41
15	Evaluation of Triphala and Trimad for their effects on adipocytes biology and lipid	Ministry of AYUSH	Rs 28.32	Rs 6.44	Rs 6.42

	metabolism				
16	Extraction of bioactive lignan and development of value added products from flaxseed	SERB and Industry (RWNLF, Pune)	Rs 25.00	Rs 25.00	Rs 1.39
17	Evaluating the anticancer activity and mechanism of action of Unani formulation Habbe Musaffi Khoon against cervical cancer	EMR AYUSH (CCRUM)	Rs 12.70	Rs 12.70	Rs 20.56
18	ICAR-AICRP-Linseed Value Addition Centre	ICAR	Rs 11.97	Rs 12.63	Rs 16.68
19	Evaluation of efficacy of novel stabilized omega-3-fatty acid and antioxidants formulation for the prevention and treatment of metabolic syndrome	SERB	Rs 9.00	Rs 9.00	Rs 9.99
20	Evaluating the anticancer activity of homeopathic preparation of *Linum usitatissimum* in breast cancer	EMR AYUSH (CCRH)	Rs 7.70		Rs 18.72
21	Evaluating the anticancer activity of different higher homeopathic potencies (200C, 1M, 10M, 50M,	EMR AYUSH (CCRH)	Rs 7.10		Rs 6.82

	CM) of *Terminalia chebula* (TC) in breast cancer cell lines and analyzing the best potency for activity against in vivo breast cancer model				
22	Evaluating the effect of Alpha linolenic acid (ALA), an omega 3 fatty acid, on modulation of epigenetic markers in cervical cancer cell lines.	DST SERB	Rs 6.00		Rs 11.18
23	"Phytochemical standardization and evaluation of anti-cancer and immunomodulatory activity of Unani formulation, Itrifal Gudadi"	EOI Unani	Rs 5.21		0
24	Orientation Training programme of Ayurveda for Non-Ayurveda doctors and scientist	National level programme Sanctioned by Rashtriya Ayurveda Vidyapeeth (RAV), Ministry of AYUSH, Govt. of India	Rs 6.00	Rs 6.00	Rs 5.91
25	Preparation of Ayurvedic claim substantiation document	Mi-lifestyle marketing global Pvt Ltd.	Rs 2.59	-	Rs 1.32
26	Medical Research Council , UK Yet to b initiated	OPTIMISE: Optimal preconception nutrition to offset inflammation and non-communicable disease risk in pregnant women and their children	18,79,830 POUNDS	-	-

27	Comparing vaginal microflora diversity between healthy and cervical cancer women for identifying isolates having probiotic and anticancer potential	DST WOS A	Nil	0	Rs 3.68
28	Repurposing cephalosporin antibiotics for oral cancer treatment	ICMR	Yet to be received		

Intramural funding:

Sr. No	Name of the Project	Funding Agency	Received (INR)	Expenditure (INR)
1	Outsourcing for biochemical investigations	Ayurvedic + Medical College	Rs 92000	Rs 60,013.00
2	ISTAM Project	ISTAM	Rs 30000	Rs 31,275.00
3	Sakal Foundation	Sakal Foundation	Rs 20000	Rs 7,340.00
4	Generated Funds	Solitarius	Rs 5,38,000	Rs 25,724.00
5	OTP - Symposium		Rs 75000	Rs 50,245.00

Student Fellowships

S.No	Funding Agency	Tilte of the project	Total grant sanctioned (In Lakhs)	Amount Received (INR) In Lakhs	Expenditure (INR) In Rs.
1	Indian Council of Medical Research	Dr. Akriti Sahay Research Associate fellowship July 2019-Jun 2021	Rs. 14.38	Rs 5.99	Rs 5.33
2	Indian Council of Medical Research	Anjali Jadhav SRF June 2018-May 2021	Rs. 16.22	Rs 5.92	Rs 5.67
3	Indian Council of Medical Research	Dr. Amrita Khaire Research Associate fellowship Sept 2018- Sept 2020	Rs. 14.96	Rs 8.37	Rs 7.82
4	Council of Scientific and Industrial Research	Juhi Nema JRF and SRF Apr 2017-Aug 2022	Rs. 18.20	0	Rs 2,900.00
5	Council of Scientific and Industrial Research	Kinjal Dave JRF and SRF July 2017-Aug 2022	Rs. 18.20	0	0
6	DST INSPIRE	Vaishali Kasture JRF and SRF	Rs. 18.20	Rs 5.42	Rs 5.27
7	UGC	Anindita Nandi	Rs. 21.00	0	0
8	DBT JRF	Amol Chaudhari	Rs 25.25	Rs 5.09	Rs 5.07
9	DBT JRF	Akanksha Mahajan	Rs 25.25	Rs 7.03	Rs 6.46
10	DST Inspire	Rama Rajadnya	Rs 22.57	Rs 4.51	Rs 4.45
11	ICMR	Ms. Mrunal Gosavi	Rs 16.22	Rs 5.41	Rs 4.10
12	CSIR HRDG JRF	Manoj M. Khavate	Rs 1.62	Rs 1.62	

Publications (Total No: 22)

Sr. No.	List of Publications	Impact factor	Scopus	Web of Science	Pubmed
1	Kamini Dangat, Amrita Khaire, Sadhana Joshi. Cross talk of vascular endothelial growth factor and neurotrophins in mammary gland development. Growth Factors 2020 Jan;38(1):16-24. doi: 10.1080/08977194.2020.1792469. Epub 2020 Jul 9.	1.54	Y	Y	Y
2	Randhir Karuna, Pisal Hemlata, Kadam Vrushali, Khaire Amrita, Malashe Nandini, Deshpande Ruma, Palkar Sonali, Lalwani Sanjay, Kumaran K, Yajnik Chittaranjan, Osmond Clive, Fall Caroline, Joshi Sadhana. Association of Preeclampsia with Anthropometric Measures and Blood Pressure in Indian Children. PLoS ONE 2020 May 5;15(5):e0231989.	2.87	Y	Y	Y
3	Khaire, A., Wadhwani, N., Madiwale, S., Joshi, S. Maternal fats and pregnancy complications: Implications for long-term health. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2020 Jun;157:102098. doi: 10.1016/j.plefa.2020.102098. Epub 2020 Apr 21.	2.88	Y	Y	Y
4	Alka Rani, Preeti Chavan-Gautam, Girija Wagh, Savita Mehendale, Narayanan S Mani, Sadhana R Joshi. Region-specific changes in the mRNA and protein expression of LCPUFA biosynthesis enzymes and transporters in the placentae of women with preeclampsia. PLACENTA. 2020 Jun;95:33-43. doi: 10.1016/j.placenta.2020.04.013. Epub 2020 Apr 27.	2.77	Y	Y	Y
5	Wadhwani NS, Sundrani DP, Wagh GN, Mehendale SS, Tipnis MM, Joshi PC, Kinare AS, Lalwani SK, Mani NS, Chandhiok N, Chandak GR, Gupte SA, Fall CHD, Joshi SR. The REVAMP study: research exploring various aspects and mechanisms in preeclampsia: study protocol. BMC Pregnancy Childbirth. 2019 Aug 23;19(1):308.	2.65	Y	N	Y

6	Sahay AS, Jadhav AT, Sundrani DP, Wagh GN, Joshi SR. Differential Expression of Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF) in Different Regions of Normal and Preeclampsia Placentae. Clin Exp Hypertens. 2020 May 18;42(4):360-364. doi: 10.1080/10641963.2019.1665677. Epub 2019 Sep 14.	1.78	Y	Y	Y
7	Kasture V, Sundrani D, Dalvi S, Swamy M, Kale A, Joshi S. Maternal omega-3 fatty acids and vitamin E improve placental angiogenesis in late-onset but not early-onset preeclampsia. Mol Cell Biochem. 2019 Nov;461(1-2):159-170. doi: 10.1007/s11010-019-03599-4. Epub 2019 Aug 16.	2.68	Y	N	Y
8	Nema J, Sundrani D, Joshi S. Role of vitamin D in influencing angiogenesis in preeclampsia. Hypertens Pregnancy. 2019 Nov;38(4):201-207. doi: 10.1080/10641955.2019.1647231. Epub 2019 Jul 24.	1.39	Y	N	Y
9	Nandi A, Wadhwani N, Joshi S. Maternal vitamin D deficiency increases the thromboxin-prostacyclin ratio through alterations in the one carbon cycle in Wistar rats. Biofactors . 2019 Jul;45(4):548-555. doi: 10.1002/biof.1510. Epub 2019 Apr 15.	3.236	Y	N	Y
10	Suryavanshi, S., Shinde, K., Raina, P. and Kaul-Ghanekar, R., Tumor retardation and immunomodulatory potential of polyherbal formulation HC9 in mouse melanoma model. *Pharmacognosy Magazine*, 2020 March *16*(68), p.181-186.	1.31	N	N	N
11	Shetty, V., Chellampillai, B. and Kaul-Ghanekar, R., Development and validation of a bioanalytical HPLC method for simultaneous estimation of cinnamaldehyde and cinnamic acid in rat plasma: application for pharmacokinetic studies. *New Journal of Chemistry*, 13 Feb 2020*, 44*(11), pp.4346-4352.	3.288	Y	Y	N
12	Gupta, E., Kaul-Ghanekar, R. and Manhas, S.S., April 2020. Evaluating the Anticancer Activity of Homoeopathic	-	N	N	N

	Preparation of Asterias rubens in Breast Cancer Cell Line on the Basis of Similia Principle. *International Journal of Health Sciences and Research*, *10*(4), pp.90-94.				
13	Salunke, M., Banjare, J. and Bhalerao, S. Sept- Dec 2019. Effect of selected herbal formulations on anthropometry and body composition in overweight and obese individuals: A Randomized, Double blind, Placebo-controlled study. Journal of Herbal Medicine 17-18, 100298.	2.221	Y	Y	N
14	Gupte, P., Harke, S., Deo, V., Bhushan Shrikhande, B., Mahajan, M. and Bhalerao, S. April-June 2020. A clinical study to evaluate the efficacy of Herbal Formulation for Obesity (HFO-02) in overweight individuals. Journal of Ayurveda and Integrative Medicine 11, 159-162.	2.1	Y	N	Y
15	Shrivastava, S., Solaskar, A., Gosavi, M., Tiraki D., Mishra AC, Arankalle VA. Evaluation of NS1-Detection-Based Cell Culture Method for Isolation of Dengue Viruses from Clinical Samples. SN Compr. Clin. Med. 2, 613–618 May 2020.	-	N	N	N
16	Kulkarni R, Modak M, Gosavi M, Wani D, Mishra AC, Arankalle VA. Comparative assessment of commercial enzyme-linked immunosorbent assay & rapid diagnostic tests used for dengue diagnosis in India. Indian J Med Res. 2020 Jan;151(1):71-78. doi: 10.4103/ijmr.IJMR_613_18. PMID: 32134017; PMCID: PMC7055168.	1.503	Y	Y	Y
17	Arankalle VA, Kulkarni R, Malshe N, Palkar S, Lalwani S, Mishra AC. Seroepidemiology of respiratory syncytial virus in western India with special reference to appropriate age for infant vaccination. J Med Virol. 2019 Aug;91(8):1566-1570. doi: 10.1002/jmv.25489. Epub 2019 May 6. PMID: 31012488.	2.049	Y	Y	Y
18	Hegde Mahabaleshwar. Unleashing the power of linseed to improve public health and combat non communicable disease menace. Indian Journal of Agricultural Biochemistry. Aug 2019, 32(1),1-9. DOI:		Y	N	N

	10.5958/0974-4479.2019.00001.7				
19	Silva CD, Israni N, Zanwar AA, Jagtap A, Leophairatana P, Koberstein JT, Modak SM. "Smart" polymer enhances the efficacy of topical antimicrobial agents. Burns. Volume 45, Issue 6, September 2019, Pages 1418-1429.	2.066.	Y	Y	Y
20	Bhise SS, Rao JR, Hegde MV, Katyare SS. Type 2 diabetes differentially affects the substrate saturation kinetics attributes of erythrocytes hexokinase and phosphofructokinase. FEBS Lett Sept 2019;594:240-50.	3.370.	Y	N	Y
21	Rahul Sonavale, Arti Narkhede, Anuradha Mulik, Bipinraj Kunchiraman and Suresh Jagtap. 2019 July. Effect of edaphic factors on major secondary metabolites of Tinospora cordifolia and Neem Guduchi with respect to their immunomodulatory effect. International Journal of Pharmaceutical Sciences and Research. 10(7): 1000-1010.	1.83	N	N	N
22	Sourav Mukherjee and Suresh Jagtap. March – April 2020. Use of Orchids in treating Diabetes and related disease: A review. The Journal of Phytopharmacology 9 (2): 130-138.	-	N	N	N

Book chapters (Total No:4)

- Ketaki Wagh and Supriya Bhalerao., Traditional and Ethnic Foods (Chapter-8) Traditional foods, Ayurveda, and diet (Pg No. 99-110) Elsevier traditional and ethnic food series. ISBN-978-0-12-820011-7
- Manohar L. Panse, Shital D. Phalke. Omega-3 Beverages In Value-Added Ingredients and Enrichments of Beverages, *Value-Added Ingredients and Enrichment of Beverages,*Academic Press, 2019, Pages 353-382, ISBN 9780128166871. https://doi.org/10.1016/B978-0-12-816687-1.00011-4

Patents:

Patent Title	**Name of Innovators**	**Patent Application No.**	**Filling date**	**Current status**
Formulation of edible oil	Zanwar AA, Hegde M.	Full Indian Patent Application Application No. 201921006187	16/02/2020	Filled
Coatings and methods for infection-resistant medical devices	Modak SM, Ghosh AK, De Silva CC, Hegde M, Zanwar AA, Dongre SH, Kadam SS	Indian patent application publication no. 201827046613	10/12/2018	Published on July 12, 2019

Awards and Honors (Faculty: 2; Students: 4)

Faculty			
Academic Year	Name of The Faculty Member	Honor	Details of Award / Honor
2019-2020	Shama R Aphale	First prize in oral presentation at state level Seminar conducted jointly organized by SGRS College of Pharmacy & Savitribai Phule Pune University.	Trophy and certificate
2019-20	Dr. Supriya Bhalerao		Contributed in developing guidelines for clinical trial protocols as a part of activities of AYUSH Task force for COVID-19
Students			
Academic Year	Name of The Faculty Member	Honor	Details of Award / Honor
2019-20	Amrita Khaire	Nanik Gurnani Award for the paper "Preeclampsia is associated with higher proportions of saturated fatty acids" by Amrita Khaire, Kamini Dangat, Hemlata Pisal, Karuna Randhir, Savita Mehendale, Sadhana Joshi at Indian Women Scientist Association held at ICMR- National Institute of Nutrition on 11^{th}-13^{th} Dec, 2019.	
2019-20	Vaishali Kasture	Travel grant Award of AUS $1,675.00 to Ms. Vaishali Kasture to attend DOHaD conference (The	

		International Society for Developmental Origins of Health and Disease) in Melbourne, Australia held from 20th -23rd October, 2019. Title: "Effect of Maternal Omega-3 Fatty Acid and Vitamin E Supplementation on Placental Angiogenic Factors in Subtypes of Preeclampsia" by Vaishali Kasture Surabhi Dalvi, Anvita Kale, Sadhana Joshi.	
2019	Ms. Sunita Bhise	Special recognition award for oral presentation held on 7th March 2020	Received certificate and medal
2018-2019 Nov 2019	Ms.Varsha Shetty	Certificate of Best Paper	International Conference on Emerging Trends in Delivery of Phytoconstituents, at Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University), Pune, 2019.

Papers Presented at International Conferences/Seminars/Workshops: (Total No: 15)

1. Ms. Vaishali Kature Oral presentation on the topic “Effect of Maternal Omega-3 Fatty Acid and Vitamin E Supplementation on Placental Angiogenic Factors in Subtypes of Preeclampsia” by Vaishali Kasture Surabhi Dalvi, Anvita Kale, Sadhana Joshi at DOHaD conference (The International Society for Developmental Origins of Health and Disease) in Melbourne, Australia held from 20th -23rd October, 2019.
2. Dr. Harshad Patil presented a poster titled “Evaluation of delivery routes and immunogenicity using non-adjuvanted or monophosphoryl lipid A adjuvanted inactivated chikungunya in mice.” At the ISV Annual Congress date
3. Dr. Ruchika Kaul-Ghanekar presented a poster titled” Homeopathic potencies of Terminalia chebula and Linum usitatissimum exhibit anticancer activity against triple negative breast cancer cells (TNBCs)” at international conference “Ayush For Future Health Challenges Strengthening Trans-Disciplinary Research” on 28th-30th November 2019; AYUSH Center of Excellence Ramkumar Rathi Patanjali Yoga Chair Savitribai Phule Pune University, Pune, India At NCCS Auditorium SPPU Campus Pune – 411 007
4. Dr. Ruchika Kaul-Ghanekar presented a poster titled” A polyherbal Unani formulation (Habbe Musaffi Khoon) exhibited anticancer activity against cervical cancer cells” at international conference “Ayush For Future Health Challenges Strengthening Trans-Disciplinary Research” on 28th-30th November 2019; AYUSH Center of Excellence Ramkumar Rathi Patanjali Yoga Chair Savitribai Phule Pune University, Pune, India At NCCS Auditorium SPPU Campus Pune – 411 007
5. Dr. Ruchika Kaul-Ghanekar presented a poster titled” Omega 3 fatty acids regulate cervical cancer growth both in vitro as well as in vivo” on All India Cell Biology Conference December 19 – 21, 2019 Indian Institute of Science Education and Research (IISER) Mohali.
6. Dr. Prerna Raina presented a poster titled “ Homeopathic potencies of Terminalia chebula and Linum usitatissimum exhibit anticancer activity against triple negative breast cancer cells (TNBCs)” at international conference “Ayush For Future Health Challenges Strengthening Trans-Disciplinary Research” on 28th-30th November 2019; AYUSH Center of Excellence Ramkumar Rathi Patanjali Yoga Chair Savitribai Phule Pune University, Pune, India At NCCS Auditorium SPPU Campus Pune – 411 007
7. Ms. Apoorva Parimoo presented a poster titled “Homeopathic potencies of Terminalia chebula and Linum usitatissimum exhibit anticancer activity against triple negative breast cancer cells (TNBCs)” at international conference “Ayush For Future Health Challenges Strengthening Trans-Disciplinary Research” on 28th-30th November 2019; AYUSH Center of Excellence Ramkumar Rathi Patanjali Yoga Chair Savitribai Phule Pune University, Pune, India At NCCS Auditorium SPPU Campus Pune – 411 007.
8. Ms. Nidhi Sharma presented a poster titled “A polyherbal Unani formulation (Habbe Musaffi Khoon) exhibited anticancer activity against cervical cancer cells” at international conference “Ayush For Future Health Challenges Strengthening Trans-Disciplinary Research” on 28th-30th November 2019; AYUSH Center of Excellence Ramkumar Rathi Patanjali Yoga Chair Savitribai Phule Pune University, Pune, India At NCCS Auditorium SPPU Campus Pune – 411 007
9. Ms. Minal Mahajan presented a poster titled “Anti-cancer and HDAC8 inhibitory activity of bioactives present in HC9, a polyherbal formulation” at All India Cell Biology Conference December 19 – 21, 2019 Indian Institute of Science Education and Research (IISER) Mohali

10. Ms. Varsha Shetty Oral presentation of paper “Synthesis, characterization and *in vitro* anticancer activity of Cinnamaldehyde loaded magnetic nanoparticles” at International Conference on Emerging Trends in Delivery of Phytoconstituents, at Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University), Pune, 2019.
11. E-poster Presentation (oral) at Asian Pacific Digestive Week (APDW) an international conference held at Kolkata on 12th -15th December 2019
12. Ms. Sunita Bhise) presented a paper titled “” at 4th International Diabetes Summit 2019, Pune Chellaram Diabetes Institute, Pune
13. Oral presentation on the topic “Preeclampsia is associated with higher proportions of saturated fatty acids” by Amrita Khaire, Kamini Dangat, Hemlata Pisal, Karuna Randhir, Savita Mehendale, Sadhana Joshi at Indian Women Scientist Association held at ICMR- National Institute of Nutrition on 11th-13th Dec, 2019.
14. Shama Aphale presented a Poster titled “Phytochemical identification and evaluation of anticancer activity of an Ayurvedic formulation Panchvalkala in cervical cancer cell lines” at State level seminar on **" Systematic Approach to Spectral Interpretation"** on 8th (Wednesday) & 9th (Thursday) January 2020, Jointly organized by SGRS College of Pharmacy & Savitribai Phule Pune University.
15. Miss. Mrunal Gosavi presented a poster titled “Evaluation of delivery routes and immunogenicity using non-adjuvanted or monophosphoryl lipid A adjuvanted inactivated chikungunya in mice. At the Virocon conference date

Invited Talks by Faculty (Total no. 22)

Sr. No	Academic Year	Date	Name of the faculty	Topic
1	2019-20	22 Feb, 2020	Dr Sadhana Joshi	Delivered a talk on the topic "Developmental Origins of Health and Disease: Placental Programming in Preeclampsia and Long Term Health" at Golden Jubilee Conference of NIRRH, Mumbai at Mumbai University, Kalina.
2	2019-20	18th January, 2020	Dr Sadhana Joshi	Delivered a talk on the topic "Role of maternal vitamin D and one carbon metabolites in pregnancy complications" at Deenanath Mangeshkar Hospital & Research Center, Erandawane, Pune.
3	2019-20	11th Dec, 2019	Dr Sadhana Joshi	Delivered a talk on the topic "Developmental Origins of Health and Disease: Clinical Implications" at Bharati Hospital, Pune,.
4	2019-20	18th October 2019	Dr Suresh Jagtap	A guest lecture delivered at B, Tech. Engineering, Ajeenkya DY Patil University, Charoli, Pune, on. Topic- 'Herbal Medicine and Biotechnology'.
5	2019-20	17 January 2020	Dr Suresh Jagtap	An invited talk was given at Prof. Ramkrishna More Arts, Commerce & Science College, Nigadi, Pune. Topic- 'Validation and Industrial Application of Medicinal Plants' under the scheme DBT STAR.
6	2019-20	**1st February 2020**	Dr. Ruchika Kaul Ghanekar	Ayurveda Research-Challenges and Opportunities
7	2019-20	**21-25, September 2019**	Dr. Ruchika Kaul Ghanekar	
8	2019-20	3rd August 2019	Dr. Sarika Mane	'Grapes, yeast, and fermentation: fungus- host interaction' arranged by Center for Complementary and Integrative Health CCIH-SPPU, Pune
9		18th August 2019	Dr. Supriya Bhalerao	'Ethical issues in Ayurveda Research' arranged by National Ayurveda Teachers Association at Pune
10		22nd August 2019	Dr. Supriya Bhalerao	'Protocol drafting' arranged By World Ayurveda Expo, Ministry of AYUSH at Navi Mumbai
11		19th October 2019	Dr. Supriya Bhalerao	'Insights from Ayurveda for research on metabolic diseases' at International Conference of Ayurveda organized by Dr. D. Y. Patil College of Ayurveda & Research Center, Pimpri, Pune
12		6th Feb	Dr. Supriya Bhalerao	'Good Clinical Practices & Ethical Guidelines for

		2020		clinical research' arranged by Bharati Hospital, Pune
13		28th Feb 2020	Dr. Supriya Bhalerao	National Science day lecture focusing IRSHA facilities and research activities, arranged by Bharati Vidyapeeth Dental College, Navi Mumbai
14		13th March 2020	Dr. Supriya Bhalerao	'Writing Research proposal- study design and Statistical considerations' arranged by Bharati Vidyapeeth Dental College and Hospital, Pune
15		12th May 2020	Dr. Supriya Bhalerao	'Immunity boosting and Ayurveda' arranged by Thakur College of Science and Commerce, Mumbai
16		20th Nov. 2019	Dr. Anand Zanwar	Invited talk at AICTE sponsored Quality Improvement Program for Pharmacy teachers entitled "Gen- Next Pharmacology: Today's Discovery Tomorrow's Medicine" at Poona college of Pharmacy, BVDU, Pune on Topic: Preclinical assessment and approaches of cardiovascular disorders
17	2019-20	November 11, 12 2019	Dr. Hegde	Key Note Speaker (Mahabaleshwar V. Hegde) 3rd International conference on food Science Technology held in London, UK Key note address on "A Road Map to Agriculture Nutrition and Health"
18	2019-20	4th - 7th December, 2019	Dr. Anand Zanwar	Delegate and Presenter 5th IUPHAR World Conference on the Pharmacology of Natural Products and 51st Annual Conference of Indian Pharmacological Society (IPS) at ICMR-NIN, Hyderabad (
19	2019-20	February 7-8, 2020	Prof. M. V. Hegde	Key note speaker ICAR-IIOR, Hyderabad National Oilseeds Mission - "Technology innovations in Oilseed Crops for Enhanced Productivity, Profitability and Nutritional Security"
20	2019-20	04 – 06 September, 2019	Dr. M. V. Hegde, Dr. Anand Zanwar, Dr. P. B. Ghorpade	Delegate and Speaker ()Annual Group Meeting on Linseed, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur
21	2019-20	2020, February 7-8, 2020	Dr. Anand Zanwar	Delegate and Presenter National Oilseeds Seminar ICAR-IIOR, Hyderabad
22	2019-20	5th October 2019	Dr. Anand Zanwar	Delegate Regulations for Food and Nutritional Products NCL Innovation Park, Pune

Ph.D. Degree Awarded: Total 6

Sr. No	Name of the Student	Name of the Guide	Topic	Month and Year of Award
1	Ms. Shruti Jawale	Dr. Preeti Chavan Gautam	Effect of omega-3 fatty acid supplementation to a maternal high dairy fat diet combined with a Vitamin b12 deficiency on cognitive performance and cardiometabolic variables in rat offspring	Dec 2019
2.	Ms. Kamini Dnagat	Dr. Preeti Chavan Gautam	Breast milk components and neurodevelopmental risk in offspring born to mothers with preeclampsia	June 2020
3	Prakash Mansara	Dr. Ruchika Kaul-Ghanekar	Exploring the role of omega-3 fatty acids in regulation of breast cancer: in vitro and clinical studies	Septemb er 2019
4	Shama Aphale	Dr. Ruchika Kaul-Ghanekar	Evaluation of anticancer and immunomodulatory activity of Panchvalkala-an ayurvedic formulation"	March 2020
5	Ms. Megha Salunke	Dr. Supriya Bhalerao	Study of oxidative stress and inflammatory markers in obesity and effect of herbal intervention on these markers	January 2020
6	Dhanashri Ingale	Dr. Suresh Jagtap	Exploring modulation of Matrix metalloproteinase through herbal and Ayurvedic formulation using cultured Synoviocytes	Jan 2020

Conference/workshops/Seminar attended

Type of the Event	Sr. No	Name of the Faculty	Date	Name of the Event	Organized By	Level (International / National / State / Institute)
Conference	1	Dr. Ruchika Kaul Ghanekar	21-25, September 2019	1st TCGA themed Conference and Workshop in India titled 'Multi-Omics Studies in Cancer: Learnings from TCGA	Centre for Translational Cancer Research (CTCR), Persistent Systems and TCGA, NIH, USA	International
Workshop	2	Kavita Shinde	21st Nov 2019	Pre conference workshop of 9th International Conference of LASA India	Indian Institute of Scince Education and Research (IISER), Pune and National Centre for Cell Science (NCCS), Pune.	
	3	Shama Aphale	1st December 2019	Workshop on Meta research and evidence – based Medicine	AYUSH Center of Excellence, Interdisciplinary School of Health Sciences, Savitribai Phule Pune University, Pune.	
	4	Dr. Ruchika Kaul Ghanekar	1st December 2019	Workshop on Meta research and evidence – based Medicine	AYUSH Center of Excellence, Interdisciplinary School of Health Sciences, Savitribai Phule Pune University, Pune.	

	5	Dr. Ruchika Kaul Ghanekar	6th February 2020	Workshop on Good Clinical Practices (GCP) and Ethical Guidelines for Clinical Research	Institutional Ethics Committee, Bharati Vidyapeeth Deemed University, Bharati Hospital & Research Centre, Pune.	
	6	Shama Aphale	6th February 2020	Workshop on Good Clinical Practices (GCP) and Ethical Guidelines for Clinical Research	Institutional Ethics Committee, Bharati Vidyapeeth Deemed University, Bharati Hospital & Research Centre, Pune.	

Invited Lectures

Sr. No	Name of the Guest	Topic	Date
1	Dr. Stephen Whitehead, National Institute of Health, USA	Challenges in dengue vaccine development: Is an effective vaccine possible?	20/02/2020
2	Dr. Tushar Patil MD (Med), DM (Onco) Eminent oncologist	Community awareness program in Cancer: 'Understanding Cancer'	4 Feb 2020
3	Dr. Jaydeep Bhat Max Planck Institute for molecular genetics (MPI-MG), Berlin, Germany.	"Towards Immunotherapy of human T-cell malignancies	14.12.2019
4	Dr. Aparna Deshmukh Prof. and Head, Dept. of Biotechnology, Thakur College of Arts and Science	Basics of Virology and immunology	15th April 2020
5	Dr. D. C. Mathangi, Professor of Integrative Medicine, Shriramchandra Institute of Higher Education and Research, Chennai	Glucose homeostasis part I and II	16th and 17th April 2020

Events Organized at IRSHA:

- **National Ayurveda Day:**
  A Guest lecture on “Ayurveda for longevity”" by Dr. Omkar Kulkarni from Pune on 24th October 2019

- **World Diabetes Day**
  Free Blood Sugar Check Up and Yoga for IRSHA staff and students 14th Nov 2019
  Academic programme on 19th Nov 2019
  1. Lecture on "Molecular mechanisms of diabetes" by Dr. Shilpy Sharma
  2. Lecture on "Experimental studies in diabetes: our experiences" by Dr. Vaishali Undale

- **International Yoga Day:**
  Awareness cum demonstrational audiovisual clip on ‘Importance of Yoga in the current pandemic COVID-19’ was developed with the help of First Year students from College of Ayurved, Bharati Vidyapeeth and was circulated through online media applications on 21st June, 2020

Extension activities:
Extension activity for screening of anticancer activity of drugs is being provided to other institutions in which we have generated funds worth Rs. 48,970/- On the occasion of the Birth Anniversary of Dr. Patangrao Kadam and Birthday of Dr. Vishwajeet Kadam “Walk for Diabetes” arranged on 9th January 2020 (Total Participants 50)

Diabetes awareness lecture on the occasion of the Birth Anniversary of Dr. Patangrao Kadam and Birthday of Dr. Vishwajeet Kadam by Dr. Vaishali Deshmukh on ‘Challenges and opportunities in the prevention of Diabetes’ on 13th Jan 2020

Any other activities

Type of the Event	Sr. No	Theme	Date	Level (International / National / State / Institute)
Orientation Training programme of Ayurveda for Non- Ayurveda doctors and scientist	1	Orientation Training programme of Ayurveda for Non-Ayurveda doctors and scientist	27th January and 1st February 2020.	National level programme supported by Rashtriya Ayurveda Vidyapeeth (RAV), Ministry of AYUSH, Govt. of India
Training at IIQM, Jaipur		Lab QMS & Internal Audit as per ISO/IEC – 17025:2017& NABL Requirements	16th July 2019– 19th July 2019	Dr. Anvita Kale; Dr. Rashmi Virkar, Mr. Shambu Pisal, Dr. Shubham Shrivastava National Level training
Training at ERTL, New Delhi		Lab QMS & Internal Audit as per ISO/IEC – 17025:2017 & NABL Requirements	15th Oct - 18th Oct 2019	Dr. Rekha Damle, Ms. Shital Nikhar National Level training
The Global Health Network-online course		ICH good clinical practice E6 (R2)	26th July 2019	All NIBEC staff National Level training
The Global Health Network-online course		Introduction to good clinical laboratory practice	05th September 2019	All NIBEC staff National Level training
Visit to Molecular Virology laboratory, IIT Roorkee		Knowledge transfer of Antiviral assessment of drugs and initiate collaborations	3rd-4th October 2019	Dr. Rekha Damle, Dr. Sudha Ramkmar
"Global Bio-India Summit 2019" organized by DBT BIRAC		Exhibiting NIBEC at Global Bio-India Summit 2019	21st-23rd November 2019	Dr. Sudha Ramkmar, Dr. Harshad Patil
An exclusive license agreement on the infection-resistant catheter coating technology has been signed		February 11, 2020	by Blue Neem Medical Devices Pvt. Ltd., Bangalore	BVDU received share of the option agreement in the amount of $ 985 pursuant to the option agreement with Blue Neem Medical Devices Pvt. Ltd. Bangalore

Flax Bio village model at IRSHA and backward linkage with linseed growing farmers of Vidarbha region of Maharashtra State under Participatory Plant Breeding, seed Production and value addition for sustainable livelihood security of linseed farmers of Vidarbha and Maharashtra. Following activities were undertaken in year 2019-20:

Sr. No.	Date	Name of the Activity	Details of the Activity
	November 2019 to March 2020	Foundation Linseed Production	400 Kg Foundation linseed of PKV-NL-260 was produced
2.	November 2019 to March 2020	Certified Linseed Production	80 Quintals of certified linseed of PKVNL-260 produced and distributed to farmers on 736 Acres
3.	22nd – 25th November 2019	Agro Vision Farmers Training	Training on "Linseed Production Technology" to 450 Farmers was given. 450 farmers attended the training/workshop

The high oleic safflower developed under ICAR-NASF Project is grown at Chikhalapur Dist. Nagpur for fixation of high oleic genes in successive generations. The range of high oleic is consistent and ranged for 77 to 81 per cent. After genetic purification and its multiplication of seed, the variety will be available for commercial cultivation in Maharashtra in due course of time.

Two students were supported for M.D. Homeopathy for partial dissertation in CINHD under guidance of Dr. Anand A. Zanwar.

a. Dr. Aarati Khatal
b. Dr. Suraj Singh

Dr. Arnab Ghosh had visited Blue Neem Medical Devices Pvt. Ltd., Bangalore (26th August to 30th August, 2019) in order to facilitate the transfer of antimicrobial catheter coating technology, to demonstrate and standardize the coating procedure in the company facility as well.

Dr. Suresh Jagtap

- Member of Examination Flying Scod for the BVDU's Mumbai campus.
- Member, Maharashtra State Biodiveristy Board, Pune.
- Expert, Project evaluation committee, DST-SERB, New Delhi for Plant Science.

Any other information or relevant photographs about the program which may be included in the report

Dr. Tushar Patil MD (Med), DM (Onco) Eminent oncologist Community awareness program in Cancer: 'Understanding Cancer'

Visitors and relevant photographs

National Ayurveda Day celebration, Dr. Omkar Kulkarni Deliver lecture on "Ayurveda for longevity" 24th October 2019

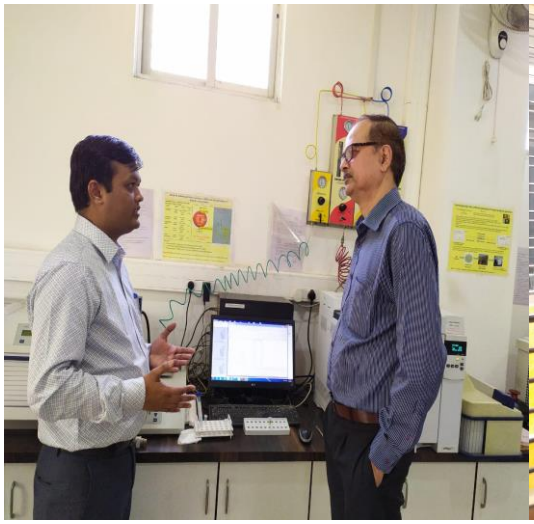
Lecture on ""Experimental studies in diabetes: our experiences" World diabetes day Academic programme by Dr. Vaishali Undale

Lecture on "Molecular mechanisms of diabetes" World diabetes day Academic programme by Dr. Shilpy Sharma

Walk for Diabetes' on the occasion of the Birth Anniversary of Dr. Patangrao Kadam and Birthday of Dr. Vishwajeet Kadam "Walk for Diabetes" arranged (Total Participants 50)

Orientation Training programme OTP2020 (27th Jan -1st Feb2020)

Visit of Dr. G. P. Dixit – PC-Linseed to AICRP-Linseed Value Addition Centre



Staff Information

Staff Category	Number
Scientific staff	18
Technical Staff	19
Ph.D. students	22
Administrative	11
Total	83

A) Name of the Teaching/ Scientific Staff:

Sr. No.	Name of the Staff	Designation	Joining Date in BV
1	Dr. Akhileshchandra Mishra	Director	22/01/2015
2	Dr. VidyaArankalle	Senior Scientist	01/01/2016
3	Dr. SadhanaRamchandra Joshi	Professor	01/01/2004
4	Dr. RuchikaKaul-Ghanekar	Associate Professor	23/07/2007
5	Dr. Suresh DnyandeoJagtap	Associate Professor	01/12/2009
6	Dr. SupriyaBhalerao	Associate Professor	12/07/2013
7	Dr. ShubhamShrivastav	Associate Professor	18/05/2016
8	Dr. HarshadPadmanabhPatil	Associate Professor	12/09/2016
9	Dr. Anvita Kale	Assistant Professor	03/04/2001
10	Dr. Ruta Kulkarni	Assistant Professor	01/08/2016
11	Dr. DeepaliSundrani	Assistant Professor	01/06/2016
12	Dr. RashmiGovindVirkar	Assistant Professor	07/02/2019

B) Name of the Technical Staff:

Sr. No.	Name of the Staff	Designation	Joining Date in BV
1	Dr. Prerna Raina	Senior Research Assistant	01/06/2016
2	Dr. Poonam Ashish Gupte	Senior Research Assistant	01/06/2016
3	Mrs. KaminiDhaneshDangat	Research Assistant	26/04/2006

4	Dr. AbhijitAvinashGhadge	Research Assistant	05/07/2012
5	Mrs. HemlataMahadeoPisal	Research Assistant	15/12/2006
6	Ms. AnuradhaRajendraMulik	Research Assistant	01/10/2016
7	Dr. Sarika S. Mane	Research Assistant	10/01/2017
8	Mr. KartikeyTanajiJagtap	Research Assistant	19/01/2017
9	Mrs. Karuna N. Randhir	Technical Assistant	01/06/2012
10	Ms. VrushaliKadam	Technical Assistant	16/08/2012
11	Ms. ShrutiVidhyadharKoparkar	Technical Assistant	07/03/2013
12	Ms. Kavita B. Shinde	Technical Assistant	01/09/2012
13	Mrs. ShamaAphale	Technical Assistant	01/04/2014
14	Ms. Shital Ashok Giramkar	Technical Assistant	01/08/2010
15	Ms. SurabhiSubhodDalvi	Technical Assistant	01/08/2015
16	Ms. RahatRizwan Ahmad Khan	Technical Assistant	12/12/2016

C) Name of the Administrative Staff:

Sr. No.	Name of the Staff	Designation	Joining Date in BV
1	Mrs. VaishaliSandipKadam	Office Superintendent	14/01/2008
2	Mr. VijaychandPandurangGavade	Sub Accountant	01/01/1993
3	Mr. AnandaDinkarJadhav	Junior Clerk	09/02/2009
4	Mr. Nitin Shankar Mote	Junior Clerk	02/05/2003
5	Mrs. Anjali RajendraGajare	Junior Clerk	20/03/2014
6	Mr. Dilip Kaka More	Trainee Clerk	03/09/2018

7	Mr. ShivajiDhondiram More	Electrician	19/03/1990
8	Mr. AnkushRambhauChandere	Driver	02/02/2015
9	Mr. JagannathTukaramYadav	Peon	18/02/1991
10	Mr. Tushar Ashok Shinde	Peon	01/02/2007
11	Mr. RavindraBalasahebMulik	Animal House Attendant	11/02/2011

D) Name of the Centre for Innovation in Nutrition Health Disease (CINHD) Staff:

Sr. No.	Name of the Staff	Designation	Joining Date in BV
A) Name of the Teaching Staff			
1	Dr. M. V. Hegade	Director CINHD	01/09/2002
2	Dr. P.B. Ghorpade	Emeritus Scientist	26/04/2013
3	Dr. AnandZanwar	Scientist	24/11/2007
4	Dr. Arnab Kumar Ghosh	Scientist	17/01/2016
5	Ms. Asavari Joshi	Scientist	10/01/2015
6	Mr. M. L. Panse	Director of Research Lab.	01/07/2013
B) Name of the Non-Teaching Staff			
1	Mr. YogeshBadhe	Project Assistant	01/06/2010
2	Ms. SunitaBhise	Project Assistant	01/03/2013
3	Mr. PramodFarde	Technical Assistant	22/06/2015

Student List

Sr. No.	Name of the Student	Designation	Sign
1	Dr. Amrita AnkushKhaire	Research Assistant-ICMR	
2	Ms. AkritiSahay	Research Assistant-ICMR	
3	Ms. VaishaliKasture	JRF-DST Inspire Fellow	
4	Mr. AmolRajendraChoudhari	JRF-DBT	

5	Ms. Anjali TukaramJadhav	SRF- ICMR	
6	Ms. Akanksha Mahajan	JRF	
7	Miss. MrunalGosavi	SRF- ICMR	
8	Ms. Rama Rajadnya	JRF	
9	Ms. Amrita Ulhe	JRF-DST SERBFellow	
10	Mrs. ShrideviGundu	SRF-DBT	
11	Ms. DakshaMunot	JRF Fellow-DST SERB	
12	Dr. AshwiniLaxmanKamble	Women Scientist-WOSA DST	
13	Mrs. Nidhi Sharma	JRF-EMR AYUSH UNANI Fellow	
14	Dr. Khan Aliya Nikhat Abdul Bari	JRF-EMR AYUSH UNANI Fellow	
15	Ms. AishwaryaRajanKharkhanis	JRF-DBT Bio-care	
16	Ms. SayaliVedpathakl	JRF-DBT Bio-care	
17	Mr. AhmedAliMandviwala	JRF-DBT Bio-care	
18	Ms. HimanshiYadav	Project Technician(III)	
19	Mrs. ApoorvaParimoo	SRF-AYUSH Fellow	
20	Ms. JuhiNema	JRF-CSIR-UGC Fellow	

Institutional Committees

SCIENTIFIC REVIEW COMMITTEE

Name and Designation	Role
Dr. Akhilesh Chandra Mishra Director IRSHA.	Chairperson
Dr. Sadhana Joshi Professor & Head, Department of Nutritional Medicine, IRSHA.	Member
Dr. Vidya Arankelle Senior Scientist, Head, Department of Infectious Diseases, IRSHA.	Member
Prof. Mahabaleshwar Hegde Scientific Advisor, Centre for Innovation in Nutrition Health Disease, IRSHA.	Member
Dr. Supriya Bhalerao Associate Professor, Department Obesity, IRSHA.	Member Secretary

INSTITUTIONAL BIOSAFETY COMMITTEE (IBSC)
Approved by DBT, India

Name and Designation	Role
Dr. Akhilesh Chandra Mishra Director IRSHA.	Chairman
Dr. Debashis Mitra, Scientist G, NCCS, Pune.	DBT nominee
Dr. Harshad Patil Associate Professor, Department of Communicable Disease, IRSHA.	Member Secretary
Dr. Kunal Lahiri, Head of the Department, Department of Microbiology, Bharati Vidyapeeth Medical College and Hospital, Pune.	Outside Expert
Dr. Supriya Bhalerao Associate Professor, IRSHA, Bharati Vidyapeeth University, Pune.	Biosafety Officer
Dr. Vidya Arankelle Senior Scientist, IRSHA, Bharati Vidyapeeth University, Pune.	Internal Experts
Dr. Preeti Chavan, Assistant Professor, IRSHA, Bharati Vidyapeeth University, Pune.	
Dr. Ruchika Kaul-Ghanekar Associate Professor, IRSHA, Bharati Vidyapeeth University, Pune.	

PURCHASE REVIEW COMMITTEE

Name and Designation	Role
Dr. Akhilesh Chandra Mishra Director IRSHA.	Chairperson
Dr. Sadhana Joshi Professor & Head, Department of Nutritional Medicine, IRSHA.	Member
Mr. Vijaychand Gavade Sub-Accountant, IRSHA.	Member
Dr. Harshad Patil Associate Professor, Department of Communicable Disease, IRSHA.	Member Secretary