Annual Report

2020-2021

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 2020- June 21

Overview of Director

It is my privilege to present the Annual report of Interactive Research School for Health Affairs (IRSHA) for the year 2020-21. All the departments of IRSHA were successful in receiving financial support of Rs. 4368.14 Lakhs from national funding agencies for carrying out their research work. This year student fellowships of Rs. 37.32 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 19 publications research articles; 1 book chapter and 1 patent application.

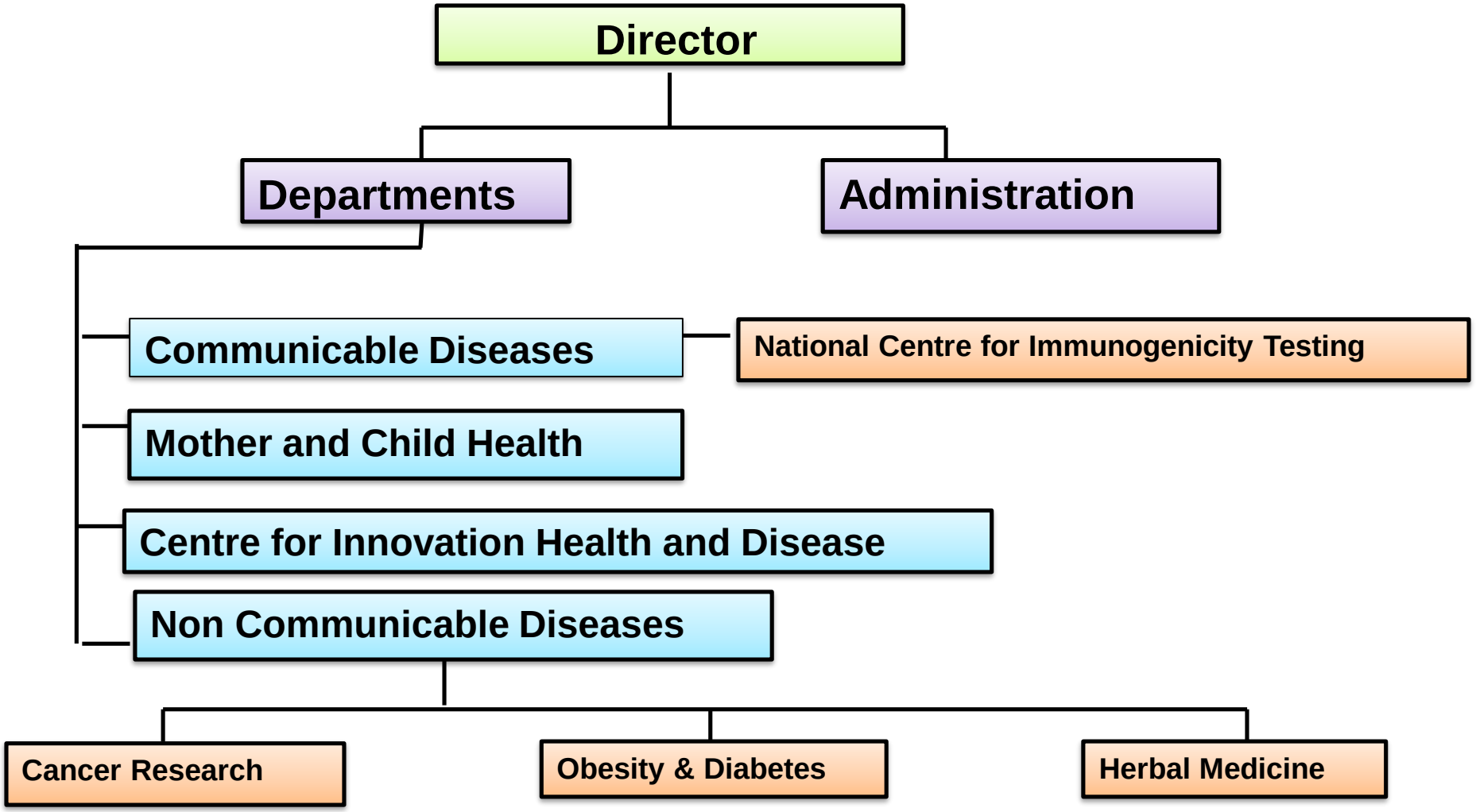
Several activities had been organized at the institute and also the staff and students participated in national and international events. A brief summary of these events, activities and achievements by all the staff members has been presented in the current report.

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
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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

Bharati Vidyapeeth Deemed University
Interactive Research School for Health Affairs (IRSHA)
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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:

Recruitment:
The total number of subjects sensitized and recruited in the study is shown in Figure 1. A total of 1154 women delivered at both the hospitals i.e. Bharati Hospital (n=555) and Gupte Hospital (n=599). Women were recruited at V1 = 11-14 wks and subsequently followed at V2 = 18-22 wks, V3 = 26-28 wks and at delivery. Information on the nutritional intake, physical activity, clinical history, medication, demographic characteristics and standard of living index (SLI) was collected. In addition, ultrasonography (USG) and color doppler measures were undertaken at 11-14 wks, 18-22 wks and at 32-35 wks of gestation. Anthropometry of all children was recorded at birth, 6, 10, 14 wks and 6, 9, 12, 15, 18, 24 months and developmental scales administered at 2 years of age.

Sample Collection:

Maternal blood was collected at four different time points (i.e. V1, V2, V3 and at delivery) for each participant. Cord blood was collected at delivery. The blood was processed to separate serum, plasma, lymphocyte, and erythrocyte fractions and are stored at − 80 °C for further analysis. These fractions are aliquoted in a number of vials for prevention of freeze thaw cycles.

Fresh placentae obtained from all pregnancies immediately after delivery were washes with 1X PBS. The fetal membranes were trimmed across the placenta edge and the umbilical cord is cut 2cm away from the insertion point. Different placental characteristics were recorded. The area around the cord insertion which was considered as the region of interest was cut into small pieces that were stored in vials and snap frozen in liquid nitrogen, and then stored at − 80 °C until analyzed.

A total number of ~90 to 95 vials for each subject across the study period are collected. The samples collected are stored at IRSHA, Bharati Vidyapeeth (Deemed to be University), Pune. Strict procedures are used to protect the confidentiality of the study participant's data and samples. A stock record (manual and computerized) is maintained for each sample, including date of sample collection and processing, sample quantity, number of aliquots for each sample, position of each aliquot in the freezer, and end user details. Access to the freezer is restricted to authorized trained personnel only.

Statistical Analysis:

A total of 1096 singleton women delivered at both the hospitals i.e. Bharati Hospital (n=546) and Gupte Hospital (n=550) and are included for analysis in the current report. Values are reported as mean and standard deviation (SD) for normally distributed continuous variables, and number (n) and percent (%) for categorical variables. All the variables are compared between the two groups (non-PE vs. PE) at each of the hospitals. Independent t-test was used to compare mean values of the various parameters between the hospitals and groups. Comparisons of categorical variables were done using Chi-square tests. Associations between the exposure (PE/non-PE as a binary variable) and predictors were analysed using linear regression. Data was analyzed using SPSS/PC+ package (Version 20.0, Chicago, IL).

General Characteristics of the Study Participants:

The mean age of the women was 28.5 years and BMI of 24 kg/m^2. 27.1% women in the present study were anemic. 7.6% women and 6.8% women conceived through IVF and IUI respectively. 60% women delivered by caesarean section and the remaining 40% delivered normally. A total number of 1154 women have delivered; 8.4% women had preeclampsia while 1.8% had preeclampsia along with gestational diabetes mellitus.

The mean gestational age at birth is 38.5 weeks. The mean birth weight was 2883.27 gms while the mean length was 48.54cm The newborns are divided as small for gestational age (SGA), adequate for gestational age (AGA) and large for gestational age (LGA) using the

INTERGROWTH-21st newborn size standards for birth weight. 21.6% children born were SGA, 75.6% were AGA and 2.8% were LGA.

Maternal Characteristics:

Maternal characteristics of Non-PE and PE women are shown in Table 3.

Women with preeclampsia (PE) had a higher BMI, systolic and diastolic blood pressure as compared to non-PE women at all the time points across gestation . Similar trend was observed at both the hospitals.

It was observed that higher percent of women were professionals and more educated in the PE group. More percent women conceived by assisted mode of conception in the PE group as compared to the non-PE group. Higher percent of women in the PE group delivered by caesarean section as compared to the non-PE group. Gravida did not differ between PE and non-PE groups. More number of nulliparous were observed in the PE group as compared to the non-PE group. Similar trends were observed at both the hospitals.

Gestational age at birth was lower in the PE group as compared to the non-PE group. Similar trend was observed at both the hospitals. Babies born to mothers with PE had a lower birth weight as compared to the non-PE group in both the hospitals. Furthermore, length and head circumference was lower in the PE group as compared to the non-PE group at Bharati hospital. The percent preterm birth in the PE group was higher than the non-PE group which was also reflected at both the hospitals. The percent SGA babies were higher whereas percent AGA were lower in the PE group as compared to the non-PE group. Similar trends were observed at both the hospitals.

Clinical Parameters

The hemoglobin levels did not differ between PE and non-PE groups at all the time points. The percent women anemic were similar between both the groups. The TSH levels at V1 were higher in the PE group as compared to non-PE group. PAPP A levels and β-hCG were not different between PE and non-PE groups at V1.

Ultrasonography and Color Doppler Assessments:

Ultrasonography and Color Doppler assessments were carried out at 11-14 wks, 18-22 wks and at 32-35 wks of gestation. All measurements were in accordance with the International Society of Ultrasound in Obstetrics and Gynecology (ISCOG) and Fetal Medicine Foundation (FMF) protocols (ISCOG guidelines, UOG, 2013).

Fetal growth measures such as Biparietal Diameter (BPD), Head Circumference (HC), Abdominal Circumference (AC), and Estimated Fetal Weight (EFW) were lower in the PE group as compared to the non PE group at 18-22 weeks of gestation while at 32-35 weeks femur length (FL) was lower in the PE group. At Bharati hospital, HC and AC were lower at 32-35 weeks of gestation in the PE group as compared to the non-PE group.

It was observed that mean uterine artery Pulsatility Index (PI) was higher at 18-22 weeks of gestation in the PE group as compared to the non-PE group and similar results were observed at Bharati hospital in addition to the higher levels at 11-14 weeks. At 32-35 weeks, umbilical artery PI and fetal Middle Cerebral Artery (MCA) PI were lower in the PE group as compared to the non-PE group.

Associations between Maternal Preeclampsia and USG and Color Doppler Measure

First we examined the univariate associations of USG and Color Doppler measures with potential confounders: mother's age, BMI, sex of the baby, SLI, hospital and GDM. Various models were then carried out for regression analyses in consultation with the experts. Three models are used for regression analyses of associations between PE and USG and Color Doppler measures: Model 1: unadjusted; Model 2: adjusted for mother's age, BMI, sex of the baby and SLI; and Model 3: adjusted for model 2 parameters plus hospital and GDM.

It was observed that fetal growth measures such as AC (-0.24 cm [95% CI -0.46, -0.03] p=0.029), FL (-0.23 cm [95% CI -0.45,-0.01] p=0.042), and EFW (-0.26 gms [95% CI -0.47,-0.04] p=0.021) at 32-35 weeks were negatively associated with preeclampsia after adjusting for mother's age, BMI, sex of the baby, SLI, hospital and GDM.

In case of Color Doppler measures, it was seen that mean uterine artery PI was positively associated with PE both at 11-14 weeks (0.20 [95% CI 0.01, 0.40] p=0.049) and 18-22 weeks (0.44 [95% CI 0.19, 0.70] p=0.001).

Placental Position, Placental Previa, Liquor and Anomalies (18-22 wks and 32-35 wks): Details of placental position, placental previa, liquor and anomalies (18-22 wks and 32-35 wks) were documented.

Placental Characteristics:

The placentae of women with PE had a lower thickness at centre, at edge and at cord insertion as compared to the non-PE placentae. At Gupte hospital, the thickness at centre of placenta was lower in the PE group as compared to non-PE group.

Placental Dimensions:

The percentage of bilobed and irregular shape was more in case of PE as compared to the non-PE group. The percentage of velamentous cord insertion was more in PE placentae as compared to non-PE placentae.

The percentage of irregular shaped placenta and velamentous cord insertion was higher in the PE group as compare to non-PE group at Bharati hospital.

Physical Activity of Women across Gestation:

The women's physical activity (1 month recall) was recorded across gestation at three different time points i.e. V1, V2 and V3 using a physical activity questionnaire. They are broadly categorized as sleeping, sedentary, light and moderate activities. The total amount of time spent in minutes per day on these activities was recorded and a daily score is calculated where higher scores indicate more activity.

The time spent on light activities was higher while time spent on moderate activities was lower in the PE group as compare to non-PE group at V1.

At V2, it was observed that the time spent for sedentary activities was more while for moderate activities it was lower in the PE group as compare to non-PE group.

At V3, similar trend was observed as seen at V1.

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), brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), phospholipids, protein carbonyl, glutathione is ongoing.

Maternal Erythrocyte Fatty Acids across Gestation and Cord Erythrocyte Fatty Acids

Maternal erythrocyte fatty acid levels at 11-14 weeks, 18-22 weeks, 26-28 weeks, and at delivery were estimated.

At V1, total saturated fatty acids (SFA) is significantly higher while omega-6 fatty acid such as linoleic acid (LA) was lower in the PE group as compared to the non-PE group. In addition to the above findings the following were also observed at Gupte hospital: lower total omega-6 fatty acids and total polyunsaturated fatty acids (PUFA). Estimated activity of Δ6 desaturase was higher in the PE group as compared to the non-PE group and similar trend was observed at Gupte hospital.

At V2, dihomogamma linolenic acid (DGLA), total omega-6 fatty acids and omega-6/omega3 fatty acid ratio were found to be higher in the PE group as compared to the non-PE group. However at Bharati hospital, docosahexaenoic acid (DHA) was lower in the PE group. At Gupte hospital, DGLA, arachidonic acid (AA), total omega-6 fatty acids, omega-6/omega3 fatty acid ratio and PUFA were higher while total SFA were lower in the PE group as compared to the non-PE group. Estimated activity of Δ6 desaturase was higher while that of Δ5 desaturase was lower in the PE group as compares to the non-PE group. At Gupte hospital, the estimated activity of Δ6 desaturase was higher in the PE group.

At V3, AA was found to be higher in the PE group as compared to the non-PE group. At Gupte hospital, DGLA, AA and total PUFA were higher while total SFA were lower in the PE group as compared to the non-PE group.

At delivery, total monounsaturated fatty acids (MUFA), α-linolenic acid (ALA) were lower while DGLA was higher in the PE group as compared to the non-PE group. Similar findings except for ALA were observed at Gupte hospital. Estimated activity of Δ6 desaturase was higher while that of Δ5 desaturase was lower in the PE group as compared to the non-PE group and similar trend was observed at Gupte hospital.

Cord and Maternal Plasma Folate, Vitamin B_{12} and Homocysteine across Gestation

There were no significant differences in the maternal (across gestation) and cord levels of folate, vitamin B_{12} and homocysteine between PE and non-PE groups. Hospital wise data shows that folate levels were higher in the cord plasma of PE group as compared to the non-PE group at Gupte hospital. While homocysteine levels at V1 were lower in the PE group as compared to the non-PE group at Bharati hospital.

The percent women with folate deficiency were very few across gestation in both the groups. The percent women with vitamin B_{12} deficiency increases as gestation advances in both the groups. The percent women with elevated levels of homocysteine were very few at each time point across gestation in both the groups. Similar trends were observed at both the hospitals.

Maternal and Cord Levels of Serum Calcium, Magnesium and Calcium/Magnesium Ratio

No significant differences were observed for calcium levels between PE and non-PE groups. Maternal serum magnesium levels were significantly lower in the PE group as compared to the non-PE group at V2. At Bharati hospital the magnesium levels were higher in maternal serum at delivery and in cord serum in the PE group as compared to non-PE group. However at Gupte hospital, the levels of magnesium were lower at V2 (p=0.057).

The Ca/Mg ratio was found to be higher at V2 in the PE group as compared to the non-PE group. Similar results were seen at both the hospitals. In cord serum the ratio was found to be lower in the PE group as compared to the non-PE group although it was not statistically significant (p=0.082). However, at Bharati hospital the cord ratio was significantly lower in the PE group as compared to the non-PE group.

There were significant associations of maternal age, BMI, gestational age, SLI score, hospital, with maternal calcium and magnesium levels and hence these were adjusted for in the regression models: Model 1: Unadjusted; Model 2: Maternal age, body mass index (BMI), standard of living index (SLI) score, hospital, gestational diabetes mellitus (GDM).

There were no associations of maternal serum calcium levels with the risk of PE in the unadjusted or adjusted models. Magnesium levels were negatively associated with the risk of PE at V2 in both the unadjusted (-2.76 mg/dl [95% CI -5.09,-0.43] p=0.021) and adjusted (-2.41 mg/dl [95% CI -4.76, -0.06] p=0.045) models. The Ca/Mg ratio was positively associated with the risk of PE in both the unadjusted (1.47 [95% CI 0.46, 2.47] p=0.005) and adjusted (1.15 [95% CI 0.17, 2.12] p=0.022) models.

hsCRP Levels Across Gestation in Maternal Serum and Cord Serum

hsCRP levels were higher at V1, V2 and V3 in the PE group as compared to the non-PE group. Similar trend was observed at Gupte hospital.

Maternal Serum Vitamin D levels across gestation

Maternal vitamin D levels were lower in the PE group across gestation however significant difference was observed at V2 and V4 in women who subsequently developed PE as compared to the women without PE. At Bharati hospital these levels were lower at V2, V3 and V4. At Gupte hospital, maternal vitamin D levels were lower in the PE group at V2 as compared to the non-PE group.

Maternal Plasma MDA Levels across Gestation

There were no significant differences observed in the MDA levels between both the groups. However, at Bharati hospital maternal plasma MDA levels were higher at delivery in women with PE as compared to women without PE.

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. Table 25 shows the anthropometric measures such as weight at various time points on children till date.

The birth weight was lower in the PE group as compared to the non-PE group. Subsequently it was observed that the weight of the child remained lower at 6 weeks, 10 weeks, 14 weeks, 6 months (trend, but no significance). The child shows catch up in weight from 15 months upto 2 years.

Developmental Scores

The Developmental Screening Test by Bharath Raj is being administered telephonically at >2 years of age by trained Psychologists at both the hospitals. The test is designed for the purpose of measuring mental development of children from birth to 15 years of age. It provides for a brief and fairly dependable assessment which checks whether the child is capable of doing a particular item or not; whether the concerned behavioral characteristic has emerged, has become explicit/ manifest in the behavioral repertoire of the child. Appraisal of the child can be done in semi-structured interview with a parent or person well acquainted with the child.

The test consists of 88 items which represent behavioral characteristics of respective age levels. At each age level, items are drawn from behavioral areas like:

- Motor development- These items have many neurological and behavioral implications.

- Adaptive behavior- These items represent sensory-motor adjustment to objects, persons and situations.
- Speech- Language- These items find a place which are inclusive of all visible and audible forms of communication like vocalizations, words etc.
- Personal- Social development- They comprise of child's personal responsiveness to the social culture of which he is a member. e.g. Play cooperativeness etc

There was a lower trend (p=0.076) observed for the Developmental Quotient (DQ) levels in the PE group as compared to the non-PE group.

Data Entry and Data Validation

Data entry for all the questionnaires administered to the subjects enrolled in the study has been completed. Furthermore, double data entry and data validation has been completed for majority of the questionnaires/parameters. The remaining double data entry and validation is ongoing.

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 2022; **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

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:

IRSHA

Findings from Preeclampsia samples

- Recruitment of subjects and sample collection [52 normotensive control (NC) and 49 women with preeclampsia (PE)] completed.
- Maternal and cord plasma levels of vitamin B12, glucose and fatty acids were estimated on all samples.
- Maternal and cord plasma levels of non-esterified fatty acids (NEFA) has been estimated on 28 samples (14 NC and 14 PE).
- The placental LAT1, GLUT1, CD320, FATP2 and FATP4 protein expression was completed on the placental tissue of 14 NC and 14 PE women

Our results suggest that preeclampsia is associated with altered expression of nutrient transporters like GLUT1 and CD320 in the syncytiotrophoblast membranes.

Findings from PRIYA samples

- Placental membrane isolation of PRIYA Trial samples has been completed (on 50 samples) and checked for enrichment.
- Fatty acids analysis has been completed on 136 maternal plasma and 120 cord plasma samples of the PRIYA trial.
- The placental GLUT1, FATP2, FATP4 and CD320 protein expression was completed on the placental tissue of 30 women (10 from each group) and analysis is on-going.

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.

Work Done:
The study has been initiated and we are awaiting for samples.

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 and 70 LBW subjects is completed.
- Subjects history and clinical information and neonatal characteristics have been recorded
- mRNA expression analysis is completed on 100 NBW and 70 LBW samples.
- Methylation levels of PPAR genes completed on 50 (25 NBW and 25 LBW) samples and remaining are on-going

Results and Conclusion

- Women in the LBW group had lower gestational age and placental weight. The LBW babies had lower birth weight, length and chest circumference.
- Placental dimensions like major axis, minor axis, breadth and trimmed placental weight were lower in the LBW group.
- 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.
- Preliminary data indicates that mean methylation of PPARD gene is higher in the LBW placentae as compared to NBW placentae.

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 at Gupte Hospital is on-going
- Recruitment and sample collection for 84 non-ART and 37 ART subjects is completed.
- Subjects history and clinical information and neonatal characteristics have been recorded.
- RNA isolation and DNA isolation is completed on collected placenta samples.
- mRNA expression of VEGF, PlGF, FLT-1, KDR genes is completed on 84 non-ART and 37 ART.
- Methylation levels of VEGF and FLT1 genes completed on 28 non-ART and 26 ART samples and remaining are on-going

Results and Conclusion

- Higher maternal age and blood pressure is observed in women of the ART group.
- Lower trend of placental mRNA expression levels of VEGF and PlGF in the ART group as compared to the non-ART group,
- VEGF promoter is hypomethylated in ART placentae.

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.

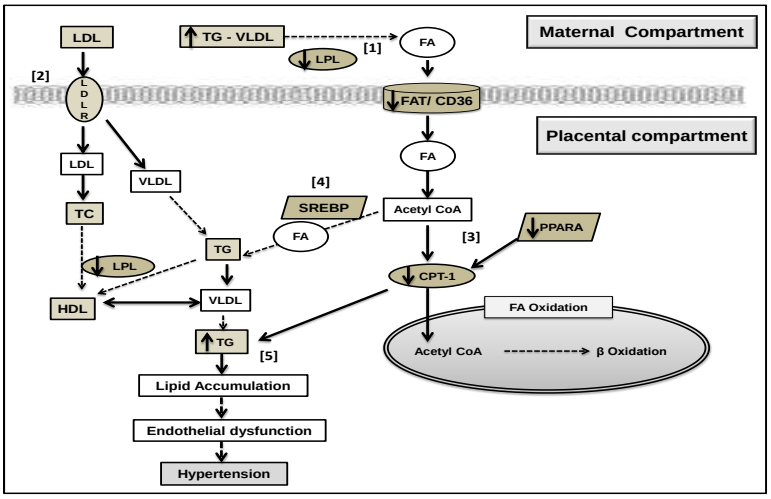
Results and conlcusion:

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].

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 :

The human study was undertaken in women with preeclampsia where various placental apoptotic markers were examined and compared with markers in the placentae from normotensive women. Further, their association with maternal blood pressure, one carbon cycle metabolites (folate, vitamin B12 and homocysteine), LCPUFA, oxidative stress marker [(malondialdehyde (MDA)], glutathione, placental dimensions and neonatal outcomes is also reported.

Study Design: The present cross-sectional study recruited 40 normotensive control (NC) and 40 women with preeclampsia at delivery. Preeclampsia was diagnosed according to the ACOG (The American College of Obstetricians and Gynaecologists) guidelines (ACOG, 2013). Maternal characteristics such as age, body mass index and clinical information such as systolic and diastolic BP, gestational age were recorded at the time of delivery. Neonatal birth weight, length and head and chest circumference were also recorded. Blood and placental samples were collected at delivery and placental dimensions were recorded.

Results and Conclusion: The human study demonstrates the association of placental apoptotic markers with one carbon cycle metabolites, LCPUFA, oxidative stress marker, glutathione, placental dimensions pregnancy and birth outcomes.

Women with preeclampsia had

• Increased levels of Malondialdehyde (MDA) and reduced glutathione in the preeclampsia placenta.

• Higher placental protein levels of proapoptotic markers (caspase-8 and caspase-3)

• Apoptotic markers were positively associated with plasma homocysteine levels and placental linoleic acid levels.

• Apoptotic marker caspase-8 and caspase-3 were positively associated with placental MDA levels.

• Protein levels of caspase-3 and Bcl-2 were negatively associated with thickness of placenta at cord insertion.

• Protein levels of BAX were negatively associated with placental glutathione levels

• Protein levels of caspase-8 were negatively associated with baby length.

The findings of the human study suggest that increased oxidative stress is associated with increased apoptotic signalling in preeclampsia. This may lead to reduced nutrient transport to the fetus resulting in growth restriction in the fetus in preeclampsia.

To summarise, the apoptotic proteins were significantly up-regulated in preeclampsia placentas. Proapoptotic markers like BAX, caspase-8 and caspase-3 were higher and antiapoptotic marker such as Bcl-2 is lower in the preeclampsia group. Changes in apoptotic markers were associated with maternal oxidative stress, placental dimensions and outcome measures. This study provides a possible mechanism to better understand the mechanisms of activation of the extrinsic and intrinsic apoptosis pathways in preeclampsia.

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.

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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.

Results and Conclusion:

The present study estimates the vitamin D status in women with and without preeclampsia. This cohort includes a total of 1154 women, of which 1096 women were with singleton pregnancy, among which 108 women developed preeclampsia. This study includes 324 pregnant women (216 Non-PE and 108 PE women). Serum vitamin D levels at V1 were found to be lower in

women who subsequently developed preeclampsia as compared to the Non-PE women ($p = 0.068$). At V2, vitamin D levels were found to be significantly lower in women who subsequently developed preeclampsia as compared to the Non-PE women ($p < 0.01$). Serum vitamin D levels at V3 were found to be comparable between the two groups. Serum vitamin D levels at V4 were found to be significantly lower in women with preeclampsia as compared to the women without preeclampsia ($p < 0.01$).

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.

Gene specific methylation analysis of selected candidate genes PlGF, FLT-1, HIF1A, HIF3A and PEMT was completed. Data showed significant hyomethylation at various CpG sites in the PlGF promoter region and FLT-1 gene and significant hypermethylation on two CpG sites in the HIF3A gene and one CpG site in HIF1A. This data indicates altered methylation of these genes in the preeclampsia placentae may influence angiogenesis, placental growth as well as intrauterine fetal development that may predispose the children to higher risk of cardiometabolic disorders in future.

Name of the Programme: Cancer Research

Title: Anti-cancer activity of homeopathic potency of *Terminalia chebula* (TC) in breast cancer cells. Project ID: CR/16 (16-19)/1/E; Funding: CCRH, AYUSH; Duration: 2016-Till present; Sanctioned Amount: Rs. 42,98,420/-; Investigators: PI: Dr. Ruchika Kaul-Ghanekar; PhD Student: Ms. Apoorva Parimoo

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. Further, 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: The effect of 6C potency of TC and its potentized distilled alcohol (6P) was evaluated on the mRNA expression of tumor suppressor genes, p53 and Rb in MDAMB231 breast cancer cells. 6C potency were tested for its effect on mitochondrial membrane potential in MCF7 cells.

Results and Conclusion:

- In MDAMB231, 6C at 1:100 dilution up-regulated the expression of p53 and Rb genes by 4.1 and 2.9 folds, respectively
- On the other hand, 6P at 1:100 dilution up-regulated p53 and Rb expression by 1.1 and 0.85 folds, respectively
- In MCF7 cells, 6C at 1:12.5 and 1:25 dilutions decreased mitochondrial membran potential of cells by 43.1 and 62.2 % compared to the control cells
- **In conclusion, 6C modulated the gene expression of tumor suppressors and induced mitochondrial depolarization in breast cancer cells**

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; Investigators: PI- Dr. Ruchika Kaul-Ghanekar, Co-PI- Nil; Ph.D. Students: Minal G. Mahajan; Human Ethical Approval: NA

Background: Matairesinol (MR) was tested for its activity against androgen- independent prostate cancer cell line PC3. MR reduced the viability and growth kinetics of PC3. It also decreased mitochondrial membrane potential of PC-3.

Work done: The effect of MR in regulating the expression of de novo cholesterol synthesis genes was determined in PC-3 cells.

Results and conclusion:

- In PC3 cells, at 80 µg/ml dose of MR, gene expression of SREBP-2 was decreased by 33.2 folds. At the same concentration, mRNA expression of HMGCR was down regulated by 68.9 folds.

- In conclusion, MR has a potential to regulate aberrant de novo cholesterol synthesis in prostate cancer cells.

Title: Evaluating the effect of lignans in regulation of lipid and cholesterol metabolism in breast cancer.

Project ID:
Funding: DST Inspire
Duration: 2019- Till present
Sanctioned Amount: Rs. 23,62,400/-
Investigators: PI – Dr. Ruchika Kaul-Ghanekar; Co PI-NA
Ph.D. Students: Prajakta Devappa Patil
Human Ethical Approval: NA

Background:
One of the most important metabolic pathways that has been found to be deregulated in breast cancer cells is de novo lipogenesis and cholesterol biosynthesis. Breast cancer cells overproduce fatty acids by de novo pathways to build new membranes and maintain active signaling for their growth, proliferation and survival. Thus, novel agents should be tested that will regulate the growth of breast cancer cells by targeting de novo lipogenesis and cholesterol pathways. Recently, dietary lignans have been shown to exhibit promising anti-cancer activity against breast cancer. Various lignans have been shown to regulate cholesterol and lipid metabolism in hypercholesterolemia and cardiovascular diseases. In this project, we propose to evaluate the anti-cancer activity of some selected lignans through regulation of de novo cholesterol and lipid metabolism in breast cancer at in vitro and in vivo levels.

Work Done: In silico approach was first used to evaluate the available lignans for pharmacokinetic, drug-likeness and pharmacodynamic properties. From molecular docking it was found sesamin has highest binding energy to most of the genes involved in lipid and cholesterol biosynthesis pathways. Sesamin was tested further at in vitro level against MDA-MB-231, triple negative breast cancer cells. The effect of sesamin on viability of MDA-MB-231 was determined.

Results and conclusion

- In silico molecular docking of 17 selected lignans was carried out with the genes involved in lipid and cholesterol biosynthesis pathways. Out of which Sesamin showed highest binding energy to most of the genes. Interestingly, for some genes [LDLR, FASN, SCD-1, ACC-1, PPAR-ϒ and SREBP-1] binding energy of sesamin was found to be higher than standard drug, doxorubicin
- The effect of sesamin was tested on the viability of triple negative breast cancer cells, MDA-MB-231. Sesamin significantly decreased the viability of MDA-MB-231. At the maximum dose of 200 μM/ml, viability of MDAMB231 was decreased to 80.6 ± 3.5%
- **In conclusion, sesamin exhibited anticancer potential and further its activity would be confirmed at mechanistic level**

Title: Evaluating the effect of selected bioactives on cytokine and chemokine regulation in prostate cancer. Project ID:; Funding: DST Inspire; Duration: 2019- Till present; Sanctioned Amount: 23,62,400/-; Investigators: PI: Dr. Ruchika Kaul-Ghanekar, Co PI- NA; Ph.D. Students: Rama Amar Rajadnya; Human Ethical Approval: NA

Background: Previously we showed that Matairesinol (MA) and 6 Gingerol (6G) decreased the growth of prostate cancer cell line PC3. MA showed better results and thus was taken forward for further studies.

Work done: In silico approach was first used to evaluate the selected (N=20) phytocompounds for pharmacokinetic, drug-likeness and pharmacodynamic properties. Out of which matairesinol (MAT) exhibited best pharmacokinetic profile with minimum toxicity. MAT was tested further for its effect on growth kinetics, mRNA expression of pro- and anti-inflammatory cytokines against androgen independent prostate cancer cell line.

Results and conclusion:

- MAT decreased the number of colonies in PC3 cell line, compared to the untreated control cells.
- MAT decreased the mRNA expression of pro-inflammatory cytokines, TNF-α, TGF-β and IL-6 expression by 4.0, 6.5and 3.3 folds, respectively, at 200 µM/ml dose.
- MAT increased the mRNA expression of anti -inflammatory cytokine IFN-γ expression by 2.02 fold at 200 µM/ml
- IL-10 is well known anti-inflammatory cytokine, but it is a well- known Pro-tumorogenic cytokine in PCa. IL-10 expression was decreased by 4.49 fold at 200 µM/ml
- In conclusion, MAT exhibited anticancer potential and further its activity would be confirmed by downstream signaling pathways.

Title: Evaluation of anti-cancer potential of selected phytochemicals against breast cancer stem cells. Project ID:; Funding: DBT; Duration: 2019-Till present; Sanctioned Amount: 23,70,000/-; Investigators: PI: Dr. Ruchika Kaul-Ghanekar, Co PI: Nil; Ph.D. Students: Akanksha V. Mahajan; Human Ethical Approval: NA

Background:

Breast cancer (BC) is the leading cause of cancer death among women worldwide. Despite of the available advanced treatment options, the problem of cancer recurrence and drug resistance remains to be a serious problem in the treatment of BC. It has been well established that CSCs lead to tumor relapse add to aggressiveness of tumor. BCSs exhibit resistance to most anti-cancer agents including chemo-and/or radiotherapy. Therefore, strategies which could inhibit CSCs must be employed complementary with the conventional treatments. Recently, naturally derived compounds are gaining more attention of the researchers as chemical drugs are associated with side effects. Thus, we propose to work on finding new phytochemicals that can potentially target BCSCs.

Work done: In the study, the photochemicals for anti-cancer study have been selected from Xanthium Strumarium. Our preliminary work showed that aqueous extract of Xanthium Strumarium seeds (XSaq) exhibited anti-cancer activity against breast cancer cells, MDAMB231. LCMS analysis of XSaq revealed the presence of phytochemicals targetting different pathwys in cancer progression.

Results and conclusion

- XSaq at 1280 ug/ml dose decreased the viability of MBAMB231 to 64.9 ± 5.7 and 55.1 ± 1.2 % at 48 and 72 h, respectively. XSaq reduced the growth kinetics of MDA MB-231 cells in dose and time dependent manner.
- LCMS analysis of XSaq showed presence of 157 components. 19 phytocompounds have previously showed to have pharmacological activity
- GR001 phytocompound showed strongest binding energy towards the markers involved in breast cancer stem cell self-renewal and maintenance.
- **In conclusion, GR001 may target the cancer stem cell markers and thus would be evaluated for its inhibitory activity against breast cancer stem cells.**

Title: Evaluating the effect of Matairesinol on Macrophage Polarization

Project ID: CR/18 (18-22)/6/P); Funding: DBT; Duration: 2019 –Till present; Sanctioned Amount: 23,64,000/-; Investigators: PI- Dr. Ruchika Kaul-Ghanekar, Co-PI- NA; Ph.D. Students: Amol Rajendra Chaudhary; Human Ethical Approval: NA

Background: 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.

Work done: THP-1 derived macrophage model was developed. The effect of Matairesinol on Macrophage polarization markers were studied at mRNA level.

Results and conclusion:

- The effect of different doses (0.25-2 µg/ml) of Matairesinol on the viability of THP-1 cells and THP-derived macrophages (M0, M1, M2) was evaluated by MTT assay for 24-72 h. The cells showed viability at 24 and 48 h in the presence of 0.25 µg/ml dose of Mat.
- Interestingly, at 24h, all the macrophages (M0, M1 and M2) exhibited viability at all the doses of the drug. However, at 0.25 µg/ml dose, all the macrophages showed viability at 48 and 72h. Since the macrophages were viable at all the doses of the drug up to 24h, we selected two doses of Mat (0.25 and 1.0 µg/ml) for further polarization studies.
- M2a macrophages were treated with 1µg/mL Matairesinol for 24h and evaluated for expression of polarization markers at mRNA level. Both M2 (CD206, TGM2, IL-10) and M1 (CD197, CD86, IL-6) markers were found to be significantly downregulated in the macrophages. CD206, TGM2 and IL-10 were significantly reduced by 18.5, and 26.3-folds, respectively. Similarly, CD197, CD86, IL-6 markers were decreased by 34, 22.86 and 2.63-folds, respectively.
- **In Conclusion, Matairesinol was found to be non-toxic for M0, M1 and M2 cells over 24h of treatment. It regulated expression of inflammatory markers in macrophages.**

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-Till present; Sanctioned Amount: 33,16,800/-; Investigators: PI- Dr. Ruchika Kaul-Ghanekar, Ph.D. Students: Ms Amrita Ulhe; Human Ethical Approval: NA

Background: Earlier, we found that ALA induced significant inhibition of HDAC1 and DNMT1 expression in cervical cancer cell lines indicating epigenetic modulation. ALA increased 5-mc content in HeLa, SiHa and C33A and decreased expression of DNMT3b in HeLa, SiHa and C33A.

Work done: The effect of ALA on DNA methyltransferase-1 (DNMT-1) and Histone Decacetylases-1 (HDAC1) was determined in HeLa, SiHa and C33A, cervical cancer cell lines.

Results and conclusion:

- ALA significantly decreased DNA methyltransferase (DNMT)-1 expression in cervical cancer cells. DNMTs facilitate hypermethylation of CpG islands in the promoter region of tumor suppressor genes leading to their silencing. Inhibition of DNMT-1 can lead to DNA demethylation and can thus restore the expression of tumor suppressor genes and cell cycle regulators. In HeLa, SiHa and C33A at maximum dose of 80 µM, DNMT1activity was decreased by ~1.5,~2.4 and ~6 folds, respectively, compared to the untreated control cells.
- ALA also decreased HDAC1 expression in cervical cancer cells. HDACs are chromatin modifying enzymes that are over-expressed in cancer. They are involved in deacetylation of histone proteins that result into inhibition of transcription of tumor suppressor genes, thereby favouring the growth of cancer cells. At 80 µM dose of ALA, HDAC activity was reduced by ~1.8, ~1.7 and ~2.4 folds, respectively, in HeLa, SiHa and C33A, compared to the untreated control cells.

- In conclusion, ALA exhibited significant inhibition of DNMT1 and HDAC1 expression in cervical cancer cell lines indicating epigenetic modulation in cervical cancer cells.

Title: Phytochemical standardisation 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; Duration: 2020-2023; Sanctioned amount: 43,57,750/-; Investigators: PI: Dr. Ruchika Kaul Ghanekar, Cancer Research Lab, IRSHA, BVDU; Co-PI: Dr BothiRaj; Human Ethical Approval: NA

Background:
In the previous year, the authentic plant material resources were identified and plant material was obtained. The preparation of traditional Unani formulation, *Itrifal Ghudadi* was optimized under the guidance of Hakim.

Work done:
The phytochemical composition of IG and its individual ingredients has been studied by LCMS. The effect of IG has been studied on the viability of cervical cancer (HeLa and SiHa), breast cancer (MCF-7 and MDA MB 231) and oral cancer (SCC-9) cell lines. The cancer cell line showing efficient response to IG has been studied for growth kinetics.

Results and conclusion:
- The LC-MS analysis of IG revealed the presence of 43 bioactives with reported pharmacological activity.
- IG significantly decreased the viability of HeLa, SiHa, MDAMB-231, and SSC-9 cells and was found to be most potent against MDAMB231. At 800 µg/ml, viability of MDAMB 231 was decreased to 43.9 %.
- IG decreased the growth kinetics of MDAMB231 in a dose and time dependent manner. At 300 µg/ml dose, growth of cells was decreased to 69.9, 88.3, 94.9 % for 24, 48 and 72 h respectively, compared to the untreated cells.
- **In conclusion, IG exhibit anticancer potential and further its activity would be confirmed at mechanistic level.**

Title: Evaluating the anticancer activity and mechanism of action of Unani formulation *Habbe Musaffi Khoon* (HMK) against cervical cancer. Project ID: (Z28015/61/2018-HPC (EMR)-AYUSH-C); Funding: CCRUM Ministry of AYUSH; Duration: 2018-Till present; Sanctioned amount: 57,56,500/-; Investigators: PI: Dr. Ruchika Kaul Ghanekar; Co-PI: Dr. Gazalla Mulla (Z.V.M, Unani Medical College, Pune, Maharashtra); Dr. Prerna Raina (Cancer Research Lab, IRSHA, BVDU); PhD Students: Nidhi Sharma; Human Ethics Approval: NA

Background: Earlier, the effect of aqueous extract of *Habbe Musaffi Khoon* (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. The mechanism of action of HMKaq was studied in terms of regulating apoptosis in cervical cancer cell lines.

Work done: The mechanism of action of HMKaq was evaluated on cervical cancer cells (HeLa, SiHa and C33A) in terms of regulating angiogenetic marker (VEGF), metastasis marker (MMP-2) , Tumor regulatory markers (p53 and MDM2) and HPV oncoproteins E6 and E7

Results and Conclusion:

- At 80 µg/ml dose, HMK downregulated the mRNA expression of VEGF in HeLa, SiHa and C33A by 1.7, 2.4, 1.7 folds. At the same dose, mRNA expression of MMP-2 in HeLa and SiHa was decreased by 1.5 and 1 folds, respectively. The mRNA expression of p53 in HeLa, SiHa and C33A was increased by 1.5, 2.9, 1.4 folds at 80 µg/ml concentration, respectively. HMKaq downregulated MDM2 mRNA expression in HeLa, SiHa and C33A by1.5, 2 and 1.3 folds.
- It can be concluded that HMKaq inhibited angiogenesis and metastasis in HeLa, SiHa and C33A by down regulating the gene expression of VEGF and MMP2 genes . HMKaq inhibited the growth of cervical cancer cells by targeting p53-MDM2 pathway.

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-WOSA; Sanctioned Amount: 32,06,000/; Project Investigator: Dr. Ashwini Kamble; Duration: 2018-2021; Human Ethical Approval: Ref: BVDU/MC/57 and BJGMC/IEC/Pharmac/ND-Dept 0119007-007

Background Abnormal cervicovaginal microflora plays an important role in HPV persistence and progression to cervical cancer. The present study aimed at isolating and identifying probiotics from vaginal swabs of healthy women and evaluating their activity against vaginal pathogens isolated from cervical cancer patients.

Work done: Cell Free Supernatants (CFS)s of the selected probiotics were evaluated for their anticancer activity against human cervical (SiHa, HeLa, C33A) cancer cell lines using MTT assay.

Results and conclusion:

- CFSs of probiotics inhibited the growth of cervical (SiHa, HeLa, C33A) cancer cells at 24 h.
- **Conclusion. The data suggest that vaginal probiotics exhibited anticancer activity.**

Title: Evaluating the anticancer activity of homeopathic preparation of *Linum usitatissimumin* breast cancer cell lines. Project ID:Z.28015/02/2018-HPC (EMR) AYUSH-D; Funding: EMR, AYUSH CCRH; Duration: 2018-2022; Sanctioned amount: 43,57,750/- ;Investigators: PI: Dr. Prerna Raina, Cancer Research Lab, IRSHA, BVDU; Co-PI: Dr Swati Shinde

Background: The effect of homeopathic potencies of *Linum usitatissimum* was evaluated on the viability of breast cancer cell lines, MCF-7and MDA-MB-231 and compared with the respective potentized D.A. 6C potency was found to exhibit cytotoxicity against both MCF-7and MDA-MB-231.

Work done: We evaluated the effect of 6C and 6P at molecular level on gene expression of tumor regulatory markers in breast cancer cells.

Results and conclusion:

- 6C at 1: 500 dilution significantly increased the expression of p53 at both gene and protein level in MDAMB231. It was observed that compared to the untreated control cells, 6C significantly increased gene expression of p53 by 1.5 folds. Moreover, 6C also increased the p53 protein levels by 1.49 folds compared to control cells. 6P did not induced any increase in p53 expression at gene and protein level.
- 6C at 1: 500 dilution significantly decreased the gene expression of MDAMB231 cells by 3.8 folds. However, 6P also decreased the MDM2 gene expression by 2 folds.
- **In conclusion, 6C inhibited the growth of MDAMB231 by targeting p53-MDM2 pathway.**

Name of the Programme: Obesity-Diabetes

1. **Title**	:	Effect of Yoga intervention on skeletal muscle linked glucose homeostasis in pre-diabetic individuals
Funding	:	DST (SATYAM)
Duration	:	March 2019-March 2022
Sanctioned Amount	:	Rs.46, 74,200/-
Received Amount	:	Rs.13,00,000/-
Principle Investigator:		Dr. Supriya Bhalerao
Co-Investigators 1	:	Dr. Pranita Ashok
Co-Investigator 2	:	Mrs. Anita Patil
Project Staff	:	Dr. Ravina Randive (SRF) from 1st Jan 2021 onwards Dr. Suresh Khadke (JRF) from 1st Jan 2021 onwards
Ethics Approval	:	IEC/2019/05 (04.03.2019) IEC/2019/35 (06.07.2019 amended) IEC/2019/05

Background:
In the present study, pre-diabetic individuals identified through community screening 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 and glycemic status. It is expected that the project will enable us to bridge the gap in existing knowledge about Yoga and its effect on skeletal muscle linked glucose homeostasis.

Objectives:
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.

Work done:
The first year of the project finished in March 2020. However, the 2nd year installment of the project was released in December 2020. During the period from April to December 2020, we explored the possibility of conducting intervention sessions online. In the first year of the project, we had observed a poor response of individuals to a multi-step screening procedure, which had resulted in a low recruitment rate. To find out solutions for this problem, we had arranged online discussions with 2 experts working in the field of yoga research.

The new project staff was appointed in January 2021. As we had lost almost 9 months of the study due to lockdown and subsequent restrictions on group activities, to cope with the loss we initiated the study at two more sites in Pune city viz., National Institute of Naturopathy (an autonomous body under Ministry of AYUSH, Government of India) and Dr. D. Y. Patil College of Physiotherapy.

In addition to promotional activities mentioned in last year's report, this year we prepared AVs to create awareness about prediabetes and to provide information about the project. Use of social media platforms like WhatsApp and Facebook is another important addition in promotional activities. The Facebook page for the project can be visited at

https://www.facebook.com/Prediabetes_IRSHA-101066471949443/ . To encourage the potential participants and to involve completed participants in propagating the project information, we felicitated the participants who had completed the study in the 1st year of the project. We have established collaboration with various clinicians in Bharati Hospital and around Pune city to provide information about the project to their patients

We resumed community screening from 2nd February 2021. The following table shows the recruitment details of the participants. Recruitment is still ongoing to complete the planned target.

Details	**Participant number**
Screened	56
Recruited	08
Complete participants	03
Dropout participants	04
Ongoing participants	01

2. **Title** **:** Evaluation of the effect of Triphala on Obesity associated Cognitive impairments (OB/15 (15-18)/8/P)

Funding : Generated funds & Sakal India Foundation 2019
Duration : 2016-2020
Investigators:
PI- : Dr. Supriya Bhalerao
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 65 individuals have been screened and 11 have been recruited (normal = 7, Obese = 4) so far.

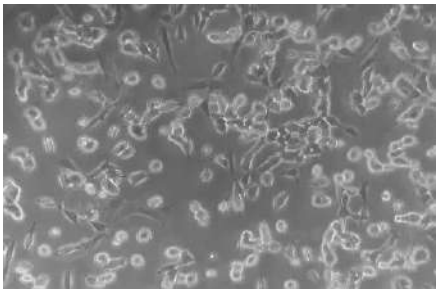
Objective 2:

Manuscript has been communicated to the Indian Journal of Pharmaceutical Sciences

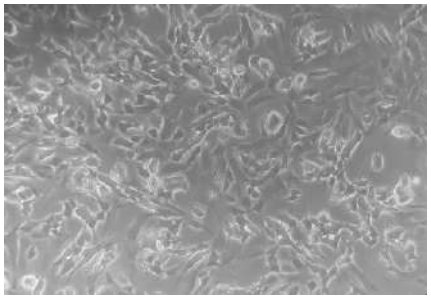
Objective 3:

Based on the finding earlier year, it was decided to use SHSY5Y cell line for the study. SHSY5Y cell line was purchased from NCCS Pune, maintained in Hams F12 K medium, 10% fetal bovine serum at 37°C in a 5% CO_2 incubator. Cell seeding density for 96 well plate ($1*10^3$) and 24 well plate ($1*10^5$) has been standardized for further experimentation.

Figure.1: Morphology assessment of SHSY5Y cells



Day 1



Day2

Cell viability assay

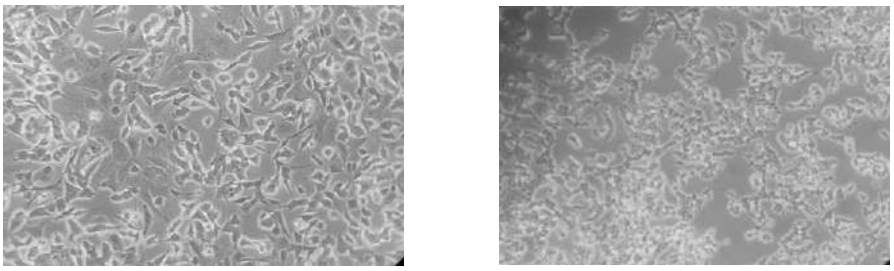
To determine non-toxic concentrations of Triphala (Aqueous extract) the cells were treated with its different concentrations (5,10,15, 25,50 and 100 µg/ml) and different time points (24, 48hrs). The viability of the cells was determined using MTT [3- (4, 5-cimethylthiazol-2- yl)-2, 5-diphenyl tetrazolium bromide] assay. Cells were observed for the changes in morphological appearance and percent cell viability was calculated by the absorbance ratio of the treatment group over the control group. Out of tested concentrations of extract 5,10,15 µg/ml were found to be non-toxic with percent viability more than 97.15%

Lipotoxicity induction and oxidative stress:

The FFA (palmitic acid)-bovine serum albumin (BSA) complex was prepared by following standard methodology & used to treat neuronal cells for 24 or 48 h according to the experimental design with different concentrations like 100, 200, 400 and 500µM. Cell viability was checked

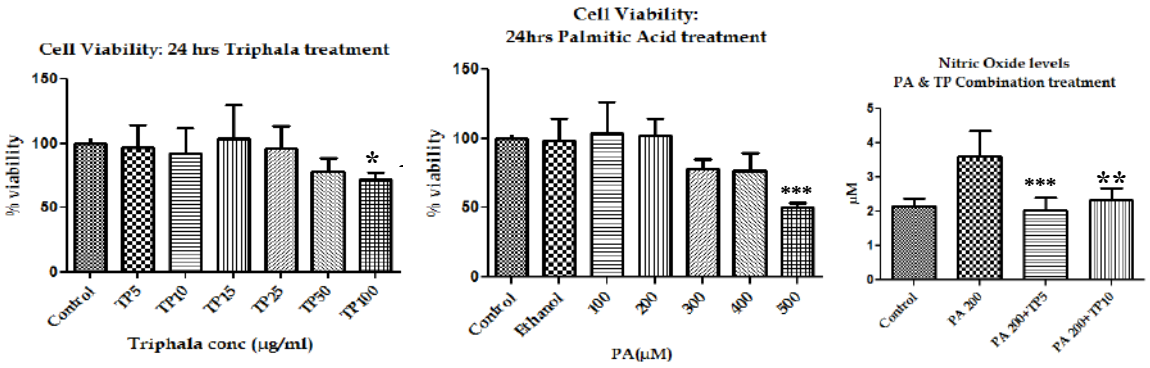
to find the toxic concentration of palmitic acid. Oxidative stress marker (nitric oxide) was estimated by using Griess method.

Figure2: Lipotoxicity of palmitic acid



Control **Palmitic acid (200µM)**

Palmitic Acid induced cytotoxicity towards SH-SY5Y was a time- and concentration-dependent.



Triphala has been found to regulate nitric oxide levels in fatty acid induced lipotoxicity.

Objective 4:

The study to achieve objective 4 has been started after Institutional Animal Ethics Committee Permission. Forty-eight male Wistar rats of 6-7 weeks old (100-110gm) have been divided in the 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)	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 (200 mg/kg bw/day) for 30 days

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

- Body Weight (weekly)
- Behavioral parameters on 90^{th} and 120^{th} day:
    - Water maze
    - Elevated Plus maze

The animals will be humanely sacrificed after completion of the experiment (i.e., Day 120). 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-cholesterol
- Oxidative stress markers (Brain homogenate): Malondialdehyde on 120^{th} day
- Acetyl choline esterase (Brain homogenate) on 120^{th} day
- Fatty acid composition of brain homogenate on 120^{th} day
- Gene expression studies from brain (BDNF, NRF2, FAS & TNFα.) on 120^{th} day
- Brain histology (Atrophy, inflammation, vascular damage etc.) histology after completion of the experiment on 120^{th} day

3. **Title** **:** Evaluation of the effect of CIT on innate and adaptive immune response in healthy individuals

Funding	:	Charak Pharma Pvt. Ltd.
Duration	:	October 2020 - ongoing
Sanctioned Amount	:	Rs.10,50,000/-
Investigators:		
PI-	:	Dr. Supriya Bhalerao
Co-PI	:	Dr. Madhavi Mahajan
Ethics Approval	:	BVDUCOA/EC/2829/2020-2021
CTRI registration	:	CTRI/2020/12/030139

Background:
The COVID-19 pandemic has emphasized the need of maintaining and boosting immunity. Though Ayurveda can offer various immunomodulating drugs and formulations, lack of scientific evidence raises a question about the potential of these medicines. The present study was therefore planned to study the effect of a patent and proprietary polyherbal formulation CIT on innate and adaptive immune responses in healthy individuals.

Objectives:
1. To study the effect of CIT on innate immunity.
2. To study the effect of CIT on cell mediated immunity.
3. To determine the safety profile of CIT.

Work done:
After obtaining Ethics Committee permission and standardization of various study parameters, the screening of individuals began in the month of January 2021. A total of 36 participants have been recruited in the study so far: 24 in the CIT Group and 12 in the Placebo group. The following parameters are being studied in these participants:

1. Vitals- Temperature, Pulse and Blood pressure
2. Respiratory health- respiratory rate, oxygen saturation and peak expiratory flow
3. Immune status, Perceived stress, Quality of Life
4. Hematological parameters- Hemoglobin, Total WBC count, ESR, Platelets, Liver function tests, Renal function tests
5. Immune parameters- absolute counts of CD4 and CD8, CD4/CD8 ratio, RBC MDA, RBC GPx, IFN gamma, TNF α, IL 10,

Communicable Diseases

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 develop adjuvanted chikungunya vaccine. During the previous years, CHIKV was propagated in Vero cell line and inactivated by treatment with β-propiolactone. Immunization studies were executed to evaluate antibody response after delivery of formulations by i.d. or i.m. routes.

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.

Antibody responses after immunization with varied amount of adjuvant

Neutralizing antibody response after immunization

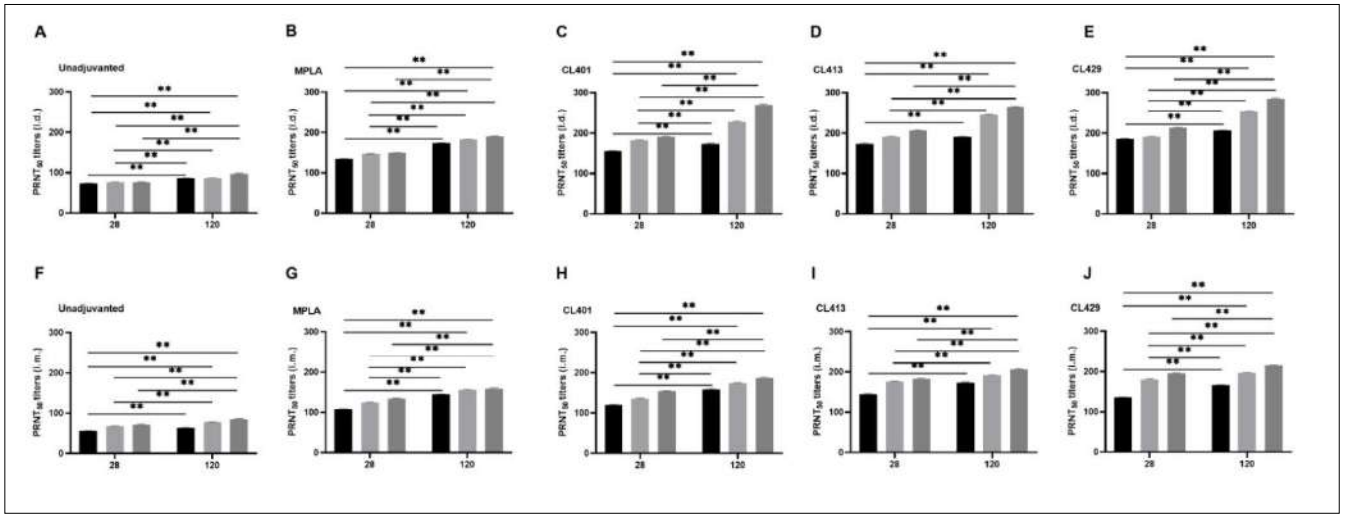


Fig 1. Mice were immunized twice with 2.5µg (black), 5µg (grey) or 10µg (dark grey) inactivated CHIKV without or with 2.5µg adjuvants by i.m or i.d. route. $PRNT_{50}$ titers were determined a week i.e. day 28 and 3 months after second dose.

MPLA and chimeric adjuvant induce higher neutralizing antibody titers than unadjuvanted CHIKV. Similar observations were seen after i.m. delivery of formulations. All chimeric adjuvants were superior to MPLA for neutralizing titers induction. I.d. delivery induced higher titers than i.m. delivery. PRNT50 titers were higher for both i.d. and i.m. deliver on day 120 compared to day 28.

Binding antibody response after immunization

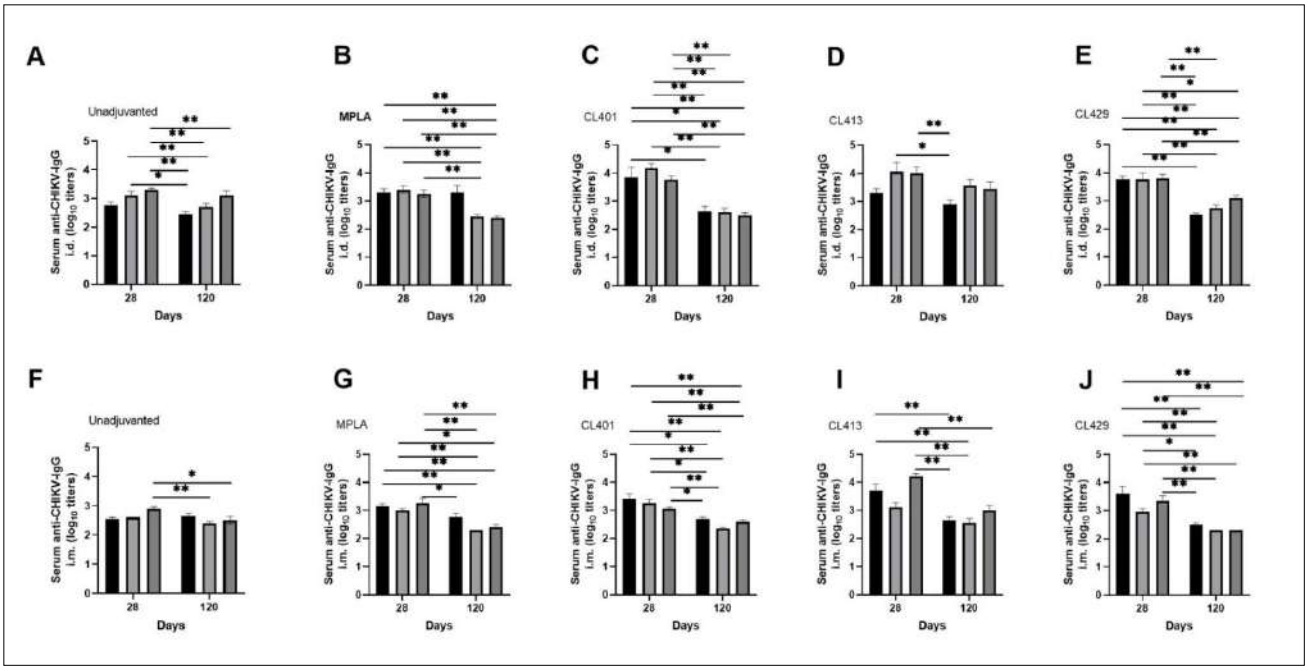


Fig 2. CHIKV specific IgG response after administration of 2.5µg (black), 5µg (grey) or 10µg (dark grey) inactivated CHIKV without or with 2.5µg adjuvants via i.m. or i.d. route.

Chimeric adjuvants induced significantly higher IgG as compared to unadjuvanted and MPLA adjuvanted inactivated CHIKV. I.d. delivery elicited higher IgG titers than i.m. delivery. CL413 induced similar titers by after administration by both routes. CL401 and CL429 induced superior response after i.d. delivery. All the chimeric adjuvant induced higher IgG titer as compared to MPLA. A significant drop in IgG titers was observed three months post second dose.

IgG antibody subclass response after immunization

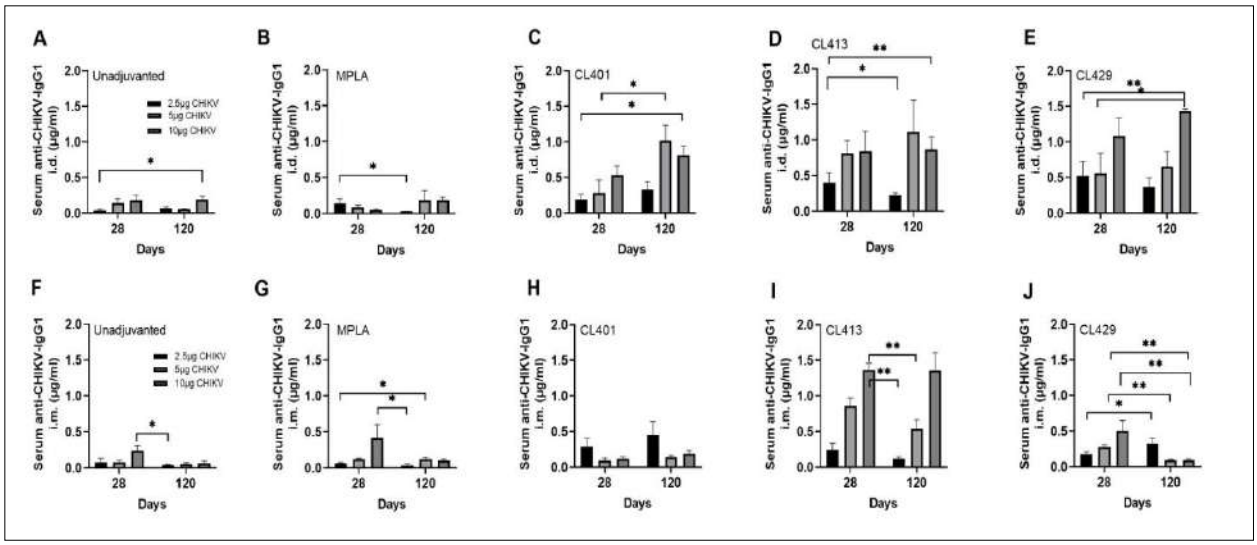


Fig 3. CHIKV specific IgG1 response after administration of 2.5µg (black), 5µg (grey) or 10µg (dark grey) inactivated CHIKV without or with 2.5µg adjuvants via i.m. or i.d. route.

Chimeric adjuvants induced increased IgG1 as compared to unadjuvanted or MPLA adjuvanted CHIKV (Fig 3) after i.d. delivery. Adjuvanted 2.5µg inactivated CHIKV induced lower IgG1 as compared to 5 or 10µg.

In case of i.m. delivery, CL413 and CL429 elicited higher response than unadjuvanted or MPLA adjuvanted inactivated CHIKV. No difference in IgG1 levels was observed in mice administered with MPLA and CL401. Inactivated CHIKV dose dependent decrease in IgG1 levels was observed for CL401. Among all adjuvants, CL413 induced higher IgG1 levels by i.d. or i.m. delivery.

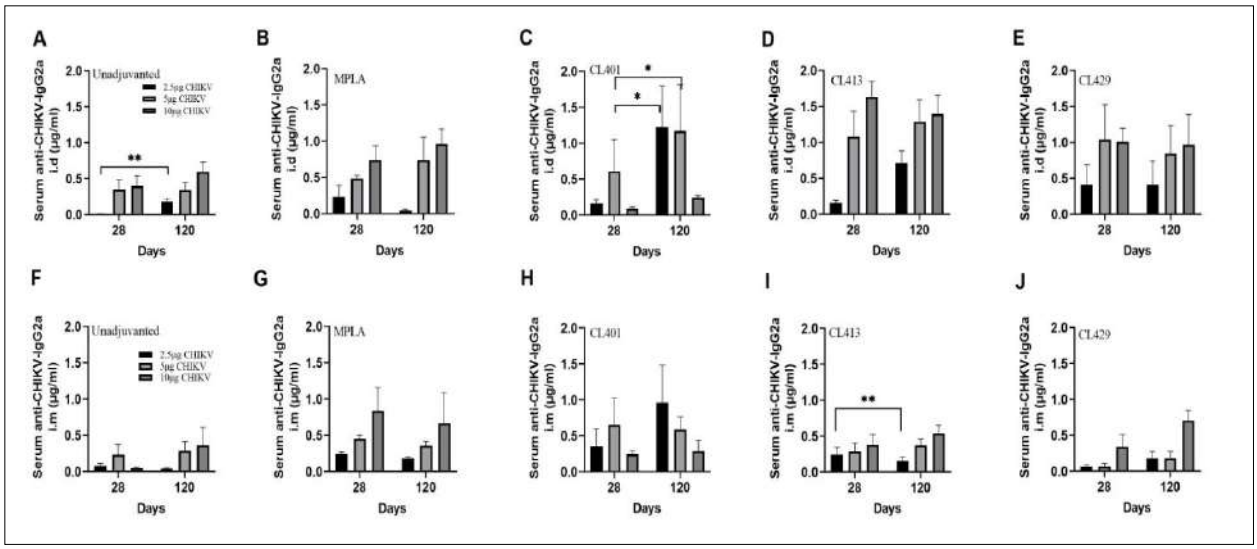


Fig 4 CHIKV specific IgG2a response after administration of 2.5µg (black), 5µg (grey) or 10µg (dark grey) inactivated CHIKV without or with 2.5µg adjuvants via i.m. or i.d. route.

No difference in CHIKV specific IgG2a levels was observed after administration of inactivated CHIKV with different adjuvants by i.d. delivery. IgG2a levels increased with inactivated CHIKV dose for MPLA and CL429. On the contrary, 10µg inactivated CHIKV induced lower IgG2a response when administered with CL401 and CL413. Similar observations were seen with i.m. delivery of various formulations. 2.5µg CL401 administered with 2.5µg CHIKV administered by i.d. route induced highest IgG2a.

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: Plan of the 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: Work on objective one out of three was carried out during the year

Recombinant bacmid for RSV M, G and F:

Recombinant bacmids specific for M, G, PreFG and PreF was extracted from DH10Bac *E. coli* by alkaline lysis method. To verify the presence of gene of interest in the recombinant bacmids, PCR anaylysis was done using primers M13F and M13R. Successful transposition gives PCR products of the size covering the mini-attTn7 site (~2300bp + size of insert), and non-recombinant bacmid alone gives a PCR product of ~300bp. Recombinant bacmids of M, G, PreFG and PreF gave PR products of sizes 3.0kb, 3.2kb, 4.0kb, 4.1 kb, 3.9kb respectively were generated as shown in Fig 5A, 4B, 4C, 4D and 4E respectively.

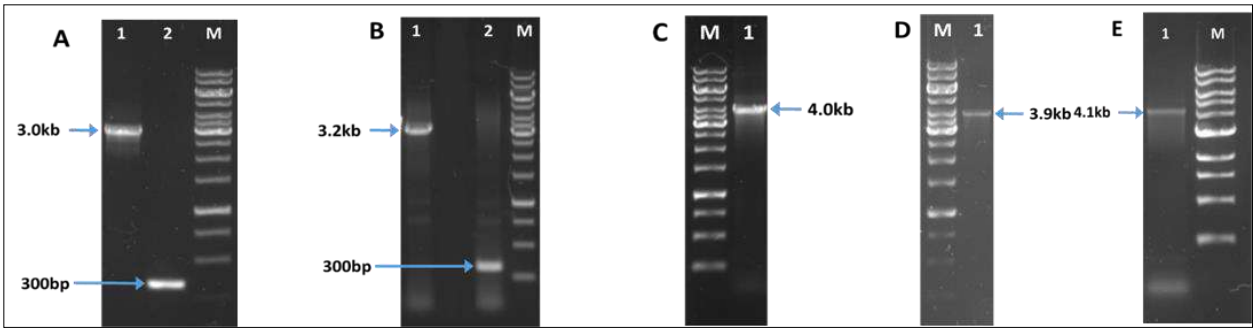


Fig 5. Analysis of recombinant bacmids by PCR. **(A)** PCR confirmation of RSV-M bacmid, which shows PCR product of 3.0kb in Lane 1. Lane 2 shows the PCR product generated by PCR of non-recombinant bacmid. **(B)** PCR confirmation of RSV-G bacmid, which shows PCR product 3.2kb in Lane 1. Lane 2 shows the PCR product generated by PCR of non-recombinant bacmid. **(C)** PCR confirmation of RSV-PreFG bacmid, which shows PCR product of 4.0kb in Lane 1. **(D)** PCR confirmation of RSV-PreF bacmid, which shows PCR product of 3.9kb in Lane 1. Lane M is loaded with 1kb DNA marker. **(E)** PCR confirmation for RSV-F0 bacmid which shows PCR product of 4.1kb in Lane 1. Lane M is loaded with a 1kb DNA marker.

Protein expression:

Expression levels of proteins of RSV M, G, F_0, PreF and PreFG were confirmed by indirect ELISA using anti-RSV immune sera. Positive control showed reactivity with expressed proteins. Secretion of all proteins was observed in cell supernatants (Fig. 6A-F). No reactivity was found using RSV-negative sera.

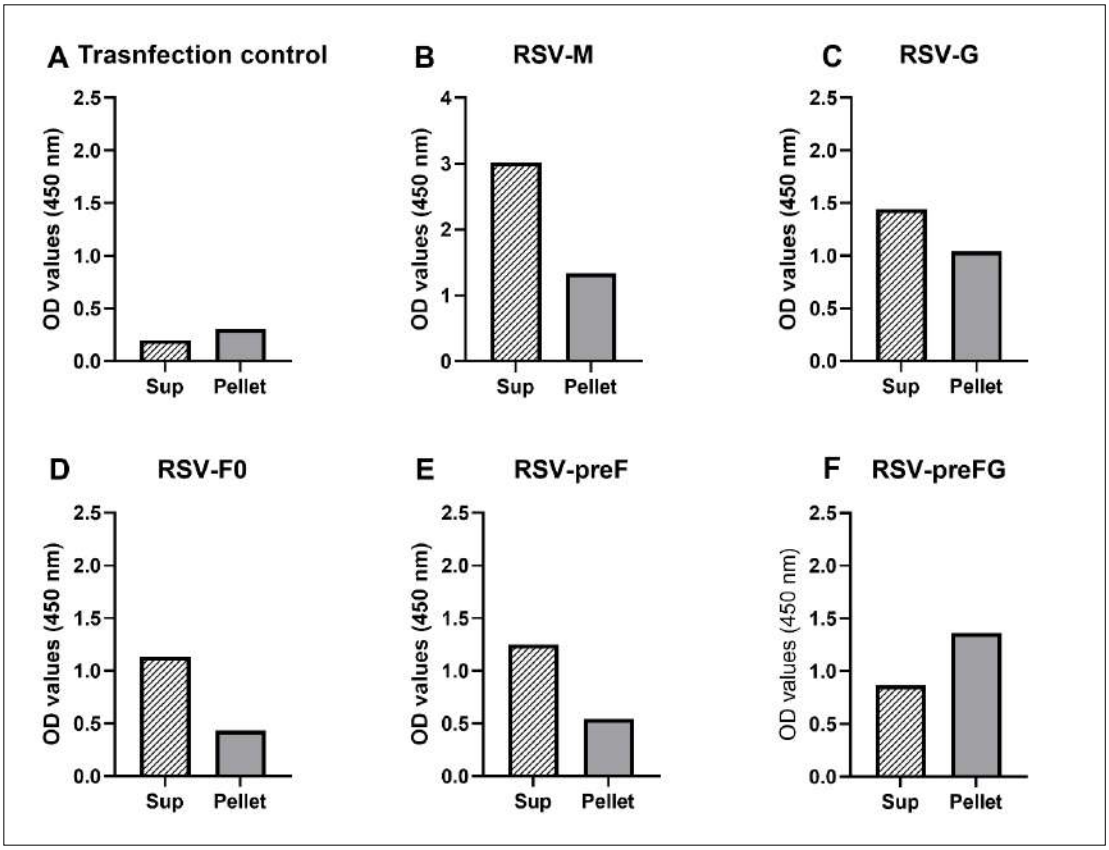


Fig 6. Expression of various RSV proteins was conferment by indirect ELISA using supernatant (sup) and lysed cell pellet of ExpiSF9 cells. Mock transfect bacmid was used as control.

Production and characterization of VLPs:

RSV-VLPs generated with either PreFG or PreF were purified by two step ultra-centrifugation. Step 1 was done on a 20% sucrose cushion (Fig 5A) to remove contaminant proteins and RSV proteins that are not associated with the RSV-VLP. The pellet (Fig 7A) was resuspended and further purified by centrifugation using discontinuous 20-60% sucrose gradient. Clear protein ring was seen between 30% and 40% sucrose gradient which are expected densities where VLP should appear (Fig 7B). Fractions of 200µl were collected and used for RSV-VLP analysis.

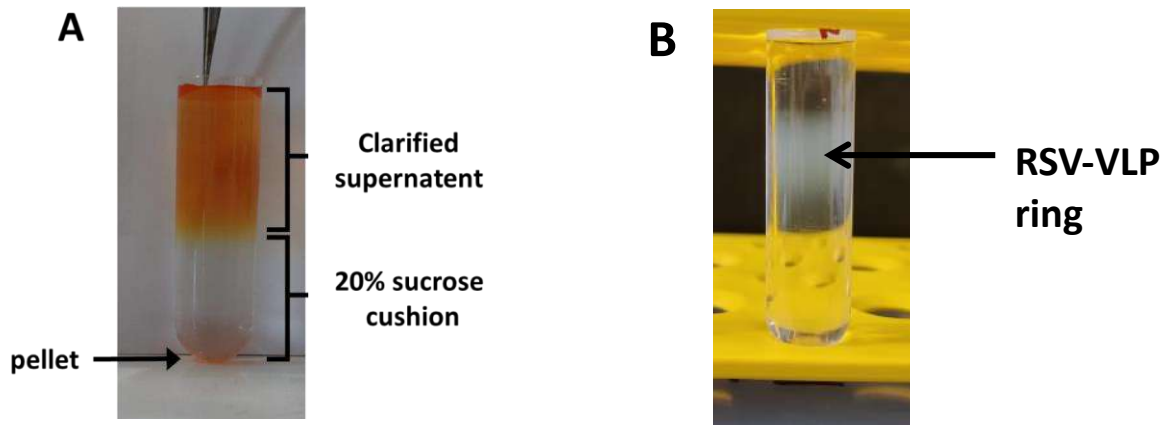
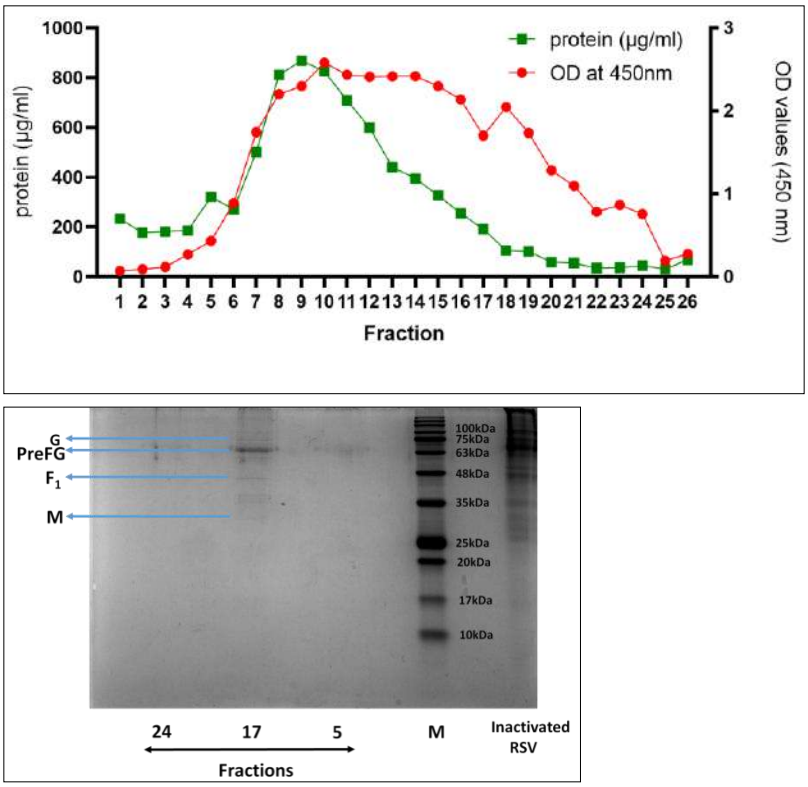


Fig 7. Purification of RSV-VLPs. **(A)** Pellet collected ultra-centrifugation of VLP harvest on 20% sucrose. **(B)** Protein ring of VLPs formed after ultra-centrifugation on discontinuous sucrose gradient.

Analysis of the collected fractions for RSV-VLP formation was done by protein estimation, indirect ELISA and SDS-gel. A peak of ELISA OD and protein content was observed at fractions 17 and 18 (fractions corresponding to ring) for VLP made using preFG and preF respectively (Fig 8A). These fractions were loaded and analyzed by SDS-gel to observe the individual protein bands formed in PreFG-VLP and PreF-VLP (Fig 8B and C). Band for G, preFG, preF, F1, M corresponding to 90, 62, 59, 50 and 28kDa were observed when peak fractions were loaded on to the gel. However, 28kDa band corresponding to M was not observed for preF. These results indicate G, preFG and M interact with each other to form VLP. Association of G and preF can be established but further studies are needed to check association with M.



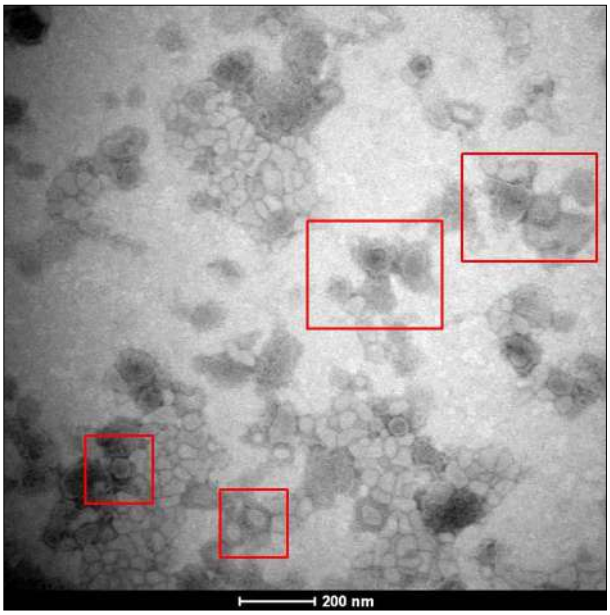


Fig 8. Analysis of RSV-VLPs. **(A)** ELISA and protein quantification of fractions PreFG and PreF VLPs by ELISA. **(B)** SDS gel analysis of PreFG VLPs

Conclusion: RSV M, G and preF and preFG specific bacmids were successfully produced using baculovirus expression system. Transfection ExpiSF9 cells resulted in expression of all proteins. Coinfection of ExpiSF9 cells with M,G and preFG resulted in formation of RSV-VLP

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, Mrs. Archana Sharma

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. In the first year, protocol for platelet isolation and platelet derived exosomes isolation was standardized.

Work done:

Due to COVID-19 pandemic in the year 2020, no samples was collected during the year 2020. We attempted to isolate platelets from healthy donors and culture in-vitro to study the platelet activation on exposure to dengue viruses.

Results:

In-vitro culture of platelets isolated from healthy donors

We collected the blood samples in vacutainer tube containing acid citrate dextrose buffer from healthy donors (n=3). All the healthy donors were negative for NS1 antigen and anti-DENV IgM antibody. After sample collection, platelet rich plasma (PRP) was obtained by centrifugation at 400 xg for 10 minutes, followed by centrifugation of PRP at 1000xg for 10 minutes to isolate platelets. The platelets were cultured in-vitro in RPMI -1640 media containing 10% exosome depleted FBS and antibiotics. Platelets were exposed to dengue viruses (DENV-1, DENV-2, DENV-3 and DENV-4) at four different time-points - 1.5hr, 6hr, 18hr and 24hr to study the activation status.

As shown in Fig. 9(A), irrespective of the dengue virus serotype, the highest level of platelet activation was achieved at 24hr post exposure to dengue viruses. Next, we examined the expression profile of CD63, exosome marker on platelet surface. As shown in Fig. 9(B), at 24hr post exposure to dengue viruses, platelets gets activated and degranulation occur indicated by the increased expression of CD63 on platelets.

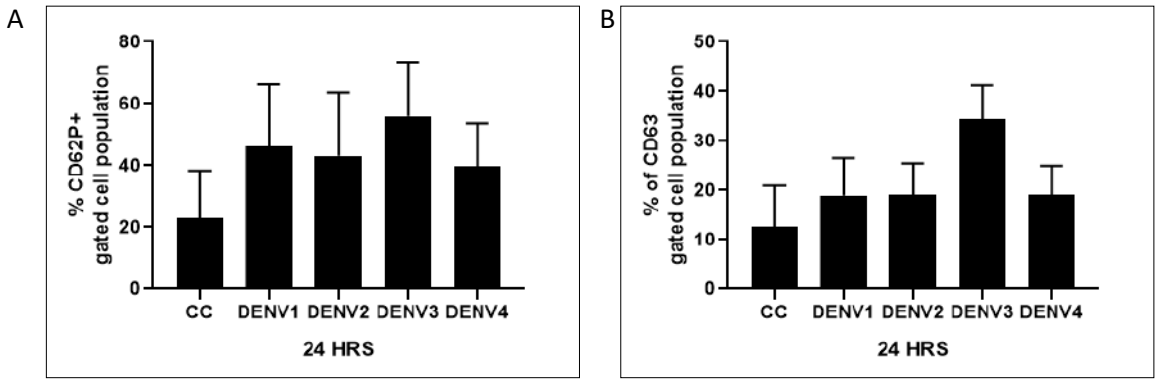
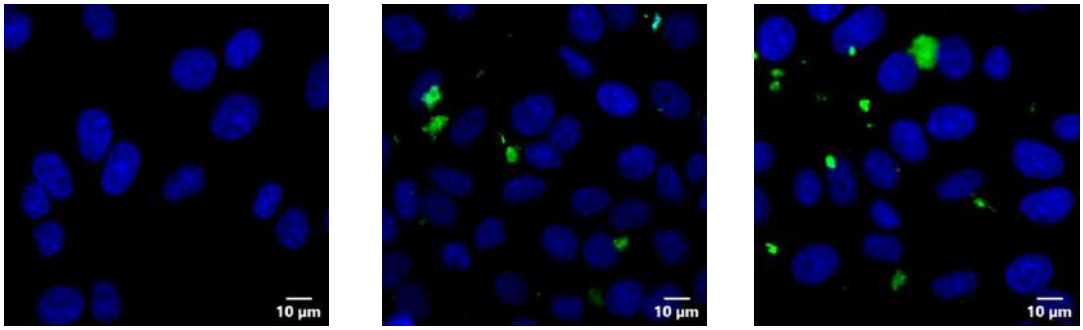


Fig 9. Percentage of CD62P (A) and CD63 (B) expression in platelets. Platelets were isolated from healthy donors (n=3) and either treated with conditioned medium as CC or exposed to DENV-1, DENV-2, DENV-3 and DENV-4 for 24hr. Platelets were harvested and stained for CD41, CD61, CD62P and CD63 to examine their activation status.

Uptake of labeled exosomes released from in-vitro cultured platelets exposed to dengue virus

The exosomes were isolated from in-vitro platelet culture either exposed to conditioned medium or dengue virus for 24hr at 37ºC in CO_2 incubator. The isolated exosomes were labeled with PKH26 dye and added to Vero cells for 2hr. As shown in Figure 10, the labeled exosomes indicated as green dots is taken up by the Vero cells.



Vero cells

Vero cells + Exosomes isolated from platelets

Vero cells + Exosomes isolated from platelets exposed with DENV-1

Fig 10. Uptake of labeled exosomes (green) by Vero cells.

Conclusion:

In-vitro cultured platelets are activated on exposure to dengue virus.

Degranulation of platelets led to increased expression of CD63 on platelet surface after dengue virus exposure.

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:

Work on second and third objectives of the project were completed during the year

Table 1: Assays available for vaccine studies

Project status	No of assay	Name of assay
NABL accredited	1	Dengue NS1 ELISA
		Dengue IgM ELISA
		DENV-1 specific PRNT
		DENV-2 specific PRNT
		DENV-3 specific PRNT
		DENV-4 specific PRNT
		Chikungunya virus (CHIKV) specific PRNT
		Chikungunya IgG ELISA
		Chikungunya IgG titration
		Dengue viremia
		Dengue virus serotyping
Standardized and validated	06	Dengue NSET
		Dengue IgG ELISA
		T cell response to Dengue by ICS (Th1 cytokines- IFN-γ,TNF-α & IL-2)
		T cell response to Dengue (CD4/CD8,memory and CTL response)
		T cell response to Dengue by ELISPOT (IFNg)
		CHIKV IgG ELISA

T**able 2: Assays available for testing of antiviral activity**

SN	**Assay Developed/Standardized**	**Guidelines used**

1	Antiviral Assessment of Drugs against SARS-CoV2 Virus and its variants	Published research article and agreed by client
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 3: Immunogenicity testing services provided during July 2020 – June 2021

Sr. No.	**Project Title (Project ID)**	**Client**	**Analytical Project Manager**	**Name of test**	**Number of samples tested**
1.	A Phase I double blind, randomized, placebo-controlled study to evaluate the safety and immunogenicity of Dengusiil in healthy adults (Dengusiil)	Serum Institute of India, Pvt. Ltd.	Dr. Shubham Shrivastava	Dengue viremia	291 720
2.	A prospective, randomized, adaptive, phase I/II clinical study to evaluate the safety and immunogenicity of Novel Corona Virus -	Zydus Cadila Healthcare Ltd.	Dr Ruta Kulkarni	SARS-CoV-2 PRNT	1053

	2019-nCov vaccine candidate of M/s Cadila Healthcare Limited by intradermal route in healthy subjects. (CoV-ZC-2013)				
3.	Testing of animal sera samples and human clinical sera samples for SARS-CoV-2 virus neutralization potential using a microneutralization and / or plaque reduction neutralization test (PRNT) assay established and validated at IRSHA as advised by Zydus (CoV-ZC-2005)	Zydus Cadila Healthcare Ltd.	Dr Shubham/ Dr Ruta	SARS-CoV-2 PRNT	311
4.	To study virus neutralization assay (PRNT) in human sera samples (CoV-ZC-2017)	Zydus Cadila Healthcare Ltd.	Dr Ruta Kulkarni	SARS-CoV-2 PRNT	177
5.	Virus neutralization assay (SARS-CoV-2 PRNT) for dog serum samples (CoV-ZC-2101)	Zydus Cadila Healthcare Ltd.	Dr Ruta Kulkarni	SARS-CoV-2 PRNT	42
6.	Determination of the SARS-CoV-2 neutralization potential of monoclonal antibodies and cytokines by using a PRNT assay (CoV-ZC-2014)	Zydus Cadila Healthcare Ltd.	Dr Ruta Kulkarni	SARS-CoV-2 PRNT	16
7.	SARS CoV-2 Plaque reduction neutralisation test (PRNT) Testing (CoV-SI-2003)	Serum Institute of India, Pvt. Ltd.	Dr Shubham/ Dr Ruta	SARS-CoV-2 PRNT	353

8.	Development of monoclonal antibody against SARS-CoV-2 (CoV-SI-2021)	Serum Institute of India, Pvt. Ltd.	Dr Ruta Kulkarni	SARS-CoV-2 PRNT	7
9.	Immunogenicity study of an mRNA vaccine candidate HGCO-19 against SARS-CoV-2 (CoV-GN-2024)	Gennova Biopharmaceuticals Ltd	Dr Ruta Kulkarni	SARS-CoV-2 PRNT	80
10.	Development of Covid-19 Antisera for therapeutic use (CoV-IS-2004)	iSERA Biological Pvt Ltd	Dr Shubham/ Dr Ruta	SARS-CoV-2 PRNT	77
11.	Testing of anti-COVID19 antiserum (in process samples & finished product) for specific antibody assessment by SARS-CoV-2 PRNT assay (CoV-PS-2015)	Premium Serums and Vaccines, Pvt Ltd	Dr Shubham/ Dr Ruta	SARS-CoV-2 PRNT	64
12.	Development of human monoclonal antibodies against SARS-CoV-2 using convalescent patient blood (CoV-BK-2020)	Bioklone Biotech Pvt Ltd	Dr Ruta Kulkarni	SARS-CoV-2 PRNT	46
13.	Pre-vaccination screening of subjects for CHIKV IgG for CHIKV vaccine Phase I trial (CHK-IL-2103)	Indian Immunologicals Ltd	Dr Ruta Kulkarni	Chikungunya IgG ELISA	46

Table 4: Antiviral testing services provided during July 2020 – June 2021

Sr. No.	Project Title	Client	Mode
1.	Determination of the antiviral activity of the drug candidate NETSP-01 against SARS-CoV2	Netsurf Research Lab private Limited and S.P. College	Service
2.	In vitro assessment of prospective formulations against SARS CoV 2 virus	Arna Immuno Ingrediants private limited	Service
3	PROTECT-C (SK-C)	Miyakawa Kogyo India Private Limited	Service
4	Godrej Consumer product testing	Godrej Consumer, Products Limited	Service
5	Anti-Virus Efficacy against SARS-CoV-2	ITC Limited	Service
6	WIPRO ENTERPRISES product testing	WIPRO ENTERPRISES (P) Limited	Service
7	In vitro antiviral assessment of NETRL-01 against SARS-CoV-2 virus	Netsurf Research Lab private Limited	Service
8	Antiviral testing of Dr. Nanoxa coated sample using SARS-CoV2 starain using ISO21702	Sicora Technologies, India	Service
9	Nano SelfClean Touch Points	Nanoselfclean Limited,UK	Service
10	Liquid Gurad	Nano-Care Deutschland AG,UK	Service
	Antiviral assesment of Sanitizer against SARS-CoV2 (EN14476)	Oman Hygienic Product, Sultanate of Oman	Service
11	Virucdal test and Persistence test for ESC brand products against SARS-CoV2 Virus	Zeta Tech, Mumbai	Service
12	Antiviral assesment of Chemical disinfectant and drugs SARS-CoV2 virus	Cadila Healthcare limited, India	Service

13	Assessment of vicudial efficacy of coatings against SARS-CoV2 (ISO21702)	TCG Centre for Research And Education in Science and Technology (TCG-CREST) , India	Service
14	*In vitro* screening of prosthetic drugs against and Dengue virus	Birla Institute of Technology, Ranchi	Collaborative

Title: **Validation of NS1 ELISA-based $TCID_{50}$ test (NSET) for titration of Dengue viruses**
Funding: DBT-BIRAC (as part of NIBEC Project CD/19/3/E)

Duration: September – December 2020

Sanctioned Amount: NA

Investigators:

PI: Dr. Ruta Kulkarni

Co-Investigators: Dr Vidya Arankalle, Dr. AC Mishra

Ph.D. Students: None

Human Ethical Approval: (IEC/2017/04, renewed IEC/2018/11)

Background: NS1 ELISA-based $TCID_{50}$ test was developed in-house at IRSHA for infectious dengue virus (DENV) titration. This method showed excellent correlation with the plaque assay and could serve as an efficient alternative for quantitation of dengue viral load in clinical samples.

Objective: This study was aimed at validation of NSET as per ICH (Q2) R1 guidelines and European Medicines Agency guideline on bioanalytical method validation.

Work done:

NSET validation was conducted individually for each dengue serotype (DENV1-4) using a set of 16 samples including undiluted DENV isolates and dengue-negative serum samples spiked with different dilutions of the virus isolates. Details of the validation parameters evaluated are given in Table 5.

Conclusion:

Dengue NSET was validated for titration of DENV-1, DENV-2, DENV-3 and DENV-4. The assay demonstrated acceptable level of accuracy, precision and linearity within the specified

range for each serotype. The stability of the study samples at different storage conditions was also demonstrated.

Table 5. Dengue NSET validation

Parameter	**Result**			
	DENV-1	**DENV-2**	**DENV-3**	**DENV-4**
Precision - Between run -Within run	Variation was within 20-25% CV			
Accuracy -Between run -Within run	Observed titer was within 20% of the expected titer.			
Lower limit of quantitation	2.01 log PFU/ml	2.7 log PFU/ml	2.6 log PFU/ml	2.42 log PFU/ml
Linearity	R^2=0.996	R^2=0.986	R^2=0.981	R^2=0.996
Stability	Stability of virus on storage for 30 minutes on ice, 24 hours at 2-8^{o}C, and after one-freeze thaw cycle was demonstrated with percent relative error within 20-25% of expected value.			

Title: **Validation of Chikungunya IgG Quantitative ELISA (Project ID: CHK-IR-2019)**

Funding: DBT-BIRAC (as part of NIBEC Project CD/19/3/E)

Duration: November 2020 – December 2021

Sanctioned Amount: NA

Investigators:

PI: Dr. Ruta Kulkarni

Co-Investigators: Dr Vidya Arankalle, Dr. AC Mishra

Ph.D. Students: None

Human Ethical Approval: (IEC/2017/04, renewed IEC/2018/11)

Background: Anti-Chikungunya IgG indirect ELISA was developed in-house at IRSHA using BPL-inactivated virus and used for quantitation of IgG titers among Chikungunya patients and for evaluation of previous exposure among healthy donors.

Objectives: This study was aimed at validation of Chikungunya IgG Quantitation ELISA (endpoint titration method) as per ICH (Q2) R1 guidelines and European Medicines Agency guideline on bioanalytical method validation.

Work done: The validation of Chikungunya IgG Quantitation ELISA (endpoint titration method) was conducted using a set of 20 samples including undiluted CHIKV IgG-positive parent samples and CHIKV IgG-negative serum samples spiked with different dilutions of the parent samples. Details of the validation parameters evaluated are given in Table 6.

Validation of CHIKV IgG quantitative ELISA by standard curve method, and using automated liquid handling system is ongoing.

Conclusion:

CHIKV IgG ELISA is validated for detection and quantitation (titration) of anti-CHIKV IgG antibodies by endpoint titration method. The assay demonstrates acceptable level of precision and accuracy within the specified range (IgG titer: 100 - 12800). The stability of the study samples at different storage and freeze-thaw conditions is also demonstrated.

Table 6. Chikungunya IgG Quantitation ELISA validation

Parameter	**Result**
Precision - Between run - Within run	Variation was within 1 $\log_2$ difference of the median titer.
Accuracy (Dilutional linearity) - Between run - Within run	Percent relative error: 0-17.7% of expected value Slope: -0.81 to -1.0
Lower limit of quantitation	IgG titer = 100
Stability	Stability of anti-CHIKV IgG on storage for 24 hours at 2-8^{o}C, and after one-freeze thaw cycle was demonstrated with percent relative error within 20% of expected value.
Robustness	No significant effect of change in incubation temperature (37^{o}C versus 36^{o}C, 38^{o}C), sample type (serum versus plasma,

	fresh versus freeze-thawed) was noted on the IgG titer (P>0.05 using Wilcoxon test).
Specificity	No cross-reactivity was observed between anti-CHIKV IgG and anti-dengue IgG antibodies in ELISA

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 Shastri, Dr. C Pawar (Kasturba Hospital, Mumbai) and Dr. A L Kakrani (D Y Patil Medical College and Hospital)

Project Staff: Dr. Durgesh Pitale, Ms. Shweta Chelluboina, Mr. Shanoong Hu, Ms. Shraddha Hatangadi

Human Ethical Approval: IEC/2020/13

Work done:

Due to the ongoing COVID-19 pandemic, collection of samples from hepatitis E patients from Pune and Mumbai could not be done. As this project is based on clinical samples, objectives could not be achieved due to unavailability of samples.

Title: Capacity enhancement of National Immunogenicity and Biologics Evaluation Center for assessing the immunogenicity of SARS-CoV-2 vaccines (**Project ID:** CD/21/1/E)

Funding: DBT-BIRAC (Under National Biopharma Mission)

Duration: February 2021 – January 2022

Sanctioned Amount: Rs. 13.41 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/2020/25

Background: Unprecedented collaborative efforts have been made all over the word to reduce duration of development of effective vaccines for COVID-19. This has resulted into development of many candidate vaccines in the country and in other places around the world. To maintain the speed and tempo of the vaccine development it is essential to develop and modernize the immunogenicity testing facilities for providing quality services matching to international standards to upcoming massive clinical trials. The present proposal is aimed at establishing such a facility with sound quality system and advance tests for assessment of COVID-19 vaccines.

Objectives:

Facility Augmentation and Upgradation of technology for existing key tests

Pseudo virus technology transfer and standardization of surrogate neutralization assay

Development of tests for CMI responses to natural COVID-19 infections and to the vaccines

Manpower training, ILC activities and develop and share standardized reagents and protocols for testing

Work done:

Table 7: Objective achieved, activities performed and status of work done

Objectives	Activities	Status

Objective1 Status report on procurement, installation and validation of high throughput systems	Procurement, installation and validation of high-throughput equipment. Validation of ACE2 based neutralization assay on high-throughput systems.	All order placed with companies. Delivery getting delayed due to current Covid-19 situations Validation kit for assessment of neutralization ability of our vaccines against variant viruses is placed with the company
Objective 2 Pseudo virus technology transfer and standardization of surrogate neutralization assay	a. Production of vesicular stomatitis virus based Pseudo-viruses VSV-SARS-S/ TFP bearing coronavirus SARS-CoV-2 S Protein b. NABL accreditation of in-house standardized neutralization assay and providing services to vaccine companies	a. Transfer of technology from IIT, Indore could not be executed as no decision is taken by them. Therefore, efforts are being made to make in-house pseudovirus and reporter virus. Standardization and validation can be undertaken once either reagents for pseudovirus development or ready to use pseudoviruses are available. The work will need another 6 -12 months for completion. b. Both PRNT and MNT for SARS-CoV-2 accreditated by NABL as per ISO-IEC 17025-2017 standards PRNT services using wild type, kappa and delta variants viruses are being provided to the companies. Services for antiviral testing using wild type and delta variant are being provided to companies.

Objective 3 Development of tests for CMI responses to natural Covid-19 infections and to the vaccines	a. Assessment of the frequency and functional profile of diverse lymphocyte subsets T and B cells by flow cytometry including intracellular cytokine secretion assays. b. In-vitro stimulation of PBMCs by inactivated whole virus and different proteins/peptide arrays of SARS CoV-2	a. A single flow cytometry based intracellular assay is validated as per CLSI-H62 guidelines to obtain enumeration of following T cell subsets, Antigen specific T cells Memory profile of Antigen specific T cells Th1 cytokine (IFN-γ, IL-2 & TNF-α) secreting antigen specific T cells Antigen specific Cytotoxic T cells (CD107a,Granzyme B & IFN-γ) b.SARS CoV-2 spike protein specific Th1/Th2 cytokine secretion employing multiplex bead array employed is standardized to quantitate cytokines in the culture supernatants of PBMCs

Title: Isolation and characterization of mutant strains (Kappa, Delta) of SARS-CoV-2. (Project ID: CD/21/1/I)

Funding: DBT-BIRAC (as part of NIBEC Project CD/19/3/E and CD/21/1/E)

Duration: April 2020 – 2021

Sanctioned Amount: NA

Investigators:

PI: Dr. Vidya A. Arankalle

Co-PI/ Co-Investigators: Dr. Shubham Shrivastava, Dr. Suhas Mhaske, Dr. Rashmi Virkar, Dr. A.C. Mishra.

Ph.D. Students: NA

Human Ethical Approval: IEC/2020/25

Background:

Coronavirus disease 2019 (COVID-19) is continued to be global public health concern in year 2021. Several countries are experiencing resurgence due to emergence of SARS-CoV-2 variants leading to second/third waves of the disease. Identification of a variant with new set of mutations could impact transmissibility and virulence of SARS-CoV-2 and therefore, it is important to monitor over time.

Work done:

Isolation of SARS-CoV-2 from clinical samples (to be filled by Rashmi)

RBD sequence analysis during the first and second wave of COVID-19 in Pune, India

Receptor binding domain (RBD) presents in the spike protein helps in the attachment of the virus to ACE2 receptor and a major target for vaccine development, monitoring of RBD sequence analysis was undertaken on quarterly basis over a period of one year (May 2020 to June 2021).

Fig 11 presents the number of patients advised and with confirmed COVID-19 diagnosis at Bharati hospital following permission of the government to provide diagnostic services. At Bharati hospital, during the first wave of disease, the number of COVID-19 cases increased steadily from June to September 2020. The highest number (n=800) was recorded in September 2020 that was followed by a sharp decline (n=285) in October 2020. An increasing trend in the number of cases was observed during March 2021 that reached the peak (n=3203) in April 2021 during the second wave of the disease.

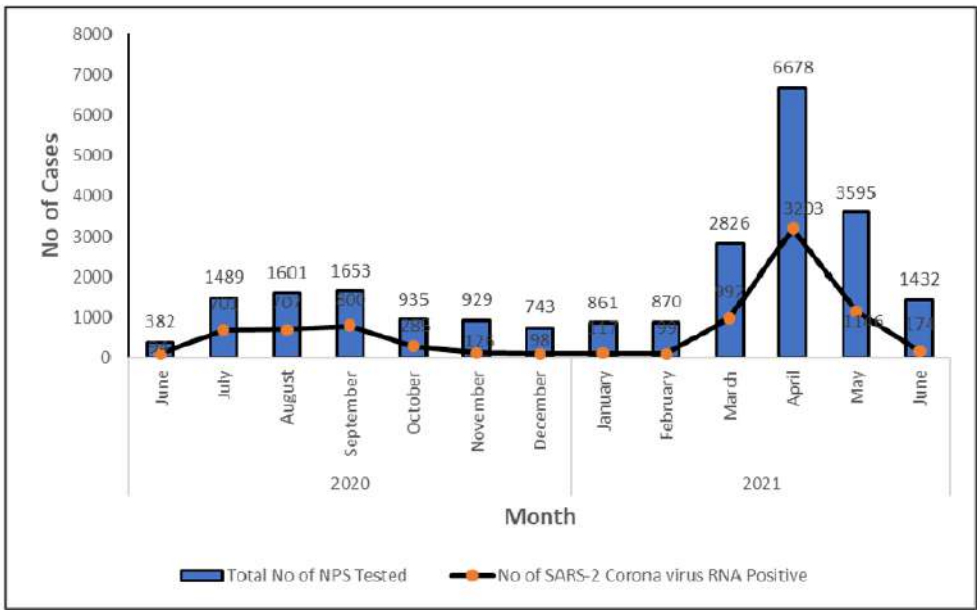


Fig 11. Number of NPS specimens from suspected COVID-19 cases tested at Bharati hospital and numbers scored positive for SARS-CoV-2 RNA by RT-PCR (June 2020 to June 2021).

Table 8 depicts the mutational analysis of the RBD region. Till December 2020, none of the samples exhibited any characteristics mutations of variants of concern (VoCs). In the month of March, 33/45 (73%) samples harbour India-specific, L452R/E484Q mutations defined later as Kappa variant. Simultaneous exponential rise in the number of COVID-19 cases revealed a clear association of emergence of this mutant with the current second wave of the disease.

Analysis of 91 RBD sequences from the samples collected during the month of April led to striking observations. At this time, 34 sequences harboured L452R/E484Q mutations (37%), characteristics of Kappa variant while 54 sequences harboured L452R/T478K mutations (59%), characteristic of the Delta variant. The increasing trend of the dominant mutation, L452R/T478K continued in the month of May and June 2021 however, we observed significant decline in the number of patients seeking COVID-19 diagnosis at the hospital. Our results indicated that the dominant clade G virus strains seems to have been replaced by the variants of concern, Delta L452R/T478K in Pune during the second wave of the disease.

Table 8. SARS-CoV-2 RBD mutant profiles during the first (2020) and second (2021) waves of COVID-19 at Pune, western India*

Collection Month (No. of samples sequenced)	Original (Wuhan)						
		UK Alpha (N501Y)	Indian**		Others		
			Kappa (L452R, E484Q)	Delta (L452R, T478K)	N440K	E484K	E484Q
May 2020 (5)	5 (100%)	0	0	0	0	0	0
Sep 2020 (10)	10 (100%)	0	0	0	0	0	0
Dec 2020 (15)	13 (87%)	0	0	0	2 (13%)	0	0
Mar 2021 (45)	4 (8.9%)	0	33 (73.3%)	2 (4.4%)	2 (4.4%)	3 (6.7%)	1 (2.2%)
Apr 2021 (91)	1 (1.1%)	1 (1.1%)	34 (37.3%)	54 (59.3%)	1 (1.1%)	0	0
May 2021 (132)	0	0	10 (7.6%)	122 (92.4%)	0	0	0
June 2021(21)	0	0	0	21 (100%)	0	0	0

*Mutants specific to Brazil/South Africa were not found. **Additionally in 2021, V382L mutation was recorded 14/289 (4.8%) in COVID-19 cases.

Analyses based on SARS-CoV-2 full genome sequences from Pune, India

During 2020-21, twenty full SARS-CoV-2 genomes from Pune were sequenced (year 2020- May: 2, September: 2; year 2021- March: 10, April: 2, May: 4). The four mutations, C241T,

C3037T, C14408T, and A23403G were observed in all the 20 genomes from the clade “G” (named after the Spike D614G mutation). Of the 10 genomes from March 2021 selected for sequencing, four were wild type while six were Kappa variant (B.1.617.1) as per RBD analysis. Two sequences from Apr 2021 and 4 sequences from May 2021 formed a distinct clade 21A and belonged to B.1.617.2 lineage (Delta variant, VoC).

Next, spike protein sequences from the Indian variants were compared with other known variants (Table 9). The Indian variants formed two distinct clusters, B.1.617.1 (Kappa) and B.1.617.2 (Delta). Mutations specific to the recently emergent variants (UK, South Africa, Brazil and California) were not shared by the Indian variants, however, signature mutations of Delta variant were observed in all six sequences obtained in Apr and May months of year 2021.

Table 9. Comparison of spike protein mutations in the Indian and other variant viruses (reference: Wuhan strain, accession number, NC045512)

Pangolin Lineages	Sequence ID*	N-terminal domain in S1 subunit AA position														RBD AA position								C-terminal domain in S1 subunit AA position							S2 subunit AA position										
		18	19	20	26	27	70	95	138	142	143	154	171	190	222	382	417	433	440	452	478	484	501	570	614	647	655	675	677	681	716	950	982	1027	1071	1101	1118	1133	1153	1176	1251
B	NC-045512, Wuhan	L	T	T	P	A	V	T	D	G	V	E	V	R	A	V	K	V	N	L	T	E	N	A	D	A	H	Q	Q	P	T	D	S	T	Q	H	D	V	D	V	G
B.1.1.306	8003-IRSHA (MT416725)																								G				H													
	8004-IRSHA (MT416726)																								G				H													
B.1.1.306	CD208550 (MW969758)																								G																	
	CD208560 (MW969757)																	I							G																	V

B.1.1	CD210922 (MW969752)										F													G				H									F			
B.1.36.29	CD210896 (EPI_ISL_1710598)																		K						G																
B.1.1.306	CD211295 (MW969753)	F				S							I									K			G			H												
B.1.1.306	CD210761 (MZ021503)	F				S																K			G			H												
B.1.617.1	CD210871 (MZ021506)							I		D										R		Q			G					R				H							
B.1.617.1	CD210927 (MZ021505)									D		K				L				R		Q			G					R				H				Y			
B.1.617.1	CD210929 (MZ021504)									D		K				L				R		Q			G					R				H				Y			
B.1.617.1	CD211290 (MW969754)									D		K				L				R		Q			G	S				R				H				Y			
B.1.617.1	CD211406 (MW969756)									D		K				L				R		Q			G					R				H				Y			
B.1.617.1	CD211294 (MW969755)									D		K								R		Q			G					R				H	D						

B.1.617.2	CD212098 (MZ574054)		R						D					V					R	K				G					R		N									
B.1.617.2	CD213366 (MZ574051)		R						D										R	K				G					R		N									
B.1.617.2	CD212095 (MZ574052)		R						D										R	K				G					R		N									
B.1.617.2	CD213570 (MZ574053)		R						D										R	K				G					R		N									
B.1.617.2	CD213522 (MZ574055)		R						D										R	K				G					R		N									
B.1.617.2	CD213523 (MZ574056)		R						D										R	K				G					R		N									
B.1.617.2	EPI_ISL_2880930 (India/HR)		R											V					R	K				G					R											
B.1.617.2	EPI_ISL_2272481 (India/DL)		R						D					V					R	K				G					R		N									
B.1.617.2	EPI_ISL_2036277 (India/WB)		R						D										R	K				G					R		N									
B.1.617.2	EPI_ISL_2897823 (India/MH)		R																R	K				G					R											
B.1.617.2	EPI_ISL_2956019 (Sweden)		R						D					V					R	K				G					R		N									

B.1.617.2	EPI_ISL_2958767 (France)		R																R	K				G					R		N								
AY.2	EPI_ISL_2923711 (USA/CA-CDPH)		R				F								V		N			R	K				G					R		N								
AY.2	EPI_ISL_2545667 (USA/HI-TAMC)		R				F			D					V		N			R	K				G					R		N								
AY.2	EPI_ISL_2929274 (USA/CA-CDC)		R				F			D					V		N			R	K				G					R		N								
AY.2	EPI_ISL_2928214 (USA/CA-OC)		R				F								V		N			R	K				G					R		N								
B.1.1.7 (UK)	EPI_ISL_601443																						Y	D	G					H	I		A				H				
B.1.1.7 (L18F)	EPI_ISL_720875	F																					Y	D	G					H	I		A				H				
B.1.1.7 (F490S)	EPI_ISL_736026																						Y	D	G					H	I		A				H				
B.1.1.7 (S494P)	EPI_ISL_741039																						Y	D	G					H	I		A				H				

B.1.1.7 (E484K)	EPI_ISL_782148																					K	Y	D	G					H	I		A				H				
B.1.351 (SA)	EPI_ISL_1012924															N						K	Y		G																
P.1 (Brazil)	EPI_ISL_792681	F		N	S				Y					S			T					K	Y		G		Y							I						F	
P.1 (Brazil)	EPI_ISL_875566	F		N	S				Y					S			T					K	Y		G		Y							I						F	
P.1 (Brazil)	EPI_ISL_875567	F		N	S				Y					S			T					K	Y		G		Y							I						F	
P.1 (Brazil)	EPI_ISL_875568	F		N	S				Y					S			T					K	Y		G		Y							I						F	
B.1.427 (California, L452R)	EPI_ISL_1620465																			R					G																
B.1.429	EPI_ISL_824555																			R					G																
B.1.525 (E484K)	EPI_ISL_1615794																					K			G																
B.1.2	EPI_ISL_824741																								G																

B.1.1.432	EPI_ISL_913915																							G																	

*Sequence IDs in bold denote sequences from India; Black: wild type and red: variant viruses

Conclusion:

Emergence of second wave of COVID-19 in India was characterized by the appearance of Kappa variant, followed by the dominance of Delta variant.

Title: Evaluation of the role of neutrophil secretory proteins, alpha-defensins and calprotectin as biomarkers of disease severity in COVID-19 patients (Project ID:CD/20/7/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. Vidya A. Arankalle, Dr. A.C. Mishra (IRSHA), Dr. Sonali Palkar, Dr. Prashant Jedge, Dr. Purwa Doke (Bharati Vidyapeeth Medical College).

Ph.D. Students: Shweta Chelluboina

Human Ethical Approval: IEC/2020/25

Background:

Being a new pathogen, understanding of the basis for severity and fatal outcome of SARS-CoV-2 infection is of paramount importance for developing therapeutic options and identification of prognostic markers. In recent studies, accumulation of neutrophils and increased neutrophil to lymphocyte ratio in peripheral blood of severe COVID-19 patients suggest that neutrophil activation may modulate immune response after SARS-CoV-2 infection.

Objectives:

To quantitate the circulatory levels of neutrophil's secretory proteins in different clinical presentations of COVID-19 patients

Work done:

Expression levels of alpha-defensins (DEFA1), calprotectin (S100A8/A9) and myeloperoxidase (MPO) proteins in COVID-19 patients

In this study, 109 COVID-19 patients were enrolled of which 23 were viral RNA positive asymptomatic contacts of COVID-19 index cases while 63 patients were symptomatic with mild (n=32) or severe (n=31) disease. Among 31 patients suffering from severe disease, 19 (61.3%) required mechanical ventilation for oxygen support and 11 (35.5%) succumbed to the infection.

Figure 12 depicts circulatory levels of (A) DEFA1, (B) S100A8/A9 and (C) MPO in serum / plasma samples of COVID-19 patients. As compared to the healthy subjects (mean 113±11), a significant increase in alpha-defensins (DEFA1) was recorded in the patients with mild (mean 230±17, $p<0.0001$) or severe disease (mean 452±46, $p<0.0001$, Figure 12A). Importantly, the rise was more pronounced in the patients with severe disease than those with mild infection ($p=0.0001$). Irrespective of disease severity, the levels of S100A8/A9 were elevated in all the individuals with asymptomatic ($p<0.0001$) and symptomatic ($p<0.0001$) infections, when compared to the healthy controls (Figure 12B). However, the rise was not significantly different among symptomatic infections with mild or severe disease ($p=0.48$). In a subset of samples, MPO levels were determined (Figure 12C). Similar to DEFA1, MPO levels were unaltered during subclinical infection ($p=0.85$) while a significant increase was observed in the patients with mild ($p=0.01$) and severe ($p=0.02$) disease. However, the difference with respect to disease severity was not significant ($p=0.29$).

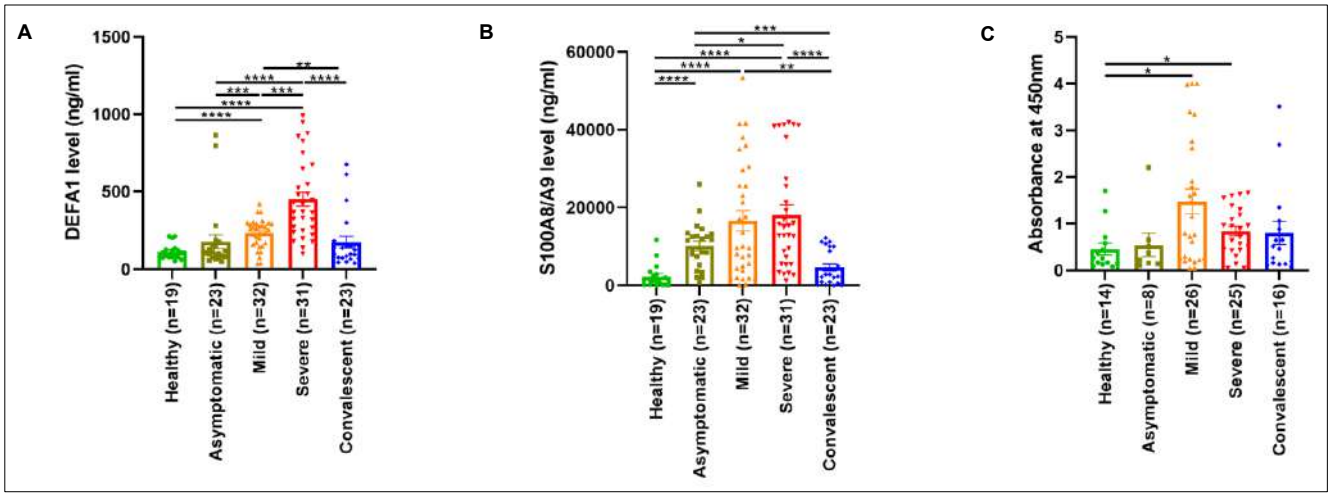


Fig 12. Expression levels of neutrophil activated proteins in sera / plasma of COVID-19 patients. Levels of (A) DEFA1, (B) S100A8/A9 and (C) MPO in COVID-19 patients with different clinical presentations in comparison to healthy controls. The data is presented as dot plots with bar representing the mean ± SEM in each group. Each dot represents a single sample. P values were calculated using Mann-Whitney test.

Expression levels of DEFA1, S100A8/A9 and MPO proteins in relation to disease severity in COVID-19 patients

Next, the levels of neutrophil secretory proteins with respect to disease severity and PODs were compared (Figure 13A-C). Interestingly, DEFA1 expression levels were significantly higher in patients with severe disease during the first week itself (p=0.004). No significant difference was observed during the second (p=0.052) and the third (p=0.97) weeks post onset of disease. S100A8/A9 and MPO levels did not differ among mild and severe disease patients at different time points post onset of disease.

To understand whether the increased expression of neutrophil secretory proteins is associated with the onset of severity, the levels of these proteins were compared with respect to the day on which severity was first identified. Of the 31 severe disease patients, 16 (52%), 9 (29%) and 6 (19%) were respectively collected within 0-3, 4-7 and 8-23 days of the identification of severity. Expression levels of all the three proteins were elevated within 7 days post identification of

severe disease (Figure 13D) suggesting that higher levels of neutrophil secretory proteins are associated with disease severity in COVID-19 patients.

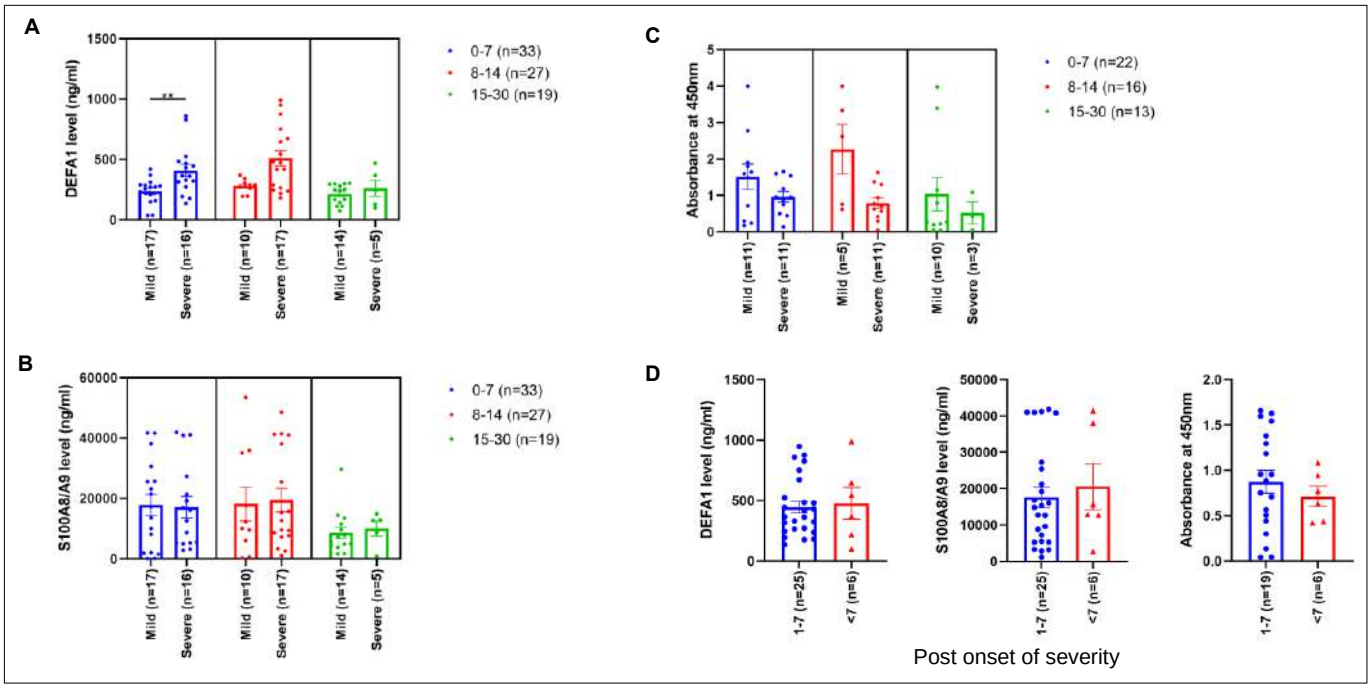


Fig 13. Expression levels of neutrophil activated proteins in sera / plasma of mild and severe COVID-19 patients. Figures A-C depict comparisons of levels of (A) DEFA1, (B) S100A8/A9 and (C) MPO among mild and severe disease patients at different PODs. Comparative levels of (D) DEFA1, S100A8/A9 and MPO among severe disease patients at different days post onset of severity. The data is presented as dot plots with bar representing the mean ± SEM in each group. Each dot represents a single sample. P values were calculated using Mann-Whitney test.

Expression levels of DEFA1, S100A8/A9 and MPO proteins in relation to disease severity and mechanical ventilation / secondary infection

In view of the fact that a large proportion of severe disease patients require mechanical ventilation and develop secondary infections, further analyses were undertaken to assess the contribution of these confounding factors in the observed rise of neutrophil secretory proteins. In our series, ~95% of the severe patients undergoing intubation developed secondary infection. As evident from Figure 14, the expression levels of all the three proteins were similar in both the groups (Figure 14A). We further compared the expression of all the three proteins in relation to

the duration between the day of secondary diagnosis and sampling (Figure 14B-D). Clearly, high expression of these proteins was not related to secondary bacterial infection but the outcome of SARS-CoV-2 infection.

Next, we compared the expression levels of neutrophil secretory proteins in the patients with severe disease in relation to the disease outcome (Figure 14E). DEFA1 and S100A8/A9 expression levels were significantly higher in patients with fatal outcome (p=0.004; p=0.03 respectively) suggesting that neutrophil activation is associated with mortality in COVID-19 patients.

DEFA1 as potential biomarker for severity

To explore, if DEFA1 levels can be used to differentiate between mild and severe disease presentations, ROC curve analysis revealed that at 314 ng/mL, the estimated sensitivity and specificity were 68% and 91% respectively (Figure 14F). The AUC value for alpha-defensin was 0.78 ± 0.06 (95% CI = 0.66 to 0.89, p<0.0002), suggesting that DEFA1 levels could act as a potential biomarker in predicting disease severity in COVID-19.

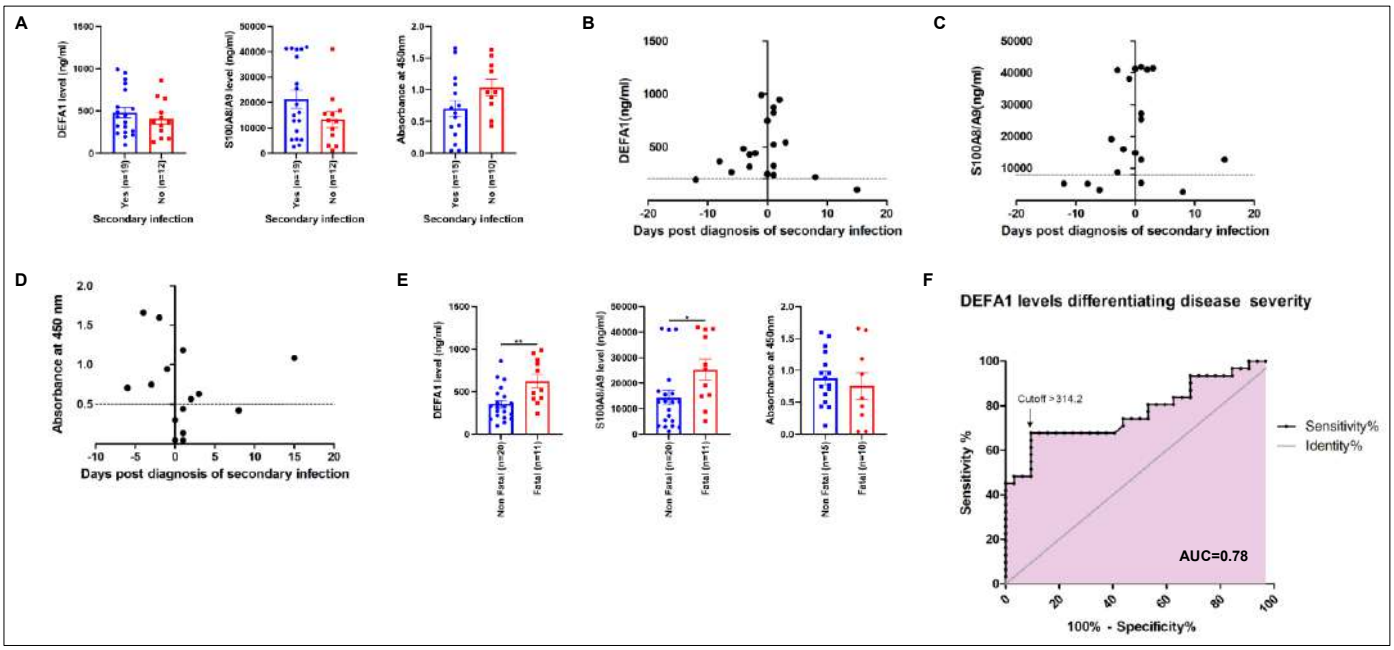


Fig 14. Expression levels of neutrophil activated proteins in severe COVID-19 patients in relation to occurrence of secondary infection and mechanical ventilation. Figure (A) depict comparative levels of DEFA1, S100A8/A9 and MPO in severe patients with or without secondary infection. The data is presented as dot plots with bar representing the mean ± SEM in each group. Figures (B-D) depicts levels of (B) DEFA1, (C) S100A8/A9 and (D) MPO on different days before or after diagnosis of secondary infection. Each dot represents a single sample. The dotted line indicates cut-off value calculated as average value of expression levels of neutrophil activated proteins in healthy controls + 2*standard deviation. Comparative levels of DEFA1, S100A8/A9 and MPO among severe disease patients with (E) non-fatal and fatal disease outcome. The data is presented as dot plots with bar representing the mean ± SEM in each group. (F) Receiver-operator characteristics (ROC) curve of DEFA1 serum levels for the prediction of COVID-19 disease severity among mild and severe disease presentations.

Conclusion:

Elevated levels of DEFA1 and MPO characterized symptomatic infections while elevated levels of calprotectin marked SARS-CoV-2 infections irrespective of clinical presentation.

DEFA1 could act as potential biomarker in predicting disease severity.

Fatal outcome was associated with a rise in the expression of DEFA1 and calprotectin levels in

the COVID-19 patients with severe disease.

Title: Long term follow-up of antibody responses in COVID-19 patients (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. Prakash Doke

Co-PI/ Co-Investigators: Dr. Purwa Doke, Dr. Jayshree Gothankar, Dr. Jayesh Patil, Dr. Purwa Doke (Bharati Vidyapeeth Medical College); Dr. Shubham Shrivastava, Dr. Vidya A. Arankalle (IRSHA)

Human Ethical Approval: BVDUMC/IEC/71

Background:

To combat the rapid spread of SARS-CoV-2 infection, several vaccines were rapidly developed and made available for immunization of different populations. To assess / predict efficacy of vaccines, it is of utmost importance to understand immunological basis for recovery from the disease as well as progression to severity. Currently, correlates of protection for COVID-19 are not known. In view of the availability of a variety of vaccines and implementation of national immunization programs, antibody dynamics in natural infection needs to be elucidated in different populations.

Objectives:

To understand the dynamics of neutralizing antibody titers in COVID-19 patients and their association with host factors.

Work done:

100 COVID-19 patients were enrolled (median 59 days of diagnosis) and 70 patients were followed up (median 106 days of diagnosis) for antibody response till 8 months post diagnosis.

Seropositivity and association of neutralizing antibody titers with the variables examined

At the time of two visits, 93/100 (93%, first) and 68/70 (97.1%, second) patients circulated IgG antibodies while neutralizing antibody (NAb) seropositivity was recorded in 91/100 (91%) and 62/70 (88.6% patients) respectively. Thus, 2/93 (2.1%) IgG positives at first visit and 6/68 (8.8%) IgG positives at the second visit lacked neutralizing antibodies. Overall, 11.4% patients were NAb negative at the time of second visit.

Figure 15 depicts modulation of NAbs titers in relation to the factors considered. At the first visit, higher NAb titers were associated with disease severity ($p<0.001$), presence of comorbidities ($p<0.005$), higher BMI ($p<0.001$), age<50 years ($p<0.001$) and male gender ($p<0.001$). Multivariate analysis identified older age ($p<0.001$), duration post-diagnosis, higher BMI and female gender as independent variables influencing NAb titers (negative correlation, $p<0.05$).

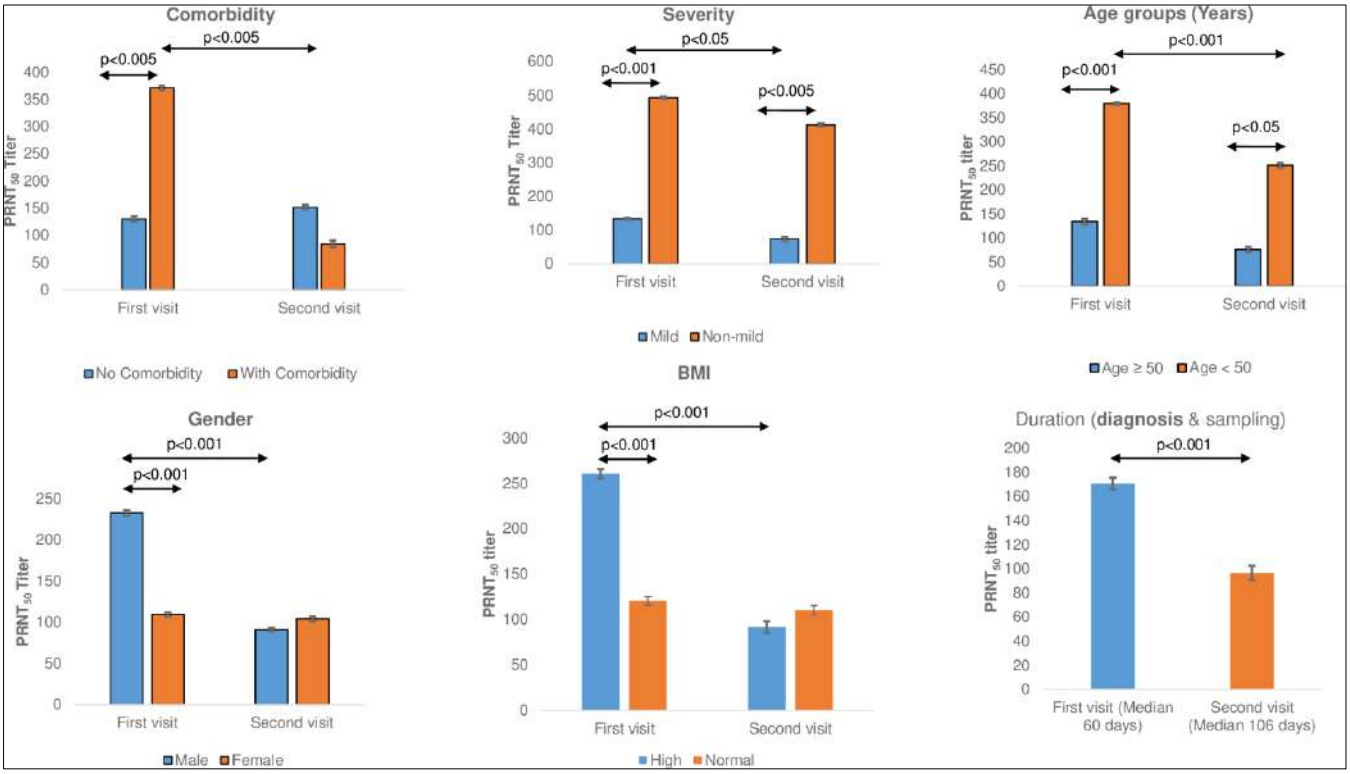


Fig 15. Neutralizing antibody titers ($PRNT_{50}$) in the patients studied at first visit (median 60 days post diagnosis) and second visit (median 106 days post-diagnosis). The variables examined include (A) Comorbidity; (B) Disease severity; (C) Age groups; (D) Gender; (E) BMI and (F) Interval between diagnosis and sampling (in days). Only significant differences between groups are marked.

Conclusion:

Despite decline of neutralizing antibody titers was observed over time, 88.6% patients continued to circulate neutralizing antibodies till 8 months post diagnosis.

Evidence of 6 reinfections suggests waning of immunity, but, probable protection from clinical disease.

Title: Perinatal transmission of SARS-CoV-2 in neonates (Project ID: CoV-BH-2007, (Project ID:CD/20/7/I)

Funding: Intramural

Duration: June 2020 to June 2021

Sanctioned Amount: NA

Investigators:

PI: Dr Nandini Malshe (Bharati Vidyapeeth Medical College)

Co-Investigators: Dr. Vidya Arankalle, Dr Ruta Kulkarni, Dr Suhas Mhaske, Dr. AC Mishra

(IRSHA),

Dr Nandini Malshe, Dr Suprabha Patnaik, Dr Sanjay Lalwani, Dr Pradeep Suryawanshi (Bharati Vidyapeeth Medical College)

Ph.D. Students: None

Human Ethical Approval: IEC/2020/47

Background: COVID-19 pandemic remains a serious public health threat worldwide. In view of the limited data on the risk of perinatal transmission of SARS-CoV-2 and transfer of maternal anti-SARS-CoV-2 antibodies, the present study was undertaken.

Objective: The objective of the study was to investigate the risk of perinatal SARS-CoV-2 transmission and transfer of maternal anti-SARS-CoV-2 antibodies among Indian neonates.

Work done:
A prospective study including 57 pregnant women with a positive SARS-CoV-2 RNA test (SARS-CoV-2-RNA+) and 59 neonates born to them was conducted at Pune, India. 39 viral RNA negative (SARS-CoV-2-RNA-negative) pregnant women and their 39 neonates were included as controls. Neonatal nasal swab/cord blood samples were subjected to SARSCoV-2 RNA detection by RT-PCR for investigation of perinatal transmission. Transfer of maternal antibodies was studied using ELISA and PRNT.

Results:
Figure 16 summarizes the clinical characteristics and laboratory investigations for neonates born to SARS-CoV-2 positive and negative mothers. 10/57 SARS-CoV-2-RNA+ mothers were symptomatic. The duration between COVID-19 diagnosis and delivery was ≤ 7 days for 82.4%.

Perinatal transmission as evidenced by viral RNA in the neonatal nasal swab/cord blood (CB) was 3.6%. IgG-anti-SARS-CoV-2 positivity was 21.6%. Of the 39 neonates born to SARS-CoV-2-RNA-negative mothers, 20 (51%) and none, respectively, were positive for IgG-anti-SARS-CoV-2 and viral RNA. Preterm deliveries were higher in SARS-CoV-2-RNA+ (18.6%) than SARS-CoV-2 RNA-negative (0/39) mothers ($p < 0.005$). Respiratory distress at birth (<4 h) was higher among neonates of SARS-CoV-2-RNA+(20/59, 33.9%) than SARS-CoV-2-RNA-negative mothers (3/39, 7.7%; $p < 0.001$). ~ 75% IgG-positives exhibited neutralization potential with mean PRNT titers of 42.4 ± 24 (SARS-CoV-2-RNA+) and 72.3 ± 46.7 (SARS-CoV-2 RNA-negative); higher in the latter ($p < 0.05$).

Conclusion: The rate of perinatal transmission was low. Transfer of maternal antibodies was lower among SARS-CoV-2-RNA+ mothers than SARS-CoV-2-RNA-negative mothers with subclinical infection during pregnancy. Presence of neutralizing antibodies in majority of IgG-positives suggests protection from SARS-CoV-2 in early life.

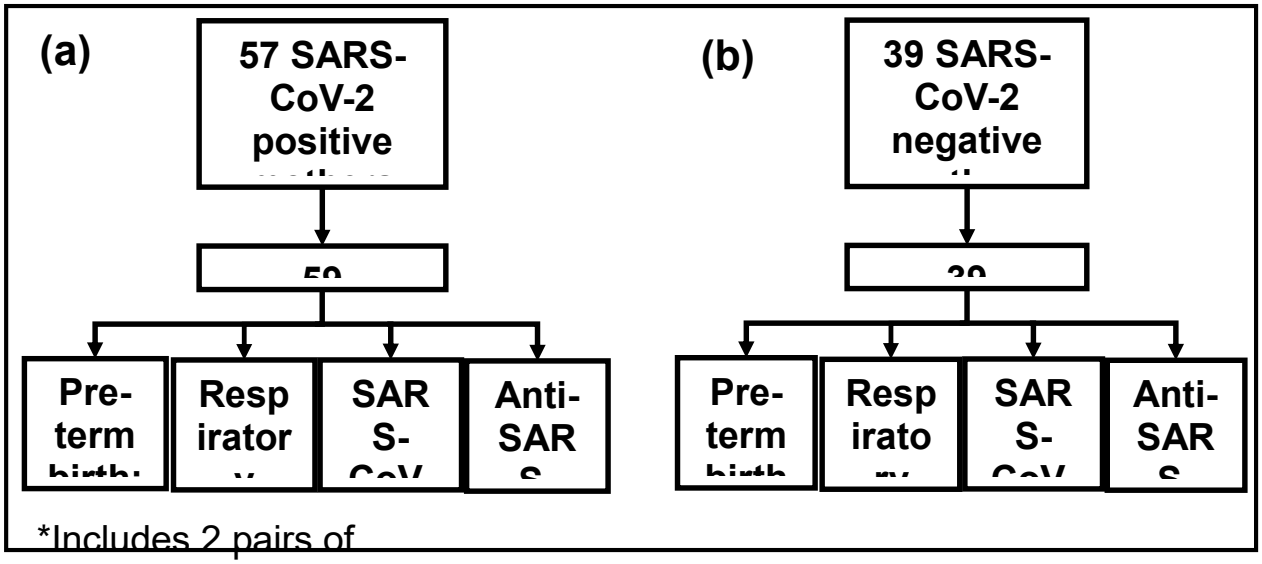


Fig. 16. Summary of clinical characteristics and laboratory investigations for neonates born to SARS-CoV-2 positive (a) and negative (b) mothers

Title: **Performance assessment of SARS-CoV-2 IgM and IgG enzyme linked immunosorbent assays in comparison with plaque reduction neutralization test (Project ID: CoV-IR-2011)**

Funding: DBT-BIRAC (as part of NIBEC Project CD/19/3/E)

Duration: August 2020 – January 2021

Sanctioned Amount: NA

Investigators:

PI: Dr. Vidya Arankalle

Co-Investigators:

Dr. Ruta Kulkarni, Dr Shubham Shrivastava, Dr. Harshad Patil, Dr. AC Mishra (IRSHA),

Dr. Sonali Palkar, Dr. Sanjay Lalwani (Bharati Vidyapeeth Medical College)

Ph.D. Students: None

Human Ethical Approval: IEC/2020/47

Background: Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome – coronavirus-2 (SARS-CoV-2) continues to be a devastating pandemic.

Objectives: This study was aimed at performance assessment of SARS-CoV-2 IgM and IgG ELISAs, and investigation of their utility for patient diagnosis and sero-epidemiologic investigations.

Work done:

Serum/plasma samples from COVID-19 patients or asymptomatic contacts (n=180) and healthy donors (n=90) were tested in parallel using 2 commercial IgM ELISAs; Erbalisa and Inbios, and 4 IgG ELISAs; Kavach, Euroimmun, Erbalisa and Inbios, along with an indigenous β-propiolactone (BPL) inactivated virus based ELISA (IRSHA-IgG-ELISA). Plaque reduction neutralization test (PRNT) was used as reference test.

Among 180 COVID-19 patients, 125 tested positive by PRNT. Inbios-IgM-ELISA showed sensitivity(Se)/specificity(Sp)/positive predictive value(PPV)/negative predictive value(NPV) of 93.6%/97.8%/98.4%/94.4% in relation to PRNT, and performed better than Erbalisa-IgM-ELISA (Se:48%,Sp:95.6%,PPV:95.2%,NPV:65.2%). During the first week of disease, only 47.4% of the COVID-19 patients tested IgM positive by Inbios-IgM-ELISA, detection improving at 2 weeks and beyond (~86-100%). Among IgG tests, Inbios-IgG-ELISA ranked first in terms of sensitivity (83.2%), followed by IRSHA (64.8%), Euroimmun (64%), Erbalisa (57.6%) and Kavach (56%)

tests. For all IgG tests, sensitivity improved during the third (73.9-95.7%) and fourth week (100%) of illness. The specificity (96.7-100%) and PPV (96.2-100%) of all IgG tests was high; NPV ranged between 71.9-87.1% with Inbios-IgG-ELISA scoring highest.

Conclusion: IgM detection by the current, most sensitive ELISAs cannot replace molecular diagnosis, but may aid as a supplement test. The available IgG tests are suitable for serosurveys for assessment of previous virus exposure.

Title: (Project ID:) Study of immune profile in SARS CoV-2 infection (CD/19/9/I)

Funding: DBT-BIRAC (as part of NIBEC Project CD/19/3/E)

Duration: May 2020-August 2020

Sanctioned Amount:

Investigators: PI -Dr. Vidya Arankalle

Co PI: Dr. Archana Kulkarni-Munje, Dr Sonali Palkar, Dr A C Mishra

Human Ethical Approval: BVDUMC/IEC/25

Background:

The ongoing pandemic of SARS-CoV-2 has turned out to be an unprecedented threat to global public health and the economy. Irrespective of the degree of industrialization or availability of medical infrastructure, all populations have been (and are being) affected. The disease, COVID-19, varies from an asymptomatic infection and self limiting mild disease to severe acute respiratory distress, multiorgan failure followed by recovery or fatal outcome. For any pathogen leading to variable clinical presentations and mortality, understanding of pathogenesis is of utmost importance. Initial studies identified older age and pre-existing chronic conditions such as diabetes, hypertension, cardiovascular diseases, cancer, etc., as high risk factors for disease severity. The major focus of the scientific community has been to unravel the immunopathogenesis of the disease requiring a clear understanding of the immune response in both mild and severe disease forms. Due to the high transmissibility of the virus and biosafety concerns, studies are predominantly limited to blood investigations. An association of cytokine storm including high levels of interleukin IL-6 production with severe disease signifies pathological immune dysregulation. Recent data suggest that antibody response is higher in

severe disease. Though studies on innate immunity are limited, T cell and B cell responses are being actively investigated. In view of the need to understand immunology of the disease in general and among the Indian population in particular, we attempted to explore the functional profile of innate immune cells (monocytes, dendritic cells, and NK cells), and adaptive immune cells (B cells, follicular T helper cells, CD4 T, and CD8 T cells) in SARS-CoV-2 infected individuals presenting with asymptomatic, mild, or severe disease. Further, the dynamics of these immune cells and, relation to neutralizing antibody titers was analyzed. We found that certain earlier modulations observed during the first week of disease could differentiate between mild and severe infections.

Work done:

This study was approved by the human ethics committee of Bharati Vidyapeeth (Deemed to be University) hospital and Medical college. A subset of patients attending Bharati Hospital, Pune, India, with confirmed positive test for SARS-CoV-2 reverse-transcriptase-polymerase chain reaction (RT-PCR), during first wave of COVID-19 (April2020- June2020), was enrolled (N=60). The patients were divided into severe (N=25) and mild groups (N=20) according to their clinical presentation. All the patients were admitted to a special COVID facility at Bharati Hospital and research center, Pune, a tertiary care hospital. Follow up blood samples collected from SD ($n = 15$) and MD ($n = 5$) patients were also included in the study.

After informed consent, approximately 5 ml of blood from all these patients and controls was collected in EDTA and processed for PBMC and plasma separation by ficoll-histopaque based density gradient method. Plasma was used for Th1 and Th2 cytokine profiling (IFNγ, TNFα, IL4, IL10, IL6, and IL2) by cytometric bead array kit (BD Biosciences) and detection/quantitation of neutralizing antibodies (Nabs) using 50% plaque reduction neutralization test (PRNT50). 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 and monocytes), natural killer cells, T cells (CD4 T cells, CD8 T cells, and follicular T helper cells [TFH]) and B cell subsets (memory B cells and plasmablast cells) by immunophenotyping for selected immune cells and their subpopulations using appropriate fluorochrome labeled antibodies (Biolegend, San Diego & BD Biosciences) by polychromatic flow cytometry approach using CytoFLEX LX platform (Beckman Coulter).

Results:

In view of the involvement of multiple immune cell subsets and numerous markers, major findings of the study and its probable effect on SARS CoV-2 immune response are outlined in Figure 17. In summary, while confirming previous findings, 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, IL2+ CD4 T cells, and follicular T helper cells. Multivariate analysis revealed a progressive association of PD1+CD4 T cells with $PRNT_{50}$ titers. Confirmation in larger cohorts and indepth functional assays to understand the underlying mechanism(s) remains the way forward.

Conclusion:

Disease severity was found to be associated with impaired maturation of mDCs and hyperactivation of NK, follicular T helper cells, and CD8 T cells. Lower IL21 receptor expression on memory B cells indicated an imbalance in IL21/IL21 R ratio. Lower BCMA positive plasmablast cells in severe cases did suggest a probable absence of long-term humoral immunity. In addition to identifying probable prognostic markers for severity, our study emphasized the definite need for in-depth viral antigen specific functional analyses in a larger patient cohort and with multiple sampling.

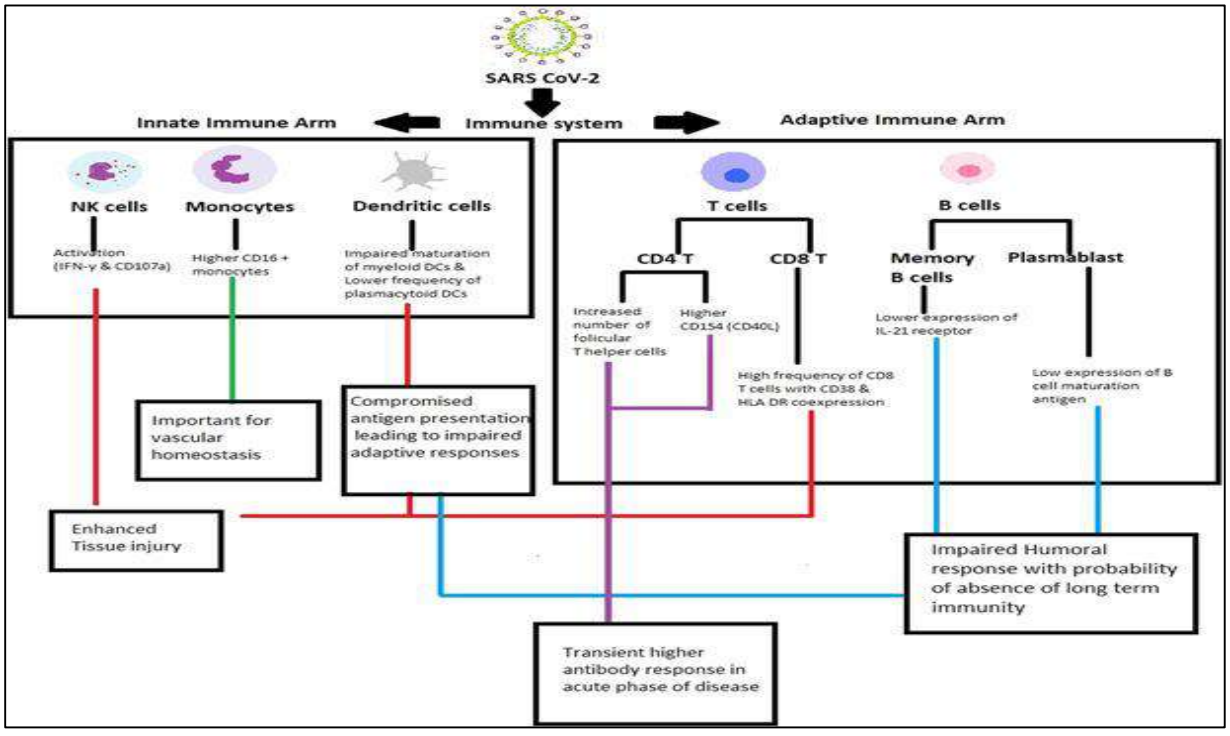


Fig 17. Schematic presentation of the major findings: Multiparametric flow cytometry was used to enumerate immune cells from innate (NK cells, DCs, and monocytes and adaptive (T cells and B cells) immunity arms in COVID-19 patients presenting with mild or severe disease. The findings pointed out major defects in the maturation (CD80 and CD86 expression) of myeloid dendritic cells and reduction in plasmacytoid dendritic cell frequency, emphasizing compromised antigen presentation. Enhanced frequency of nonclassical monocytes could be an important event in maintaining vascular homeostasis in the inflammatory cytokine milieu formed due to the infection. Activated NK and CD8 T cell subsets observed in severe cases may enhance lung tissue injury and disease severity. Likewise, activation of TFH, and CD4 T cell compartments in these cases may lead to a transient higher humoral response observed during the acute phase of infection. However, whether these antibodies are protective or aid in disease severity remains unclear. Lower IL21 receptor expression on B cells may lead to impaired proliferation and differentiation of B cells. Moreover, plasmablast cells showed a comparatively lower BCMA expression, a long term survival marker for plasma cells. Collectively, long term immunity is likely to be hampered, another observation being reported recently.

Title: (Project ID:) Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) viremia and anti SARS CoV-2 antibodies in the blood donors from Pune (CD/20/8/I)

Funding: DBT-BIRAC (as part of NIBEC Project CD/19/3/E)

Duration: December 2020-April 2021

Sanctioned Amount: NA

I**nvestigators**: **PI** – Dr. Vidya Arankalle

Co PI: Dr. Archana Kulkarni-Munje, Dr. Suhas Mhaske, Dr.D. B.Wani, Dr. A C Mishra,

Human Ethical Approval: BVDUMC/IEC/01

Background:

The pandemic of Coronavirus disease 2019 (COVID-19) continues to affect global population causing significant mortality and economic loss. As severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causing COVID-19 disease is a respiratory pathogen, strict compliance to the relevant public health measures was recommended and enforced worldwide.

During the pandemic, shortage of blood supply remained a major concern. In view of the large number of asymptomatic infections and possibility of blood donations during the symptom-free phase, possibility of transfusion-associated transmission of SARS-CoV-2 was considered.

So far, viral RNA positivity during the later time points of the pandemic and among Indian blood donors are not reported. For understanding epidemiology of different pathogens, screening of age-stratified general population and high risk groups remains the ideal method. Nonetheless, blood donor screening is often used as a reflection of adult population and preferred due to easy accessibility. Blood donor screening has proved useful in assessing potential magnitude of transfusion associated transmission of viruses with predominant non-parenteral routes.

Objective:

To assess prevalance of anti-SARS-CoV-2 antibodies in blood donors

Work done: To assess the potential risk of parenteral transmission and prevalence of anti-SARS-CoV-2 antibodies, blood donors recruited by the blood bank of Bharati Hospital, Pune, India were studied. Sampling was done at two time points (1) December-2020 when the first wave was at minimum (n=435) and (2) April-2021 when the second wave was at the peak (n=223). All the 658 plasma samples were screened for IgG-anti-SARS-CoV2 antibodies (ELISA). IgM testing was done only for samples collected in 2020. A total of 274 plasma pools and 15 IgM alone positives were tested for viral RNA; pools of 2 for IgG-negatives and pools of 3 for IgG-positives. None of the 658 donors gave history of COVID-19 or contact with COVID-19 patients.

Results:

Figure 18 summarize the findings.IgG-anti-SARS-CoV-2 positivity rose from 39.3% (2020) to 60.1% (2021, $p<0.0001$). The rise was significant in < 35 years age group ($P<0.001$). Viral RNA was detected only in antibody negatives. Overall, 2/435 (0.46%, 2020) and 0/223 donors (2021) donors were viral RNA positive. The proportion of donors with high OD values in ELISA (>2) decreased from 72.5% to 55.3% ($p<0.001$) suggestive of decrease in antibody titers. In Indian blood donors, SARS-CoV-2 viremia remains infrequent. Rampant asymptomatic infections and significant increase in exposure during 2months of the second wave are noteworthy.

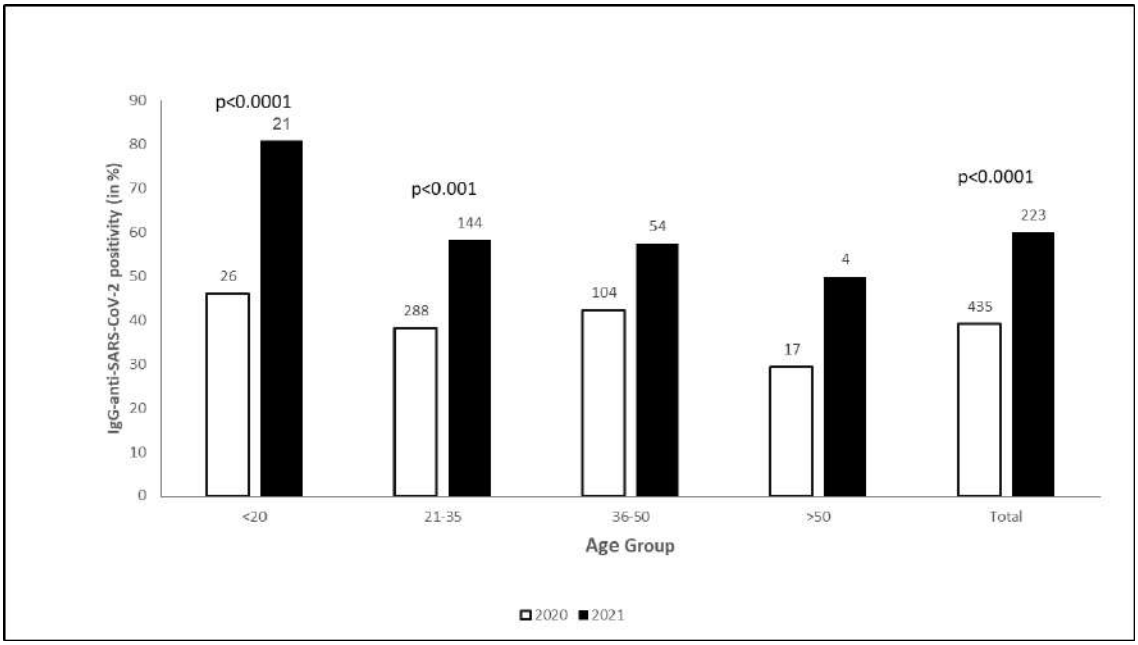


Figure 18 Depicts age wise comparison of IgG anti-SARS-CoV-2 antibody positivity among blood donors examined at the end of the first wave (December 2020) and during the 2nd month of the on-going second wave (April 2021). Values at the top of each bar represent total number of blood donors included in the respective age group.

Conclusion:

In summary, proportion of viremic blood donors in Pune remains low. Exposure to SARS-CoV-2 as evidenced by IgG-anti-SARS-CoV-2 positivity was high in December 2020 (39.3%) increasing further during the second wave to 60% in April 2021, through asymptomatic infections.

Title: (Project ID:) Immune response of adult COVID-19 vaccine recipients with special reference to immunological memory, T cell response and persistence of neutralizing antibodies (CD/21/2/I)

Funding: Intramural

Duration: January 2021-December 2022

Sanctioned Amount:

Investigators: PI – Dr. Vidya Arankalle

Co PI: Dr. Archana Kulkarni-Munje, Dr. Ruta Kulkarni, Dr Sonali Palkar, Dr Sujata Rege, Dr. Jitendra Oswal, Dr.Prakash Doke, Dr Sanjay Lalwani, Dr A C Mishra

Human Ethical Approval: BVDUMC/IEC/185A

Background: The current pandemic has witnessed the unprecedented spread of SARS-CoV-2 across the globe and efforts of the scientific community to develop an effective and safe vaccine at the earliest. From Jan-2021, emergency use of Covishield (adenovirus-based, developed by Oxford University and AstraZeneca) and manufactured by Serum Institute of India Pvt Ltd, SIIPL, Pune) and Covaxin (inactivated, whole virus-based, developed by Bharat Biotech International Ltd, BBIL, Hyderabad in collaboration with ICMR and manufactured by BBIL) was approved by the DCGI. Health care workers and frontline workers were given the deserving first priority. As contemporary guidelines eldery (+60yrs) were also among the priority group hence a cohort of these individuals is also included to assess the efficacy of vaccine despite of high risk of severity and mortality.

The satisfactory results of AstraZeneca vaccine trials conducted in three countries have been reported. So far, data from the Indian population immunized with the vaccine manufactured by SIIPL is not in the public domain. With BBIL vaccine, virus challenge studies in vaccine is reported to be safe in humans. An important concern has been the observed rapid decline in antibody titers following natural infection with SARS CoV-2. During serologic follow up of COVID19 patients, we did observe declining antibody titers. Therefore, the duration of antibody response after immunization and generation/persistence of T cell immune response needs to be carefully examined. According to the government of India guidelines, the vaccines were given irrespective of H/O COVID-19 infection. Hence understanding the dynamics of immune response among individuals with prior SARS CoV-2 exposure was possible.

In view of the above, we proposed undertaking a study evaluating cell-mediated immunity and persistence/titers of neutralizing antibody titers in the immunized individuals. As the vaccine was administered to anti-SARS-CoV2 antibody positive individuals, we assessed the extent of boosting of neutralizing antibody titers. Furthermore, we also assessed development of Immunological memory in vaccine recipients by enumerating virus specific T cells and their functionality in the form of Th1 cytokine generation in presence of viral protein stimuli. Such studies are not reported and are likely to yield valuable, novel information.

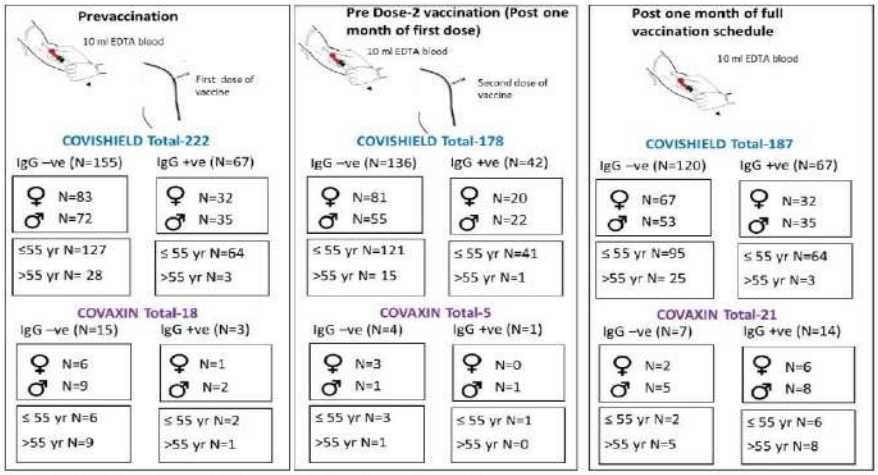
Objectives

To assess immunologic memory and T cell responses in vaccine recipients with and without prior exposure to SARS CoV-2

To compare neutralizing antibody titers and persistence in vaccine recipients with and without prior exposure to SARS CoV-2 till one- year post-vaccination.

To compare the cellular and humoral response to Covishield and Covaxin

Work done: The study started in January 2021 after the approval by Human Ethics committee of Bharati Vidyapeeth medical college. History of (H/O) COVID was obtained before each sampling. For the subjects included in the final analysis, H/O comorbidities/smoking and alcohol intake was obtained telephonically. Blood samples were collected before vaccination, before 2nd dose and one-month post-dose-2, in EDTA tubes. PBMCs were separated within 4hours of blood collection by ficoll- histopaque based density gradient method. PBMCs and plasma samples were stored at -80°C in aliquots.We have proposed whole blood sample collection from Covishield and Covaxin recipients at baseline, pre dose-2, one month after second dose, six months after second dose and one year after second dose. Figure 19 depicts the status of sample collection and types of analyses performed till June 2021. Sample collection from the patients at six months and one year is planned. The results included in the report comprise the analyses performed on the samples collected till June 2021.



Serological analyses- IgG ELISA & $PRNT_{50}$ (All study participants)
T cell mediated response analyses in vaccine recipients

- Pre vaccination IFN-γ ELISPOT & Flow cytometry based ICS assay for IFN-γ & IL-2 (N=16; all COVISHIELD)
- Post one month of Full schedule of vaccine [COVISHIELD(N=63); COVAXIN(N=13)]

1. IFN-γ ELISPOT
2. Flow cytometry based ICS assay for IFN-γ & IL-2 secretion by T cells

Fig 19: Study disposition.The figure depicts age and gender wise details of the study participants at different time points -baseline (prevaccination), Pre-dose 2 and post dose-2 for both the vaccines. Categorization based on the presence of SARS CoV-2 IgG positivity prior to vaccination is provided.

All the samples were tested for the (1) presence of IgG-anti-SARS-CoV-2 antibodies by ELISA (SCoV-2 Detect IgG ELISA, Inbios International, Inc., USA). This ELISA determines antispike antibodies and (2) presence/titers of neutralizing antibodies (Nabs) by 50% plaque reduction neutralization test (PRNT50) using live virus. Based on testing of plasma samples from 61 blood donors collected during 2017-19, the cut off value for a positive test was decided to be a titer of >10. T cell responses against SARS CoV-2 spike protein were assessed by Human IFN-γ ELISPOT^{Pro} kit (Mabtech) following the manufacturer's instructions. Additionally, IFN-γ and IL-2 secreting spike specific T cells were quantified by flow cytometry based intracellular cytokine assay.

Results:

In COVISHIELD recipients, pre-vaccination-antibody negatives (pre-negatives, n=120), %Nab seroconversion (median, IQR Nab titers) increased from 55.1% (16, 2.5-36.3) post-dose-1 to 95.6% (64.5, 4.5-154.2, p<0.001) post-2nd-dose and were independent of age/gender/BMI/smoking/alcohol intake. Comorbidities reduced Nab titers (p=0.004). Postdose-

1 Nab titer was the only independent variable influencing post-dose-2 Nab titers (p<0.001, r=0.84). In pre-positives, Nab titers rose from 75 (29-129) pre-vaccination to 3050 (1282-3998, p< 0.001, n=42) post-first-dose, but declined to 1740 (911-3116, p=0.037) post- 2nd-dose. Nab response in prepositives was independent of age/gender/BMI/Comorbidities. Post-dose-2-seroconversion (50%, p<0.001) and Nab titers (6.75, 2.5-24.8, p<0.001) in COVAXIN-recipients were lower than COVISHIELD. COVAXIN elicited a superior IFN-γ- T cell response as measured by ELISPOT (100%; 1226, 811-1532 spot forming units, SFU/million PBMCs v/s 57.8%; 21.7, 1.6-169.2; p<0.001) and ICS (56.9%, 30.1-73.6 v/s 32.1%, 15.1-56.7, p=0.03).

Conclusion:

This first-time, systematic, and real-world assessment revealed a higher humoral response by COVISHIELD and stronger cellular responses by COVAXIN. Relation of dose interval and decline in Nab titers post-2nd-dose in pre-positives (COVISHIELD) and lower humoral response (COVAXIN) need further assessment. Immunogenicity/efficacy of vaccines will change with the progression of the pandemic needing careful evaluations in the field-settings. Clearly, a larger

series of COVAXIN recipients need to be studied. With increasing numbers of infections, majority of the population should develop high antibody titers that can protect from reinfections/progression to severe disease needing hospitalizations.

Title: Breakthrough infection of SARS-CoV-2 in Pune City (Project ID: CD/21/1/I)

Funding: DBT-BIRAC (as part of NIBEC Project CD/21/1/E)

Duration: March 2021 – June 2021

Sanctioned Amount: NA

Investigators:

PI: Dr. Vidya Arankalle

Co-Investigators: Dr. Suhas Mhaske, Dr. Ruta Kulkarni, Dr. Prakash Doke, Dr. AC Mishra,

Ph.D. Students: None

Human Ethical Approval: BVDUMC/IEC/185A

Background:

COVID-19 Vaccination drive has been carried out though out world with number of approved vaccines developed with different efficacy. In India, national COVID19 vaccination program started on 16th January 2021 for health care workers and subsequently for citizens above age 60 and 45 having comorbidities like asthma, diabetes, heart diseases etc with the two vaccines: Covishield (Oxford-AstraZeneca) and Covaxin (BBV152, Bharat Biotech). Despite of vaccination, large number of population is getting infected with SARS-CoV2, to be termed as breakthrough infection, may be due to the mutations in genome leading to variants exhibiting more infectivity and immune escape. Though, breakthrough infection presents clinically mild but may lead to severe due to emergence of new variants. In this study, breakthrough infection of SARS –CoV-2 have been documented in two tertiary care hospitals (Bharati hospital and Kashibai Navale Hospital) in Pune city.

Objective:

To investigate the variants of SARS-Cov2 in breakthrough infections in second wave of COVID19

Work done:

In this study 261 cases were noticed to be infected with SARS-CoV2 after receiving one or 2 doses of Covishield or Covaxin in samples collected in two different tertiary care hospitals in Pune during March 2021 to first week of July 2021. Total 126 samples with full or partial vaccination and 368 samples of non-vaccinated with COVID19 infection were analyzed for sequence of RBD region of spike gene.

This study identified the predominant presence of signature mutants of Kappa (L452R and E484Q) and delta (L452R and T478K) variants of SARS-CoV2 in circulation in demographically similar vaccinated and non-vaccinated controls individuals. At the initial of second wave of COVID-19, in the month of March, Kappa variant (B.1.617.1) was predominant over Delta variant (B.1.617.2) and subsequently April onwards occurrence of Delta found to be more in circulation over Kappa in irrespective of vaccine status. In the month of June and early July, only Delta variant was in circulation in both the groups (Table 10). These mutations occurring in receptor binding domain region of spike provides partial resistance to vaccine elicited antibody neutralizations and could be the responsible factor for breakthrough infection.

Month of NPS collection	Partially/Fully vaccinated (n=126)				Non-vaccinated (n=168)					
	Kappa (E484Q)	Delta (T478K)	No mutation	Total	Alpha (N501Y)	Kappa (E484Q)	Delta (T478K)	No mutation	N440	Total
Mar-21 (n=46)	10 (76.92 %)	1 (7.6%)	2 (15.38 %)	13	0	27 (81.8 %)	1 (3%)	3 (9%)	2 (6%)	33
Apr-21 (n=92)	19	18	0	37	1	15	36	1 (1.8%)	1	54

	(51.35 %)	(48.64 %)			(1.8%)	(27.27 %)	(67.92 %)		(1.8 %)	
May-21 (n=137)	10 (15.87 %)	53 (84.12 %)	0	63	0	0	74 (100%)	0	0	74
Jun-21 (n=16)	0	9 (100%)	0	9	0	0	7 (100%)	0	0	7
Jul-21 (n=3)	0	3 (100%)	0	3	0	0	0	0	0	0

Table 10: Month-wise infection of SARS-CoV-2 in vaccinated and non-vaccinated individuals

% Calculated with respect to no. of samples of respective category

High sero-positivity for SARS-CoV2 IgG and neutralization (100% & 95%) in fully vaccinated individuals as compared to partially vaccinated (90.32% & 87.5%) demonstrated the efficacy of complete vaccination, though the difference in PRNT titers of partially and fully vaccinated individuals is non-significant. The significant high PRNT titers after infection in vaccinated (partially or fully) individuals highlights the disease protection potential of vaccination. This may be the cause of reduction in severity and hospitalization in case of breakthrough infection.

Conclusion:

The current study demonstrated the variants of SARS-CoV-2 leading to breakthrough infection in second wave of COVID19 in Pune and importance of surveillance to identify the circulating variants and breakthrough infection assisting to monitor the surge of vaccine escape variants of concern.

CINHD

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 101.08 Lakh (2015-21), 15.66 Lakh (2020-21)

Background:

Broad objective is linseed value addition. Following objectives for 2020-21 were planned and approved during Annual Linseed Group meeting held at ICAR-IIOR, Hyderabad in virtual mode during 13 – 14 August, 2020.

Objectives:

a) Blending of linseed oil with edible oil

b) Development of value added bread with linseed

c) Nutritional evaluation of released linseed varieties in India

d) Development of linseed derived omega-3 health supplements

Work done:

a) Blending of linseed oil with edible oil Following fresh blends of edible oils with linseed oil were prepared

- Base oil: Palm olein (PO), Coconut oil (CO), Rice bran oil (RBO)
- Blended oils: Palm Olein+ linseed oil (PO+LO-80:20), coconut oil + linseed oil (CO+LO-80:20), rice bran oil + linseed oil (RBO+LO-80:20)
- Antioxidant used: TBHQ+Tri E, AP+TBHQ, AP+Tri E, Rosemary extract, Green Tea extract

Three liters of each type of oil/blends (with/without antioxidants) were placed into stainless steel 2500W electric deep fryer and heated to 180 °C for 8 hr continuously. The oil samples were collected initially (before heating) and at the end of 30 min, 1 hr, 2 hr, 4 hr, 6 hr and 8 hr. These oil samples were analyzed for peroxide value, p-anisidine value, Totox value, free fatty acid content and fatty acid profile to understand thermo-oxidative stability.

The present study involved primary and secondary oxidation parameters along with fatty acid estimation after 8 hrs heating. It is observed that, blending of linseed oil with rice bran oil was

better in controlling the primary and secondary oxidation parameters with marginal alterations in the fatty acid profile. In case of palm olein oil blending, antioxidants such as AP+TBHQ, AP+Tri E, Rosemary and Green Tea were comparative more effective in controlling the thermal oxidation. Coconut oil blend showed comparatively more oxidation and significant alterations in fatty acid profile with or without antioxidants during 8 hrs heating study. This thermal stability study will be continued for evaluating the fate of the degraded products during thermal cycles in blended oil.

b) Development of value added bread with linseed

In order to incorporate omega-3 and other linseed based bioactive ingredients in bread the composition of the oil and flour was formulated using linseed and blended oil (rice bran oil + linseed oil). The flours were evaluated for its functional properties such as bulk density, water absorption capacity, water solubility index and oil absorption capacity. The breads were prepared using fully automatic bread maker using standard operative procedure provided by manufacture. Then breads were evaluated for proximate analysis, fatty acid composition and microbial load. Omega-3 bread were subjected for its functional assessment using universal texture analyzer for various parameters such as hardness, area, cohesiveness, springiness, springiness index, gumminess, chewiness, fracture force and adhesive force etc. Finally sensory evaluation using 9-point hedonic scale was carried out to understand the consumer acceptability of these breads.

Based on functional properties of flour and nutritional, microbial, texture analysis and sensory evaluation of bread, it can be concluded that, 5% linseed powder with or without blended linseed oil can be considered for developing the value added bread for fortification of omega-3 fatty acid in bread.

c) Nutritional evaluation of released linseed varieties in India

TL-99 variety recorded highest protein (27.72 g/100g), total ash (4.55 g/100g) content. Crude fibre was highest is Divya 8.85 g/100g followed by Shekhar 7.84 g/100g. Padmini recorded highest carbohydrate levels i.e. carbohydrate 24.85 g/100g. The energy values were comparable in all varieties ranging from 516.09 Kcal/100 in TL-99 to 554.5 Kcal/100g in Garima. Calcium ranged between 190 mg/100g (Shekhar) to 260 mg/100g (Padmini). Iron was ranging between 86.86 mg/kg (Divya) to 565.6 mg/kg (TL-99). Potassium ranged between 720 mg/100g (Padmini) to 1070 mg/100g (TL-99) and zinc ranged between 53.9 mg/kg (Shekhar) to 78.35 mg/kg (TL-99). The saturated fatty acid ranged between 10.55±0.33% (JLS-95) to 13.88±0.05% (Shekhar).

The mono-saturated fatty acid levels ranged between 20.38±0.11% (Garima) to 26.62±1.56% (TL-99). Linoleic acid (omega-6 fatty acid) was highest in TL-99 (53.64±1.92%) and lowest in Divya (11.76±0.11%). α-linolenic acid (omega-3 fatty acid) was highest in JLS-95 (56.52±0.40%) and lowest in TL-99 (7.26±0.64%). The functional assessment (bulk density, water absorption capacity, oil absorption capacity, emulsion activity, emulsion stability and least gelation concentration) showed wide variation in the tested linseed varieties. Variations in functional properties of linseed powder may be due to variation in its nutritive contents, the relative ratio of different constituents like protein, carbohydrates and fat. Based on the particular properties and the nutrient profile, these varieties can be explored/recommended for developing the value added food formulations.

d) Development of linseed derived omega-3 health supplements

Oil-in water type emulsion was prepared by using linseed oil, water and sucrose ester as an emulsifier. Total 3 emulsified formulations were prepared i.e. emulsion fortified with vitamins (fat and water soluble vitamins), emulsion fortified with protein (whey protein concentrate) and plain emulsion (does not contains protein/vitamin). The omega-3 fatty acid was present in all three formulation in similar quantity in all the formulations. The prepared emulsified formulation was added to milk. Milk added with different emulsion formulations was mixed, homogenized at room temperature using homogenizer at 2500–3000 rpm. Control milk without added emulsion was treated similarly. The plain milk and fortified milk was evaluated from proximate analysis. In order to understand the stability of omega-3 fatty acid upon heating for domestic use, thermal stability study using 3 heating cycles were carried out. In this study, plain milk/fortified milk was heated and cooled to room temperature, for 3 times. The milk samples were collected initially (no heating) and at the end of each cycle and subjected for fatty acid analysis using gas chromatography.

In the present experiment, emulsified methodology was used to fortify milk with omega-3 fatty acid along with vitamins/protein using emulsification method. Formulations containing vitamins and protein were prepared and the concentration of linseed derived omega-3 fatty acid was kept similar in the formulation. The saturated fatty acid content was comparatively reduced in all fortified milk as compared to plain milk. There was significant increase in poly-unsaturated fatty acid and particularly omega-3 fatty acid content in all fortified milk as compared to plain milk. Ratio of omega-6 to 3 was approximately 2:1, indicating healthy fatty acid profile of the milk. Stability of omega-3 fatty acid during heating was studied, comparatively across the groups,

average O3 loss in all the three groups was same, however O6 and PUFA was more stable in case of linseed emulsion fortified with whey protein concentrate as compared to control milk, plain linseed emulsion and linseed emulsion fortified with vitamin Pre-mix. O6 to O3 ratio was towards healthy side at the end of 3rd heating cycle, indicating the nutritive quality of milk was maintained. In this experiment, suitable method of fortification of milk with omega-3 fatty acid along with vitamins and protein was developed maintaining healthy omega-6 to omega-3 ratio i.e. 2:1. Omega-3 fatty acid in milk is stable to normal heating for home consumption. Omega-3 fatty acid is essential component of human milk, the process developed humanizes the cattle milk more suitable for human consumption.

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 efficacy of formulation containing omega-3 fatty acid along with vitamins and micronutrients was evaluated in animal model of metabolic syndrome and single dose in bioavailability study was completed. This year's objective was to carry out repeat dose tissue distribution study of formulation as compared to fish oil.

Work done:

In this study, comparative analysis of the uptake, incorporation and conversion of α-linolenic acid (ALA) (from flax oil based emulsified formulation) to its metabolites with preformed eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (fish oil) in animal model was performed. The animals were administered the formulation and fish oil for the period of three months and at the end of study fatty acid profiling of plasma, RBC and vital organs (liver, kidney, heart, pancreas, adipose and brain) lipids was performed and data is presented in figure 1 and 2. The bioavailability of an individual fatty acid is influenced by the presence of other fatty acids, in the present study diet was similar for all experimental rats and dose of total omega-3 fatty acid kept constant in both groups.

In the present study, considering the overall fatty acid profiling of blood (plasma and RBC) and vital organs (liver, kidney, heart, adipose, brain and pancreas), it can be concluded that the formulation group showed improved the omega-3 levels which was equivalent to fish oil group. Comparable efficiency of ω-3 FA from formulation group to convert ALA to DHA as compared to fish oil in RBC and vital organs lipids (liver, brain and heart), additionally ALA remain available in pancreas and adipose tissues. Both ALA and DHA has different mechanisms in different diseases conditions. So the developed formulation is able to provide ALA and DHA both together to show the desired effect.

Figure 1: Repeat dose bioavailability study of formulation as compared to fish oil (Plasma and RBC fatty acid analysis)

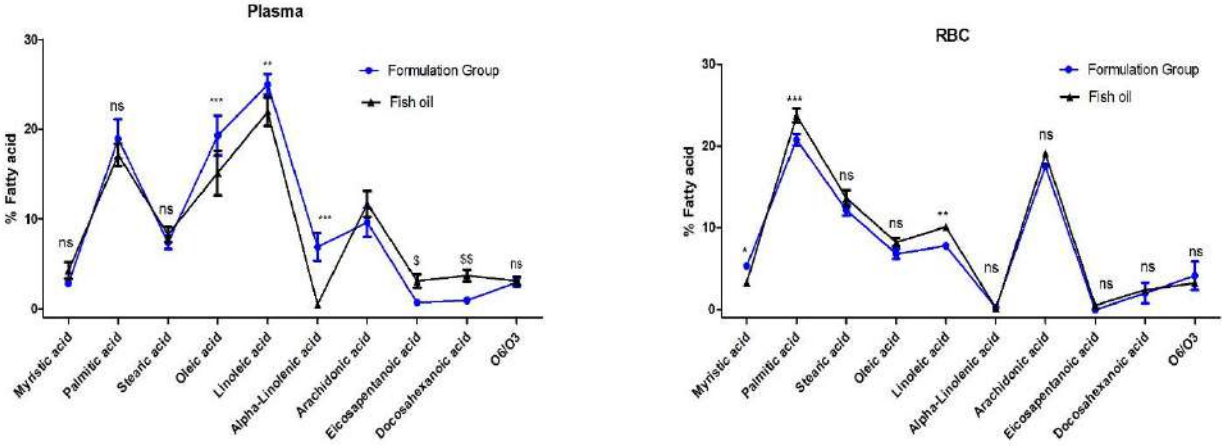
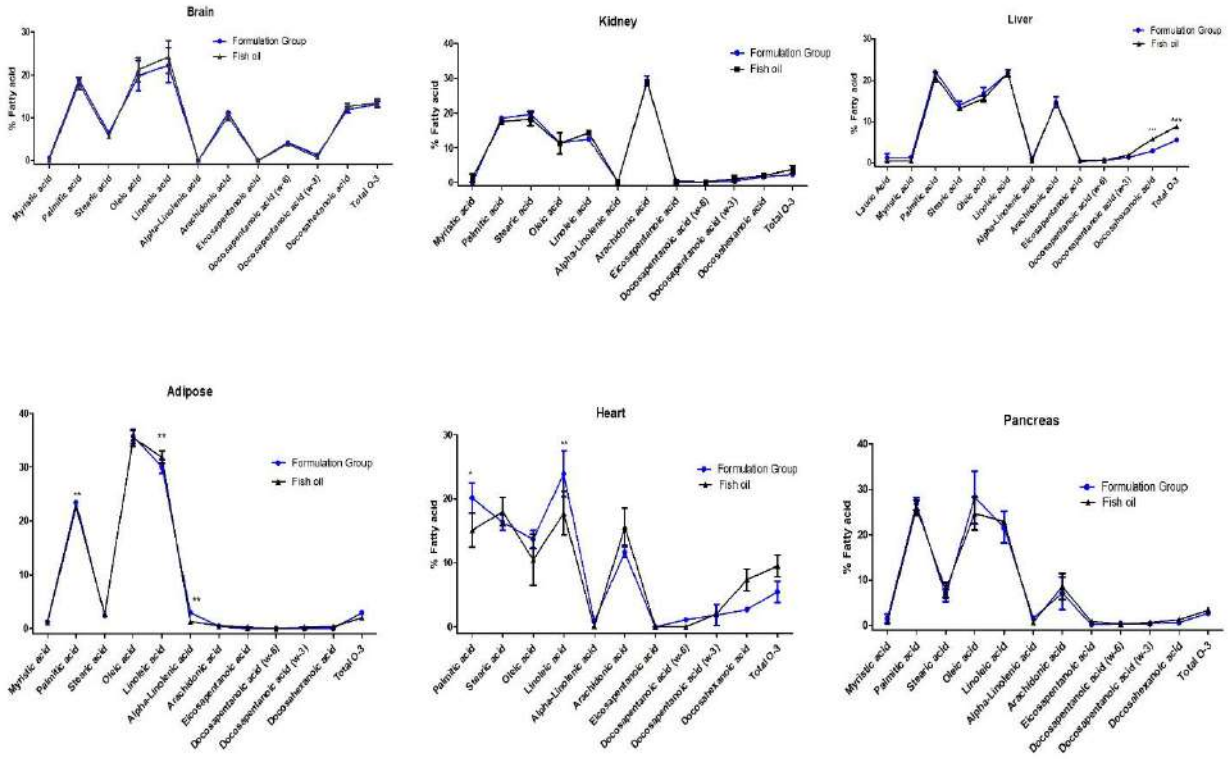


Figure 2: Repeat dose bioavailability study of formulation as compared to fish oil (Tissue fatty acid analysis)



Conclusion:

- The total absorption of omega-3 fatty acid from formulation group was comparable to that of fish oil.
- Formulation is able to provide all the forms of omega-3 fatty acid i.e. ALA, EPA, DPA and DHA, as against fish oil which provides only EPA and DHA.

3. Title: Extraction of bioactive lignan and development of value added products from flaxseed

Project ID: INHD/19/1/E

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:

Last year project was started and only administrative formalities such as animal ethics committee approval, appointment of project staff and purchase of equipment was processed. Further as per first objective, lab scale trials of extraction of lignan from flaxseed was initiated.

Work done:

In order to develop solvent extraction method of secoisolariciresinol diglucoside (SDG) lignan from flaxseed, various pre-treatment (Table 1) were carried out and subjected for determination of SDG-lignan content by high performance thin layer chromatography (HPTLC). Extraction of lignan from flaxseed: Accurately weighed differently processed flaxseed samples (Table 1) were hydrolysed with 1 N sodium hydroxide for 1 hr at room temperature with intermittent shaking, followed by extraction with 50% ethanol. Then the filtrate was acidified to pH 4 using 1 M hydrochloric acid, followed by filtration and recovery of the sample using hot plate/tray dryer.

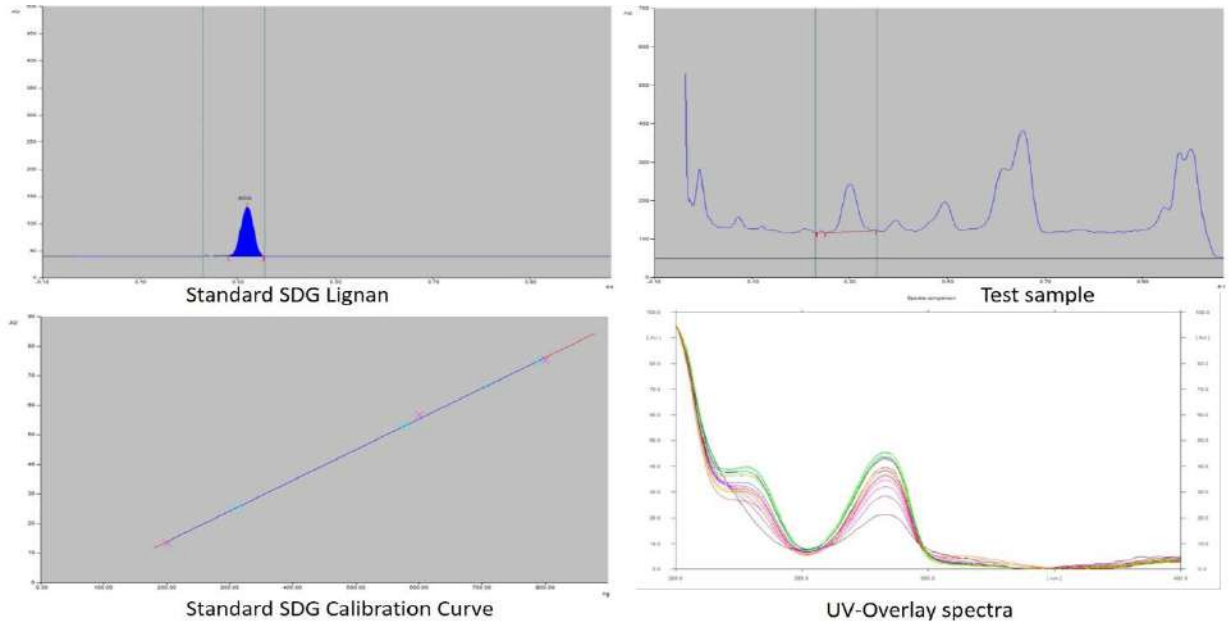
Table 1: Comparative assessment of SDG-lignan content in different fractions of flaxseed:

	Description of sample/pre-treatment		**Description of sample/pre-treatment**
A	Whole seed powder	G	De-mucilage, defatted whole seed powder
B	Hull powder	H	De-mucilage, defatted hull powder
C	De-hull powder	I	De-mucilage, defatted de-hull powder
D	De-mucilage whole seed powder	J	Defatted whole seed powder
E	De-mucilage hull powder	K	Defatted hull powder
F	De-mucilage de-hull powder	L	Defatted de-hull powder

Fig. 3: Fractionating flaxseed parts separating lignan rich fraction

Various solvents such as pure alcohol, 70% alcohol, methanol, 1, 4-dioxane, acetone were tried for improved extraction of SDG-lignan and it was observed that 70% ethanol was most effective for extraction of SDG-lignan from flaxseed. Flaxseed samples were subjected for pre-hydrolysis and post-hydrolysis by NaOH and it was observed that pre-hydrolysis step (hydrolysis followed by alcoholic extraction) gave significantly higher yield of the SDG-lignan concentrate than that of post-hydrolysis. As per standardized methodology and also literature review it has been reported that, 1 N sodium hydroxide is required for effective hydrolysis for releasing the lignan from the polymer. But in the present modified methodology, since hydrolysis and extraction is simultaneous and continuous, we tried 3 different normality of sodium hydroxide i.e. 0.25 N, 0.5 N and 1 N and it was observed that there was non-significant difference in SDG-lignan content between 0.25 N and 1 N sodium hydroxide. Interestingly post-extraction acid neutralization step was also not necessary as p^H was closer to 6 to 7 and there was non-significant difference in SDG-lignan content between conventional extraction and modified methodology used in the present study. So the developed method in the present study was optimized w.r.t. solvent, hydrolysis normality, post-acid neutralization and standardization on lab scale soxhlet apparatus.

Fig. 4: HPTLC characterization of SDG.lignan



Various parameters were optimized to reduce the solvent consumption, simultaneous hydrolysis and extraction helps in reducing the time of extraction, number of steps of extractions are also reduced which ensures the minimum loss of the bioactive SDG-lignan content. The developed

method is operational at room temperature so no thermal degradation is expected. Fractionation of flaxseed in lignan and non-lignan helps in better utilization of every part of seed in different food formulations and also helps in increased yield and improved nutritional value of the of the product. This lab scale method will be up-scaled on pilot plant (solid liquid extractor).

4. Title: Development of infection-resistant urinary latex 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:

Catheter-associated urinary tract infection (CAUTI) is a very common healthcare associated infection (HAI) of the urinary tract. It is the most prevalent nosocomial infection in ICU patients. CAUTI is associated with high morbidity, mortality, prolonged hospitalization and increased healthcare costs. Common complications associated with urinary catheters include infection and inflammation in the lower urinary tract, often beginning with colonization of bacteria followed by bio-film formation on the surface of the device.

Currently antimicrobial coated "BactiguardTM" BIP Foley catheter, commercially available in India, has been demonstrated significantly ineffective in preventing microbial adherence and biofilm formation on device surface. Therefore, effective antimicrobial coating technologies are needed in order to prevent biofilm formation on the surface of urinary Foley catheter. Thus, the research objective was to develop a novel antimicrobial coated urinary Foley catheter which would provide a prolonged and broad-spectrum antimicrobial efficacy to prevent biofilm formation.

Work Done:

- A proprietary novel antimicrobial composition in combination with a bio-compatible matrix forming polyurethane polymer was formulated to coat on the surface of latex urinary Foley catheter.
- Latex Foley catheters were successfully coated with the antimicrobial composition. The newly developed infection resistant urinary catheter has been named as "AntiBac-L catheter". Integrity of antimicrobial coating on urinary catheter surface was found to be stable.

- Surface of AntiBac-L catheter was more smooth after coating with antimicrobial composition, compared to uncoated catheter. Atomic force microscopy (AFM) studies demonstrated that surface smoothness of AntiBac-L catheter was 12.5 times higher than uncoated catheter.
- Coated AntiBac-L catheter showed broad spectrum antimicrobial activity against the microorganisms, including Gram-negative (E. coli, P. mirabilis, K. pneumoniae, P. auruginosa), Gram-positive (S. aureus, S. faecalis) bacterial strains and fungal isolate (C. albicans).
- In vitro studies showed that the newly developed infection resistant AntiBac-L catheter was found to be more effective in preventing adherence of uropathogens like E. coli (clinical isolate), K. pneumonia, P. aeruginosa (clinical isolate), S. faecalis, and C. albicans (clinical isolate), compared to the commercially available "BactiguardTM" BIP Foley catheter and uncoated catheter.
- Inner luminal surface of coated AntiBac-L catheter was found to be effective in preventing adherence of uropathogen P. mirabils, a common causative agent for blockages in catheter.
- Scanning electron microscopy (SEM) studies demonstrated that coated AntiBac-L catheter prevented biofilm formation against uro-pathogenic E. coli (clinical isolate).
- Dry weights of C. albicans biofilm formed on coated AntiBac-L catheter surface after 24h, 48h and 72h of incubation, was found to be lower compared to uncoated catheter surface.
- Sustained release of active drug molecule like chlorhexidine was observed from the coated AntiBac-L catheter surface, signifies antimicrobial efficacy and durability of the coated catheter.
- Compared to the uncoated catheter, antimicrobial coating did not significantly alter the physico-mechanical properties [i.e., tensile strength, Young's modulus and elongation at break (%)] of the coated AntiBac-L catheter, before or after soaking in artificial urine.
- After 6 months of storage at room temperature, coated AntiBac-L catheters have retained antimicrobial activity against the uropathogenic bacterial and fungal isolates.

Project outcome:

- Antimicrobial coating technology on latex Foley urinary catheter has been developed and exclusively licensed to Blue Neem Medical Devices Pvt. Ltd., Bangalore.

- Antimicrobial coating technology has been successfully transferred to Blue Neem Medical devices Pvt. Ltd., Bangalore and Dr. Arnab K. Ghosh, PI of the project, has joined at Blue Neem Medical Devices to conduct pre-clinical and clinical studies prior to commercialization.
- Coatings and methods for infection-resistant medical devices; Indian Patent Application No. 201827046613; Filed on December 10, 2018; Indian Patent Application Publication No. 201827046613, Published on July 12, 2019 (patent pending).
- A research manuscript write up on the "Development of a novel anti-microbial coated urinary catheter resistant to biofilm formation" is under preparation.

5. Title: Development of a novel antimicrobial hand sanitizer

Project ID: INHD/20/1/I

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, hand sanitizer formulations proposed by WHO does not show long lasting antimicrobial activity. Hence, 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:

We developed a novel antimicrobial hand sanitizer, "Germ-free hand sanitizer" 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. We mentioned about this invention in the Annual Report 2019-2020.

- **Evaluation of the bactericidal efficacy of the hand sanitizer using *ex-vivo* porcine skin:**

Ex-vivo porcine skin model was used to evaluate the effectiveness of our newly developed hand sanitizer, according to the standard ASTM E2897-12 method. This work was carried out at the

laboratory of Dr. Shanta Modak (our collaborator), Columbia University, USA. Porcine skin has widely been used as a surrogate for human skin. Antimicrobial efficacy was evaluated after applying 150 µl of the test hand sanitizers on a pair of sterilized porcine skins inoculated with 100µl of 10^7 cfu/ml *S. aureus* culture (ATCC 6538), followed by immediately rubbing the skins together for 30 seconds. Phosphate buffered saline (PBS) was used as the control and applied on the porcine skins in the same manner. The porcine skins were kept at room temperature for 30 seconds.

Quantitative viable bacterial counts were determined by using cup scrub technique. A circular cylinder (4 cm diameter) was pressed firmly over the porcine skin surface and 1mL of sterile Drug Neutralizing Fluid (DNF) was added into the cylinder. The porcine skin was then scrubbed with moderate pressure for 60 seconds by using a sterile polytetrafluoroethylene (PTFE) scraper. After scrubbing, the sampling fluid was transferred into a sterile tube. The samples were serially diluted and spread over the TSA plates with spreader. The plates were incubated at 37°C for 24 hours. Viable colonies were enumerated and the $\log_{10}$ reduction values were determined with respect to the control growth.

- **Evaluation of the virucidal efficacy of the hand sanitizer against SARS-CoV2 virus (COVID-19):**

This experiment was conducted according to EN 14476:2013+A2 guideline, at the laboratory of NIBEC, IRSHA. Briefly, in a sterile 15 ml culture tube, 1 ml of interfering substance (0.3 gm/l bovine albumin solution) was added to 1 ml of SARS-CoV2 virus suspension ($TCID_{50}$ / ml = 10^6) and mixed well. Then, 8 ml of the Germ-free hand sanitizer was added to the tube and mixed well. The culture tube was incubated at 20°C in a water bath for 1 min. Immediately at the end of incubation, 0.5 ml of the test mixture was added into 4.5 ml ice cold maintenance medium, i.e. 2% MEM (minimum essential medium) and placed into an ice bath. A series of 10 fold dilutions of this mixture from 10^1 to 10^{10} was prepared within 30 minutes. About 0.1 ml of each dilution was added into the wells of a microtitre plate containing a confluent (>90%) Vero cell monolayer. After 1 h of incubation at 37 °C, 0.1 ml of cell culture medium was added to each well. The plates were incubated for 7 days at 37 °C. The viral cytopathic effect (CPE) was read by using an inverted microscope. After incubation, viral infectivity titre ($TCID_{50}$) was determined by Spearman-Karber method. Reduction in virus infectivity was determined from the differences of $\log_{10}$ virus titres before and after treatment with the test product.

Results**:**

- **Evaluation of the effectiveness of hand sanitizers using *ex-vivo* porcine skin:**

Antibacterial efficacy of the hand sanitizers (*viz*. Germ-free sanitizer, WHO sanitizer and Dettol sanitizer) is presented in Table 2, using *ex-vivo* porcine skin model. According to ASTM E2897-12 method, Germ-free hand sanitizer was more effective against *S. aureus*, compared to the Dettol and WHO hand sanitizers.

Table 2: Antibacterial activity of hand sanitizers using *ex-vivo* porcine skin

Test products	**Antibacterial activity against *S. aureus* [Log_{10} reduction]**
Germ-free sanitizer	3.88 ± 0.84
WHO sanitizer	2.30 ± 1.28
Dettol sanitizer	2.51 ± 1.02

Values are represented as mean ± SD of three independent experiments (n=3). Control counts ranged from $1X10^5$ to $1X10^6$ cfu/ml

1. **Virucidal activity of the hand sanitizer against SARS-CoV2 (COVID 19):**

Efficacy of the Germ-free hand sanitizer against SARS-CoV2 (COVID 19) is presented in Table 3.

Table 3: Virucidal activity of Germ-free hand sanitizer against SARS-CoV2

Products	**Concentration**	**Interfering substance**	**Test temperature**	**Level of cytotoxicity**	**Log $TCID_{50}$ after contact time**		**Log_{10} reduction**
					0 min	**1 min**	
Germ-free sanitizer	100%	0.3g/L BSA	20°C	3.5	5.5	< 3.5	>2
Virus Control	NA	0.3g/L BSA	20°C	NA	5.5	5.5	NA

Conclusions:

1. Germ-free hand sanitizer was found to be more effective against *S. aureus* compared to the Dettol and WHO hand sanitizers, according to ASTM E2897-12 method using *ex-vivo* porcine skin model.

2. Germ-free hand sanitizer exhibited more than 99% reduction (Log_{10} reduction >2) of SARS-CoV2 (COVID-19) viral titre in suspension.

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:

Our preliminary studies on the activities of major enzymes of the glycolytic pathway in erythrocytes of patients with T2D have shown very significant alterations. Keeping this in mind it was proposed to find out whether the diabetic state influences the kinetic properties of the key enzymes of the glycolytic pathway and their corresponding glycolytic intermediates. The morphological evaluation of RBCs including qualitative and quantitative analysis of diabetic erythrocyte cell membrane components viz. proteins and lipids and their interrelationships in T2D are studied and established. It is anticipated that the observed altered metabolic patterns may offer a new biomarker for research in T2D based on RBCs and related diabetic complications. This project has been successfully completed and closed as all the objectives of the project are achieved.

Work done:

- All the university requirements for the 2nd Ph.D. presentation were fulfilled and submitted the progress report of the same, after successful presentation of the work in front of internal evaluation committee.
- All the university requirements for the 3rd Ph.D. presentation were fulfilled and submitted the progress report of the same, after successful presentation of the work in front of internal evaluation committee.
- At the time of 3rd Ph.D. presentation, the first draft of PhD thesis was presented and was approved by the internal evaluation committee for the submission.

- The final draft of Ph.D. thesis was submitted to the PhD section of Bharati Vidyapeeth (Deemed to be University) under the faculty of Pharmaceutical Sciences.

Conclusion:

The present research was carried out to understand how persistent higher amount of glucose is handled by RBC, in uncontrolled diabetic patients. Alterations in enzyme kinetics, consequently, lead to changes in cytosolic metabolite levels. The alterations in substrate kinetic studies of enzymes involved in glycolytic pathway, and subsequent changes in concentrations of glycolytic intermediates in RBCs of T2D patients can very well be the beginning of diabetic complications These alterations in glycolytic pathway, possibly leads to alterations in levels of metabolites and morphological changes in RBC membrane, loss of deformability, and diminishing the normal biochemical functions of vital proteins by excessive non-enzymatic glycation.

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 2019-2020, Groundnut oil blends (GNO) with Flax seed oil (FO) were prepared and analyzed for nutritive quality, oxidative stability, cell viability. Storage stability at room temperature for palm olein, groundnut and coconut oil blends was determined. Thermal stability of the PO and GNO blends was also determined. Similar studies were done for Coconut oil (CO) and FO blends.

Work done:

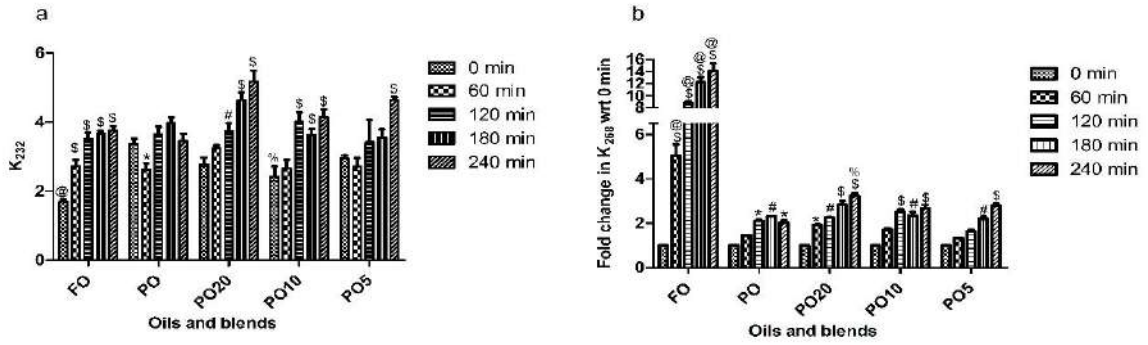
1. Thermal stability for PO and GNO blends was evaluated
2. Storage stability for palm olein (PO) blend containing 20 % FO (P20) was determined
3. Storage stability for groundnut oil blend containing 20 % FO was determined
4. Coconut oil blends containing 20,10 and 5% FSO had been prepared and indicated as C20, C10 and C5 respectively

5. Nutritive quality, oxidative stability and thermal stability of the CO blends has been analyzed

Results:

- Thermal stability for PO and GNO blends

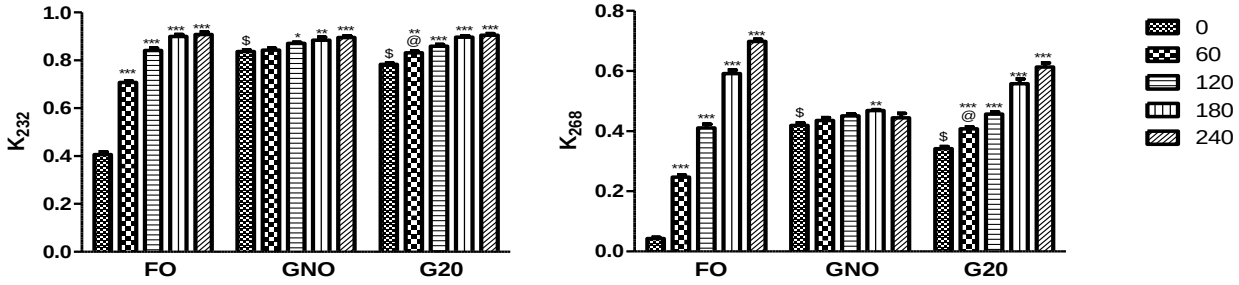
Fig. 5



Effect of continuous heating of the oils and blends at 180 °C on K_{232} and K_{268}. Oils or their blends were heated at 180 °C for 240 min. Samples were collected at specified intervals and UV absorbance was recorded at 232 nm and 268 nm . K_{232} (A) and K_{268} (B) were calculated. Two-way ANOVA and Bonferroni posttests were used for statistical analysis. $:($p<0.001$vs 0 min), #: ($p<0.01$vs 0 min), *:(($p<0.05$vs 0 min), @: ($p<0.001$vs PO) and %: ($p<0.01$ vs PO)

As shown in Fig. 5a and b, heating FO at all the four time points showed a statistically significant increase in K232 and K268 values once again highlighting the thermal instability of FO. But PO with initial high K232 value, did not show significant rise, indicating robust nature of PO. Notably, different blends tolerated different durations of the heating before showing significant deterioration in the form of K232 and K268. It is important to note that, the thermal deterioration accelerated as the percentage of FO in the blends increased. It is reflected as level of significance and time required to achieve that significance level.

Fig. 6



Effect of continuous heating of the oils and blends at 180 °C on K_{232} and K_{268}. Oils or their blends were heated at 180 °C for 240 min. Samples were collected at specified intervals and UV absorbance was recorded at 232 nm and 268 nm . K_{232} and K_{268} were calculated. Two-way ANOVA and Bonferroni posttests were used for statistical analysis. *; $p < 0.05$ vs respective 0 h, **; $p < 0.01$ vs respective 0 h, ***; $p < 0.001$ vs respective 0 h, $; $p < 0.001$ vs FO 0 h, @; $p < 0.001$ vs GNO 0 h

As shown in Fig. 6, heating FO resulted in a statistically significant increase in K_{232} and K_{268} values. GNO with initial high K_{232} and K_{268} values, though did not show significant rise at initial time point, rise in both parameters was significant at later time points. Notably, for G20 there was significant deterioration in the form of K_{232} and K_{268} at all the time points. It is important to note that, the thermal deterioration accelerated as the duration increased for FO and G20. Thus the data reflects that GNO is unable to impart thermal stability to the FO containing blend.

- Storage stability for palm olein blend (PO20)

Table 4. Storage stability of PO20 in terms of peroxide value and acid value

Oil/Blend	0 Month	3rd Month	6th Month	9th Month
		Peroxide value		
PO	1.98±0.0	3.63±0.057***	5.3±0.0***	9.26±0.06***
FO	0.6±0.05	3.3±0.00***	5.4±0.1***	12.73±0.05***
PO20	2.5±0.0	4.33±0.057***	7.2±0.0***	11.43±0.11***
		Acid value		
PO	0.89±0.0	0.89±0.0	0.89±0.0	1.34±0.0***
FO	0.48±0.00	0.75±0.02***	1.59±0.00***	2.01±0.03***
PO20	0.89±0.0	0.89±0.0	0.89±0.0	1.79±0.0***

PO, FO and PO20 were stored at room temperature upto nine months. Peroxide value and acid value were at mentioned time intervals. Data is represented as Mean±SD of three experiments. Two-way ANOVA and Bonferroni posttests were applied to determine statistical significance. ***: $p<0.001$vs 0 month

Nine month storage stability study for the blend, PO20 which had highest percent of oxidatively susceptible FO was conducted. Table 1 represents fold change in the PV and AV at indicated time points compared to initial (0 month) readings. Table 4 clearly indicates that PV for individual oils and PO20 increased significantly throughout study period ($p<0.001$ VS 0 month). But rise in the PV for FO was very high (≈21 fold) as compared to PO and PO20 (≈4.5 fold rise) by nine months. Fold rise in the PV for PO and PO20 showed very similar trend indicating minimum or limited peroxidation of oil or blend for the period of nine months at room temperature. Table 1 also represents fold rise in the AV compared to initial (0 month) readings. Similar to PV, FO showed gradual and significant fold rise in the AV ($p<0.001$) while PO and PO20 did not show significant change in the values until nine month. As seen in case of PV, PO and PO20 followed similar trends but fold rise in AV for PO20 at nine months was slightly higher than PO.

- Storage stability for groundnut blend (G20)

Table 5. Storage stability of G20 in terms of peroxide value and acid value

Oil/Blend	**0 Month**	**1rd Month**	**3th Month**	**5th Month**
		Peroxide value (meq O2/ kg oil)		
FO	0.43 ± 0.01	1.98 ± 0.25***	3.45 ± 0.24***	13.00 ± 0.14***
GNO	0.40 ± 0.00	2.10 ± 0.48***	3.93 ± 0.13***	6.20 ± 0.28***
G20	0.90 ± 0.14	2.91 ± 0.56*** %	7.42 ± 0.88***@	12.53 ± 0.18***@
		Acid value (KOH/ g oil)		
FO	0.81 ± 0.00	0.98 ± 0.04***	0.70 ± 0.009***	0.38 ± 0.00***
GNO	1.72 ± 0.14	2.16 ± 0.09***	1.68 ± 0.06***	0.44 ± 0.02***
G20	1.77 ± 0.07	2.25 ± 0.07***	1.58 ± 0.07***	0.37 ± 0.01***

Two Way ANOVA and Bonferroni Posttests were applied . ***; *p<0.001* vs 0 Month, %; *p<0.05* vs GNO respective time point ,@; *p<0.001* vs GNO respective time point

Table 5 indicates that GNO alone had lowest deterioration in terms PV. Blends showed high PV similar to FO. After initial rise, AV displayed declining trend. Fatty acid analysis of oils/blend did not show significant alterations upon storage.

- Initial physicochemical characterization for CO blends

Table 6. Fatty acid composition of the blends

Fatty acid	**FO**	**CO**	**C5**	**C10**	**C20**
Caprillic acid	0.00 ± 0.00	6.69 ± 0.03#	5.78 ± 0.04#,ω	4.89 ± 0.01#, ω,μ	4.28 ± 0.04#,ω, μ

Capric acid	0.00 ± 0.00	5.5 ± 0.04$^{\#}$	4.99 ± 0.06$^{\#, \Delta}$	4.4 ± 0.01$^{\#, \omega,\mu}$	3.98 ± 0.02$^{\#,\omega,}$ $^{\mu,*}$
Lauric acid	0.00 ± 0.00	47.94 ± 0.07$^{\#}$	45.2 ± 0.07$^{\#, \omega}$	42.26 ± 0.2$^{\#,}$ $^{\omega,\mu}$	36.36 ± 0.02$^{\#,\omega,}$ $^{\mu, *}$
Myristic acid	0.00 ± 0.00	20.64 ± 0.04$^{\#}$	19.7 ± 0.04$^{\#, \omega}$	19.21 ± 0.03$^{\#,}$ $^{\omega,\blacklozenge}$	16.05 ± 0.08$^{\#,\omega,}$ $^{\mu, *}$
Palmitic acid	6.61 ± 0.08	8.7 ± 0.23$^{\#}$	8.72 ± 0.06$^{\#}$	8.98 ± 0.04$^{\#,\blacklozenge}$	8.7 ± 0.01$^{\#}$
Stearic acid	6.59 ± 0.07	3.76 ± 0.02$^{\#}$	3.91 ± 0.03$^{\#}$	4.22 ± 0.01$^{\#,\Delta,\blacklozenge}$	4.68 ± 0.04$^{\#,\omega,}$ $^{\mu,\alpha}$
Oleic acid	21.61 ± 0.01	5.54 ± 0.04$^{\#}$	6.61 ± 0.02$^{\#, \omega}$	7.39 ± 0.22$^{\#,}$ $^{\omega,\mu}$	9.09 ± 0.11$^{\#,\omega,}$ $^{\mu, *}$
Linoleic acid	14.47 ± 0.1	0.87 ± 0.03$^{\#}$	1.65 ± 0.08$^{\#, \omega}$	2.37 ± 0.02$^{\#,}$ $^{\omega,\mu}$	3.97 ± 0.02$^{\#,\omega,}$ $^{\mu, *}$
Alpha Linolenic acid	50.72 ± 0.23	0.00 ± 0.00$^{\#}$	3.14 ± 0.01$^{\#, \omega}$	5.9 ± 0.01$^{\#, \omega,\mu}$	12.1 ± 0.02$^{\#,\omega,}$ $^{\mu, *}$
ΣS	13.2 ± 0.16	93.22 ± 0.42$^{\#}$	88.29 ± 0.3$^{\#, \omega}$	83.95 ± 0.25$^{\#,}$ $^{\omega,\mu}$	74.03 ± 0.09$^{\#,\omega,}$ $^{\mu, *}$
ΣM	21.61 ± 0.01	5.54 ± 0.04$^{\#}$	6.61 ± 0.02$^{\#, \omega}$	7.39 ± 0.22$^{\#,}$ $^{\omega,\mu}$	9.09 ± 0.11$^{\#,\omega,}$ $^{\mu, *}$
ΣP	65.19 ± 0.13	0.87 ± 0.03$^{\#}$	4.24 ± 0.83$^{\#, \omega}$	8.26 ± 0.03$^{\#,}$ $^{\omega,\mu}$	16.06 ± 0.04$^{\#,\omega,}$ $^{\mu, *}$
LA: ALA	0.29 ± 0.00	ND	0.52 ± 0.03$^{\#}$	0.40 ± 0.00$^{\#,\beta}$	0.33 ± 0.00$^{\$,}$ $^{\mu,\&}$

The FA composition of the oils and their blends was determined by GC-FID. The data is represented as Mean ± SD (n=3). Two Way ANOVA and Bonferroni Posttests were applied to determine statistically significant differences between and among different FA present in the oils and blends. ΣS represents total saturated fatty acids, ΣM represents total monounsaturated fatty acids and ΣP represents total unsaturated fatty acids. #; $p< 0.001$ vs FO, $; $p< 0.05$ vs FO, ω; $p< 0.001$ vs CO, Δ; $p < 0.05$ vs CO, μ; $p< 0.001$ vs C5, ♦; $p< 0.05$ vs C5, β; $p< 0.01$ vs C5, * ; $p< 0.001$ vs C10, α; $p < 0.05$ vs C10, &; $p< 0.01$ vs C10

As presented in Table 6, FA composition of CO and blends were statistically different than FO which was also reflected in the total SFA, MUFA and PUFA content (#; $p< 0.001$ vs FO, $; $p< 0.05$ vs FO). FO had highest PUFA content while CO had highest SFA content. CO had meager amounts of both MUFA and PUFA especially essential FA; LA and no ALA. When compared with CO, blends had significantly modified FA composition except palmitic acid content. It is important to note that as the percentage of FO increased, ALA content also increased resulting in

significant lowering of LA: ALA. FA percentages were significantly different among the blends which was also reflected in the total SFA, MUFA and PUFA content.

Table 7. Physico-chemical parameters of the blends

Oil/Blend	AV (mg KOH/ g oil)	% FFA (as Lauric acid)	PV (milliequivalent O2/ kg oil)	SP (˚C)
FO	2.52 ± 0.48	0.49 ± 0.01	0.80 ± 0.00	104.0 ± 1.41
CO	$0.84 \pm 0.08^{**}$	0.59 ± 0.09	$0.00 \pm 0.00^{***}$	$205.0 \pm 1.41^{\mu}$
C5	$0.90 \pm 0.00^{**}$	0.67 ± 0.06	$0.65 \pm 0.07^{\#}$	$198.5 \pm 0.71^{\mu,\pi}$
C10	$0.90 \pm 0.00^{**}$	0.73 ± 0.11	$0.60 \pm 0.00^{*,\#}$	$198.5 \pm 0.71^{\mu,\pi}$
C20	$1.09 \pm 0.04^{**}$	$0.85 \pm 0.00^{*}$	$0.85 \pm 0.07^{\#,@,\%}$	$196.5 \pm 0.71^{\mu,\pi}$

Acid Value (AV), % Free Fatty Acid (% FFA), Peroxide Value (PV) and Smoke point (SP) of the blends were determined immediately after the blend preparation. The data is represented as Mean ± SD (n=3). One-way ANOVA and Tukey's Multiple Comparison Test was applied to determine statistically significant differences among the oils and blends. *and **and ***; $p< 0.05$ vs FO, #; $p< 0.05$ vs CO, @; $p< 0.05$ vs C5, %; $p< 0.05$ vs C10, µ; $p < 0.01$ vs FO and π; $p< 0.01$ vs CO

There were no significant differences between and among the AV of the CO and blends as represented in Table 7. % FFA values were similar for FO, CO and the blends except C20 which had significantly higher value than FO. No peroxides were detected in CO but blending with FO resulted in the significant increase in PV compared to CO. SP of FO was significantly lower than CO and their blends. When compared with CO, blends had significantly lower SP but they were still significantly higher than that of FO (*$p<0.01$*).

Conclusion

Based on the nine month storage stability study and thermal stability study, it can be stated that, PO had imparted storage stability to PO20 at least up to nine months while thermal stability was limited to some extent. In case of blends of FO and CO, FA profile especially LA: ALA ratio, total SFA, MUFA and PUFA were significantly altered just by addition of 5 % FO in the blend. It can be stated that, upto 20% FO can be easily used in the blend with CO without affecting its suitability as cooking oil.

Other Information

Budget

Extramural Grants (newly sanctioned and ongoing) Total Projects:6

S. No	**Name of the Scheme/Project/ Endowments/ Chairs**	**Name of the Funding agency**	**Year of Award**	**Funds provided (INR in lakhs)**	**Duration of the project**	**Funds Received in April 2020-March 2021**
1	Influence of Maternal One-carbon (1C) Metabolism in Placental Function, Fetal Growth and Programming	DBT	2018	Total Amount Awarded: 161.98 ; IRSHA SHARE: Rs. 6.85	3 yrs	720518
2	Investigating Mechanisms Leading to Preeclampsia	ICMR	2017	Rs. 757.95	5 yrs	141.33
3	Epigenetic regulation of angiogenic factors in assisted reproductive technology (ART) and non-ART derived placentae	DBT	2019	Rs. 59.91	3 yrs	2020 577
4	Epigenetic regulation of placental peroxisome proliferator activated receptor (PPAR) in women delivering low birth weight babies	DBT	2019	Rs. 35.50	3 yrs	1263 185
5	Capacity enhancement of National Immunogenicity and Biologics Evaluation Center for assessing the immunogenicity of SARS CoV Vaccine	DBT-BIRAC 2	2021	134500 000/-	Feb 2021 - 2022	6707 9000

6	Evaluation of circulatory biomarkers for disease severity in hepatitis E	Indian Council of Medical Research (ICMR)	2020	43.63	Jan 2020 -Dec 2021	4358205
7	Platelet derived exosomes and their role in endothelial dysfunction in dengue infection	DBT-BIOCARE	2019	16.01	June 2019 - 2022	1518262
8	Establishment of National Centre for Immunogenicity Testing, NCIT to evaluate vaccines in clinical trials	DBT-BIRAC 1	2019	1250+350(bvdu)	May 2019 - 2023	37500000
9	Development of potent adjuvanted respiratory syncytial virus vaccine for mucosal delivery	DBT-Wellcome India Alliance	2019	168.93	Jan 2019 - Dec 2023	1921624
10	Evaluation of different adjuvants for development of potent chikungunya vaccine	DST-SERB	2018	32.22	May 2018 - 2021	750000
11	Phytochemical standardization and evaluation of anti-cancer and immunomodulatory activity of Unani formulation, Itrifal Gudadi".	EOI AYUSH CCRUM	2020	43.57	2020 - 2023	1216000
12	Evaluating the effect of Alpha Lenolenic acid (ALA), on omega 3 fatty acid,on modulation of epigenetic markers in the cervical cancer cell lines	DST SERB	2018	33.16	2018 - 2021	1000000
13	Comparing vaginal microflora diversity between healthy and cervical cancer women for identifying isolates having probiotic and anticancer potential	DST WOS	2018	32.06	2018 - 2021	1055000
14	Evaluating anticancer activity and mechanism of action of Unani formulation Habbe Mussafi Khoon against cervical cancer	EMR AYUSH CCRUM	2018	57.56	2018 - 2021	0

15	Evaluating anticancer activity of homeopathic preparation Linum usitassium in breast cancer cell lines	EMR AYUSH CCRUM	2018	42.02	2018 - 2020	0
16	Effect of Yoga intervention on skeletal muscle linked glucose homeostasis in prediabetic individuals	DST-SATYAM	2018	46.74	2018 - 2020	1300000
17	ICAR- AICRP-Linseed value addition centre	ICAR	2015	101.08	Centre awarded From April - 2015 onwards	1566400
18	Evaluation of efficacy of novel stabilized omega-3-fatty acid and antioxidants formulation for the prevention and treatment of metabolic syndrome	SERB	2017	40.36 Lakh for 3 years	3 Years	353745
19	Extraction of bioactive lignan and development of value added products from flaxseed	SERB	2019	23.45	3 Years	0
	Endowments, Chairs ,	Charak Pharma Pvt. Ltd	2020	9.45	6 mo	3.53
	Extraction of bioactive lignan and development of value added products from flaxseed	Real World Nutrition	2019	27.85	3 Years	0

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	Amol Chaudhary DBT - SRF	Evaluating the effect of Matarisenol on macrophage polarization	23,64,000/-	2,76,732/-	2,75,409/-
2	Akanksha Mahajan DBT - JRF	Evaluation of anticancer potential of selected phytochemicals against breast cancer stem cells	23,70,000/-	5,41914/-	4,91758/-
3	Rama Rajadnya DST Inspire JRF	Evaluating the effect of selected bioactive on cytokine and chemokine regulation in prostate cancer	23,62,400/-	4,81,280/-	4,81,280/-
4	Prajkta Patil DST Inspire JRF	Evaluating the effect of lignans inregulation of lipid and cholesterol metabolism in breast cancer	23,62,400/-	4,81,280/-	4,81,269/-
	CSIR HRDG	Chemometric analysis and development of methodology for quality standardization of 'Vidanga'	4.92	3.60	3.60
	ICMR	Ms. Mrunal Gosavi	Rs 16.22	Rs 10.08	Rs 10.08
	UGC-NET	Miss. Sayali Vedpathak	Rs. 9.46	Rs. 5.91	Rs. 3.13

Publications (Total No: 3)

	Title of paper	Name of the author/s	Name of journal	Year of publication
	Cross talk of vascular endothelial growth factor and neurotrophins in mammary gland development	**Kamini Dangat, Amrita Khaire, Sadhana Joshi**	**Growth Factors**	**2020 Jul**
	Omega-3 fatty acids differentially influences embryotoxicity in subtypes of preeclampsia.	**Kasture V, Dalvi S, Swamy M, Kale A, Joshi S.**	**Clin Exp Hypertenses**	**2020 April**
	Association of Preeclampsia with Anthropometric Measures and Blood Pressure in Indian Children	**Randhir Karuna, Pisal Hemlata, Kadam Vrushali, Khaire Amrita, Malashe Nandini, Deshpande Ruma, Palkar Sonali, Lalwani Sanjay, Kumara**	**PLoS ONE**	**2020 May**

		n K, Yajnik Chittaranjan, Osmond Clive, Fall Caroline, Joshi Sadhana		
	Maternal fats and pregnancy complications: Implications for long-term health	Khaire, A., Wadhwani, N., Madiwale, S., Joshi, S.	Prostaglandins, Leukotrienes and Essential Fatty Acids.	2020 Jun
	Region-specific changes in the mRNA and protein expression of LCPUFA biosynthesis enzymes and transporters in the placentae of women with preeclampsia.	Alka Rani, Preeti Chavan-Gautam, Girija Wagh, Savita Mehendale, Narayanan S Mani, Sadhana R Joshi.	PLACENTA	2020 Jun
	Differential Expression of Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor	Sahay AS, Jadhav AT, Sundrani DP,	Clin Exp Hypertenses	2020 May

	(BDNF) in Different Regions of Normal and Preeclampsia Placentae.	**Wagh GN, Joshi SR**		
	Role of neurotrophins in pregnancy and offspring brain development.	**Sahay A, Kale A, Joshi S.**	**Neuropeptides**	**2020 Oct**
	Prenatal vitamin D supplementation reduces blood pressure and improves placental angiogenesis in an animal model of preeclampsia	**Nema J, Sundrani D, Joshi S**	**Food Funct.**	**2020 Jul**
	Exploring the role of LC-PUFA metabolism in pregnancy complications.	**Godhamgaonkar AA, Wadhwani NS, Joshi SR**	**Prostaglandins Leukot Essent Fatty Acids**	**2020 Nov**
	Tumor retardation and immunomodulatory potential of polyherbal formulation HC9 in mouse melanoma model	**Suryavanshi, S., Shinde, K., Raina, P. and Kaul-Ghanekar, R**	**Pharmacognosy Magazine**	**2020 March**
	Development and validation of a bioanalytical HPLC method for simultaneous estimation of cinnamaldehyde and cinnamic acid in rat	**Shetty, V., Chellampillai, B. and Kaul-Ghanekar, R**	**New Journal of Chemistry**	**Feb-20**

	plasma: application for pharmacokinetic studies			
	Evaluating the Anticancer Activity of Homoeopathic Preparation of Asterias rubens in Breast Cancer Cell Line on the Basis of Similia Principle.	**Gupta, E., Kaul-Ghanekar, R. and Manhas, S.S**	**Internatio nal Journal of Health Sciences and Research**	**Apr-20**
	A clinical study to evaluate the efficacy of Herbal Formulation for Obesity (HFO-02) in overweight individuals	**Gupte, P., Harke, S., Deo, V., Bhushan Shrikhande, B., Mahajan, M. and Bhalerao, S**	**Journal of Ayurveda and Integrative Medicine**	**April-June 2020**
	Evaluation of NS1-Detection-Based Cell Culture Method for Isolation of Dengue Viruses from Clinical Samples	**Shrivastava, S., Solaskar, A., Gosavi, M., Tiraki D., Mishra AC, Arankalle VA.**	**SN Compr. Clin. Med**	**May-20**
	Comparative assessment of commercial enzyme-linked immunosorbent	**Kulkarni R, Modak M, Gosavi**	**Indian J Med Res**	**2020 Jan**

	assay & rapid diagnostic tests used for dengue diagnosis in India	**M, Wani D, Mishra AC, Arankalle VA**		
	Use of Orchids in treating Diabetes and related disease: A review	**Sourav Mukherjee and Suresh Jagtap**	**The Journal of Phytopharmacology**	**March – April 2020**
	Compositional alterations in erythrocyte membranes in Type II diabetes	**Sunita S. Bhise, Janhavi R. Rao, Mahabaleshwar V. Hegde, Surendra S. Katyare**	**IJEB**	**Oct-20**
	Standardization of ELISA for anti-chikungunya-IgG antibodies and age-stratified prevalence of anti-chikungunya-IgG antibodies in Pune, India	**Patil HP, Rane PS, Gosavi M, Mishra AC, Arankalle VA**	**Eur J Clin Microbiol Infect Dis**	**Oct-20**
	Correlation of serostatus and viraemia levels among Indian dengue patients at the time of first diagnosis.	**Kulkarni R, Shrivastava S, Patil HP, Tiraki D, Mishra AC,**	**Trans R Soc Trop Med Hyg**	**2020 Jul**

		Arankalle VA		

Book chapters (Total No:0)

Asavari A. Joshi, Anand Arvind Zanwar. Effect of Olive oil on Metabolic Syndrome. In Olives and Olive Oil in Health and Disease Prevention. Academic Press- Elsevier Inc. 2021, Chapter no. 22; 261-272. https://doi.org/10. 1016/ B978-0-12-819528-4. 00038-9

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 No. 201921006187	16/02/2020	Application published on 21-08-2020

Awards and Honors (Faculty: 0; Students: 0)

Faculty			
Academic Year	**Name of The Faculty Member**	**Honor**	**Details of Award / Honor**
Students			
Academic Year	**Name of The Faculty Member**	**Honor**	**Details of Award / Honor**

Papers Presented at International Conferences/Seminars/Workshops: (Total No: 0)

1. Mr. Kartikey T. Jagtap- Presented research paper entitled “A comparative assessment of morphological and phytochemical variation among authentic and market samples of ‘Vidanga’ at conference Shri Pancham Khemraj Mahavidyalaya, Sawantwadi, (MS) on

International Conference on 'Research Interventions and Technological Advancements in Plant Sciences (RITAPS, 2021)' Jointly Organized with Association of Plant Science Researchers, Dehradun (26th and 27th March 2021).

2. Mr. Manoj M. Khawate- Presented research paper entitled "Qualitative and quantitative determination of secondary metabolite Embelin from traditional medicinal plant from Myrsinaceae family using HPTLC method. at conference Shri Pancham Khemraj Mahavidyalaya, Sawantwadi, (MS) on International Conference on 'Research Interventions and Technological Advancements in Plant Sciences (RITAPS, 2021)' Jointly Organized with Association of Plant Science Researchers, Dehradun (26th and 27th March 2021).
3. Mr. Mayur Aswani- Presented paper entitled as "Prebiotic profiling of indigenous selected *Dioscorea* spp. using *in-vitro* techniques" at International Conference on Biotechnology and Biological Sciences, held between 18th – 20th November 2021 organized by University of Engineering & Management, Kolkata, India.
4. Amit A. Jagtap, Asavari A. Joshi, Yogesh S. Badhe, Pramod D. Farde, Mahabaleshwar V. Hegde, Anand A. Zanwar. Bioavailability of Omega-3 Fatty Acid in Normal and High Fat High Carbohydrate Diet Induced Animal Model, at 18th Annual World Congress Insulin Resistance Diabetes & Cardiovascular Disease, USA held during December 3-6, 2020.
5.

Invited Talks by Faculty (Total no.)

Sr. No	Academic Year	Date	Name of the faculty	Topic
1		17-06-2021	Dr. Supriya Bhalerao	Auyrveda & Allopathy – Common ground, common goals (Dr. KK's MED talks)
	2020-2021	20/05/2021	Dr. Harshad Patil	Delivered talk on Development of adjuvanted influenza

				vaccine for pulmonary delivery at 11th Annual Vaccines World Summit 2021
	2020-21	24th Dec 2020	Dr. Anand Zanwar	Linseed technologies and value addition
	2020-21	19th Dec 2020	Dr. Anand Zanwar	Metabolic Syndrome-Pharmacological Investigations

Ph.D. Degree Awarded: Total 0

Sr. No	Name of the Student	Name of the Guide	Topic	Month and Year of Award

Collaborations:

International Collaborations: Nil

Sr. No	Name of the Collaborator	Period of Collaboration	Objectives	Status

National Collaborations:

Sr. No	Name of the Collaborator	Period	Objectives	Status
1	Dr. Girish Tillu, CCIH, University of Pune	2016 till date	Scientific and technical inputs for developing project proposals, Network pharmacology of Ayurvedic formulations	Ongoing
2	Dr. Yogesh Shouche, NCMR-NCCS, Pune	2016 till date	Microbiome analysis	Ongoing
4	Dr. Vaishali Deshmukh, Pune	2016 till date	Expert opinion for all ongoing projects from endocrinology perspective Diabetes/obesity awareness Activities	Ongoing
5	Dr. D. C. Mathangi, Professor of Integrative Medicine, Shriramchandra	Feb 2020 to till date	Scientific and technical inputs for developing project proposals	Ongoing

	Institute of Higher Education and Research, Porur, Chennai			

MOU's and Linkages:

Sr. No	Name of the Partner	Objectives	Status
1	D Y Patil college of Physiotherapy, Pimpri Pune	DST- SATYAM project activity	Ongoing

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. Harshad Patil	19th-20th May 2021	11th Annual Vaccines World Summit 2021	Imapac	International Conference
		Dr. Anand Zanwar	13-14 August 2020	Annual Group Meeting on Linseed	ICAR-IIOR, Hyderabad	National
		Dr. M. V. Hegde	13-14 August 2020	Annual Group Meeting on Linseed	ICAR-IIOR, Hyderabad	National
		Dr. P. B. Ghorpade	13-14 August 2020	Annual Group Meeting on Linseed	ICAR-IIOR, Hyderabad	National

Workshop	2	Dr. Harshad Patil	15th October 2020	National Biopharma Mission, Environment, Health and Safety Webinar series	BIRAC	National
		Dr Ruta Kulkarni	15-20 March 2021	Online Faculty Development Programme on Outcome-based Education	BV(DU), Pune	National
Workshop	3	Dr Ruta Kulkarni	29-30 April 2021	Training on Laboratory system and Internal Audit as per ISO/IEC 17025:2017	Quality Council of India	National
Workshop	4	Dr Archana Munje	29-30 April 2021	Training on Laboratory system and Internal Audit as per ISO/IEC 17025:2017	Quality Council of India	National
Workshop	5	Dr Suhas Mhaske	29-30 April 2021	Training on Laboratory system and Internal Audit as per ISO/IEC 17025:2017	Quality Council of India	National
Workshop	6	Dr Ruta Kulkarni	13 May 2021	Online training on Good clinical practice	Institutional Ethics Commitee, BV(DU)	Dr Ruta Kulkarni

Invited Lectures

Sr. No	Name of the Guest	Topic	Date
1	Dr. Akshay Anand, Professor (NonMedical), Department of Neurology, PGIMER Chandigarh	Quality assurance in Yoga research	10th July 2020
	Dr. Sriranjini Jaideep, Vaidya Scientist Fellow, Consultant, Ayurveda Association, Canada	Diet and Nutrition in Ayurveda	24th April 2021
	Dr. D.C. Mathangi, Professor & HOD, Mind Body Medicine & Lifestyle sciences, Shri Ramchandra Institute of Higher Education & Research, Chennai Dr. Vaishali Deshmukh, Honarary Consultant, Deenanath Mangeshkar Hospital, Pune	Mind body medicine- scope & opportunities	22nd May 2021

Events Organized at IRSHA:

Events Organized at IRSHA

1. National Ayurveda Day & World Diabetes Day: Both these occasions were celebrated on 23rd November, 2020 in collaboration with Charak Samhita Research, Training & Skill Development Center, Institute of Training and Research in Ayurved (ITRA), Jamnagar, Gujarat and the theme was "Ayurveda and Diabetes".
2. Dr. Mandip Goyal, Dept. of Kayachikitsa and Dr. Vaishali Deshmukh, Honarary Consultant, Deenanath Mangeshkar Hospital, Secretary (SPHERE) Pune delivered talks on; 'Diabetes in Ayurveda' and 'Need for Integrative approach in Diabetes management'

respectively. The event concluded with expert comments from Prof. Ganapathy Bantwal, newly elected President of Endocrine Society of India.

3. International Yoga Day: It was celebrated on 21st June 2021 through online mode. Dr. Nilangi Sardeshpande delivered a talk on "Importance of Yoga in daily life" followed by words of encouragement and blessings from Bapu Borotikar.
4. Inauguration of National Immunogenicity and Biologics Evaluation Center (NIBEC) established under DBT-BIRAC, National Biopharma Mission by Dr. Renu Swarup Secretary, Department of Biotechnology, Government of India on 3rd September 2020.
5.

Extension activities:

- M. L. Panse served as Expert for online review of Biotechnology Ignition Grant project proposals for total seven (7) projects -12/10/2020
- **Seed Production by Dr. P. B. Ghorpade:**

Foundation and certified seed was produced with the help of "Gajanan Maharaj Jawas Utpadak Gat Samuh, Chikhlapar, District – Nagpur" under Participatory Plant Breeding and Seed Production of PKVN-NL-260

	Foundation seed	**Certified Seed**
1.	**5 Qtl.**	**60 Qtl.**
2.	**50 Acres**	**600 Acres**
		
	Foundation Seed plot – PKV-NL-260	**Certified Seed plot – PKV-NL-260**

The monitoring was done for genetic purity of seed at different stages of crop growth in January and February 2021.

- **Seed Distribution:**

The certified seed was processed, bagged (600 bags, 10 kg each) and distributed to farmers of Eastern Vidarbha for its commercial cultivation through different agencies of Govt. of Maharashtra e.g. KVK, ATMA and State Department of Agriculture.

- **Development of High Oleic Safflower variety:**

The high oleic Safflower variety developed under ICAR-NASF Project was grown at Chikhlapar, Nagpur district for genetic purification and its stability for oleic content and multiplication of its seed for its commercial utilization.

Varieties	**Oleic content (%) 2020**	**Oleic content (%) 2021**	**Varieties**	**Oleic content (%) 2020**	**Oleic content (%) 2021**
NASF-1	59.92	74.59	**NASF-21**	58.88	47.12
NASF-2	67.82	61.10	**NASF-26**	76.88	75.02
NASF-3	79.98	74.01	**NASF-29-2**	68.78	76.76
NASF-4	76.35	77.44	**NASF-29-4**	75.58	78.17
NASF-5	78.66	72.26	**NASF-29-6**	71.63	78.45
NASF-6	78.69	77.71	**NASF-29-7**	69.16	74.43
NASF-7	80.53	79.29	**NASF-29-8**	72.76	78.59
NASF-8	80.04	78.36	**NASF-29-9**	74.89	78.14
NASF-9	72.15	----	**NASF-29-10**	77.32	77.88
NASF-10	74.49	68.24	**NASF-29-11**	76.90	77.52
NASF-11	80.00	78.26	**NASF-29-12**	75.39	73.28
NASF-12	80.80	81.30	**NASF-29-13**	66.06	76.95
NASF-16	79.09	75.75	**NASF-29-14**	71.39	---
NASF-17	74.68	65.98	**Check variety PBNS-12**	14 to 15	

Any other activities

Type of the Event	Theme	Date	Level (International / National / State / Institute)	Role
SYNAPSE 2021	International conference on Mind Body Medicine in Endocrinology	27- 28 Feb 2021	International	Dr. Supriya Bhalerao as Panelist in the panel Discussion

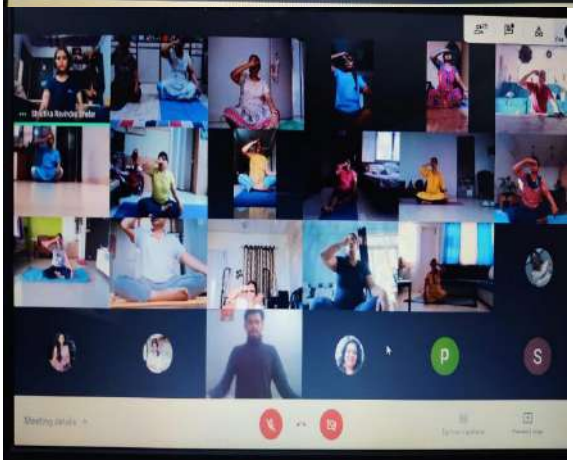
Any other activities:

1. Dr. Supriya Bhalerao is nominated on Experts Committee for Products Approval, FSSAI, Ministry of Health & Family Welfare, Govt. of India
2. Dr. Supriya Bhalerao is appointed as Member, Data Safety Monitoring Board (DSMB) for COVID studies, Ministry of AYUSH, Govt. of India
3. Effective team building workshop conducted by Dr. Renuka Joshi, Director, School for Soft Skills Development on 2nd May 2021

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

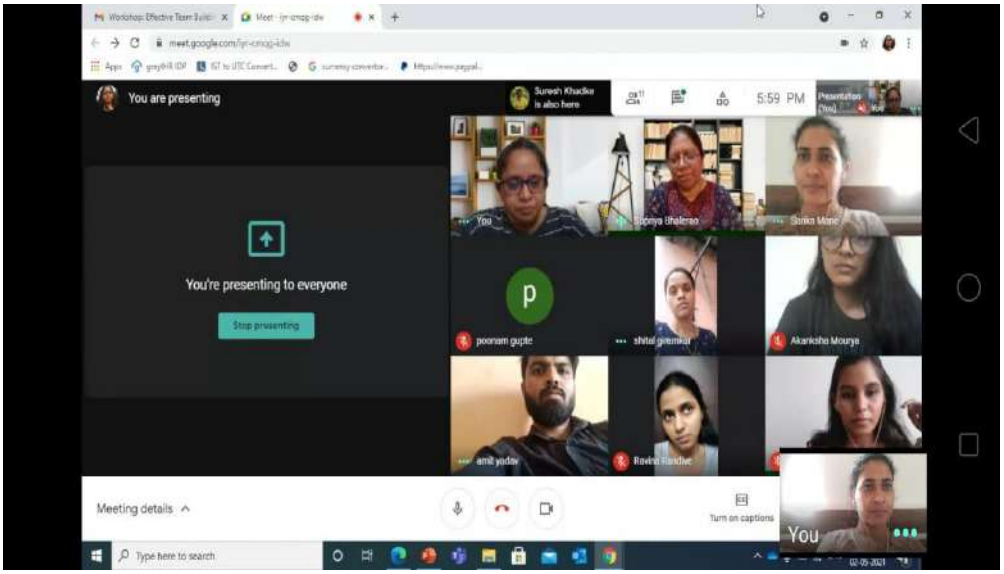
Screening camp at Katraj Dairy on Women's Day

International Yoga Day 2021



National Ayurveda Day Celebration

Online Effective Team Building workshop



Staff Information

Staff Category	Number
Scientific staff	1
Technical Staff	2
Ph.D. students	8

Administrative	**0**
Total	**11**

A) Name of the Teaching/ Scientific Staff:

Sr. No.	Name of the Staff	Designation	Joining Date in BV
1	Dr Ruchika Kaul-Ghanekar	Associate Professor	

B) Name of the Technical Staff:

Sr. No.	Name of the Staff	Designation	Joining Date in BV
1	Dr Prerna Raina	SRA	MAY 2016
2	Kavita Kadam	TA	SEP 2012

C) Name of the Administrative Staff:

Sr. No.	Name of the Staff	Designation	Joining Date in BV
1			

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			
B) Name of the Non-Teaching Staff			
1			

Student List

Sr. No.	Name of the Student	Designation	Date of Joining Ph D programme
1	Apoorva Parimoo	SRF	
2	Minal Mahajan	PhD fellow	
3	Amrita Ulhe	JRF	
4	Amol choudhary	SRF	
5	Akanksha Mahajan	JRF	
6	Rama Rajadnya	JRF	
7	Prajakta patil	JRF	
8	Nidhi Sharma	SRF	

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,	Member

IRSHA.	
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