वार्षिक प्रतिवेदन

Annual Report

2020 - 21

ICMR - NATIONAL INSTITUTE OF NUTRITION

Indian Council of Medical Research

Hyderabad, Telangana, INDIA

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आई सी एम आर – राष्ट्रीय पोषण संस्थान

ICMR - NATIONAL INSTITUTE OF NUTRITION

भारतीय आयुर्विज्ञान अनुसंधान परिषद

Indian Council of Medical Research

हैदराबाद, तेलंगाना, भारत

Hyderabad, Telangana, INDIA

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RESEARCH HIGHLIGHTS

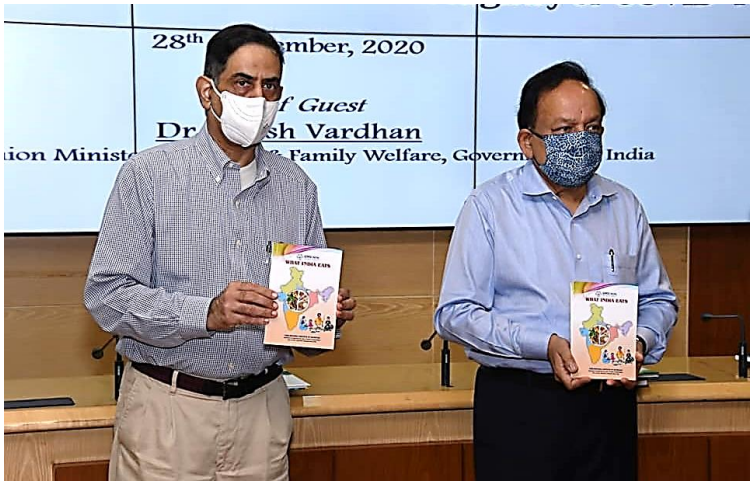
RELEASE OF PUBLICATIONS

Dr. Harsh Vardhan, Hon'ble Union Minister for Health & Family Welfare, Science & Technology and Earth Sciences released three publications "Nutrient Requirements for Indians 2020 - Recommended Dietary Allowances (RDAs) & Estimated Average Requirements (EARs)"; 'What India Eats" and "100 Years History of ICMR-NIN". He also launched a unique crowd sourcing data collection programme "'Mapping of nutrition and health status – A national level participatory real-time data generation programme". Dr. Hemalatha R, Director, ICMR-NIN and Dr. Balram Bhargava, Secretary, DHR & DG ICMR were present on the occasion.

Nutrient Requirements for Indians – Recommended Dietary Allowances (RDAs) and Estimated Average Requirements (EARs): New Nutrient Requirements for Indians that includes the Estimated Average Requirements (EAR) and Tolerable Upper Limits (TUL) of nutrients alongside Recommended Dietary Allowances (RDAs) has been released keeping in view the changing food habits, physical activity, nutrition transition and health status. These recommendations are the basis for defining the nutrient levels in policies and programs. The

EARs and TUL are not only useful in evaluating the nutritional adequacies of populations or groups but also in defining the regulation on food fortification and nutrient supplementation etc. Dr. Harsh Vardhan, Hon'ble Union Minister for Health and Family Welfare along with the Director-General, ICMR and Director, ICMR-NIN released this document at ICMR, New Delhi on Sept 2020.

The report - What India Eats, which gives an overview of the dietary patterns across the country was released during the event. “What India Eats” shows that despite regional disparity in consumption, cereals were being consu-med the highest across the country. The report, being first of its kind, also highlighted the rural–urban divide, which confirmed that urban areas had better dietary diversity with more people consuming more food groups. Also, lower consumption of vegetables and fruits was associated with increased risk of diabetes and lower intake of milk was associated with increased risk of hypertension. Increased availability of inexpensive staple cereal crops has reduced hunger, but impaired dietary diversity by displacing local ingredients and protective foods.



PUBLIC HEALTH AND COMMUNITY NUTRITION

Assessment of situational analysis of food and nutrient intakes of young children to enable to develop local food models for improving infant and young child feeding in the states of Chhattisgarh, Gujarat, Madhya Pradesh and Uttar Pradesh: Among NPNL women, except proteins energy, iron, vitamin C and thiamin, intake of other nutrients were below EAR, iron was below EAR in Chhattisgarh. The availing of ANC services was observed to be good but availing at least 4 ANCs and registration before the first trimester was low. Also, consumption of at least 100 IFA tablets during pregnancy was not satisfactory. IYCF practices such as initiation of breast feeding within 1 hour of birth, initiation of complementary feeding at 6-7 month of age, number of Complementary Feeds and quantity of Complementary Feeds were all sub-optimum. There is a need to increase awareness among mothers about these practices as well as hygienic practice while feeding the children. Also there is a need to improve knowledge of AWWs about ICDS services and improving coverage for supplementary nutrition for mothers as well as children. The dietary diversity scores of the children were also less than 50% in the states except Gujarat (89%). The prevalence of underweight and stunting were also found to be high.

Taking the agricultural production patterns and based on FGDs that assessed availability, acceptability and accessibility of foods in the region for each state, model food plates were constructed, which will be useful to help improve awareness among community & grass root level workers.

Adaptations to health and nutrition service delivery in COVID-19, India: Phone survey with frontline workers: Modeling studies estimated severe impacts of potential service delivery disruptions due to COVID-19 pandemic on maternal and child nutrition outcomes. Although anecdotal evidence exists on disruptions, little is known about the actual state of service delivery at scale. We partnered with other organization and studied disruptions and restorations, challenges and adaptations in health and nutrition service delivery by frontline workers (FLWs) in 7states, including Telangana during COVID-19 in 2020. We conducted phone surveys with 303 FLWS (AWW: 99, ASHA:102, ANM: 102) in 3 districts (Hyderabad, Janagoan, Medchal) of Telangana state between August–October 2020, asking about service delivery during April 2020 and in the August-October , and analyzed changes in the service delivery between April and August –October 2020.To summarize, services continued during lockdown were counselling, Take Home Ration (THR) distribution and home visits. Services disrupted during lockdown were hot-cooked meal, immunization, Village Health and Nutrition Day (VHND), Antenatal Care (ANC), growth monitoring, distribution of Oral Rehydration Solution (ORS). Most of the services were resumed by September/October. Adaptations to combat the situations were home delivery and usage of phones. Major challenges faced were lack of transport, lack of beneficiary support.

Validation of capillary hemoglobin estimation using autoanalyzer for community level screening of anemia: In this study, we developed a novel Point of Care (POC) technique for haemoglobin (Hb) estimation using a pooled capillary blood sample coupled with a portable autoanalyser and validated it for the first time, by comparing with Hb measurements in a venous blood sample by two reference methods: the same autoanalyser and the direct cyanmethemoglobin method.

- In a cross-sectional study which included participants with a wide range of Hb values, we demonstrated that the Hb measurements by our POC method are in close agreement with venous blood Hb estimated by the reference methods.

- The blood sample related bias measured by comparing Capillary haemoglobin estimated by Autoanalyser (Hb-C-AA) with Venous haemoglobin estimated by Autoanalyser (Hb-V-AA) was trivial (mean difference 0.1 g/dL) with <3 percentage point difference in estimated anaemia prevalence by the two methods.
- The analytical method related bias (Hb-V-AA vs Hb-V-CM) was also negligible (mean difference < 0.1g/dL).

  (Hb-V-CM- Venous haemoglobin estimated by cyanmethemoglobin method)
- Another novel aspect of the present study is the additional validation of our POC method in a longitudinal study by comparing the treatment related changes in Hb-C-AA with the changes in Hb-V-AA and Hb-V-CM measured at similar time points.
- The lack of significant difference between the post-treatment Hb increments estimated by the POC method and simultaneously measured Hb increments by the reference methods offers further confirmation of the validity of our POC method.
- Hence, our study demonstrates that a pooled capillary blood sample measured by an autoanalyser can estimate Hb values without significant bias as compared to the venous blood measurements by reference methods.
- The use of portable POC autoanalyser in population level anemia screening programs can also help in more appropriate treatment. The RBC indices available from the autoanalyzer can assist identification haemolytic or megaloblastic anaemia where IFA supplementation may be contraindicated or of limited value.

Effect of Maternal Iron deficiency anaemia on Iron metabolism in placenta

The aim of the study was to understand the effects of maternal iron deficiency anaemia on major transport proteins of iron in the placenta.

Of the 200 mothers recruited in their third trimester of pregnancy and admitted for their delivery, 59% were found to be anaemic with 60.35% having moderate anaemia. Most of red cell parameters were observed to be higher in cord blood of newborns of anaemic mothers. A significant positive correlation was observed between maternal hemoglobin and ferritin and cord blood hemoglobin and ferritin. All the 3 iron transport proteins (Ferroportin1/FPN1, Divalent metal transporter1 / DMT1 and Zyklopen) showed a statistically significant increased expression at both m-RNA level and protein level, proportionate to the severity of maternal anaemia. Multivariate analysis showed that higher cord blood ferritin, newborn weight and cord blood hemoglobin were associated with higher DMT1, FPN1, and Zyklopen expression.

Our study, for the first time shows increased expression of the placental iron transporters FPN1, DMT1 and Zyklopen at both mRNA and protein in maternal iron deficiency anaemia, thereby facilitating increased transport of iron from the mother to the foetus to replenish foetal iron stores.

Vitamin A deficiency among under-five year children in India: an analysis of national data sets:

Biochemical vitamin A deficiency (VAD) is believed to be a serious public health problem (low serum retinol prevalence >20%) in Indian children, necessitating a nation-wide vitamin A supplementation (VAS) program that delivered high doses of retinol every 6 months to children of under 5 years of age. We evaluated the risk of biochemical VAD in under-five Indian children from the Comprehensive National Nutrition Survey.. The dietary vitamin A inadequacy and excess from national and sub-

national surveys factored in fortification and VAS. The biochemical VAD was found to be much lower than anticipated. Only 3 states had a prevalence of VAD that was significantly greater than 20%. Further there is potential for a considerable overlap of ongoing vitamin A fortification (oil and milk) and VAS programs in India, with the possibility that the cumulative intake of vitamin A along with the high dose of the VAS, might exceed the tolerable upper limits. This raises the need for considering a targeted state-based VAS program, unlike the nation-wide VAS program that is currently the norm.

Carotenoid status in type 2 diabetes patients with and without retinopathy: Diabetic retinopathy (DR) is one of the leading causes of blindness. We evaluated the dietary intake and blood carotenoid levels in type 2 diabetes (T2D) patients with no DR (NDR) and with DR. The plasma levels of carotenoids were significantly lower in DR patients compared to Control and NDR groups. Dietary intakes of zeaxanthin, lycopene, α-carotene and β-carotene were significantly lower in NDR compared to Controls, and a further decrease in the DR compared to NDR group. Plasma carotenoids levels were significantly inversely associated with duration of diabetes and HbA1c, but positively associated with HDL. This study demonstrated a decrease in plasma levels and lower dietary intakes of carotenoids in DR subjects compared to diabetes patients without retinopathy.

FOOD TOXICOLOGY AND NUTRITION

Mycotoxin exposure, intestinal inflammation and childhood stunting in India

An evaluation of the number of samples contaminated with mycotoxin levels showed that out of a total of 27623 samples subjected for analysis of various mycotoxins, prevalence of contamination was about 47.7% (Table-1). Cereals and millets, tree nuts, spices and oilseeds showed prevalence of mycotoxin contamination above 50%. Milk had 43% prevalence in contamination with AFM1. Among the cereals, maize had the highest contamination prevalence and wheat lowest. Among the cereals maize had the highest prevalence of contamination. The prevalence of trichothecene mycotoxins namely DON and T-2 toxin contamination was observed to be highest in maize (29%) followed by rice (24%) and wheat (21%) while that of fumonisin contamination was largely reported in maize and sorghum with a prevalence of 75%. In spices, most of the data reported concerned aflatoxins in red chillies, black pepper, turmeric and ginger with an overall prevalence of 68% in contamination. The number of mycotoxins detected was highest in cereals, millets and spices thus signifying these as high risk foods.

An attempt was made to assess the extent of mycotoxin contamination reported in the database in relation to the maximum permissible limits set by the FSSAI for different mycotoxins in different food categories (Table-3). The percentage of samples exceeding the maximum limits of 10 and 15 µg/kg for aflatoxin B1 and total aflatoxins set by FSSAI in cereals and millets was highest in maize (30.8%), 10% in rice and lowest in sorghum (5.7%). In milk samples, 11.2% of the samples exceeded the FSSAI MLs of 0.5µg/kg for AFM1 in milk. Among the oilseeds, FSSAI maximum limits for AFB1 exceeded in 25% of groundnut samples. In wheat, FSSAI maximum limits of 1000 µg/kg for DON were exceeded in 8.5% of samples and maximum limits of 20 µg/kg for OTA in 1% of wheat samples respectively that were investigated in the studies included in the database. In apples and apple juice 7% of samples exceeded the FSSAI maximum limits of 50 µg/kg for patulin. The above data indicated that considerable number of food commodities of dietary importance particularly for young children such as cereals and milk contained mycotoxins above the food safety limits set by the FSSAI.

The Indian mycotoxin database has considerable potential to be used as a template for planning surveillance and monitoring programmes on mycotoxins by the FSSAI or any regulatory agency concerned with quality and safety of food commodities.

Based on the outcome of dietary mycotoxin exposure modelling using population level estimates of food intakes and prevalence of stunting in Indian children and the systematic review, there is a scope for further investigations in high mycotoxin risk regions and regions with high prevalence of stunting to assess the role of mycotoxin exposure on child stunting in India.

Development of New Bio-Marker for Quantification of Acetylcholinesterase (Ache) Enzyme Activity, Organophosphate, Carbamates, and Nerve Agent in Biological and Ambient Matrices

a) Developed an assay utilizing HPLC and UV detector for determination of AChE activity, conversion of 1-napthol acetate to 1-napthol is measured to estimate AChE activity in blood samples and/or environmental water samples.

b) The performance was judged based on reproducibility, sensitivity, accuracy and to screen enzyme activity within 30 minutes.

c) A series of experiments were performed varying the concentration of blood and substrate with optimal sensitivity using 50-µmole substrate and50-µL blood. The reaction was complete within 30 minutes.

d) Further trials to reduce volume of blood to 25-µL and reaction time to 15 minutes has also shown positive results, validation for the same is in progress.

e) In addition, we adapted the method for use as a general screening tool against organophosphates and carbamate pesticides in ambient samples.

f) This method was validated on blood drawn from OP poisoning patient admitted in Osmania hospital, Hyderabad, Telangana and method was found sensitive, accurate and less time consuming for determination of AChE.

g) 23 patients were identified and blood samples were collected from these patients until they are discharged or deceased.

h) Six patients had expired during the process of treatment and very low levels or decreasing trend of AChE enzyme concentration levels were observed.

i) However, of the 6 expired patients, 2 patients had shown increased concentrations of AChE enzyme levels but respiratory failure was the reason found for their death. Thus, this result has proved the efficacy of our developed method

Impact of *Salmonella* killing lytic bacteriophages on probiotic microbiota: The studies on the impact of *Salmonella* killing lytic bacteriophages on probiotic microbiota (*Lactobacillus bulgaricus, Lactobacillus rhamnosus, Lactobacillus acidophilus, Streptococcus thermophilus*, and *Bifidobacterium breve)* showed no spots and inhibition zone both in spot test assay and agar well diffusion assays. The results of the turbidometric assay showed that even after incubation up to 24h, the growth of probiotic microbiota remained unaffected. The results of this study clearly showed that the administration of lytic bacteriophages will not harm the probiotic microbiota and are likely to be safe for use in food preservation.

BASIC STUDIES

Impact of chronic hyperglycemia on small heat shock proteins in diabetic rat brain: Small heat shock proteins (sHsps) are a family of proteins involved in fundamental cellular processes, including protein folding, apoptosis, and maintenance of cytoskeletal integrity. Hyperglycemia created during diabetes leads to neuronal derangements in the brain. We investigated the impact of chronic hyperglycemia on the expression of sHsps and their role in neuronal apoptosis in a diabetic rat model. Hyperglycemia decreased Hsp27, and increased HspB5 and Hsp22 at transcript and protein levels. Diabetes induced the aggregation of HspB5, Hsp22, α-synuclein, and pTau and impaired the interaction of HspB5 with α-synuclein and pTau. Moreover, diabetes reduced the interaction of HspB5 with Bax. Together, these results indicate that chronic hyperglycemia induces differential responses of sHsps by altering their expression, solubility, interaction, and apoptosis.

Molecular mechanism(s) involved in vitamin D deficiency induced muscle atrophy: Vitamin D deficiency (VDD) is known to lead to muscle wasting in both humans and rodents. Hence, we studied the effects of VDD in the muscle using a VDD rat model. VDD or insufficiency led to a decrease in expression of myosin and actin-associated proteins which are essential for muscle contraction. There was a decrease in expression of glycolytic and oxidative enzyme genes indicating a low oxidative capacity of skeletal muscle in the VDD state. Furthermore, expression of muscle specific micro RNAs involved in myogenesis, were reduced in the VDD muscle. These results demonstrates that chronic VDD or insufficiency reduced the size of skeletal muscle fibres, altered their composition, and decreased their oxidative potential. Most of the changes observed were reversible, either partially or completely, by restoring vitamin D to the diet of the deficient rats.

Vitamin D deficiency induced neurodegeneration: Role of protein homeostasis pathways: A growing body of evidence from epidemiology and neuroscience links vitamin D deficiency (VDD) with a range of neurodegenerative disorders. In this pilot study we examined the effect of VDD on protein homeostasis pathways [ubiquitin proteasome & ER stress] in the brain of rats. The data indicates that the ubiquitin proteasome system appears to be down-regulated while the ER stress pathway seems to be up-regulated in the VDD brain compared to control brain.

Phytochemical, Pharmacognostic And Nutritional characterisation of the *Panax* species (Araliaceae) from the Eastern Himalayan region, India for addressing medicinal, trade and regional livelihood security issues: The study was intended to analyse the nutritional potential and acute toxicity assessment of *Panax bipinnatifidus* and *P. pseudoginseng* (which belong to root and tubers food group available in the Eastern Himalayan region of India. The proximate composition indicated higher content of protein, dietary fibre and carbohydrate. Dominant content of arginine and glutamic acid were found with cysteine and methionine as limiting amino acids in both the *Panax* samples. High level of palmitic, oleic and linoleic acid was observed with higher values poly-unsaturates in *P. pseudoginseng*. Sucrose, fructose and raffinose were detected in both the samples. Analysis of minerals and trace elements reflects high content of phosphorus, potassium, magnesium and selenium in *Panax* samples. Acute toxicity study and histopathological analysis confirm the safety profile for the consumption of both the Panax samples. The present findings thus highlight the nutritional potential and safety for the consumption of the east Himalayan *Panax* species for therapeutic uses.

I. PUBLIC HEALTH NUTRITION

1. ASSESSMENT OF SITUATIONAL ANALYSIS OF FOOD AND NUTRIENT INTAKES OF YOUNG CHILDREN TO ENABLE TO DEVELOP LOCAL FOOD MODELS FOR IMPROVING INFANT AND YOUNG FEEDING IN THE STATES OF CHHATTISGARH, GUJARAT, MADHYA PRADESH AND UTTAR PRADESH IN INDIA

Despite several nutrition intervention programmes being in operation for several decades, undernutrition among vulnerable groups, still continues to be an important public health problem in India. National Nutrition Monitoring Bureau (NNMB) surveys revealed that inadequate dietary intakes especially in young children was one of the major causes for persistent undernutrition in the state. Therefore, focusing on a package of interventions is crucial during the first 1000 days of life i.e., from conception to the first two years of life.

Inadequate knowledge among mothers of young children on Infant and Young Child Feeding (IYCF) practices - quantity, quality and frequency of foods to be given to the growing children and feeding during illnesses; coupled with poor counseling skills of the Frontline Workers (ANMs, AWWs and ASHAs) on 'nutrition specific practices' could be the reasons for the persistent problems of undernutrition.

The nutrition intervention programs are primarily providing Take Home Ration (THR) for 6-35 months children and hot cooked supplementary foods for 3-6 years children, but none of these programs includes region or state specific balanced diets acceptable to the majority of the population in the respective geographical areas. Therefore, it is proposed to develop an evidence-based region or state specific food model as India eats diverse foods based on socio-economic status and culture.

OBJECTIVES

1. To assess state-wise nutritional intake at the household level based on intake of calorie, protein and consumption of important micronutrient rich food groups.
2. Document the seasonal variation in local food production, availability in market and food price.
3. Describe the types of foods prepared as well as the quantity, quality and frequency of food consumption at the household level
4. Illustrate locally available foods at household level and their sources, choices and consumption pattern, and

5. Explain the current prevalent practices of food behaviour (enablers and barriers) with respect to IYCF practices of mothers of young children, especially in terms of food consistency, quantity, frequency and diversity.

METHODS

A Community based cross sectional study was carried out in the selected states during Jan 2020 to March 2020 and from Jan 2021 to March 2021. For this survey, four regions were selected randomly and from each region, 1 district was selected. From each district, 4 blocks were selected from the list and from each block, 2 villages were selected using systematic sampling procedure in order to get equal chances of selection of village from the block. Thus 8 villages were covered from each district/ region and 32 from the state (4 regions of states).

The sampling of households (HHs) within the clusters was done by dividing the clusters into natural segments of approximately 150-200 households each. One segment from each of these segments was selected randomly. The selected segment was house-listed and households with a mother in the age group of 15-49 years of age with a child under two years of age (0 to 23 months) were numbered. From this list, 20 HHs were selected by systematic random sampling from each cluster/village. In the selected households, the heads of the households were interviewed to understand the agricultural practice, production, and household income, affordability of different food items and seasonality of production among other things.

Data on HH socioeconomic variable, food frequency, 24 hr recall diet survey, anthropometric measurements and interview with AWWs, ASHA, ANM, village *Sarpanch*, agriculture officers, fair price shop dealer and PRI members were carried out in each village.

In addition to quantitative data collection, qualitative data was collected through focus group discussion (FGDs) among mothers of <2 year children, and farmers in the villages. Also in depth interview was conducted with village *sarpanch*, shop keeper, fair price shop owners, and agriculture officers in the district.

RESULTS

- A total of 639 HHs and 664 children in Uttar Pradesh, and 650 HHs and 645 children in Chhattisgarh were covered for anthropometry and dietary analysis.
- In Gujarat and Madhya Pradesh, secondary data of NIN survey carried out in 2012 and 2015 were used for anthropometry and NNMB (2012) data was used for dietary data analysis.
- A majority of HHs in Chhattisgarh and Madhya Pradesh belonged to Scheduled tribe community (45% & 37% respectively), while 40% in Gujarat belonged to forward community and 49% in Uttar Pradesh belonged to backward caste.
- About 80-88% fathers of index children were literate, while 72% mothers in Madhya Pradesh and 90% in Chhattisgarh were literate.
- Per capita monthly income was lowest in Gujarat (Rs. 1249; secondary NIN data 2012) and was highest in Chhattisgarh (Rs. 2191).
- Proportion of pucca houses was more in Uttar Pradesh (62%) and low in Chhattisgarh (26.5%).
- Sanitary latrine was present in 68-72% of HHs in Chhattisgarh & Uttar Pradesh and 45% in Gujarat (secondary data 2012)
- Nutrient intakes were observed below the recommended levels in all the states among

1-2 year children.

- Dietary diversity score of 4 or more food groups was 45% in Uttar Pradesh, 48% in Chhattisgarh, 47% in Madhya Pradesh and 89% in Gujarat.
- Among pregnant women, nutrient intakes of energy, proteins, calcium, iron, Vitamin A, Riboflavin and folate were below recommended levels.
- Majority of mothers had availed ANC services during pregnancy; however only 15% had availed at least four ANCs in Madhya Pradesh and 69% in Chhattisgarh.
- Registration of pregnancy in the first trimester was 845 in Chhattisgarh and 52-54% in Gujarat and Uttar Pradesh.
- Regular growth monitoring was reported by 89% in Gujarat and only 5% in Uttar Pradesh.
- Full immunization was more in Gujarat and Madhya Pradesh (100%) and 72% in Chhattisgarh.
- The prevalence of underweight was 30-32% in Chhattisgarh, Gujarat and Madhya Pradesh while it was 25% in Uttar Pradesh, the prevalence of stunting was 29-33% in Chhattisgarh, Gujarat and Madhya Pradesh and 41% in Uttar Pradesh, while wasting was 11% in Uttar Pradesh and 24-25% in Chhattisgarh and Madhya Pradesh.
- The prevalence of undernutrition (BMI>18.5) among women was 26-28% in Uttar Pradesh & Madhya Pradesh and 36-45% in Chhattisgarh and Gujarat.

Fig 1. State wise distribution (%) of mothers according to ANC services

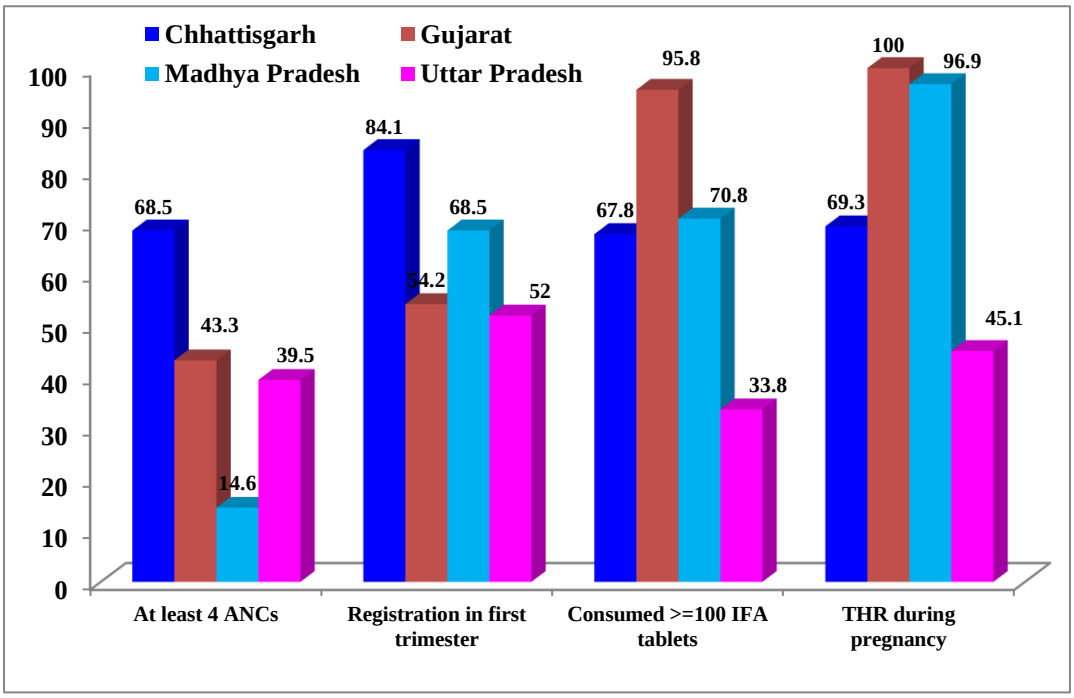


Fig 2. State wise distribution (%) of mothers according to delivery practices

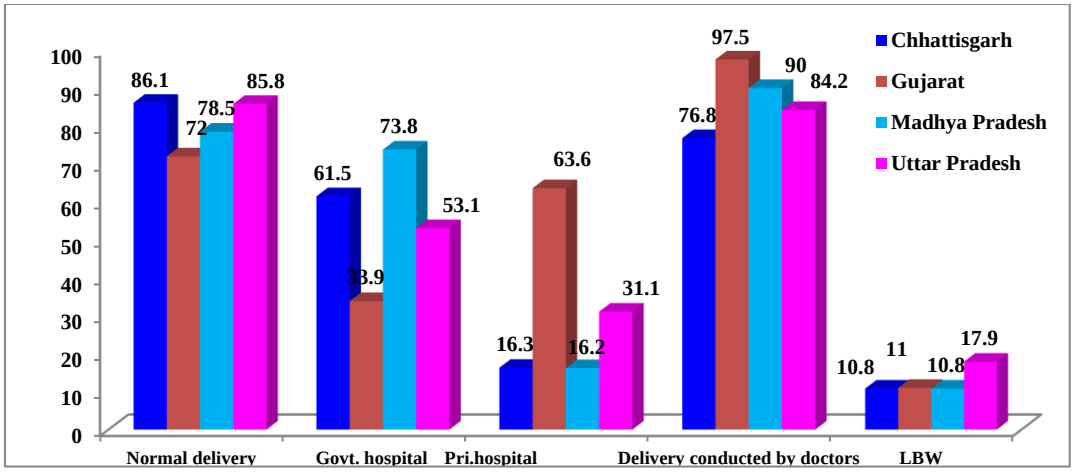


Fig 3. State wise distribution (%) of 0-11 month children according to infant feeding practices

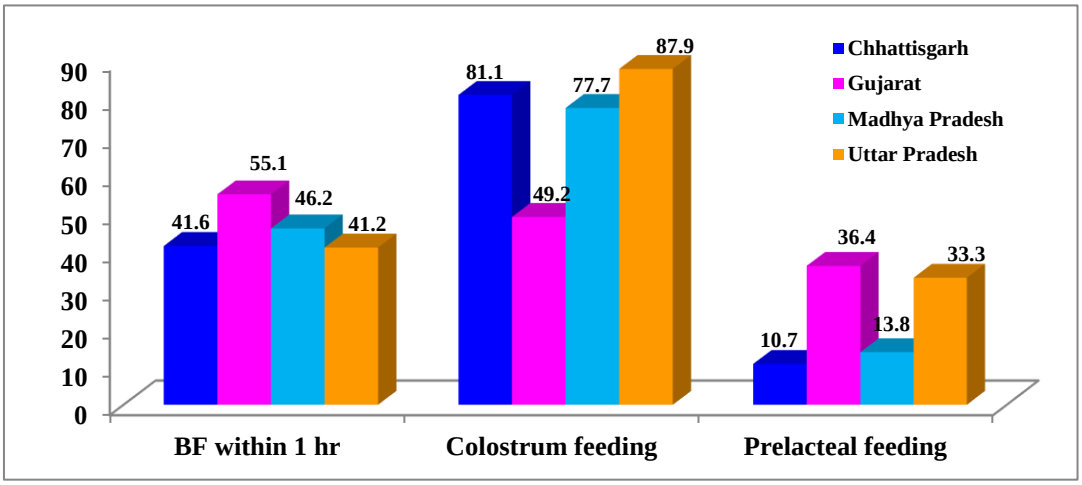
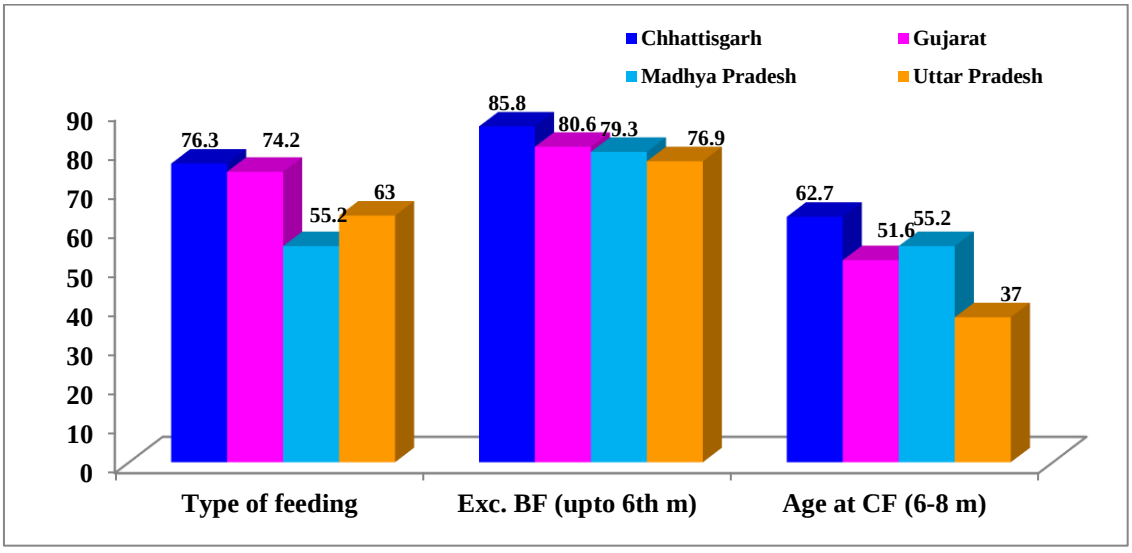


Fig 4. State wise distribution (%) of 0-11 month children according to feeding practices



CONCLUSION

In conclusion, food and nutrient intakes were less than recommended levels among 1-2 year children, while intakes of some important micronutrients such as vitamin A, Calcium and iron were below 50% of RDA. The availing of ANC services was observed to be good but availing at least 4 ANCs and registration before the first trimester was low. Also, consumption of at least 100 IFA tablets during pregnancy was not satisfactory. IYCF practices such as initiation of breast feeding within 1 hour of birth, initiation of complementary feeding at 6-7 month of age, number of Complementary Feeds and quantity of Complementary Feeds was sub-optimum. There is a need to increase awareness among mothers about these practices as well as hygienic practice during feeding the children. Also there is a need to improve knowledge of AWWs about ICDS services and improving coverage for supplementary nutrition for mothers as well as children. The dietary diversity scores of the children were also less than 50% in the states except Gujarat (89%). The prevalence of underweight and stunting were also found to be high.

RECOMMENDATIONS

The agricultural production patterns and FGDs were assessed to look into the availability, acceptability and accessibility of foods in the region and for each state, model food plates were constructed taking them into account.

II. MATERNAL AND CHILD NUTRITION

1. ADAPTATIONS TO HEALTH AND NUTRITION SERVICE DELIVERY IN COVID-19, INDIA: PHONE SURVEY WITH FRONTLINE WORKERS

India's lockdown approach to contain COVID 19 pandemic has impacted the lives in multiple ways. The AWWs, ANMs, ASHAs are the primary contact points at the village-level for all health and nutrition related services in the first 1000 days. These services include antenatal care, food supplementation, micronutrient supplementation, health and nutrition education, growth monitoring, immunization, care for sick and malnourished children. The AWWs deliver these services through a network of Anganwadi centers at the village-level. The policy response to curtail the spread of COVID-19 led to lockdown measures, which resulted in the closing down of the Anganwadi centers. This led to changes in the delivery modalities of core health and nutrition services. There is anecdotal evidence of ground level positive adaptations to the policy changes in few states. There is, however, no systematic evidence on the local adaptations to health and nutrition service delivery during the pandemic. Even less is known about the uptake of these services. Therefore, a learning agenda is needed to systematically document on the ground adaptation, challenges faced by the frontline workers (FLWs) as well as beneficiary utilization of services. Based on the need for systematic documentation, a multi-state phone survey with FLWs across nine states in India (Uttar Pradesh, Bihar, Chhattisgarh, Madhya Pradesh, Gujarat, Jharkhand, Odisha, Tamil Nadu and Telangana) in collaboration with partners was conducted. The goal of this initiative was to use common survey instruments across multiple states to generate findings that are comparable across states.

OBJECTIVES

a) Identify frontline or local management adaptations to health and nutrition service delivery during COVID19, with a focus on positive adaptations.

b) Analyze adaptations to identify a range of feasible solutions that have the potential to strengthen delivery and uptake of essential health and nutrition interventions in the context ofCOVID-19 and beyond.

METHODOLOGY

STUDY DESIGN: Descriptive cross-sectional study.

***Total Number of samples*:** 303 (AWW: 99, ASHA: 102, ANM: 102)

SELECTION OF SUBJECTS

Three districts chosen (Hyderabad, Janagoan & Medchal) included 36 PHCs and 296 sub-centers. The number of sub-centers varied between 3 to 26 under each PHC. All PHCs was selected and a probability proportionate to size sampling was employed to select the number of sub-centers under each PHC. One ASHA from each sub-center was selected and

the corresponding ANM, AWW was selected. In collaboration with the government, contact information of the FLWs was obtained. The FLWs were reached over phone and if they consent, then they were interviewed. In case if the FLW is not available, then the subsequent FLW was contacted.

Method of Survey/ Survey technique: Telephonic survey (hence the use of mobile phones).

DATA COLLECTION

The study was conducted between June and December 2020. Data was collected using pretested survey instruments. The survey instrument was tailored to conduct the survey over phone. All the instruments were translated from English to Telugu. Topics included in each of the data collection tools are presented in Table 1. Each survey was capped between 20-30 minutes.

Table 1. Frontline worker and supervisor survey modules

Module	Domains of enquiry
Adaptations to service delivery	Provision of THR, growth monitoring, health and nutrition education, facilitation of VHND, community-based events, ANC, immunization; adaptations to the delivery of these services.
Communication channels	Ways of communicating with supervisors and beneficiaries in the context of COVID. Beneficiary behaviour toward frontline worker.
COVID responsibilities, incentive and training	New responsibilities and perceptions related to COVID-19 Training and incentives for COVID related duties, FLWs worker access to PPE.
COVID knowledge and its influence	Knowledge of COVID 19 symptoms and prevention, knowledge channels Impact of COVID on FLWs and their household: employment, income, food supply, travel, health, etc.

Statistical Analysis: All the categorical variables were summarized as frequencies and percentages with 95% Confidence Interval and the continuous variables were summarized as mean (SD) or Median (IQR) depending on the normality of the data.

Informed Consent: The enumerators read out the consent form in the local language, and only when the respondent agrees to participate, through verbal consent, the survey proceeded.

Potential risks and benefits: There were no risks involved in participating in this study as it is a non-invasive telephonic survey. If the respondent at any time indicates discomfort responding to the questions, the respondent will immediately be excused from the survey.

RESULTS

Table 1. Number of respondents sampled, interviewed, and response rate

	Sampled	Interviewed	Response Rate
Type of respondent			
ASHA	184	102	55.4
ANM	122	102	83.6
AWW	106	99	93.4

Table 2.1. Services delivered during the lockdown and in the previous month – AWW

Services	April 2020 (% AWW)		September 2020 (% AWW)		Change between April & Sep 2020 (pp)	p-value
Overall	**N**	**%**	**N**	**%**		
Opened AWC (include option 'some days' as well)	93	93.9	97	98	4.1	0.279
Conducted VHND	47	47.5	89	89.9	42.4	0.00
Made home visits	77	77.8	98	99	21.2	0.00
Counselling on health and nutrition	77	77.8	99	100	22.2	0.00
Preconception						
IFA supplementation for adolescent (for IFA questions, exclude 'no such beneficiary' from the denominator, no one reported in our case)	15	15.1	28	28.9	13.8	0.039
Pregnant and lactating mothers						
ANC for all women	55	55.6	65	65.7	10.1	<0.001
ANC for 3rd trimester or high-risk pregnancy	22	22.2	25	25.3	3.1	
No ANC Visit	21	21.2	9	9.1	12.1	
Immunization services (include all beneficiaries + only PW options)	70	70.7	88	88.9	18.2	0.003
IFA supplementation for pregnant women	49	49.5	56	56.6	7.1	0.393
IFA supplementation for lactating women	44	44.4	51	51.5	7.1	0.393
Helped in institutional delivery*	75	75.8	NA	NA	NA	NA
Children's health and nutrition						
Growth monitoring	19	19.2	90	90.9	71.7	0.000
Referred malnourished cases (exclude 'no identified malnourished children in the catchment area' from denominator) N=55 & 51 respectively	18	32.7	32	62.7	30	0.030
Immunization services (include all beneficiaries + only children options)	60	60.6	74	74.7	14.1	0.048
IFA supplementation for children	19	19.2	29	29.3	10.1	0.136
ORS/ORS and Zinc to diarrhea	32	32.3	40	40.4	8.1	0.301
Social protection						
Provided THR	99	100	97	98	-2	0.477

Provided hot cooked meal to children	0	0	2	2	2	
Provided hot cooked meal to pregnant women	0	0	3	3	3	
Provided hot cooked meal to lactating women	0	0	3	3	3	
Village level services for school children						
Hot cooked meal	NA	NA	27	27.3	NA	NA
Dry ration	2	2	68	68.7	66.7	0.00
Cash	NA	NA	98	99	NA	NA

Table 2.2. Services delivered during the lockdown and in the previous month – ASHA

Services	**April 2020 (% ASHA)**		**Sep 2020 (% ASHA)**		**Change between April and Sep 2020 (pp)**	**p-value**
Overall	**N**	**%**	**N**	**%**		
Opened AWC (include option 'some days' as well)	NA	NA	NA	NA	NA	NA
Conducted VHND	58	56.9	96	94.1	37.2	000
Made home visits	87	85.3	100	98	12.7	0.00
Counselling on health and nutrition	91	89.2	102	100	10.8	0.002
Preconception						
IFA supplementation for adolescent (for IFA questions, exclude 'no such beneficiary' from the denominator)	55	53.9	71	69.6	15.7	0.031
Pregnant and lactating mothers						
ANC for all women	46	45.1	62	60.8	15.7	<0.001
ANC for 3rd trimester or high-risk pregnancy	39	38.2	36	35.3	-2.9	
No ANC Visit	17	16.7	4	3.9	12.8	
Immunization services (include all beneficiaries + only PW options)	88	86.3	101	99	12.7	0.001
IFA supplementation for pregnant women	88	86.4	101	99	12.6	0.001
IFA supplementation for lactating women	77	75.5	92	90.2	14.7	0.001
Helped in institutional delivery	98	96.1	NA	NA	NA	NA
Children's health and nutrition						
Growth monitoring	NA	NA	NA	NA	NA	NA
Referred malnourished cases (exclude 'no identified malnourished children in the catchment area' from	NA	NA	NA	NA	NA	NA
Immunization services (include all beneficiaries + only children options)	78	76.5	92	92.2	15.7	0.015
IFA supplementation for children	56	54.9	77	75.5	20.6	0.003

ORS/ORS and Zinc to diarrhea	65	63.7	92	90.2	26.5	0.000
Social protection						
Provided THR	NA	NA	NA	NA	NA	NA
Provided hot cooked meal	NA	NA	NA	NA	NA	NA
Village level services for school children						
Hot cooked meal	NA	NA	NA	NA	NA	NA
Dry ration	NA	NA	NA	NA	NA	NA
Cash	NA	NA	NA	NA	NA	NA

Table 2.3. Services delivered during the lockdown and in the previous month – ANM

Services	April 2020 (% ANM)		Sep 2020 (% ANM)		Change between April & Sep 2020 (pp)	p-value
Overall						
Opened AWC (include option 'some days' as well)	NA	NA	NA	NA	NA	NA
Conducted VHND	48	47.1	92	90.2	43.1	0.000
Made home visits	88	86.3	98	96.1	9.8	0.026
Counselling on health and nutrition	89	87.3	100	98	10.7	0.007
Preconception						
IFA supplementation for adolescent (for IFA questions, exclude 'no such beneficiary' from the denominator)	69	67.6	77	75.5	7.9	0.277
Pregnant and lactating mothers						
ANC for all women	49	48	61	59.8	11.8	<0.001
ANC for 3rd trimester or high-risk pregnancy	40	39.2	39	38.2	-1	
No ANC Visit	13	12.7	2	2.0	10.7	
Immunization services (include all beneficiaries + only PW options)	88	86.2	100	98	11.8	0.004
IFA supplementation for pregnant women	92	90.2	99	97.1	6.9	0.085
IFA supplementation for lactating women	95	93.1	96	94.1	1	1.00
Helped in institutional delivery	101	99	NA	NA	NA	NA
Children's health and nutrition						
Growth monitoring	NA	NA	NA	NA	NA	NA
Referred malnourished cases (exclude 'no identified malnourished children in the catchment area' from denominator)	NA	NA	NA	NA	NA	NA
Immunization services (include all beneficiaries + only children options)	76	74.5	92	92.2	17.7	0.006
IFA supplementation for children	67	65.7	80	78.4	12.7	0.061

ORS/ORS and Zinc to diarrhea	78	76.5	93	91.2	14.7	0.008
Social protection						
Provided THR	NA	NA	NA	NA	NA	NA
Provided hot cooked meal	NA	NA	NA	NA	NA	NA
Village level services for school children						
Hot cooked meal	NA	NA	NA	NA	NA	NA
Dry ration	NA	NA	NA	NA	NA	NA
Cash	NA	NA	NA	NA	NA	NA

Table 3. Adaptations made to provide services to beneficiaries during the lockdown

Adaptations to service delivery	AWW		ASHA		ANM	
	% (N = 99)		% (N = 102)		% (N = 102)	
In conducting VHND	**n**	**%**	**n**	**%**	**n**	**%**
Conducted VHND/CBE as usual	11	11.1	22	21.6	14	13.7
VHND/CBE was conducted for some beneficiaries only	29	29.3	34	33.3	22	21.6
Organized VHND/CBE for different groups of beneficiaries at different times of the day	14	14.1	13	12.7	11	10.8
VHND/CBE session was conducted over multiple days to cover all beneficiaries	5	5.1	12	11.8	10	9.8
All beneficiaries were given masks	23	23.2	31	30.4	21	20.6
Beneficiaries were asked to maintain distance	33	33.3	37	36.3	32	31.4
Marked areas for seating	27	27.3	28	27.5	18	17.6
AWW/ASHA/ANM wore masks	29	29.3	28	27.5	26	25.5
Kept sanitizer/soap and water ready	22	22.2	29	28.4	26	25.5
Disinfected the VHND premises	1	1	7	6.9	4	3.9
Counselling						
During home visits	68	68.7	79	77.5	73	71.6
During ANC/PNC visit	13	13.1	33	32.4	51	50
At a community event/VHND	17	17.2	23	22.5	21	20.6
Using a phone	11	11.1	16	15.7	22	21.6
make an Audio call	11	100	15	93.7	22	100
Use of phone for counselling women						
send a SMS message	1	9	3	18.7	0	0
send Audio messages on WhatsApp	2	18.1	1	6.2	2	9.1
Make a Video call	1	9	0	0	0	0
Send Video messages on WhatsApp groups	3	27.3	1	6.2	1	4.5
ANC						
No one asked for help	2	2	0	0	0	0
Made an appointment for ANC at the health center	33	33.3	57	55.9	73	71.6
Arranged for transport to visit the health facility	11	11.1	61	59.8	45	44.1

Adaptations to service delivery	AWW		ASHA		ANM	
	% (N = 99)		% (N = 102)		% (N = 102)	
Coordinated with ASHA/ANM to arrange for the visit at the health center	49	49.5	57	55.9	57	55.9
Coordinated with supervisor to arrange for the visit to the health facility	1	1	13	12.7	11	10.8
Reminded through WhatsApp message/ phone call	43	43.4	32	31.4	57	55.9
Visited beneficiaries' home to call for ANC	23	22.2	42	41.2	38	37.3
IFA						
During VHNDs	9	9.1	25	24.5	24	23.5
I/AWH delivered IFA to beneficiary homes	30	30.3	60	58.8	61	59.8
Beneficiaries were asked to collect it from AWC	9	9.1	19	18.6	30	29.4
Beneficiaries were asked to collect it from health facilities	17	17.2	23	22.5	41	40.2
Other FLW/ community volunteer delivered it to beneficiary homes	21	21.2	7	6.9	32	31.4
Delivery						
No deliveries	8	8.1	1	1	1	1
Did not know about the deliveries until delivery	0	0	3	2.9	2	2
I was not able to help	15	15.2	4	3.9	4	3.9
Accompanied to the hospital	22	22.2	87	85.3	53	52.0
Arranged for transport to the hospital	47	47.5	86	84.3	90	88.2
Coordinated with AWW, ASHA or ANM to accompany them to the hospital	57	57.6	54	52.9	85	83.3
Visited home in case of home delivery	3	3.0	1	1.0	0	0
Facilitated child delivery in case of home delivery	1	1.0	1	1.0	0	0
Immunization						
Could not help	2	2	0	0	0	0
No one asked for help	0	0	1	1	0	0
Reminded through WhatsApp message/ phone call	48	48.5	39	38.2	66	64.7
Visited beneficiaries' home to call for immunization	42	42.4	68	66.7	38	37.3
Made an appointment for immunization	35	35.4	47	46.1	70	68.6
Arranged for transport to visit the immunization venue	13	13.1	33	32.4	23	22.5
Coordinated with AWW/ASHA/ANM to arrange for the visit at the immunization venue	47	47.5	49	48	62	60.8
Coordinated with supervisor to arrange for the visit at the immunization venue	1	1	17	16.7	15	14.7
ORS and Zinc ORS diarrhoea						
Provided ORS/ORS and zinc during VHNDs	3	3	27	26.5	19	18.6
I/AWH delivered ORS/ORS and zinc to beneficiary homes	21	21.2	46	45.1	49	48

Adaptations to service delivery	AWW		ASHA		ANM	
	% (N = 99)		% (N = 102)		% (N = 102)	
Beneficiaries collected ORS/ORS and zinc from AWC	15	15.2	10	9.8	22	21.6
Beneficiaries collected ORS/ORS and zinc from my house	5	5.1	21	20.6	12	11.8
Beneficiaries collected ORS/ORS and zinc from health facilities	11	11.1	10	9.8	25	24.5
Other FLW or community volunteer delivered ORS/ORS and zinc to beneficiary homes	5	5.1	4	3.9	23	22.5
I told beneficiaries how to prepare ORS at home	9	9.1	24	23.5	30	29.4
THR						
As usual at the AWC on designated days	77	77.8	NA	NA	NA	NA
I/AWH delivered THR to beneficiary homes	65	65.7	NA	NA	NA	NA
Provided cash transfer instead of THR	0	0	NA	NA	NA	NA
Provided dry ration (e.g., rice, dal, wheat, etc.) instead of THR	2	2	NA	NA	NA	NA
Hot cook meal						
Nothing	3	3	NA	NA	NA	NA
Provided THR in lieu of HCM	27	27.3	NA	NA	NA	NA
Dry ration (e.g., rice, dal, wheat etc.) instead of HCM	68	68.7	NA	NA	NA	NA
Provided money in lieu of HCM	0	0	NA	NA	NA	NA
Other locally prepared nutritious supplements	0	0	NA	NA	NA	NA

INFERENCE AND CONCLUSION

The response rate is quite low (55.4%) in comparison with ANM (83.6%) and AWW (93.4%). The mean (SD) age of the responded FLWs was 40 (7) years, experience was 14 (7) years. Majority (82.2%) of them had access to smart phone of which 14.9% received from the Government. All the AWW who received smart phones from the Government had ICDS-CAS in their phones.

Less than half of AWWs (47%) were able to conduct the VHNDs and only about one-fifth of them were able to conduct ANC visits (22%), Growth monitoring and IFA supplementation (19%). None of them was able to provide hot cooked meals and only 2% of them were dry ration. However, 99% of them provided THR. About 70% of them reported other services like immunization, house visits and counselling were happening even during the lockdown. However, in September 2020, most of the services almost resumed except IFA supplementation and hot cooked meal.

ASHAs as well as ANMs reported that Conducting VHNDs and providing ANC care and IFA supplementation was the most affected service during the lockdown. A higher proportion of the participants reported they asked people to follow social distancing and wore masks while conducting VHND as an adaptive measure. Though, higher proportion of the participants visited the beneficiaries for counselling, the usage of mobile phones was found to be higher during the lockdown time. For ANC visits and immunization, a higher proportion of them reminded the pregnant women through WhatsApp/ phone call. Majority of them supplied

IFA and ORS packets to the homes of beneficiaries. THR was supplied either at AWCs or at beneficiary's home. As the AWCs were not functional, hot cooked meals were not provided at all and hence dry rations were provided to about 68% of the participants.

Personal protective equipment were the most common resource asked by most of the participants followed by incentives, training and travel support. Contact with beneficiaries' and the superiors was mainly through phone during lockdown. Majority of the participants had good knowledge regarding the preventive measures needed to be taken during COVID and they heard about it mainly from WCD/ health and family welfare. Fear of family members getting sick, burden of additional work and travelling long distance without proper transport facilities were the aspects that affected frontline worker's during lockdown.

To summarize, services continued during lockdown were counselling, THR distribution and home visits. Services disrupted during lockdown were hot-cooked meal, immunization, VHND, ANC, growth monitoring, distribution of ORS. Most of the services were resumed by September. Adaptations to combat the situations were home delivery and usage of phone. Major challenges faced were lack of transport, lack of beneficiary support.

2. VALIDATION OF CAPILLARY HEMOGLOBIN ESTIMATION USING AUTOANALYZER FOR COMMUNITY LEVEL SCREENING OF ANEMIA (SUB STUDY DONE UNDER AN ON-GOING ICMR TASK FORCE PROJECT: IMPACT EVALUATION OF "SCREEN AND TREAT" APPROACH FOR ANAEMIA REDUCTION: A CLUSTER RANDOMISED TRIAL IN RURAL TELANGANA

In addition to iron-folate prophylactic supplementation, the Intensified National Iron Plus Initiative (I-NIPI) program advocates opportunistic facility-based screening of persons referred by frontline health workers and treatment with appropriate doses of iron folate based on grade of anemia. However, only a small proportion of anaemic individuals can access facility-based care and receive adequate treatment for anaemia.

In a recent pilot study, we tested the feasibility of collecting 150-250 µL of capillary blood in a microtainer (0.5 mL K2EDTA conical tube) followed by Hb measurement in a haematology analyser that was made portable in a vehicle. This sample collection method/coupled with auto analyser based Hb measurements were found to be comparable to that of venous blood Hb, measured by auto-analyser. Nevertheless, this method requires validation in the context of anemia prevalence assessment, particularly different grades of anemia. Therefore, this validation study was carried out as a sub-study under the "Screen and Treat" Anaemia project to inform the sample collection method to be used for community level screening of anaemia.

AIMS AND OBJECTIVES

To compare the haemoglobin (Hb) concentration of capillary blood measured by auto analyzer with that of venous blood Hb levels measured by auto analyser and direct cyanmethamoglobin method; and to document the variability in prevalence estimates of anemia and response to iron/folate intervention.

METHODOLOGY

In a cross-sectional study in 748 participants (aged 17 - 86 yr, 708 women) from Hyderabad, India, were compared capillary blood Hb and anaemia prevalence measured by a POC (point of care) autoanalyzer (Horiba ABX Micros 60OT) (CbAHb) with venous blood Hb and anaemia prevalence measured by two reference methods: autoanalyzer (VbAHb) and direct cyanmethemoglobin method (VbCynHb). Additionally, a longitudinal study was conducted in a sub-sample of participants (n=426, age 17-21y) to measure discrepancies in Hb increments by the three methods in response to iron folate (IFA) treatment.

RESULTS

In the cross-sectional study, Hb values ranged from 5.1 to 18.2 g/dL and the mean difference between CbAHb and VbAHb or VbCynHb were lower than the coefficients of variation of their estimates, and thus trivial. Bland Altman analyses showed that CbAHb was higher than VbAHb (mean difference, limits of agreement: 0.123, -0.772 - 1.019 g/dL) and VbCynHb (0.116, -1.636 -1.867 g/dL). However, the difference positively correlated with the Hb values. Anaemia prevalence estimated from VbCynHb, CbAHb and VbAHb (72.1%, 65.1% and 67.8%, respectively) did not differ significantly. In the longitudinal study, the Hb increment (mean± SD) in CbAHb in response to IFA intervention (1.57 ± 1.74 g/dL) was slightly, but not significantly, lower than increments in VbAHb (1.70 ± 1.70) and VbCynHb (1.66 ± 1.74 g/dL).

INFERENCE AND CONCLUSION

Our study demonstrates that a pooled capillary blood sample measured by an autoanalyzer can provide Hb values that are very close to venous blood values. The use of portable autoanalyzer in population level anemia screening programs can also help in more precise treatment based on diagnostic RBC indices, such as the exclusion of haemolytic or megaloblasticanemia where IFA supplementation would be contraindicated or of limited value.

III. CLINICAL EPIDEMIOLOGY

1. EPIDEMIOLOGICAL INVESTIGATION OF MYSTERIOUS ILLNESS IN ELURU, ANDHRA PRADESH

A sudden outbreak was reported with neurological symptoms like seizures, loss of consciousness and mental confusion etc. among few clusters from Eluru town since 4th December, 2020. The action plan was made under the supervision of Director, ICMR-NIN with information to Secretary, DHR & Director General, ICMR. A Team constituted of Clinical Epidemiologists, Anthropologist, Nutritionist, Food Chemistry, Toxicologist and Microbiologist visited the site during 7th - 8th December, 2020.

OBJECTIVES

1. To describe the distribution of the outbreak in terms of time, place and person.
2. To identify the contaminant if any, and its source which is causing the outbreak.
3. To propose the measures to control the outbreak and prevent further occurrences.

METHODS

From the epidemic curve with a sudden onset, steep rise, peaking and subsequent fall in number of cases observed by 8th December, it was concluded that the outbreak could likely be a common source single exposure outbreak. In the absence of fever, the possibility of infectious origin was unlikely, and toxicity of unknown origin was the primary suspicion. From the clinical picture, the following possible differential diagnoses were made: 1. Acute Heavy Metal poisoning, 2. Acute pesticide poisoning (Organophosphate / organochlorine compounds), 3. Mycotoxin exposure.

For the observed distribution of cases within a short duration of time, to identify the source of contamination, the samples of drinking water at various levels of distribution and also milk, rice/dal samples, vegetables from community and market, were collected for analyses.

RESULTS

Initial Clinical Examination was undertaken in the District Government Hospital, Eluru. Biological samples, water samples and other food Samples collected from active cases, recovered cases and controls. Clinical findings were Seizures and loss of consciousness, mental confusion, and pinpoint pupil. The blood and urine samples were tested for pesticides, out of which, Triazophos (organophosphorus) was present in blood and urine samples. Vegetables like tomatoes and brinjals were tested for pesticides including herbicides, out of which metribuzin (herbicide) was present. Heavy metals were tested; all are nearly within permissible limits which needs further evaluation. Water samples from households were having pesticides (Triazophos) above permissible limit. No mycotoxins were observed.

Table 1. Percentage of pesticide identified in blood, urine and household water samples of cases and controls

S. No.	Type of Sample	Cases			Controls		
		Number of samples analysed	Samples above permissible limits	Mean concen-tration	Number of samples analysed	Samples above permissible limits	Mean concen-tration
1	Blood	90	67 (74 %)	10.48ng/ml	11	1(9 %)	2.82 ng/ml
2	Urine	51	50 (98%)	0.92 ug/L	Nil	Nil	Nil
3	Water	13	12 (92 %)	1.00 ug/L	7	1(14 %)	0.08ug/L

INFERENCE AND CONCLUSION

The acute clinical signs recorded were correlating with pesticide poisoning. The presence of triazophos in blood, urine and water samples, despite its short half-life, and the time lapse curve showing characteristic decrease with time, suggests the diagnosis of organophosphate poisoning. The presence of heavy metals in few samples of water/food and in blood may be explained by their chronic exposure, which is more common. The clinical picture and epidemic curve characteristic of a common source single exposure outbreak point also are in favour of organophosphate poisoning.

2. IMPACT OF MEASLES RUBELLA (MR) VACCINATION CAMPAIGN ON POPULATION IMMUNITY IN INDIA [IMRVI STUDY]

While MR surveillance in India generates useful information, understanding population immunity against measles and rubella viruses could support strategic decision making and evaluation of the progress of the measles elimination and rubella control program. Apart from coverage evaluations of immunization initiatives and generating surveillance data, well-planned, large-scale measles and rubella seroprevalence studies would help to enhance our understanding of population immunity and susceptibility profiles of communities in different risk settings in India. The India Expert Advisory Group (IEAG) on measles in its first meeting in February 2017 recommended measles serosurveys to guide the program.

The current study proposed estimation of measles and rubella antibody seroprevalence stratified by age groups in various geographic areas. The project compared results generated from community and facility-based surveys.

OBJECTIVES

Primary Objectives

1. Estimate age-specific population immunity to measles and rubella viruses within a specified precision of ±10% within three age strata (children 9 months to 4 years and

5 to 14 years of age, and women 15 to 49 years of age) in India using serological surveys.
2. Compare the accuracy, precision and cost of estimating age-specific measles and rubella population immunity using convenience samples from health care facilities versus community-based serosurveys.

Secondary objectives

3. Validate and calibrate seroprevalence estimates obtained by using dried blood spots (DBS) with results of capillary serum samples in a subset of survey samples.
4. Assess the risk profile of measles and rubella/CRS through mathematical modelling by extrapolating the data generated through the study.
5. Estimate the seroprevalence for other vaccine preventable diseases or other diseases of national interest if the residual samples of study subject's permit.

METHODS

A Community based serosurvey for estimation of (a) population immunity against measles and rubella and (b) coverage of MR vaccine campaign in the community in 9 sites – 7 districts where MRHRUs are located and 2 purposively selected districts where rubella vaccine has been in use in private sector.

ICMR NIN coordinated a study at MRHRU, Tirupathi and Urban Hyderabad. In Both these areas study was conducted Post-campaign of Measles vaccination.

STUDY DESIGN AND SAMPLING STRATEGY

Nine cross-sectional serosurveys were conducted in five districts in India. This included four districts where pre and post measles and rubella (MR) campaign serosurveys could be included and one district where only the post MR campaign serosurvey was completed. Four districts were selected based on geographic diversity, presence of an established Model Rural Health Research Unit (MRHRU), and the ability to conduct serosurveys before and after the MR vaccination campaign. MRHRUs are government research facilities located in rural areas of India set up since 2013 by the Government of India to promote health research activities among rural populations. In one district, use of rubella vaccine in the private sector was also considered. The surveys were conducted among three age groups (9 months to less than 5 years, 5 to less than 15 years, and women 15 to less than 50 years [post MR campaign only]).

Using guidance from the WHO Vaccination Coverage Cluster Survey Reference manual and Demographic and Health Survey manuals, a three-stage cluster design was adopted in which first villages and wards were selected, then one cluster or Census enumeration Block (CEB) was randomly selected from each village and ward, and third, all age-eligible individuals in the selected cluster were enumerated and then randomly selected for the study. In the first stage, in each district 30 villages in rural areas or wards in urban areas were selected based on the 2011 nationwide census using the probability proportional to size. In the second stage, from each village or ward, one cluster was randomly selected and then all individuals in the cluster were enumerated. The generic term "cluster" was used for a CEB which per the India census is a well-defined area in a village or ward with 120-150 households (approximately 600-750 population) (Census of India, 2011) allotted to an enumerator at the time of the decennial census. In the third stage, thirteen eligible individuals per age group were selected by simple random sampling.

Biospecimen collection, transport, storage, testing and analysis

Up to 2 mL of venous blood was collected in a serum separator tube (Becton Dickinson 367983). Samples were left at ambient temperature for 30 minutes after collection, centrifuged at 3000 revolutions per minute for 10 minutes using a portable centrifuge, and stored at 4-8° C in cold boxes until transported back to the site laboratory at the end of the day. In the laboratory, specimens were re-centrifuged, sera were aliquoted and stored at -20° C. At the end of the survey, sera were transported to the ICMR- National Institute of Virology, Pune under cold-chain for serologic testing.

All sera were tested using commercial anti-measles IgG and anti-rubella IgG enzyme immunoassay kits. Equivocal samples were retested in duplicate using the same assay and the qualitative result most observed out of the three results was selected as final.

RESULTS

Data collected from ten clusters each of Hyderabad and Chittor respectively. Due to covid pandemic study was terminated, however data from other MRHRUs have been analyzed and yet to be published. The challenges faced and recommendations to facilitate the implementation of serosurveys with the experience of Hyderabad and Chittor, has been described in table 1.

Table 1: Challenges faced and recommendations to facilitate the implementation of serosurveys

Serosurvey Activity	Challenge	Recommendations

Community Mobilization	Lack of cooperation from certain settings and populations.[a]	• Establish a community mobilization plan and initiate mobilization activities prior to fieldwork. Consider the following:
		  ○ Review the list of selected clusters and compile general cluster-level characteristics like location, urban/rural status, and presence of slum areas.
		  ○ Identify CHWs and influencers prior to serosurvey initiation
		  ○ Account for additional time and effort required to obtain permission from affluent neighborhoods.
		  ○ Plan serosurvey timings with input from local community members to improve response rates
		• Involve influencers within and outside the health system depending on setting to improve acceptance. This includes sensitizing them about the study so that they can help address questions and concerns from the community. For example, in affluent urban communities permissions from residents' associations helped build community support to the study while among migrant poor populations and minority groups support from local WHO representatives or religious leaders was helpful.

Serosurvey Activity	Challenge	Recommendations
		• Provide compensation to local influencers for their time to ensure sustained support. In our surveys, providing compensation to local CHWs like ASHAs was important because they typically receive performance and service-based compensation for their regular work. No monetary compensation was expected or provided to local or religious leaders. • Collect qualitative data on cluster level characteristics during survey conduct (e.g., urban or rural, slum or non-slum areas, and common reasons for non-response) to assess patterns of non-response across clusters. This type of data was useful for planning and interpretation of the results.
Mapping of clusters	Outdated census lists and reference maps.	• Identify the most recent comprehensive list of communities with household enumeration for the sampling frame. If sampling is based on an outdated census, use census maps as a reference and prepare updated sketch maps of the clusters.[5] • Engage with local leaders, health workers, and other community members to physically locate selected cluster and its boundaries.
	Variability in quality and completeness of reference maps.	• Review and identify low quality and missing reference maps prior to field implementation. • In the case of low-quality or missing maps, use alternative spatial sampling methods or geographic units that align with cluster depending on setting[15,16]
Enumeration of households and its members	Inaccessible households (due to terrain or permission).	• Devote additional time and effort to include inaccessible households in enumeration such as alternate methods of contact. For example, in some situations field teams were able to contact households that were initially inaccessible over the phone for information with support from CHWs and other locals.
	Households not enumerated because they were locked, no competent respondent at home or refusal.	• Document non-response rates (e.g., percentage of households not enumerated) and reason for non-response (e.g., locked households, refusal to provide any information, or no competent respondent at home) at enumeration. • Regularly monitor non-response rates and adapt. Adjust timing based on holidays (e.g., local festivals and summer holidays) or local events (e.g., elections and harvest), with input from CHWs, or other community members supporting the field team. Be flexible with the time and day of household visits.

Serosurvey Activity	Challenge	Recommendations
		• Report non-response rates at enumeration in dissemination reports and publications to provide context when interpreting seroprevalence estimates.
Enrollment of selected participants	Low participation rates due to refusal or unavailability from certain settings and populations or during certain time periods.[a]	• Document and report non-response rates (e.g., percentage of selected participants not enrolled) and reason for non-response (e.g., locked households, refusal to provide blood, or other reason) at enrollment. • Regularly monitor non-response rates and reasons for non-response and adapt survey activities. For example, if eligible individuals refuse blood collection, increase community mobilization efforts and engage with CHWs to explain the survey procedures and address questions and concerns. • Devote additional time and effort to enroll unavailable participants, including adjusting timing of visits, scheduling follow-up visits, and returning when parents or guardians are available.
Data Collection	Miscommunication or non-standardized administration of survey questions to participants. Low vaccination card retention and reliance on parental recall. Data entry errors (e.g. date of birth)	• Design questionnaire and data collection tools to prevent or resolve quality issues. For example, limit the questionnaire to the questions of interest and avoid extraneous questions, consider photographing vaccination cards for use in resolving data errors, and use examples based on local context when probing for vaccination recall (e.g. dates of holidays or festivals). • Regularly monitor data and fieldwork to identify data quality issues and provide feedback or retraining as needed. For example, near real-time data monitoring was conducted through generation of weekly cluster summary reports which highlighted cluster level response rates, data for key variables (e.g., vaccination coverage) and potential data entry errors (e.g., date of vaccination prior to date of birth). These reports were circulated and discussed weekly to identify and rectify any data quality issues. Other monitoring activities included daily oversight by site investigators, daily reporting from field teams, weekly conference calls and frequent site monitoring visits.

Serosurvey Activity	Challenge	Recommendations
	Complex infrastructure of tablet-based application and poor internet connectivity can lead to data upload issues and temporary data loss.	• Hire staff with prior experience using mobile phones or tablets. If not possible, include additional hands-on practice sessions during training and piloting. • Pilot test the functionality and performance of data collection application in the setting representative of where the survey will be conducted prior to initiation. • In case of issues with tablet, use IT based solutions for recovery of data from tablet. Alternatively, may also develop back up procedures for data collect (for example, paper forms) as a back up.
Biospecimen collection, transport and storage	Improper blood collection in the field and improper packaging of blood specimens from field to laboratory can lead to hemolysis of specimens.	• Monitor hemolysis in the community and laboratory after transport. Adapt procedures, if needed to minimize hemolysis. • Assess causes of hemolysis during collection. Use recommended good practices to minimize hemolysis of blood specimens, including: o Let liquid blood specimens sit undisturbed for at least 30 minutes after collection. o Use conditioned ice packs[b] when storing samples in field to prevent the samples from freezing leading to hemolysis. o Centrifuge specimens in field before transporting, if possible. A portable centrifuge could be used to centrifuge samples in the field site. o Carefully pack samples for transport including using conditioned ice packs and not letting blood tubes come in direct contact with ice packs. • Use recommended good practices when handling blood specimens, including: o Have experienced phlebotomists collect blood from infants or younger children. Use of butterfly needles (instead of needle and syringe) may be easier when collecting blood from younger children but should only be considered for use by experienced technicians. o Reduce mislabeling issues through use of pre-printed labels, reconfirming participant ID labels in the community and laboratory.
	Specimen mislabeling	• Centrally generated participant identification number and preprinted labels could avoid mismatch between participant information and laboratory data.

Serosurvey Activity	Challenge	Recommendations
	Frequent power outages in laboratories can impact cold chain storage conditions	• Ensure 24-hour power back up in laboratory where samples are stored.
Biospecimen Testing & Analysis	Assay validity and other operational issues.	• Monitor assay quality using in-house controls or commercial serum panels in addition to EIA kit controls to assess quality of the assay over time and across runs. All EIA plates should include a minimum set of controls and a more comprehensive validation should be conducted when the EIA lot is changed or a new technician or equipment is used. • Consider feasibility of testing a subset of specimens on another EIA or gold standard assay, such as a plaque reduction neutralization assay for measles. Seek support from a reference laboratory, if needed. • Routinely retest specimens (2-5% of total) to assess intra-plate (within the same plate) and inter-plate (on different plates) variability using metrics of test reliability.
Overall planning, coordination and logistics	Identifying implementation partners	• Identify and collaborate with experienced local implementation partners. Partners can leverage prior experiences, local knowledge and relationships to inform all aspects of a serosurvey.
	Inadequate training of staff	• Conduct intensive in classroom training for survey teams to review background and steps of the survey objectives and methodology. In addition to didactic lectures, interactive sessions involving role-play of informed consent procedures and data entry into tablet questionnaires were practiced. Trainings also included field sessions in nearby communities, to practice survey mapping and enumeration steps in the field. During the training for survey teams, dedicate a day to include field practice of field to practice identifying a cluster using census maps, confirming it's boundaries and mapping the area. • Trainers to be present on site for supportive supervision of field teams for the first cluster in each survey.
	Safety of teams in the field	• Field teams to always enter field sites with local health workers or authorities and always inform local leaders or entities the purpose and period of their stay. If necessary, field teams can also inform local police authorities in case of anticipated issues.

Serosurvey Activity	Challenge	Recommendations
		• Teams must not remain in the field after sunset.
	Insufficient communication between core and field teams	• Social messenger groups were found to be useful in connecting core team of study investigators with field teams to get regular updates from the field and address any issues in real time.
	Logistical, ethical and budgetary challenges when returning results to participants (if applicable)	• If serology results will be returned to participants, the process, timing, ethical requirements and budget need to be considered prior to the start of the survey. Additional considerations may be needed for different settings. For example, participants living in rural areas may prefer to receive results in person whereas in urban areas via postal mail or electronically.

INFERENCE AND CONCLUSION

Data has been collected from ten clusters each of Hyderabad and Chittor respectively. Due to covid pandemic study was terminated, however data from other MRHRUs have been analysed and yet to be published. the challenges faced and recommendations to facilitate the implementation of serosurveys with the experience of Hyderabad and Chittor, has been published.

IV. DIETETICS STUDIES

1. ASSESSMENT OF IODINE STATUS AMONG PREGNANT WOMEN IN SELECTED DISTRICTS OF INDIA – AN ICMR TASK FORCE STUDY

Iodine is an essential component of the thyroid hormones, which regulate metabolic processes in most cells, besides playing a vital role in the development of most organs, in particular the brain. The period of pregnancy is associated with parallel increase in iodine and thyroid hormone requirements indicating the need for additional iodine intake during this period to prevent potential iodine insufficiency. Adequate iodine concentration in breast milk is essential for optimal neonatal thyroid hormone synthesis and neurological development in breastfed infants. During pregnancy, the requirement of iodine increases to 250 µg/day when compared to normal adult (150 µg/ day) to meet the higher metabolic demands of thyroxin (T_4) production, transfer iodine to fetus and increased renal iodine clearance by the mother. According to the Coverage Evaluation Survey (CES) conducted in all the states and union territories of India in 2009, every year nine million pregnant women and eight million new borns are at risk of Iodine Deficiency Disorders (IDD) in India, based on household-level coverage of adequately iodized salt. With an intention to generate population level estimates on iodine status among pregnant and lactating women including growth in their infants, representing different parts of the country, the Indian Council of Medical Research (ICMR) has undertaken the task force study under report. ICMR-National Institute of Nutrition (ICMR-NIN) is the collaborating centre, representing South India to generate such longitudinal data on iodine status from pregnancy to two years postpartum and infant growth from birth to two years of age with the following objectives.

AIMS AND OBJECTIVES

- To carry out longitudinal studies to assess urinary iodine level, the serum/plasma micronutrient level and thyroid profile of pregnant women during first, second and third trimester as well as at the time of delivery, at 6 months and at one year after delivery.
- To assess the new-borns for birth weight and growth up to one year of age.
- To estimate the iodine content in edible salt samples collected from households of all study volunteers at all the six time points.

METHODS

A longitudinal study in 200 pregnant women was carried out in the Yadadri/ Bhuvanagiri District of Telangana State. The district composed of two revenue blocks. From each block, 50 villages were selected by probability proportional to size (PPS) sampling and from each village two pregnant women were randomly recruited from households and included in the study. The women were followed up six times (1st, 2nd and 3rd trimester, at delivery, at 6 months

and 12 months postpartum). Their Infants were followed up at birth and at 6 and 12 months after birth. Socio demographic and wealth status, previous and current obstetric particulars and other household particulars were collected at recruitment. Anthropometry (maternal height at recruitment, weight, mid upper arm circumference and blood pressure in the women and weight and length in their infants at every follow-up), diet (multiple 24-hour diet recall), Ante-natal care, post-natal care related information, biological samples (blood and urine), household salt samples and other health related information were collected at recruitment and at every follow-up. The biological samples, salt samples and the raw data scrutinized at ICMR-NIN has been sent to ICMR-Centre for promotion of nutrition research and training (ICMR-CNRT), for the estimation of iodine, thyroid profile and selected micronutrients as well as for data entry.

RESULTS

The rate of attrition was about 20%. The task as per the intended protocol is completed on part of ICMR-NIN. We will be able to prepare the site-specific report/manuscript, once the data is shared by ICMR-CNRT after they analyse the data from all sites are report.

INFERENCE AND CONCLUSION

Inferences and conclusions will be made once the site-specific data is shared by ICMR-CNRT, after they complete the sample and data analysis of all the coordinating centres and make the primary technical report.

V. BASIC STUDIES

1. VITAMIN A DEFICIENCY AMONG UNDER-FIVE YEAR CHILDREN IN INDIA: AN ANALYSIS OF NATIONAL DATA SETS

Vitamin A (VA) is an essential nutrient which must be provided in the diet as it cannot be synthesized by humans. Young children are more vulnerable to its deficiency. Vitamin A deficiency (VAD) along with nutritional blindness was prevalent in India in 1950s and 60s. In 1970, the National Prophylaxis Program against Nutritional Blindness, providing mega-doses of VA, was initiated to prevent nutritional blindness due to Keratomalacia. Later, with studies reporting a beneficial impact of mega-dose VA supplementation (VAS) in reducing all-cause mortality by 23% , the focus of this program shifted to decreasing mortality rates, although this move is widely debated. However, whether VAD still a significant public health problem in India is a question. This is so, given national progress with reduction of infant and child mortality rates, immunization coverage, poverty reduction, and recent initiation of oil and milk fortification with vitamin A, underpinned by an almost complete reduction in clinical signs of VAD in children.

Until recently in India, there was no nation-wide survey which examined the serum retinol levels simultaneously with inflammatory biomarkers in young children. The Comprehensive National Nutrition Survey (CNNS, 2016-18) offered this primary evidence for assessing VAD across India in 1-5y old children. The CNNS serum retinol data, in conjunction with dietary intake assessments and the recently mandated cooking oil and milk fortification with VA, provided an opportunity for a critical assessment of VAD and the need for the VAS program in India. We evaluated biochemical VAD on a national level in 1-5 year old Indian children, in parallel with their risk of dietary deficiency measured against a factorial estimate of the distribution of VA requirements. The risk was also evaluated with theoretical enhanced VA intakes coming from fortification. Finally, an evaluation of the risk of excess intake of retinol from fortification and VAS over 6 months was made against the tolerable upper limit (TUL) of intake.

METHODOLOGY

Three national survey data sets were used in the present analysis; the CNNS 2016-18 for serum retinol, and specific rounds of dietary surveys from the National Nutrition Monitoring Bureau (NNMB) and the National Sample Survey Office (NSSO) for dietary intake assessments. The number of 1-5 years children with serum retinol measurements was 9563; among these 3978 (41.6%) of them had received VAS within the past 6 months. Serum retinol concentrations were measured by reverse-phase HPLC. The serum CRP levels were measured by nephelometry and particle-enhanced immunonephelometry. Serum retinol values were adjusted for the CRP concentration by the probability method of correction for inflammation.

A diet survey was carried out by the NNMB in 10 Indian states in 2011-2012 covering 800 households for dietary assessment in each state. A one-day individual 24th dietary recall was collected from children from each household, to yield their reported vitamin A intakes as Retinol Activity Equivalents (RAE)/ d. The NSSO survey covered the entire India during 2011-2012 across all socioeconomic strata, in 7469 villages and 5268 urban blocks, and collected data on monthly per-capita expenditure on household food purchases of 223 food items with a recall period of 30 days. The quantities of different foods purchased by the household were converted to their VA equivalent as RAE/day, using the Indian Food Composition and this was adjusted for the number of members in the household and the use of consumption units (CU) for individual quantity of intake. For children aged 1-3yr and 4-5yr, the CU used was 0.5 and 0.7 respectively, to obtain the vitamin A intake (RAE) per CU per day.

The risk of dietary inadequacy of VA among children was assessed using dietary intakes from the NNMB and NSSO surveys. These were calculated as the sum of pre-formed retinol in the diet and that coming from the transformation of β-carotene in foods (total retinol activity equivalents, RAE, µg/d), using a conversion of 8:1, based on the Indian RDA estimation. The risk of inadequacy of VA intake was calculated by comparing the distribution of intakes with the distribution of requirement of VA, for any specific age group, using the probability approach. To calculate a theoretical reduction of the risk of dietary inadequacy with the fortified VA intakes, the oil and milk consumption of children were estimated in the NSSO survey at the national level and then multiplied into their fortified retinol content respectively, to evaluate the additional preformed retinol intake. The risk of toxicity with supplementary vitamin A through MD-VAS and mandatory fortification at a national level was also assessed separately in 1-3yr and 4-5yr old children in the NSSO survey.

RESULTS

The geometric mean of serum retinol in the national group was 31.2 µg/ dL and the prevalence of VAD, based on the cutoff of 20 µg/ dL, was 15.7% (95% CI 15.2, 16.3%). When compared with the method of censoring those serum retinol values associated with high serum CRP values, the precision was lower and the VAD prevalence was 17.5% (95% CI 15.3, 20.0%). There were very few children (0.4%; n=35, normal CRP) with serum retinol values <10 µg/ dL. There were no significant differences in serum retinol values between urban and rural children, and boys and girls, or in the prevalence of VAD between them, (Table 1). VAD prevalence in children who reported receipt of VAS within the previous 6 months was 11.3% and in those who did not, the prevalence was 16.9%. The geometric mean of serum retinol concentration was 34.0 µg/ dL (95% CI 33.3, 34.6) in the former group, while in the latter, it was 30.4 µg/ dL (95% CI 30.0, 30.8). When disaggregated by geographical state, 7 states had VAD point prevalence ≥20% by the modified BRINDA method (Figure 1). Two-thirds of the 35 children with very low serum retinol (<10 µg/ dL) came from these 7 states. Of these 7 states, only 3 states, Jharkhand, Mizoram and Telangana had a significantly higher than 20% prevalence, that is, the lower 95% CI of the estimated VAD prevalence was >20%.

The average intake of VA in 1-5yr old children from the NNMB survey was 98.2 µg RAE/day (geometric mean, GM), with a geometric SD (GSD) of 3.34 µg RAE/ day. The risk of inadequate dietary intake of VA calculated from the distribution of NNMB dietary intakes for 1-5 y old children was 70%. The average intake of VA of 1-5yr old children from the NSSO survey was 134.0 (GM) with GSD of 2.0 µg RAE/ day. This survey had a wider coverage and used a food frequency approach. The average risk of inadequate intake based on the NSSO estimate of usual intake was 69% (Table 2).

Table 1. Serum retinol values and prevalence of vitamin A deficiency among children (1-5yr) stratified based on locality, sex and age groups

Characteristic	Serum retinol (µg/dL) Mean (95% CI)	VAD % (95% CI)
Urban (n=4145)	31.6 (31.0, 32.3)	15.1 (14.0, 16.2)
Rural (n=5418)	31.1 (30.7, 31.5)	16.0 (15.3, 16.6)
Boys (n=5034)	31.1 (30.6, 31.5)	16.1 (15.3, 16.9)
Girls (n=4529)	31.5 (31.0, 31.9)	15.4 (14.6, 16.2)

Table 2. Risk of inadequacy and excess vitamin A intake with fortification and supplementation in children from the NSSO survey (n = 100,547 Households)

Dietary intake	Risk of inadequate intake[1] (%)	Risk of excess intake[1] (%)	
	1-5y	1-3y	4-5y
Normal diet[2]	68.8	0.9	0.5
Normal diet + Fortified oil	32.5	1.8	0.9
Normal diet + Fortified oil and milk	24.9	2.1	1.0
Normal diet + Fortified oil and milk + VAS	1.1	30.0	7.8

[1]Risk of excess intake was calculated separately for 1-3yr and 4-5yr as TUL is different for these age groups. For the VAS condition, risk of excess intake was calculated from the cumulative intake over 6 months.

If it is assumed that, in a normal population, the risk of dietary inadequacy will be 50% (or lower); then in the current CNNS survey, the expected proportion with biomarker-based risk (low serum retinol) would be about 20%, reasonably similar to the observed biochemical VAD prevalence. The average oil and milk consumption in all children from the NSSO survey (GM±GSD) was 14.5±1.0 g/day and 83.0±2.3 ml/day, respectively. The theoretical additional intake from these sources was calculated based on the individual intake of oil alone, or oil and milk together, and the resultant total vitamin A intake was then evaluated for the risk of dietary inadequacy in children in each case, by SES quintile (Figure 2). With the added intake of retinol from oil fortification alone, the risk of dietary inadequacy dropped to 33% in all children, and with oil and milk intake considered together, it dropped to 25%. In children from the upper 2 quintiles of SES, it was 16% and 10% respectively, while in the lower 2 SES quintiles, it was 52% and 45% respectively. For 1-3yr old children in the NSSO survey, the risk of excess with oil and milk fortification was low and was 2%, 0.2% and 3% for all children and those from the lower and upper 2 SES quintiles, respectively (Table 2 and Figure 2). In 4-5y old children, the corresponding proportions were 1%, 1% and 0% respectively (Table 2 and Figure 2). In the six-month cumulative framework, the supplementary retinol from VAS and food fortification contributed to about 79% and 52% of the cumulative six-month TUL of 108000 µg in 1-3y and 162000 µg 4-5yr old children. Then, the proportion of 1-3yr old children at risk of exceeding the TUL was 30% for all children, and 44% and 12% for children from the highest and lowest 2 SES quintiles, respectively. In 4-5y old children, the corresponding values were 8%, 16%, and 1%, respectively.

Figure 1: Prevalence of vitamin A deficiency among children (1-5y) across geographical states of India

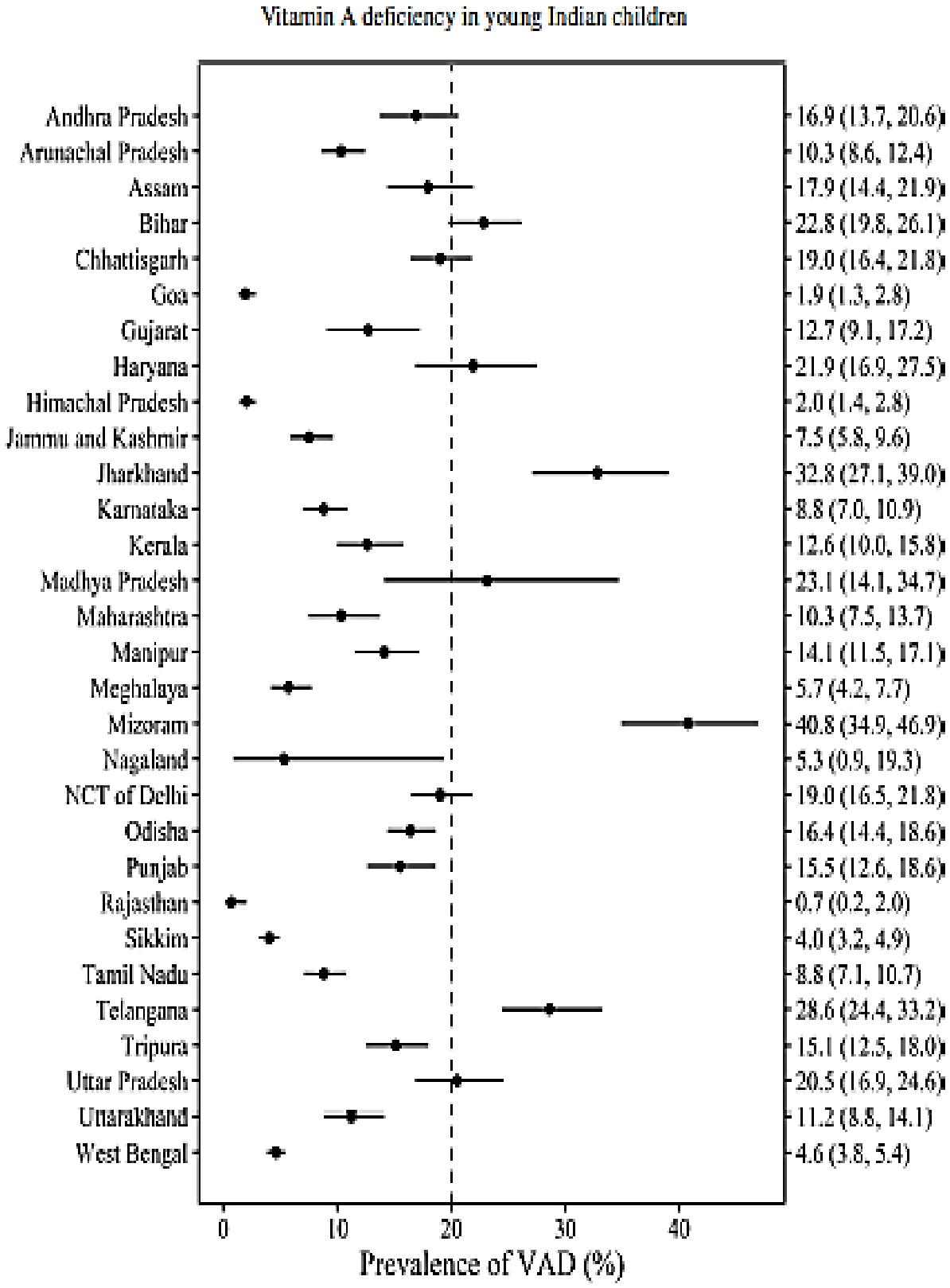
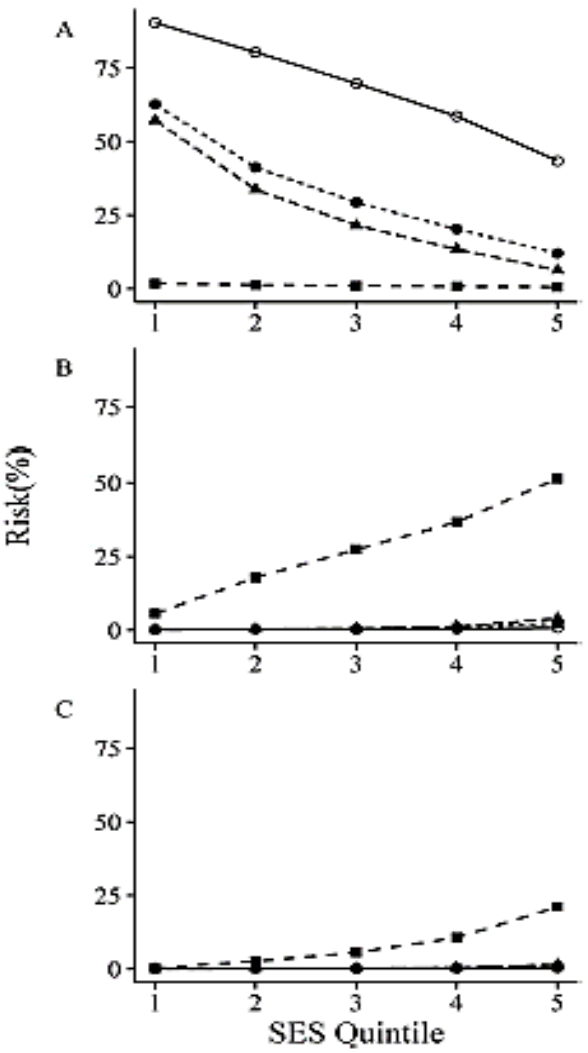


Figure 2: Estimates of the risk of inadequacy and excess vitamin A intake, stratified by SES quintiles from the NSSO survey. Panel A: Risk of inadequacy of vitamin A with habitual diet alone, or with fortified oil and milk, or added VAS. Panel B: Risk of excess in 1-3y old children with habitual diet alone, with fortified oil, with fortified oil and milk combined, or all with added VAS. Panel C: Risk of excess in 4-5y old children with habitual diet alone, with fortified oil, with fortified oil and milk combined, or all with added VAS.



Habitual diet; Habitual diet + fortified oil; Habitual diet + fortified oil + fortified milk; Cumulative (six months) risk for habitual diet + fortified oil + fortified milk + VAS.

CONCLUSION

The evidence from national surveys on serum retinol and dietary VA intakes, and an analysis of survival benefit indicates the need for serious consideration for adopting a targeted approach instead of continuing with the universal VAS in India. This should be accompanied by careful monitoring of a) 6mo-5yr mortality trends through the ongoing Sample Registration System; b) keratomalacia case-load data from ophthalmic hospitals or sentinel sites; and c) serum retinol and retinyl esters from repeat national surveys or regional studies in high burden states if cost is a limitation. Continuing universal VAS, along with recently introduced fortification, is likely to result in the risk of exceeding the TUL of VA intake in a proportion of children, especially those in higher SES, as well as wastage of scarce financial and logistic resources.

2. CAROTENOID STATUS IN TYPE 2 DIABETES PATIENTS WITH AND WITHOUT RETINOPATHY

Diabetic retinopathy (DR) is the most frequent microvascular complication of diabetes, and is a leading cause of blindness and visual impairment affecting one-third of people with diabetes worldwide. Established risk factors for DR are poor glycaemic control, duration of diabetes, hypertension, and dyslipidaemia. Moreover, increasing evidence has emphasized the critical involvement of oxidative stress in the pathogenesis of DR. Elevated oxidative stress in DR can affect endothelial vascular function, survival of retinal cells, neurodegeneration and blood-retinal barrier. Studies have shown ethnic differences in the prevalence and severity of DR even after controlling for systemic risk factors. In addition to ethnic and genetic factors, other environmental factors such as nutritional and lifestyle factors could play a major role in the development of DR. Further, studies demonstrated that patients with diabetes are at higher risk for deficiency of micronutrients.

Recently, we demonstrated a high prevalence of multiple subclinical micronutrient deficiencies and dietary inadequacies in apparently healthy adults (30–70 years old). Previous studies indicated that vitamin B12 deficiency might be an independent risk factor for DR. Carotenoids are plant-derived pigments required for the general health, particularly in embryonic development, immunity, reproduction and vision. Carotenoids are broadly classified into carotenes and xanthophylls. Lutein, zeaxanthin and lycopene are the principal carotenoids present in the human ocular tissues, which are essential for normal vision in humans. Carotenoids act as potential antioxidants to protect cells from free radical damage and hence may prevent or delay the development of DR. Therefore, in this study, we assessed the plasma carotenoid levels and their dietary intakes in type 2 diabetes (T2D) patients with no DR (NDR) and with DR and compared with apparently normal healthy controls

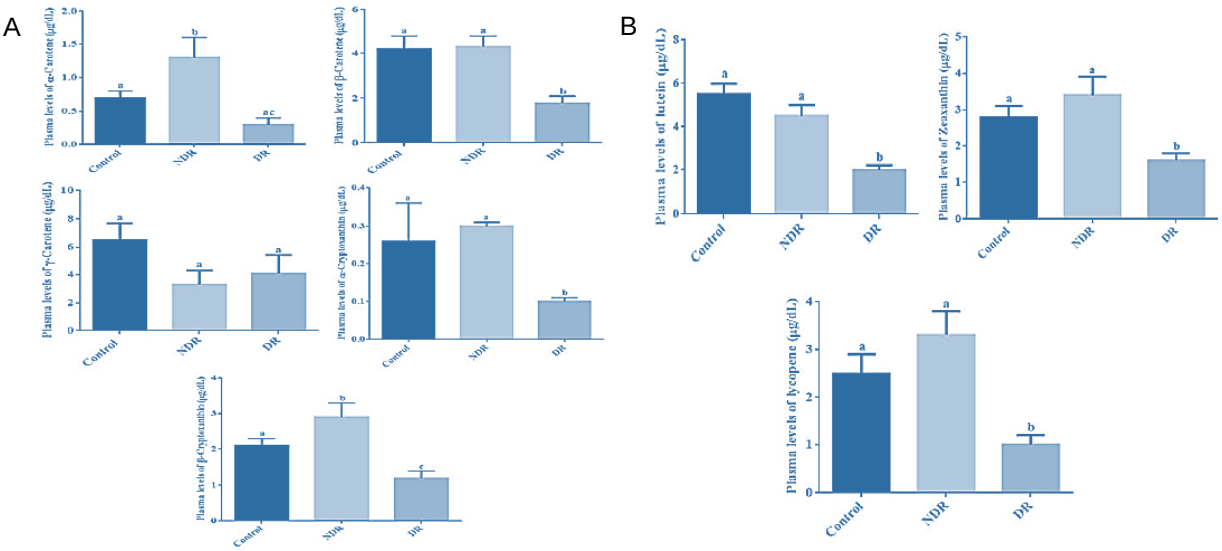
METHODOLOGY

A hospital-based cross-sectional case-control study was conducted on 344 T2D patients, with no DR (NDR; n=150) and with DR (DR; n=194). In addition, apparently healthy individuals (Control; n=151) matched for age, sex and other socioeconomic status were recruited as the normal controls. Patients with T2D, without and with DR, were matched for duration of diabetes and were recruited from patients attending the Pushpagiri Vitreo Retina Institute, Hyderabad. The study was carried out in compliance with the guidelines of the Helsinki Declaration of 1975 and approved by the Institutional Ethics Committees of the respective institutes. Estimation of plasma carotenoids was carried by HPLC. Dietary intake nutrients including carotenoids was assessed in a subset of the samples using a raw food-based food frequency questionnaire of 1-year duration.

RESULTS

The gender distribution was approximately the same among the groups. Therefore, the data for both men and women were pooled in the respective groups. Further, the duration of diabetes was matched for NDR and DR and also HbA1c levels were comparable between DR and NDR. The plasma levels of lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, lycopene, α-carotene and β-carotene were significantly lower in DR compared to NDR and Control (Figure 1). Further, the plasma levels of all the carotenoids (except β-cryptoxanthin and α-carotene) were similar between NDR and Control groups.

Figure 1. Plasma concentration of (A) provitamin A (PVA) carotenoids and (B) non-PVA carotenoids across the groups. NDR, diabetes no retinopathy; DR, diabetes with retinopathy.



Values are mean ± SE. Mean values across groups were compared by one-way analysis of variance F test with least significant difference. Significant differences ($p < 0.01$) of mean values between the groups are indicated by different superscript letters (a,b & c).

Among the food groups, the intakes of pulses & legumes, other vegetables, roots & tubers, nuts & oilseeds, fruits, fish, milk & milk products and sugars were found to be significantly lower in DR compared to NDR and Control. The dietary intakes of the majority of nutrients (except carbohydrate, thiamine, iron, magnesium and manganese) were significantly lower in DR compared to NDR and Control. Most importantly, the mean intakes of zeaxanthin, lycopene, α-carotene and β-carotene were found to be significantly lower in NDR compared to Controls, and a further decrease in the intakes (except zeaxanthin) was found in the DR group compared with the NDR group (Table 1).

Table 1. Dietary intake of carotenoids in the study subjects

Carotenoid intake (µg/day)	**Control (n=54)**	**NDR (n=50)**	**DR (n=52)**	**F-value**	**p-value**
Lutein	$1862^{a} \pm 252$	$1245^{a} \pm 140$	$1760^{a} \pm 230$	2.31	0.103
Zeaxanthin	$39.7^{a} \pm 6.3$	$16.6^{b} \pm 2.0$	$28.8^{ab} \pm 4.2$	6.17	0.003**
β-Cryptoxanthin	$50.2^{a} \pm 7.7$	$41.0^{a} \pm 5.4$	$33.6^{a} \pm 4.4$	1.91	0.152
Lycopene	$2721^{a} \pm 223$	$2320^{a} \pm 206$	$1596^{b} \pm 174$	8.04	<0.001**
α-Carotene	$1017^{a} \pm 123$	$772^{b} \pm 95.0$	$605^{b} \pm 121$	3.36	0.037*
β-Carotene	$3508^{a} \pm 384$	$2652^{b} \pm 234$	$2486^{b} \pm 278$	3.21	0.043*

NDR, diabetes no retinopathy; DR, diabetes patients with retinopathy.
Values are mean ± SE. Mean values across groups were compared by one-way analysis of variance F-test with least significant difference. **Significantly different at $p < 0.01$. *Significantly different at $p < 0.05$. Significant differences of mean values between the groups are indicated by different superscript letters (a, b).

Interestingly regardless of groups, lutein, zeaxanthin, β-cryptoxanthin, lycopene and β-carotene levels were significantly inversely associated with duration of diabetes but positively associated with HDL. Random blood glucose and HbA1c showed a significant negative correlation with levels of lutein and lycopene.

CONCLUSION

In summary, for the first time, we have shown that the rTL and mtCN decline with age and both are associated with each other in the Indian population. The plasma folate and vitamin B12 levels may influence aging by stabilizing the TL and mtCN. By deciphering the role of these factors in aging may aid in developing effective therapeuticstrategies to prevent or delay age and age-associated disorders.

3. IMPACT OF CHRONIC HYPERGLYCEMIA ON SMALL HEAT SHOCK PROTEINS IN DIABETIC RAT BRAIN

Small heat shock proteins (sHsps) are a family of proteins induced in response to multiple stressful events. There are 10 sHsps: HSPB1 (Hsp27), HSPB2 (MKBP), HSPB3, HSPB4 (αA-crystallin; αAC), HSPB5 (αB-crystallin; αBC), HSPB6 (Hsp20), HSPB7 (cvHsp), HSPB8 (Hsp22), HSPB9, and HSPB10 (ODF1). One of the physiological functions of sHsps is chaperone-like activity where the sHsps bind to improperly folded protein-substrates and transfer them to the ATP-dependent chaperones or protein degradation machines like proteasomes. In addition, sHsps are involved in the maintenance of cytoskeletal integrity and other cellular physiological processes, including programmed cell death, development, cell differentiation, platelet function, and vasorelaxation. Diabetes and its complications are alarming public health problems globally. The long-term complications of diabetes are thought to be a result of the accumulation of tissue macromolecules that have been modified by various posttranslational modifications (PTMs). However, the pathogenic mechanisms underlying diabetic neuropathy (DN) are still largely unknown, although oxidative stress, advanced glycation end products, inflammation, and neurodegeneration are implicated. Hyperglycemia and oxidative stress created during diabetes have detrimental effects on the brain, which result in brain atrophy or neurodegeneration.

Some studies reported the differential expression and induction of sHsps in rat brain and cultured hippocampal neurons under heat shock, glial expression of sHsps in immature rat brain following an excitotoxic lesion. It was reported that Hsp27, HSPB2, HSPB3, αBC, Hsp20, and Hsp22 are expressed in the adult rat brain while Hsp27, αBC, Hsp20, HspB7, and Hsp22 in the mouse brain at the transcript level. Furthermore, it was reported that expression of HSPB2 and HSPB3 is very low in the rat brain. The remaining sHsps are tissue-specific and are expressed in tissues, such as muscle, heart, and testis. Multiple studies have reported the upregulation of sHsps in Alzheimer's disease, multiple sclerosis lesions, amyotrophic lateral sclerosis, Parkinson's disease, and Alexandra disease. Diabetes also poses a risk for developing neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. Amyloidogenic proteins, such as α-synuclein and Tau, are major constituents of Lewy bodies (LBs) and neurofibrillary tangles (NFT), a neuropathological hallmark of synucleopathies and tauopathies respectively. sHsps prevent the aggregation of

amyloidogenic proteins by directly interacting with them. However, the response of sHsps in the brain under chronic hyperglycemia remains poorly understood. Previously, we and others have reported the altered expression of sHsps, their phosphorylation status, role in cytoskeletal protection and apoptosis in the retina, heart, lens, and skeletal muscle in hyperglycemia. Herein we report the effect of chronic hyperglycemia on the expression and solubility of sHsps and their interaction with amyloidogenic proteins and apoptotic mediators in the cerebral cortex (CC) of diabetic rats.

METHODOLOGY

Diabetes was induced in two-month-old male SD rats by a single dose of intraperitoneal injection of streptozotocin. All experimental procedures were approved by the Institutional Animal Ethical Committee. Control and diabetic animals were fed the AIN-93 diet *ad libitum*. At the end of 4 months, rats were sacrificed and the cerebral cortex (CC) of brain tissue was collected. The tissue was, fixed, embedded in paraffin blocks, and cut into sections. The sections were used for H&E and Nissl body staining.

Total RNA was extracted from control and diabetic rat CC using Tri-reagent and reverse transcribed to get cDNA. Quantitative real-time PCR was performed with cDNA templates using SYBR green master mix using gene-specific primers. Normalization and validation of data were carried out using β-actin as an internal control. Data were compared between control and diabetic samples according to the comparative threshold cycle ($2^{-\Delta\Delta ct}$) method. CC was homogenized and the protein concentrations were estimated. Equal amounts of protein were resolved by 12% SDS-PAGE and transferred to nitrocellulose membranes and incubated overnight at 4°C with respective primary antibodies diluted in PBST. After washing with PBST, membranes were incubated with secondary antibodies conjugated to HRP. The immunoblots were developed with an enhanced chemiluminescence detection kit using an Image analyzer. An *in-situ* Cell Death Detection Kit was used to assess apoptosis. Co-immunoprecipitation (Co-IP) was carried out using a Co-IP kit (26149PR; Thermo Scientific Pierce, Rockford, IL, USA) according to the manufacturer's instructions as previously reported. Briefly, CC was homogenized in TNE buffer containing 0.5% TritonX-100, and the homogenate was centrifuged at 12,000 ×*g* at 4°C for 20 min for interaction studies.

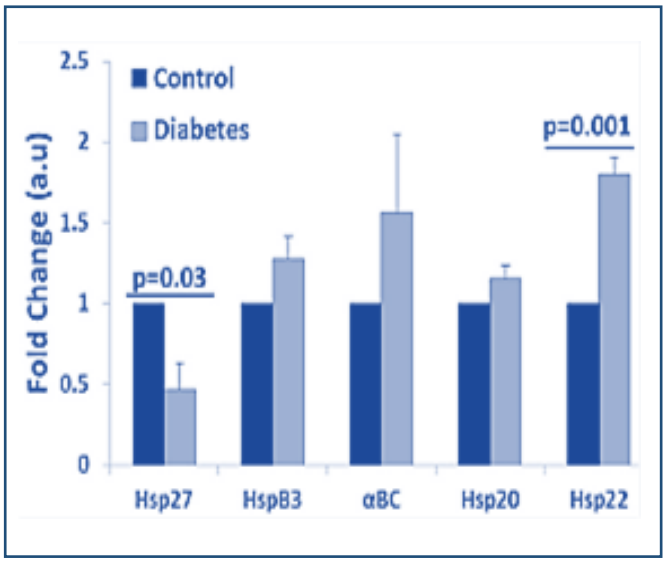
RESULTS

To show the impact of hyperglycemia on morphology and neuronal degeneration, we performed H&E, Nissl body, and TUNEL staining in sections of control and diabetic CC. H&E staining of the CC in diabetic rats revealed halos around the cells. Neurons also showed eosinophilic degeneration indicated by the loss of eosinophilic cytoplasm and contracted cells. Nissl body staining using Cresyl violet revealed cellular degeneration indicated by chromatolysis of cells and by condensed and lightly stained Nissl body substance. The staining findings indicated cellular degeneration in the CC of diabetic rats. In support of this, diabetes increased the number of TUNEL positive cells compared to controls. We analyzed the expression of Hsp27, HSPB3, αBC, Hsp20, and Hsp22 in diabetes using qRT-PCR (Fig. 1) and immunoblotting (Fig. 2). Hsp27 was significantly downregulated in diabetic rats compared to that in the control. HSPB3 and Hsp20 were unaltered in diabetes compared to the control, while αBC showed an increasing trend. Interestingly, Hsp22 expression was significantly upregulated in diabetes compared to the control. To correlate transcript expression with protein levels, we investigated the protein levels by immunoblotting using specific antibodies. Hsp27 levels were downregulated in diabetic rats. The downregulation correlated with mRNA levels. HspB3 protein was undetectable, whereas αBC protein showed increased expression, consistent with its transcript levels. Hsp20 protein levels were

unaltered, whereas Hsp22 levels were significantly increased, corroborating its mRNA levels. Additionally, Hsp70 levels were also significantly decreased in diabetic rats compared to controls.

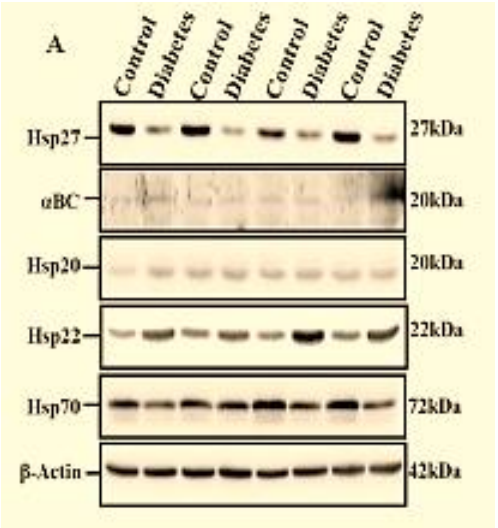
The solubility of predominant sHsps, including Hsp27, αBC, Hsp22, as well as α-synuclein and Tau in CC was assessed by differentiating TritonX-100 soluble and insoluble fractions. Interestingly, the Hsp27 content was significantly reduced in TritonX-100 soluble and insoluble fractions of diabetic rat CC compared to the control. The αBC content was significantly increased in both TritonX-100 soluble and insoluble fractions. In addition, Hsp22 content was also significantly increased in both TritonX-100 insoluble and soluble fractions. Surprisingly, α-synuclein levels were unaltered in the TritonX-100 soluble fraction, although it was significantly increased in the TritonX-100 insoluble fraction. Tau levels were significantly decreased in the TritonX-100 soluble fraction and insoluble fraction. Interestingly, phosphorylation of Tau (S199/S202) was significantly increased in the TritonX-100 soluble and insoluble fractions. We also assessed the interaction of αBC with α-synuclein and pTau (S199/S202) in TritonX-100 soluble and insoluble fractions. Interestingly, diabetes significantly reduced the interaction of αBC with α-synuclein and pTau in the soluble and insoluble fractions compared to the control. We speculated that chronic hyperglycemia could induce neuronal cell death. To assess this, we investigated neuronal cell apoptosis using apoptotic markers Bax and Bcl-2. Bax was significantly increased and Bcl-2 was significantly decreased in diabetic rats compared to controls (Fig. 3). Since αBC prevents apoptosis by interacting with the pro-apoptotic mediator Bax, we investigated the interaction of αBC with Bax under chronic hyperglycemia using co-IP. Interestingly, diabetes reduced the interaction of αBC with Bax compared to controls (Fig. 3).

Figure 1. Effect of hyperglycemia on the expression pattern of sHsps in CC of control and diabetic rats.



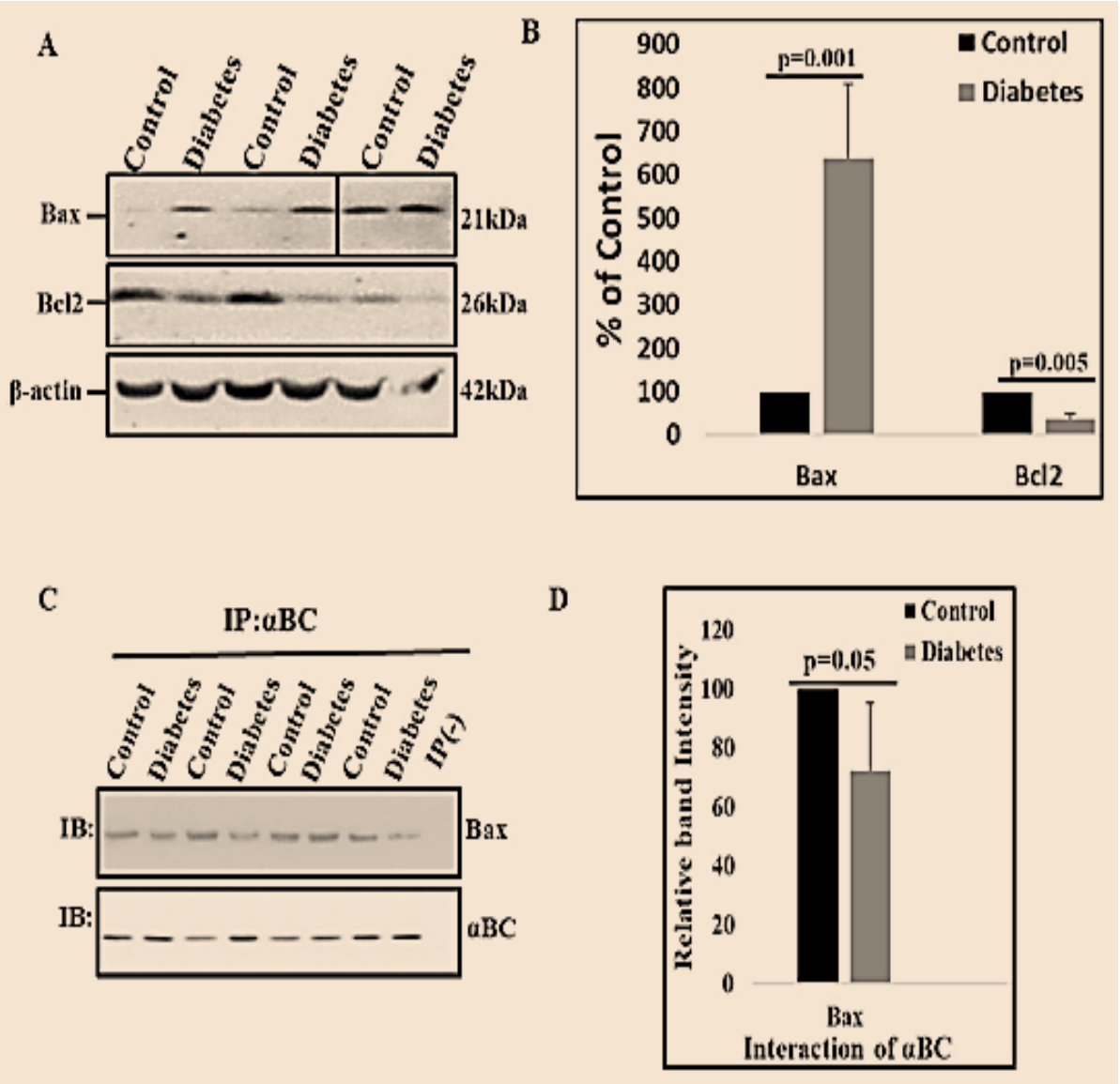
Data represent mean± SEM of three independent experiments.

Figure 2. Effect of hyperglycemia on the protein levels of sHSP in CC of control and diabetic rats.



Data represent mean ± SEM of four independent experiments for sHSP and three for HSFs.

Figure 3: Neuronal cell death in CC of control and diabetic rats



(Panel A) Immunoblots of apoptotic markers. (Panel B) Respective quantitative bars demonstrating the apoptotic markers. (Panel C) Interaction of αBC with apoptotic mediator including Bax analyzed by co-immunoprecipitation. (Panel D) Quantitative bars demonstrating the interaction of αBC with Bax. IB, immunoblotting antibody; IP, immunoprecipitation; IP (-), no antibody control immunoprecipitation. Data represent mean ± SEM of four independent experiments.

CONCLUSION

In summary, we report that the differential expression, reduced solubility, and impaired interaction of sHsps with amyloidogenic proteins and Bax increased neuronal cell death in chronic hyperglycemia. In conclusion, diabetes induces differential responses of sHsps by affecting their expression, solubility, and interaction with other proteins.

4. MOLECULAR MECHANISM(S) INVOLVED IN VITAMIN D DEFICIENCY INDUCED MUSCLE ATROPHY

Vitamin D deficiency is reported to be highly prevalent worldwide. Vitamin D deficiency results from impaired vitamin D action and leads to bone disorders such as rickets [in children] and osteomalacia [in adults]. It also leads to “muscle wasting” or “muscle atrophy” in both animals and humans. Administration of low-dose vitamin D in elderly subjects prevented muscle atrophy and reduced the incidence of falls and hip fractures in them. On the other hand, studies have found a significant positive correlation between reduced 25(OH)D_3 levels and reduced muscle function in the older population. Therefore, clinical evidence suggests a role for vitamin D in muscle metabolism and function. Muscle wasting or muscle atrophy is caused by increased protein degradation and/or decreased protein synthesis. Although protein degradation occurs via three different pathways, namely – the lysosomal, the Ca+ activated pathway and the ubiquitin-proteasome pathway (UPP), the major pathway responsible for excessive protein loss in various diseased states is reported to be the UPP. In this context, we have earlier reported that vitamin D deficiency induced muscle protein degradation also occurs via the ubiquitin proteasome pathway and calcium alone in the absence of vitamin D could reverse majority of the muscle changes observed in vitamin D deficiency. Based on this data, it appears that vitamin D and calcium play an important role in muscle protein metabolism. The mechanisms/pathways involved in the muscle wasting seen in vitamin D deficiency have not been worked out. In the present work, using a vitamin D deficient rat model, we have studied the molecular mechanism(s) involved in vitamin D deficiency induced muscle atrophy. The following are the broad objectives:1) To identify the muscle genes modulated in vitamin D deficiency induced muscle wasting using qRT-PCR based muscle-specific gene array, 2) To examine the role of mTOR pathway in regulation of protein synthesis in vitamin D deficient muscle. 3) To study the role of muscle specific miRNAs in vitamin D deficiency induced muscle wasting.

METHODOLOGY

Vitamin D deficiency or insufficiency was induced in a rat model using different levels of vitamin D3 in the diet. Serum levels of 25(OH)D3 & parathyroid hormone [PTH] were estimated by using commercial kits. Serum parameters such as Ca, P and alkaline phosphatase were done using standard methods. Total protein degradation in muscle was measured by the rate of tyrosine released into the media. 3-methylhistidine in the urine was estimated by HPLC. Profiling of muscle genes was done using RT2 profiler gene array specific for rat skeletal muscle myogenesis and myopathy. mRNA levels of muscle genes were studied by qPCR and expression of muscle proteins was done by western blotting using specific antibodies.

RESULTS

- Vitamin D deficiency and insufficiency was confirmed by the serum 25(OH)D3 levels which were observed to be significantly lower in the deficient group [VDD] compared to control [Table 1] while the low vitamin D [LVD] group showed levels higher than the deficient group but lower than vitamin D sufficient group. As expected, the PTH levels were significantly ($p<0.05$) higher in the deficient group compared to control group, while the levels in LVD group were similar to that of control group [Table 1]. Rats in the VDD group were hypocalcemic, while the rats in LVD and CONT groups showed normal serum calcium levels [Table 1]. The lean body mass [LBM] was

significantly lower in the VDD group compared to CONT, while the LVD group had LBM similar to CONT. The levels of serum Ca, 25(OH)D3 and PTH in the pair-fed [PF] group were slightly lower than the CONT group. All the serum parameters appeared to be normalized in the group rehabilitated with control diet [RD] [Table 1].

- mRNA levels of all the genes that were found to be altered in the RT2 microarray experiment in the VDD and LVD groups were measured in all the five different dietary groups by RT-qPCR. Our RT2 array data showed that the expression of key oxidative enzyme genes citrate synthase (*Cs*) & pyruvate dehydrogenase kinase4 (*Pdk4*) were significantly ($p \leq 0.05$) decreased in the LVD group compared to +D-AL group. Pdk4 deficiency is known to inhibit fatty acid oxidation. Therefore, we hypothesized that the enzyme *β-Had* involved in fatty acid oxidative metabolism and the key glycolytic enzymes (*Hk2 & Pfkm*) may also be altered in VDD. Indeed, the expression of all the glycolytic and oxidative enzymes was observed to be significantly ($p<0.05$) decreased in the VDD muscle (Fig 1a). Rehabilitation with vitamin D appeared to normalize the expression of these genes to that of controls (Fig 1a). In line with the RT2 data, negative regulators of muscle mass genes i.e., *Fbxo32*, *Trim63 & Mstn* were observed to be up regulated in the VDD muscle compared to +D-AL control muscle (Fig 1b); whereas expression of *Cryab,* a gene that protects muscle from atrophy was significantly ($p<0.005$) suppressed in both the VDD and LVD groups (Fig 1b). While the muscle contractility gene *Myh2* appeared to be down regulated in both the VDD ($p<0.005$) and LVD ($p<0.05$) groups, *Myh1* and *Myh7* were significantly lower in the VDD group only (Fig 1c). Again, in confirmation of the RT2 data the expression of the troponin genes: *Tnnc1 & Tnnt1* was found to be significantly decreased in both the VDD ($p<0.005$) and LVD ($p<0.05$) groups compared to the +D-AL group. (Fig 1c). On the other hand, the expression of the transcriptional coactivator genes peroxisome proliferator activated receptor gamma (PPAR) coactivator (*Ppargc-1α*) and *Ppargc-1β* was reduced in both the VDD and LVD groups compared to +D-AL control and their expression appeared to be reversed upon supplementation with control diet (Fig 1d). The mRNA levels of *Vdr*, and *MyoG* genes were significantly ($p<0.005$) decreased in the VDD group only while the mRNA level of *Mb* gene was reduced ($p<0.005$) in both the VDD and LVD groups (Fig 1d & b). Rehabilitation with the adequate vitamin D (2000IU D_3) diet appeared to correct the expression of majority of genes.

- In order to assess if the reduced gene expression of myosin proteins (*Myh1 & Myh2*) and myoglobin (*Mb*) also resulted in decreased expression levels of their protein products the protein levels of these genes were assessed using specific antibodies. In line with the gene expression the myosin and myoglobin protein levels were significantly reduced in the VDD ($p<0.005$) and LVD ($p<0.05$) groups compared to +D-AL group (Fig 2a-c). Further, to confirm that decreased mRNA expression of Cs enzyme also leads to decrease in activity; the Cs enzymatic activity was monitored in muscle homogenates from the different experimental groups. Cs activity was significantly ($p<0.05$) less in the VDD group compared to +D-AL (Fig 2d). In the LVD group the activity appeared to be lower than +D-AL group but higher than the VDD group. Rehabilitation with the adequate vitamin D (2000IU D_3) diet did not completely normalize the Cs activity.

- Further, the expression of muscle specific micro RNAs [myomirs] was assessed in the skeletal muscle of the different experimental groups. The expression of four

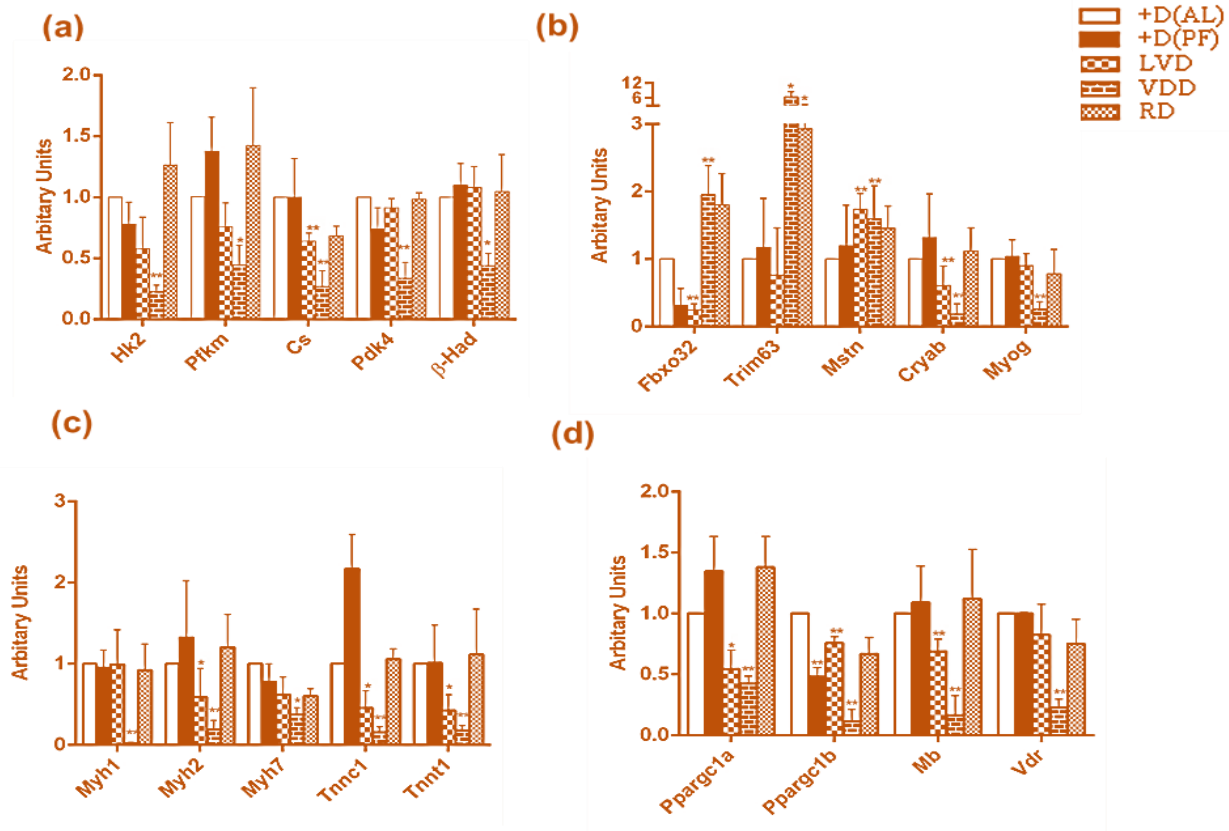
different myomirs, namely: miRNA133b [panel A], miRNA206 [panel B], miRNA499 [panel C] and miRNA23a [panel D] were significantly ($p<0.05$) decreased in the VDD group compared to CONT group [Fig 3A-D]. The levels of these miRNAs appeared to be reduced in the LVD group too in comparison to the CONT group. Rehabilitation with the adequate vitamin D (2000IU D_3) diet appeared to normalise the levels of the myomirs to that of the CONT group [Fig 3A-D].

Table 1. Serum Vitamin D dependent and lean body mass in different experimental groups

Parameter	CONT	PF	LVD	VDD	RD
Calcium (mg/dL)	9.47 ± 0.33^{a}	8.8 ± 0.43^{a}	9.3 ± 0.26^{a}	5.9 ± 0.2^{b}	9.3 ± 0.1^{a}
PTH (pg/mL)	37.68±16.15^{a}	42.08±20.322^{a}	33.81±16.09^{a}	406.82±41.96^{b}	91.02±46.75^{a}
25(OH)D_3(ng/mL)	59.42±5.04^{a}	50.66±1.68^{a}	20.11±2.42^{c}	4.52±0.70^{b}	52.80±7.53^{a}
LBM (g)	373.9±16.1^{a}	314.4±14.3^{b}	356.0±11.2^{ab}	250.0±25.2^{c}	335.9±14.4^{ab}

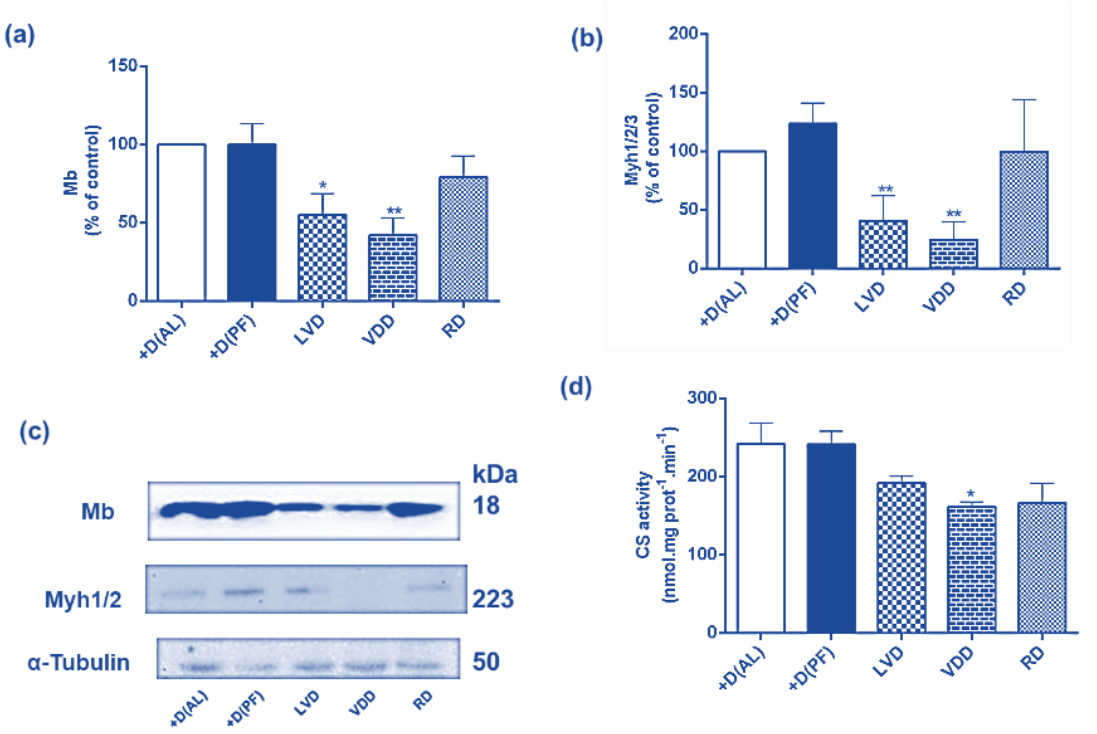
Data are shown as mean ± SEM of measurements from six rats in each group. Data with different superscripts are significantly different from each other at $p \leq 0.05$.

Figure 1. Vitamin D deficiency alters expression of glycolytic, muscle atrophy, contractility and muscle growth genes



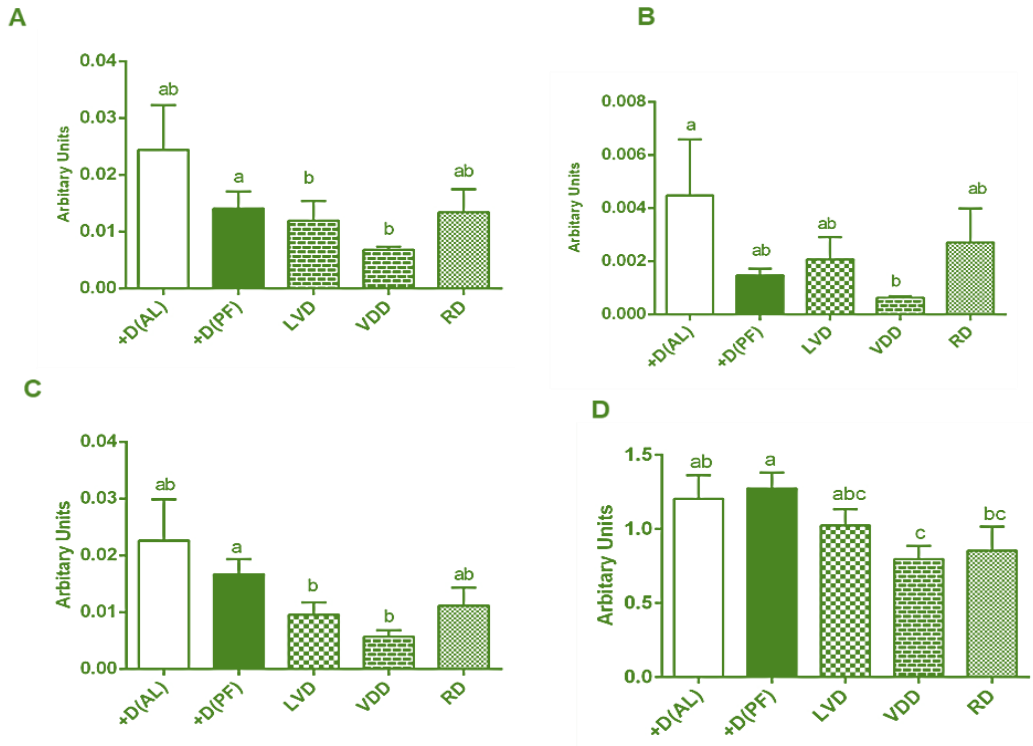
Panel a, expression of glycolytic and fatty acid oxidation genes (*Hk2, Pfkm, Cs, Pdk4 & βHad*); Panel b, expression of the atrophy genes (*Fbxo32 & Trim63*), alpha-crystallin (*Cryab*), myostatin (*Mstn*) and myogenin (*MyoG*) genes. Panel c, mRNA expression of the muscle contractility genes (*Myh & Tnn),* while Panel d, expression of vitamin D receptor (*Vdr*) and mitochondrial biogenesis (*Ppargc1a, Ppargc1b & Mb*) genes.

Figure 2. Vitamin D deficiency reduces myoglobin and myosin protein levels and enzymatic activity of citrate synthase in the gastrocnemius muscle



Panel a, depicts the protein levels of Mb, while panel b shows the protein levels of Myh1/2. Panel c, shows a representative picture of blots with Mb, Myh1/2 and α-tubulin antibodies; while panel d, shows the CS activity in the different experimental groups.

Fig 3. Vitamin D deficiency reduces the expression of myomirs in skeletal muscle



Panel A-D shows the levels of miRNA133b, miRNA206, miRNA499 and miRNA23a respectively.

INFERENCE & CONCLUSION

In conclusion, this study demonstrates that Vitamin D deficiency and insufficiency impairs skeletal muscle contraction, composition, and energy metabolism. Further, the expression of muscle-specific miRNA appears to be reduced in the VDD muscle. A significant outcome of our work is the deleterious effect of low amounts of circulating vitamin D on functional parameters in muscle such as contraction, energy metabolism and mitochondrial biogenesis; thereby indicating that subclinical deficiency as seen in humans, already impairs muscle function. Hence, our work gives insight into the molecular mechanism(s) being governed by Vitamin D in the skeletal muscle and provides evidence that higher circulating level of Vitamin D is essential for optimal skeletal muscle functions than for maintaining calcium homeostasis.

5. VITAMIN D DEFICIENCY INDUCED NEURODEGENERATION: ROLE OF PROTEIN HOMEOSTASIS PATHWAYS

Vitamin D deficiency is increasing in prevalence worldwide with a significant impact on health. Several studies have shown that relatively high proportions of people around the world have inadequate levels of vitamin D. Vitamin D is well known for its role in the maintenance of calcium homeostasis and bone development. The presence of the vitamin D receptor and enzymes involved in the hydroxylation of vitamin D (25OHase and 1α-OHase) in the brain implicates a role for this hormone in cognitive function and dementia. A growing body of evidence from epidemiology and neuroscience links vitamin D deficiency with a range of neuropsychiatric disorders and neurodegenerative disease. Human studies strongly support a correlation between low levels of circulating 25-hydroxyvitamin D (25 (OH) D) and cognitive impairment or dementia in aging populations. In parallel, animal studies show that supplementation with vitamin D is protective against biological processes associated with Alzheimer's disease (AD) and enhances learning and memory performance in various animal models of aging and AD. Epidemiological evidence from cross-sectional studies provides support for a link between vitamin D deficiency and incidence of Parkinson's disease (PD). Furthermore, a longitudinal study investigating the association between vitamin D status and subsequent occurrence of PD showed that low serum vitamin D levels predicted an elevated risk of PD. Subsequent studies have shown that vitamin D deficiency is also associated with more advanced severity of disease. Neurodegenerative diseases are a class of proteopathies characterized by the accumulation of misfolded and/or aggregation-prone proteins. Neurodegenerative disorders such as AD and Parkinson's disease are characterized by pathogenic accumulation of misfolded proteins. Two major pathways regulating protein removal include autophagy-lysosome (AL) and the ubiquitin–proteasome system (UPS) to promote the physiological degradation of marked proteins, either through a lysosome-dependent or ubiquitination-dependent manner. Any aberrant event on these pathways can lead to protein deregulation and result in pathological onset. In this context AD is associated with the build-up of two proteins namely: β-amyloid and tau, which aggregate into extracellular plaques and neurofibrillary tangles. Both the AL and UPS are known to be involved in the degradation of the β-amyloid and Tau proteins. The endoplasmic reticulum (ER) is the organelle needed for proper folding of proteins to make them biologically functional. The misfolded proteins are subject to ER-associated degradation (ERAD). Malfunction of ERAD

results in the accumulation of misfolded proteins in the lumen and membrane of the ER, causing ER stress. The present study assessed the effect of vitamin D deficiency or insufficiency on the protein homeostasis pathways in the brain and its possible impact on the development of neurodegenerative disorders.

OBJECTIVES

- To study the effect of vitamin D deficiency/ insufficiency on different components of the ubiquitin proteasome pathway in the brain.
- To examine the effect of vitamin D deficiency/ insufficiency on the lysosome-autophagy and ER stress pathways in the brain.

METHODOLOGY

Brain tissue lysates were prepared from Vitamin D control and deficient rats from an earlier experiment. The catalytic activities of the 20S proteasome namely chymotrypsin-like [Ch-L], trypsin-like [T-L] and caspase-like [Cp-L] were measured using fluorogenic substrates specific to each activity. mRNA expression was studied using gene-specific primers by qPCR.

RESULTS

- Vitamin D deficiency was confirmed by serum calcium and 25(OH)D3 levels. The deficient animals were observed to be hypocalcemic, [9.5 vs 5.9 mg/dL] while the 25 (OH) D3 levels were significantly reduced in the deficient group [59.4 vs 4.52ng/mL].
- As shown below in figure 1, all the three enzymatic activities of the proteasome were observed to be significantly decreased in the deficient brains compared to control brains [panel A]. The mRNA expression of proteasome subunit genes: PSC8 & PSC2 was observed to be higher in the -D group compared to the +D or control group [panel B]. On the other hand, the mRNA levels of the deubiquinating enzymes [UCHL1 & UCHL5] were decreased in the deficient group compared to control group [panel C].
- The gene levels of ER stress markers [Bip, CHOP & PERK] were observed to be significantly ($p<0.05$) higher in the brains of the deficient group than the control group [figure 2].

Figure 1. Proteasomal enzyme activities

[A], mRNA levels of proteasome subunits [B] and deubiquitinating enzymes [C] in brain lysates from control and vitamin D deficient rats.

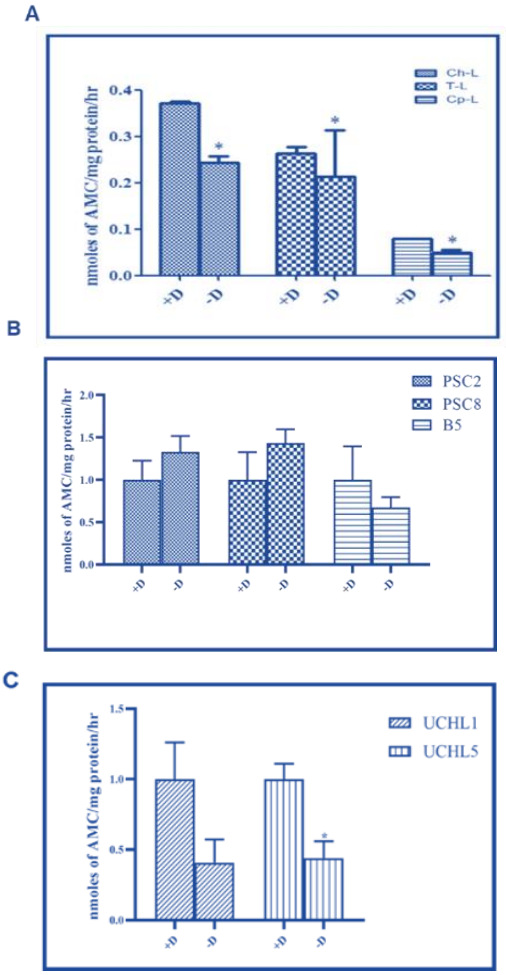
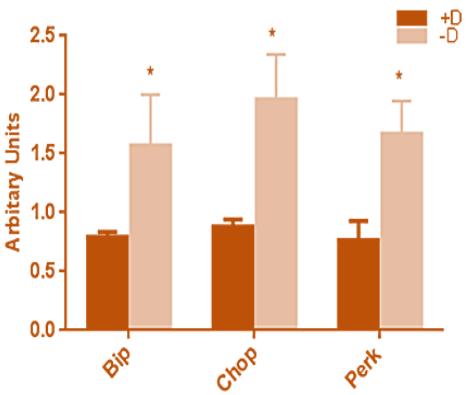


Figure 2. mRNA levels of ER stress marker genes in brain lysates from control and vitamin D deficient rats



INFERENCE AND CONCLUSION

This was a pilot study to examine the effect of Vitamin D deficiency on proteostasis pathways in the brain of rats. The preliminary data generated indicates that the ubiquitin proteasome system appears to be down-regulated while the ER stress pathway seems to be up-regulated in the brain during vitamin D deficiency. A full proposal has been submitted for extramural funding, which will enable us to decipher the in-depth role of protein homeostasis pathways during Vitamin D deficiency in the brain.

VI. PATHOLOGY AND MICROBIOLOGY

1. EFFECT OF MATERNAL IRON DEFICIENCY ANAEMIA ON IRON METABOLISM IN PLACENTA

It is observed that the amount of iron transferred from mother to fetus across the placenta is directed against a concentration gradient, the mechanism of which has been little studied. Although many proteins involved in iron influx and efflux across the placental syncytiotrophoblasts (Transferrin receptor 1/TfR1, Hemochromatosis protein/HFE, Divalent metal transporter 1/ DMT1, Ferroportin/FTN1 and Multicopperferroxidase/ Zyklopen) have been identified and the mechanisms behind iron transfer from mother to fetus are also beginning to be clarified, there are still many unanswered questions regarding the increased influx of iron from mother to the foetus, in the condition of maternal iron deficiency which need to be answered. In order to determine these mechanism(s) of adaptation in the placenta, we in the present study, examined the expression of the above mentioned iron transport proteins in placentas of iron deficient mothers and compared them with those in normal controls, which would probably help us to understand the mechanism behind the increased influx of iron to the fetus in spite of maternal iron deficiency.

OBJECTIVES

- To evaluate iron deficiency status in pregnant mothers, placenta and cord blood.
- To determine the expression levels of iron transport proteins in placenta.
- To correlate mother's iron status to that of cord blood and to levels of iron transport proteins in placenta.

METHODOLOGY

Two hundred pregnant women, in their third trimester of pregnancy, were recruited after taking written informed consent and divided into anaemic (Haemoglobin / Hb<11g/dl) and non-anaemic groups (Hb ≥ 11g/dl). After delivery, blood was collected from the mothers, and umbilical cord along with the placentas. Neonatal anthropometry was also performed to study neonatal development. Expression of the three crucial iron transport proteins (DMT1, FPN1 and Zyklopen) were studied in placentas by mRNA analysis and immunohistochemistry.

RESULTS

Of the 200 subjects recruited, 59% were anaemic with 60.35% having moderate anaemia. Most of red cell parameters were higher in cord blood of newborns of anaemic mothers. All the iron transport proteins (Ferroportin1, DMT1 and Zyklopen) showed a statistically significant increased immunohistochemical staining, proportionate to severity of maternal anaemia. Similarly, mRNA expression levels of genes of the proteins were higher in anaemic mothers in comparison to non-anaemic mothers.

2. IMPACT OF *SALMONELLA* KILLING LYTIC BACTERIOPHAGES ON PROBIOTIC MICROBIOTA

Bacteriophages are bacterial viruses that have great potential to use as biocontrol agents in foods (Hungaro et al, 2013). Lytic bacteriophages offer number of desired properties like, specificity for target bacteria, self-replication and self-limiting and ubiquitous presence in nature that makes bacteriophages an excellent tool for food safety (Mahony et al, 2011).

A healthy human intestinal microbiota is composed of symbionts, commensals and some pathobionts. Generally gutmicroflora comprised of several bacterial communities that involved in many functions such as metabolic barrier effect, and trophic function etc. In dysbiosis conditions, the composition of the intestinal microbiota is altered resulting in reduction of symbionts, commensals or increase in number of pathobionts (Mills et al, 2013). Dysbiosis in gut microflora leads to Irritable bowel syndrome (IBS), ulcerative colitis and crohn disease (Collins et al 2009; Rediff et al, 2010).

Recently we have isolated *Salmonella* killing lytic bacteriophages from sewage and tested its effectiveness as a biocontrol agent against *Salmonella* sp. Since these lytic bacteriophages are consumed along with the food, its effect on selective gut microflora has to be studied. Hence this study is proposed to see the impact of *Salmonella* killing lytic bacteriophages on selective probiotic microflora.

OBJECTIVES

1. To see the effect of *Salmonella* killing lytic bacteriophages isolate on probiotic microbiota.
2. To see the synergistic effect of probiotic microbiota on *Salmonella* killing lytic bacteriophages.

METHODOLOGY

For this experiment standard cultures of gut microflora like *Lactobacillus acidophilus, Lactobacillus lactis, Lactobacillus rhamnoses, Streptococcus thermophilus, and Bifidobacterium breve were* procured from American Type Culture Collection (ATCC). The double Agar Overlay technique was used to isolate *Salmonella* killing lytic phages from sewage. In vitro assays like turbidometric assay, spot test assay, and agar well diffusion assays were performed to see the effect of phages on probiotic microbiota. The experiment on the **s**ynergistic effect of probiotics on phages was also carried out. T-test was used to compare phage treated sample and its control at different time points. The same data were analyzed using univariate ANOVA with repeated measurements under each probiotic microflora at three-time points under the control and experiment groups.

RESULTS AND INFERENCE

The results of this study clearly showed the lytic bacteriophages are specific to *Salmonella* and will not harm the probiotic microflora and are likely to be safe for use in for food preservation.

VII. FOOD CHEMISTRY

1. PHYTOCHEMICAL, PHARMACOGNOSTIC AND NUTRITIONAL CHARACTERISATION OF THE *PANAX* SPECIES (ARALIACEAE) FROM THE EASTERN HIMALAYAN REGION, INDIA FOR ADDRESSING MEDICINAL, TRADE AND REGIONAL LIVELIHOOD SECURITY ISSUES

Plants are used as therapeutic medicine in many countries including India. Application of plants for the medicinal purpose is an ancient tradition, far older than the contemporary sciences of medicine, pharmacology and chemistry. The WHO has estimated that over 75% of the world's population still relies on plant derived medicines, usually obtained from traditional healers, for its basic health care needs. Mankind has been dependent for many centuries on various plants as nutrient, beverage, cosmetics, dye and medicine to maintain health and to improve quality of life. Among these plants, 'ginseng' (called by the local people), is one of the world's most widely used medicinal plant and has a long history. In Asia, *Panax ginseng* C.A. Meyer is considered as most precious plant among herbs, and the ginseng has been in the spotlight worldwide.

Panax L. (Araliaceae) consists of 19 species, of which 17 species are from eastern Asia and 2 from eastern North America. In India, the genus is represented by *Panax sikkimensis* Ban., *P. bipinnatifidus* Seem., *P. bipinnatifidus* Seem. var. *angustifolius* (Burkill) J. Wen and *P. pseudoginseng* Wall., found mostly in the north and North-eastern regions of India. *P. sikkimensis* has been reportedly described from the Shillong peak area (Meghalaya) of India. It is found wild in the hills of Arunachal Pradesh, Manipur, Meghalaya, Nagaland and also in Darjeeling area of West Bengal. The rhizome is used as a tonic and vitalizer by the local herbal practitioners. The local people collect rhizomes from the forest area and supply to the practitioners or sell in the local market. As a result these plants has become rare and endangered especially in Manipur and Nagaland.

There are no validated reports available regarding the *Panax* species and landraces of Eastern Himalaya to date. The *Panax* species namely *P. bippantifidus* Seem., *P. peudoginseng* Wall. and *P. sikkimensis* Ban. found in East Himalayan Biodiversity hotspots have been left without agronomic care for centuries due to unawareness among the local communities. As a result, three *Panax* species have been found in the Arunachal Himalayan region. The current project was undertaken to work out these particular species of *Panax* found in Arunachal Himalayan Region to establish its identity as a separate species both taxonomically as well as biochemically. It also aims to validate pharmacognostic and pharmacological characters of the plants to establish its efficacy. Evaluation of active secondary metabolites content and, ecological adoptive potential by taking original *Panax ginseng* as marker species so that medicinal, nutritional and nutraceutical characters of the

East Himalayan species could be validated for future drug development and molecular medicine.

Rationale

Due to immense medicinal and commercial prospects, the pharmacognostic studies, biomedical, biochemical validation studies of *Panax* species and landraces of East Himalaya (India) is urgently required to ensure sustainable harvesting, trade and conservation practices of these threatened taxa. It is therefore required that proper pharmacognostics and documentation is done besides studying its nutritional and phytochemical aspect especially taking into consideration the landraces of Arunachal Pradesh which has not been thoroughly explored till date. Since the soil condition and agro-climatic factors play a significant role in the geographical variation of a particular species of a plant, it can be assumed that *Panax* landraces of Arunachal Pradesh would show a distinct phytochemical and biochemical fingerprinting. This will pave a way for establishing distinct identity of the landraces to assert sustainable trade and conservation practices at regional and global level. Validation study of the major active principles called ginsenosides present in the *Panax* landraces of Arunachal Himalaya at qualitative and quantitative level would ensure the potency, safety and efficacy of its application.

Sample area and collection

Samples of *Panax bippantifidus* Seem collected at Ziro Lower Subansiri district, Arunachal Pradesh and *Panax sikkimensis* was brought from Gangtok, Sikkim. Taxonomic authentication was done by Dr. Hui Tag, Associate Professor and Head, Department of Botany, Rajiv Gandhi University, Arunachal Pradesh. Herbarium specimens for all collected species were prepared and the voucher specimen were properly prepared, labelled and deposited in the herbarium of the Department of Botany, Rajiv Gandhi University for future references. Rhizome of both the species were dried under the shade and supplied for the nutrient analysis.

OBJECTIVES

1. Survey of *Panax* species and landraces from Eastern Himalayas.
2. Pharmacological evaluation of crude extracts and phytochemical screening of bioactive compounds from the selected plants for authentication of bioactive principles and its pharmacological activity.
3. Determination of nutritive value and anti-nutrition factors in the selected species.
4. Germplasm multiplication through development of appropriate agrotechnology research by already identified prospective local cultivators (NGOs) and formulation of energy drink and herbal tea for local trade and uses.

RESULTS

Proximate composition

The proximate composition of the two different *Panax* species was analysed and given in the table 1. *Panax bipinnatifidus* had higher moisture and ash content (12.19 and 4.55 g respectively) than *P. sikkimensis* (10.11 and 3.88g respectively) whereas the protein content was comparable in both the species (9.25 and 9.87). Ash, carbohydrate and fat and energy were highest in *P. bipinnatifidus* (4.55, 49.73, 12.93 g and 341 kcal, respectively) and lowest in *P. sikkimensis* (3.88, 45.85, 8.55g and 320 kcal, respectively). It was inferred that total dietary fibre was higher in *P. sikkimensis* (17.88g) due to the presence of higher amount of

insoluble (15.70g) and soluble dietary fibre (2.18g) and it was lower in *P. bipinnatifidus* (15.23g).

Water soluble vitamins

The results of the water soluble vitamins analyzed using HPLC technique showed that *Panax sikkimensis* had a higher content of riboflavin (0.81 mg), niacin (4.10 mg), pyridoxamine (0.38 mg), pyridoxine (0.24 mg), total B6 (0.65 mg) and total folates (0.10 mg) compared to *P. bipinnatifidus*. It was also observed that the total ascorbic acid content was 1.58 mg in *P. sikkimensis* while it was below deductable limit (BDL) in *Panax bipinnatifidus*. There were no difference in the pantothenic acid (1.00 mg) and pyridoxal (0.03 mg) in both the species.

Table 1. Proximate composition and water-soluble vitamins in the Indian *Panax* species

Sl. No.	Nutrients	*P. sikkimensis*	*P. bipinnatifidus*
	Proximate composition and dietary fibre (g/100g)		
1	Moisture	10.11	12.19
2	Protein	9.87	9.25
3	Ash	3.88	4.55
4	Total Fat	8.55	12.93
5	Total Dietary Fibre	17.88	15.23
6	Insoluble Dietary Fibre	15.70	13.20
7	Soluble Dietary Fibre	2.18	2.03
8	Carbohydrate	49.73	45.85
9	Energy (Kcal)	320	341
	Water soluble vitamins (mg/100g of samples)		
10	Vitamin B2	0.81	0.20
11	Vitamin B3	4.10	3.21
12	Vitamin B5	1.00	1.00
13	Vitamin B6	0.65	0.20
14	Vitamin C	1.58	0.00
15	Vitamin B9	0.10	0.06

Minerals

Mineral and trace elements was analysed is tabulated in table 2. Iron content in both the species were 12.62 and 19.62 mg while, sodium was 7.58 and 12.62 mg. The trace elements such as arsenic, mercury, lead, cadmium and antimony were higher in *P. sikkimensis* while the minerals such as sodium and iron content was higher in *P. bipinnatifidus.*

Free sugars and oligosaccharides

Free sugars and oligosaccharides of the two *Panax* species were analysed. Out of the four different free sugars analysed, fructose and sucrose were present in both the *Panax* species and it ranged from 0.05 g to 0.08 g and 0.18 g and 0.21 g respectively with *Panax sikkimensis* showing the highest. Raffinose was the only oligosaccharide deducted in both the species which ranged from 0.05 and 0.10 g with the highest concentration in *P. sikkimensis*. Stachyose and Verbascose were BDL in both.

Table 2. Minerals and trace elements in the Indian *Panax* species

Sl. No.	Nutrients	Units	*P. sikkimensis*	*P. bipinnatifidus*
1	Iron (Fe)	mg	12.62	19.62
2	Magnesium (Mg)	mg	6.03	3.17
3	Manganese (Mn)	mg	366	273
4	Phosphorus (P)	mg	160	152
5	Potassium (K)	mg	1217	1078
6	Sodium (Na)	mg	7.58	12.62
7	Zinc (Zn)	mg	2.87	2.19
8	Copper	mg	0.62	0.59
9	Lithium (Li)	µg	14.40	29.86
10	Chromium (Cr)	µg	34.8	65.90
11	Cobalt (Co)	µg	16.66	6.80
12	Nickel (Ni)	µg	62.2	85.08
13	Arsenic (As)	µg	5.01	3.10
14	Selenium (Se)	µg	12.36	3.04
15	Molybdenum (Mo)	µg	11.60	11.15
16	Cadmium (Cd)	µg	3.60	1.39
17	Antimony (Sb)	µg	0.90	0.26
18	Mercury (Hg)	µg	1.26	0.69
19	Lead (Pb)	µg	6.82	1.72

Fatty acid composition

Fatty acid composition (fatty acid methyl esters, FAME) of the both *Panax sikkimensis* and *P. bipinnatifidus* is tabulated in table 5. From the results of the analysis, it was inferred fatty acids such as stearic (4.35 and 4.32%), arachidic (3.45 and 3.46%), lignoceric (1.71 and 1.68%), palmitoleic (0.65 and 0.65%) and eicosaenoic (0.44 and 0.46%) are found to have no significant difference between *P. sikkimensis* and *P. bipinnatifidus.* However, there was a difference observed in the concentration of palmitic acid (31.12 and 32.23%) and oleic acid (9.78 and 10.66%) among the two species. Total saturated fatty acids (TSFA) ranged from 43.07 to 45.33% and total mono unsaturated fatty acids (TMUFA) ranged from 10.87 to 11.78% with *P. bipinnatifidus* having the highest concentration. The amount of behenic acid ranged from 3.63 to 4.15%, linoleic acid ranged from 37.44 to 40.31%, a-linolenic acid ranged from 3.82 to 4.04% with total poly unsaturated fatty acids (TPUFA) ranged from 41.25 - 46.06% with the highest concentration in *P. sikkimensis*.

Amino acid composition

Eighteen amino acids were quantified (g/100g of protein) in both the *Panax* species andd summarised in table 4. Total amino acid content in *P. sikkimensis* and *P. bipinnatifidus* was 72.03 and 69.82 g respectively while the amount of total essential amino acids was found to be 23.73 and 24.79 g respectively. Sulphur containing amino acid cystine and methionine was

found to be the limiting amino acid in both the species. Among the 18 amino acids quantified, arginine was the highest in both *P. sikkimensis* (15.47 g) and *P. bipinnatifidus* (10.47 g). Glutamic acid was the second highest amino acid both in *P. sikkimensis* and *P. bipinnatifidus* (9.49 and 10.10 g respectively). Lysine content of *P. sikkimensis* was little higher (4.40 g) compared to *P. bipinnatididum* (3.73 g). Threonin, serine, glutamic acid, proline, glycine are found to be slightly higher in *P. bipinnatididum* than in their counterpart.

Table 3. Fatty acid composition (% of FAME) in Indian *Panax* species

Sl. No.	Fatty acids	*P. sikkimensis*	*P. bipinnatifidus*
1	Palmitic (C16:0)	31.12	32.23
2	Stearic (C18:0)	4.35	4.32
3	Arachidic (C20:0)	3.45	3.46
4	Behenic (C22:0)	4.15	3.63
5	Lignoceric (C24:0)	1.71	1.68
6	Palmitoleic (C16:1)	0.65	0.65
7	Oleic (C18:1n9c)	9.78	10.66
8	Eicosaenoic (C20:1n9)	0.44	0.46
9	Linoleic (C18:2n6c)	40.31	37.44
10	A-Linolenic (C18:3n3)	4.04	3.82
11	Total Saturated Fatty Acids (TSFA)	43.07	45.33
12	Total Mono Unsaturated Fatty Acids (TMUFA)	10.87	11.78
13	Total Poly Unsaturated Fatty Acids (TPUFA)	46.06	41.25

Polyphenol profile

The individual polyphenols in both the *Panax* species were quantified using HPLC technique. *P. bipinnatifidus* had little elevated levels of protocatechuic acid (0.68 mg), vanillic acid (1.23 mg), gallic acid (20.22 mg), chlorogenic acid (3.52 mg), luteolin (0.42 mg), quercetin (1.20 mg), myricetin (0.12 mg), hesperetin (0.34 mg), daidzein (0.21 mg). Whereas, *P. sikkimensis* found higher content of 2-coumaric acid (0.03 mg), caffeic acid (0.73 mg), ferulic acid (0.13 mg), luteolin 7 O glucoside (0.20 mg), kaempferol (0.04 mg). Gallic acid was found to be the highest polyphenol followed by chlorogenic acid and vanillic acid. There was no difference in sinapic acid content in both the species. It was also observed that even though traces of 2-coumaric acid and kaempferol were present in *P. sikkimensis*, it was BDL in *P. bipinnatifidus*.

Table 4. Amino acids composition (g/100g of protein) in Indian *Panax* species collected from Northeast India.

Sl. No.	Amino acid content	*P. sikkimensis*	*P. bipinnatifidus*
1	Tryptophan	1.1	1.1
2	Aspartic acid	7.55	6.86
3	Threonin	2.93	3.28
4	Serine	3.30	3.79
5	Glutamic acid	9.49	10.10
6	Proline	2.52	3.62
7	Glycine	2.87	3.29
8	Alanine	4.26	3.66
9	Cystine	0.85	0.89
10	Valine	2.90	3.10
11	Methionine	0.69	0.69
12	Isolucine	2.34	2.46
13	Luecine	4.22	4.22
14	Tyrosine	2.51	3.13
15	Phenylalanine	2.90	3.30
16	Histidine	1.75	2.15
17	Lysine	4.40	3.73
18	Arginine	15.47	10.47
19	Total Amino Acids	72.03	69.82
20	Total Essential Amino Acids	23.73	24.79
21	Limiting Amino Acid	Cys&Met	Cys&Met

VIII. FOOD TOXICOLOGY

1. MYCOTOXIN EXPOSURE, INTESTINAL INFLAMMATION AND CHILDHOOD STUNTING IN INDIA

Mycotoxins, the toxic secondary metabolites of certain species of fungi, are globally recognized for their adverse effects in humans and animals. Among the known mycotoxins, aflatoxins, fumonisins, trichothecenes and ochratoxin A are toxicologically significant. Aflatoxins are considered to be the most important mycotoxins globally because of their wide occurrence in various important food and feed commodities and their toxic and carcinogenic properties in human populations. Recently, the WHO has concluded that aflatoxins may be involved in growth impairment in young children based on various epidemiological studies in regions of Africa with high dietary aflatoxin exposure.

In India, mycotoxins assume a significant public health problem as highlighted by various incidents of acute mycotoxin poisoning chiefly from aflatoxins, ergot alkaloids, trichothecenes, and fumonisins. Unseasonal rains, improper post-harvest drying and storage of commodities, and insect infestation are some of the critical factors leading to mycotoxin contamination of food commodities in India. Mycotoxin contamination of important food commodities such as cereals, millets, groundnuts and spices is well documented in India. The possible role of chronic mycotoxin exposure in the high prevalence of child stunting in India is as yet unknown and warrants investigation. The Food Safety and Standards Authority of India (FSSAI) has established maximum limits for mycotoxins namely aflatoxins, aflatoxin M1, ochratoxin A, deoxynivalenol and patulin in cereals, nuts, oilseeds, spices, apple juice and milk.

On the basis of the above background a collaborative research project between the University of Aberdeen (UoA) (Lead PI UK) and the ICMR-National Institute of Nutrition (ICMR-NIN) (Co-PI India), has been undertaken under the Development stage grant of the Medical Research Council (MRC) UK Research and Innovation (UKRI) Global Challenges Research Fund (GCRF) to explore the mechanistic link between mycotoxin exposure, intestinal inflammation and child stunting in India. The study has been proposed with the hypothesis that exposure to dietary mycotoxins increases the risk of childhood stunting, involving a mechanism whereby mycotoxins act on the gut by decreasing intestinal barrier function, and inducing low-grade intestinal inflammation. This will impair nutrient utilisation and increase the risk of intestinal infections, thereby exacerbating the risk of malnutrition and stunting in a vulnerable group of children.

AIMS AND OBJECTIVES

- ✓ Establish mycotoxin contamination of foods frequently consumed by mothers and children
- ✓ Assess the ditary exposure to mycotoxins in children upto 5 years of age through chemical analysis of food and mycotoxin biomarkers in biological samples (urine, blood).

- ✓ Assess the prevalence of stunting in children up to 5 years of age, and establish its association with mycotoxin exposure and intestinal inflammation as well as assessing nutritional status and microbiome composition in a longitudinal study.

METHODS

The present project made an attempt to explore this association through a systematic review, and modelling of dietary mycotoxin exposure and stunting in young children in India using mycotoxin dataset collated from published and grey literature and population level food intake estimates from representative large scale food consumption databases from India i.e., the National Nutrition Monitoring Bureau dataset of rural children. The ICMR NIN was assigned the responsibility of compiling the Indian mycotoxin and food intake databases under the project to facilitate mycotoxin dietary exposure modelling using different scenarios of how dietary mycotoxin exposure and overall diet quality could affect adverse health outcome such as stunting. The UoA undertook the work on systematic review and modelling of mycotoxin exposure which is under progress at the UoA.

RESULTS

An evaluation of the number of samples contaminated with mycotoxin levels showed that out of a total of 27623 samples subjected for analysis of various mycotoxins, prevalence of contamination was about 47.7% (Table 1). Cereals and millets, tree nuts, spices and oilseeds showed prevalence of mycotoxin contamination above 50%. Milk had 43% prevalence in contamination with AFM1. Among the cereals, maize had the highest contamination prevalence and wheat lowest. Among the cereals maize had the highest prevalence of contamination. The prevalence of trichothecene mycotoxins namely DON and T-2 toxin contamination was observed to be highest in maize (29%) followed by rice (24%) and wheat (21%) while that of fumonisin contamination was largely reported in maize and sorghum with a prevalence of 75%. In spices, most of the data reported concerned aflatoxins in red chillies, black pepper, turmeric and ginger with an overall prevalence of 68% in contamination. The number of mycotoxins detected was highest in cereals, millets and spices thus signifying these as high risk foods.

An attempt was made to assess the extent of mycotoxin contamination reported in the database in relation to the maximum permissible limits set by the FSSAI for different mycotoxins in different food categories (Table 2). The percentage of samples exceeding the maximum limits of 10 and 15 µg/kg for aflatoxin B1 and total aflatoxins set by FSSAI in cereals and millets was highest in maize (30.8%), 10% in rice and lowest in sorghum (5.7%). In milk samples, 11.2% of the samples exceeded the FSSAI MLs of 0.5µg/kg for AFM1 in milk. Among the oilseeds, FSSAI maximum limits for AFB1 exceeded in 25% of groundnut samples. In wheat, FSSAI maximum limits of 1000 µg/kg for DON were exceeded in 8.5% of samples and maximum limits of 20 µg/kg for OTA in 1% of wheat samples respectively that were investigated in the studies included in the database. In apples and apple juice 7% of samples exceeded the FSSAI maximum limits of 50 µg/kg for patulin. The above data indicated that considerable number of food commodities of dietary importance particularly for young children such as cereals and milk contained mycotoxins above the food safety limits set by the FSSAI.

Table 1- Mycotoxin data in different food items

Food item / Description	No. of studies	Total No. of samples analysed	Total number positive	% positive
Rice & rice products	14	3586	1353	37.7
Wheat & wheat products	13	2446	496	20.3
Maize & maize products	23	4481	2737	61.0
Barley and oats	3	95	18	18.9
Millets	15	3045	2216	72.8
Oilseeds-Groundnut	14	4510	1735	38.5
Other oilseeds/oils	13	1038	325	31.3
Oilseed cake (Groundnut and others)	6	528	369	69.8
Tree nuts and dried fruit	10	543	444	81.8
Spices	17	1681	1153	68.6
Fruits/fruit juice-Apple	2	170	33	19.4
Pulses	4	397	88	22.2
Milk	21	4337	1899	43.8
Milk products	3	207	21	10.1
Dried vegetables	2	60	51	43.3
Dried morels-Morchella species	1	78	38	48.7
Infant foods	1	29	25	86.2
RTE Fruit jam	1	40	20	50
Chewing products - Pan masala & Tobacco	1	29	29	100
Non-food items – animal/poultry feed	4	323	139	43
Total*		27623	13189	47.7

**Total represents number of samples analysed and positive as reported in 108 studies*

Table 2 - Number of samples with mycotoxin levels exceeding the FSSAI maximum limits

Food item	Mycotoxin	No. analysed	FSSAI maximum limits (µg/kg)	No.> FSSAI maximum limits*	Percent
Rice	AFB1	3406	10	327	9.6
Sorghum, millets	AFB1	2071	10	116	5.7
Maize	AFB1	4177	10	1284	30.7
Wheat	AFB1	2175	10	365	16.8
	DON	164	1000	14	8.5
	OTA	221	20	2	0.9
Treenuts	AFB1/AFs	543	10	76	14.7
Spices	AFB1	1681	15	181	12.0
Groundnut	AFB1	4423	10	1139	25.8
Other oilseeds	AFB1	1038	10	161	15.9
Pulses	AFB1	397	10	34	8.6
Fruits-Apple	PAT	170	50	12	7
Milk	AFM1	4337	0.5	482	11.2

**Based on available/reported data on mycotoxin levels in the Database*

INFERENCE AND CONCLUSION

The Indian mycotoxin database has considerable potential to be used as a template for planning surveillance and monitoring programmes on mycotoxins by the FSSAI or any regulatory agency concerned with quality and safety of food commodities.

Based on the outcome of dietary mycotoxin exposure modelling using population level estimates of food intakes and prevalence of stunting in Indian children and the systematic review, there is a scope for further investigations in high mycotoxin risk regions and regions with high prevalence of stunting to assess the role of mycotoxin exposure on child stunting in India.

2. DEVELOPMENT OF NEW BIO-MARKER FOR QUANTIFICATION OF ACETYLCHOLINESTERASE (ACHE) ENZYME ACTIVITY, ORGANOPHOSPHATE, CARBAMATES, AND NERVE AGENT IN BIOLOGICAL AND AMBIENT MATRICES

There is general concern about residues of pesticides in drinking water and food products and their impact on human health. The use of pesticides in agriculture, especially organophosphorus (OP) and carbamate pesticides, which are the most widely and commonly used insecticides all around the world. There is strong evidence that acute and chronic exposure to these compounds have adverse health effects. Acetylcholinesterase (AChE) is a key enzyme in the nervous system and its activity is essential to normal neuromuscular and brain function as the principal enzyme responsible for the breakdown of the neurotransmitter,

acetylcholine (ACh) (Ondrej Holas 2012). The inhibition of AChE activity has been used widely as a biomarker of exposure to organophosphorus pesticides (OPs).

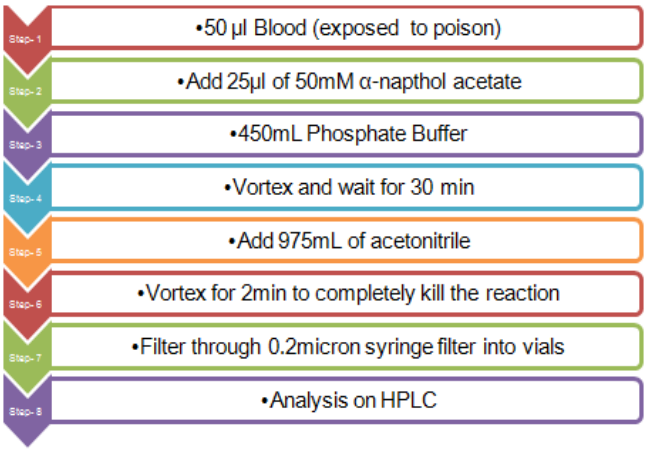
Thus, the focus of this project is on development of prototype method for estimation of AChE, OP and carbamate exposure using few µl amounts of blood sample, is technically very simple to perform, is rapid and robust while providing excellent sensitivity. In addition, with only small modification the method can be adapted to rapid measurement of water, soil and surface OP and carbamate contamination.

AIMS AND OBJECTIVES

- To develop a macro scale fluorescence /UV method for acetylcholinesterase (AChE) determinations in biological samples using 1µl of blood.
- To develop a new biomarker for the determination of toxic compounds in biological/ambient matrices.
- To establish a dose-response relationship between toxicants and AChE activity in biological matrices.
- To promote this method for general use in the treatment of exposed people

METHODS

Extraction Procedure: The extraction procedure is shown systematically as below:



Instrumentation

The Shimadzu Ultra-Fast Liquid Chromatography system, (Shimadzu, Japan) was utilized for separation of 1-naptho, 1-napthol acetate, 2-napthol and 2-napthol acetate. The instrument was provisioned with High-Speed Pumps (Shimadzu 20AD Pump), a PDA (Photo Diode Array) detector (SPD-M20A), an auto sampler (SIL-HTC), a degasser (DGU-20A) and a control module. The chromatographic separation was executed on a Waters XTerra MS - C18 (100mm x 4.6mm) 3.5µm column (Milford, Massachusetts, USA). The software installed in the system was LC solutions for data analysis and evaluation.

Liquid chromatography conditions (LC)

Chromatographic separation was achieved for the all compounds using a liquid chromatograph equipped with a C18 reversed-phase column (Waters XTerra MS - C18 100mm × 4.6µm, 3.5µm) 10 µL were injected into the instrument using the Schimazdu auto-sampler.

Assay optimization in water

In 100-µL clean water (LC-MS grade), a mixture of 50-µmole of each 1-napthol acetate and 2-napthol acetate (100-µl) was added and followed by AChE (2 unit/µl) in phosphate buffer (pH 7.0) (450 µl). After different time interval, the reaction was stopped using 975µL acetonitrile and then filtered through 0.2µm syringe filter. 10 µL reaction mixture was injected in HPLC on the same condition mention above.

Assay optimization in blood

The basic methodology is simple. Blood samples are incubated at room temperature with the substrate in the buffer for 30 minutes at which time the reaction is stopped by the addition of acetonitrile, the samples filtered and injected into the HPLC system. The conversion of 1-naphthol acetate to 1-naphthol catalyzed by the AChE in the blood is followed by the simultaneous disappearance of the 1-napthol acetate and appearance of the 1-napthol on the chromatograms.

Validation of methods in poisoning cases

During the experiment, it was observed that the 1-napthol acetate was completely converted in 1-napthol at 30 minutes of initiation of reaction which proves that the enzyme is very reactive and responsible for the conversion of 1-napthol acetate to 1-napthol. Similarly, in 1-mL phosphate buffer, 1µL enzyme was added and ran on HPLC after 30 minutes; no peak was observed which acts as a control.

RESULTS

Subject

1. ***Healthy volunteer blood*:** Six healthy volunteers were selected for blood donation and 2 mL blood has drawn from each healthy volunteer for performing the experiment for conversion of 1-napthal acetate to 1-naphthol and 2-naphtal acetate to 2- naphthol.
2. ***Poisoning Cases:*** For validation of the method, the target subject of this study was the organophosphate poisoning cases referred to the Osmania hospital in Hyderabad, Telangana, India. 23 patients were identified and blood samples were collected from these patients until they are discharged or deceased.

Liquid chromatography conditions (LC): Compositions of water (gradient-A) and acetonitrile (Gradient-B) were used, and best sensitivity and separation for all of the compounds of interest were achieved using the isocratic compositions of mobile phase water–acetonitrile (60: 40, v/v) was pumped at a flow-rate of 1 mL/min. The column oven temperature was held constant at 25 °C. The wavelength was measured at 275 nm. All measurements were carried out in phosphate buffer (pH 7.0) as well as acetonitrile at room temperature and result integrated by an integrator.

Correlation Coefficient (R2): The obtained correlation coefficient for all compounds was ≥0.999 and ≥0.997 for all compounds of our interest. ***Assay optimization in blood***

Healthy individuals were enrolled for control groups and individuals with medical complications were excluded from the study. Blood samples collected from the subjects were analyzed on HPLC for the determination of the normal range of AChE. The reaction of 1-napthol acetate conversion to 1-napthol at different time intervals until the time of total conversion is described below in figures 1a-h and table 1.

Table 1. Rate of 1-napthol Product formation

Time	Area	Product
0	7786	0.083
5	636020	6.761
10	1073904	11.416
15	1416443	15.057
20	1641148	17.445
25	1725160	18.338
30	1794759	19.078
35	1794759	19.078
40	1794759	19.078

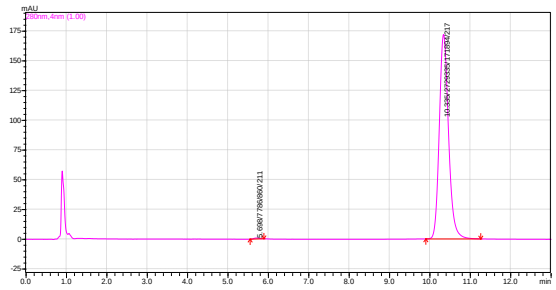


Fig 1a: Reaction of 1-napthol acetate conversion to 1-napthol at 0 min

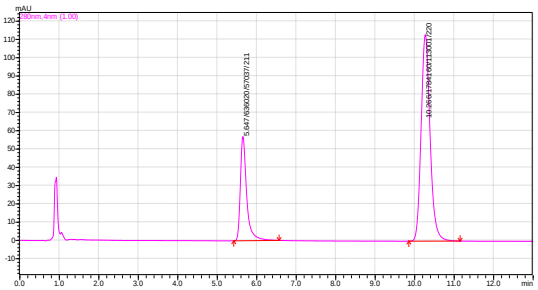


Fig 1b: Reaction of 1-napthol acetate conversion to 1-napthol at 5 min

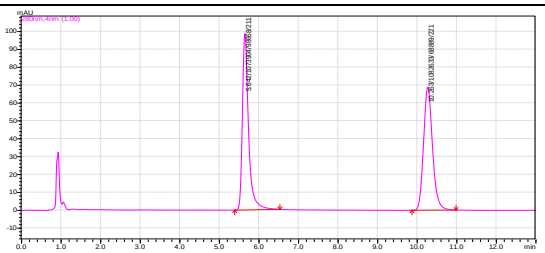


Fig 2c: Reaction of 1-napthol acetate conversion to 1-napthol at 10 min

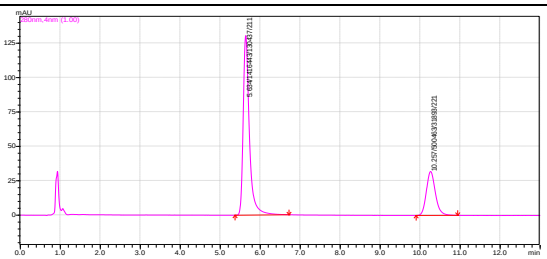


Fig 1d: Reaction of 1-napthol acetate conversion to 1-napthol at 15 min

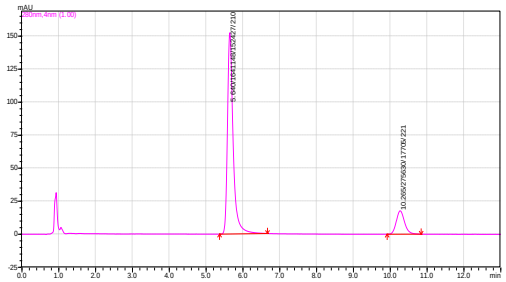


Fig 1e: Reaction of 1-napthol acetate conversion to 1-napthol at 20 min

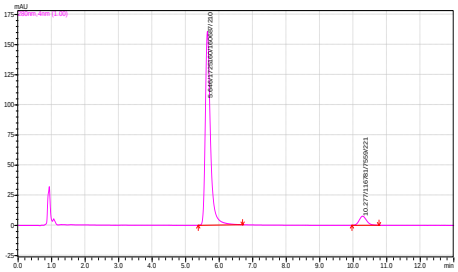


Fig 1f: Reaction of 1-napthol acetate conversion to 1-napthol at 25 min

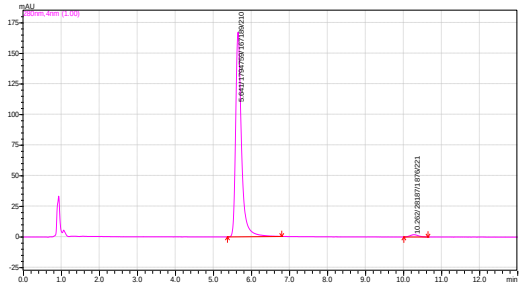


Fig 1g: Reaction of 1-napthol acetate conversion to 1-napthol at 30 min

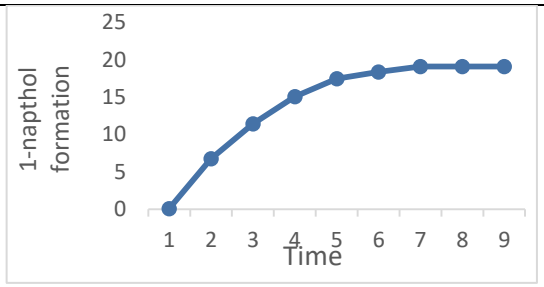
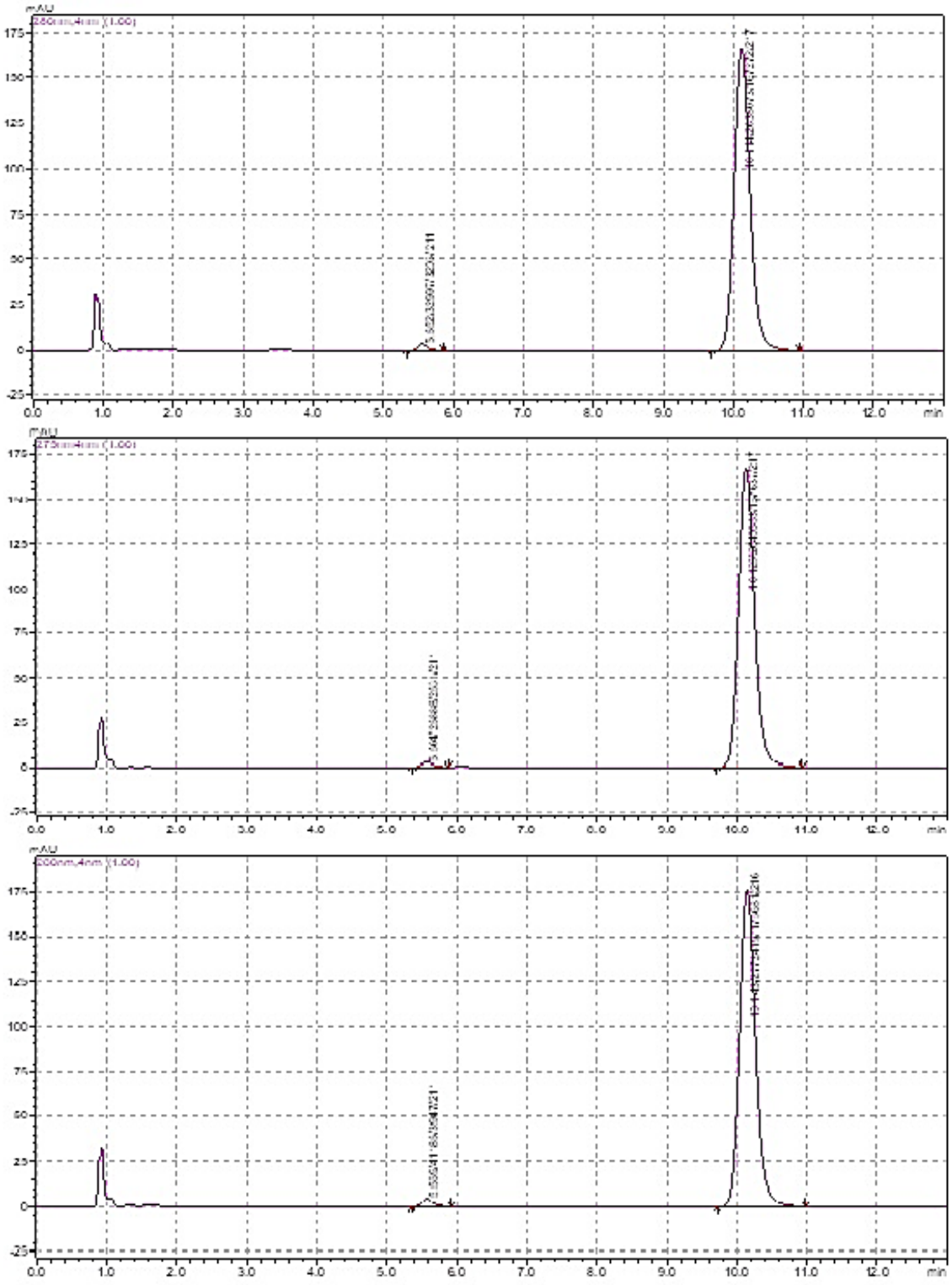


Fig 1h: Curve showing 1-napthol acetate conversion to 1-napthol at different time intervals

To establish a dose-response relationship between toxicants and AChE activity in biological matrices.

The chromatograms showing the reaction are shown below (Fig-2) and also this experiment has showed the inhibition of ACHE by chlorpyrifos.

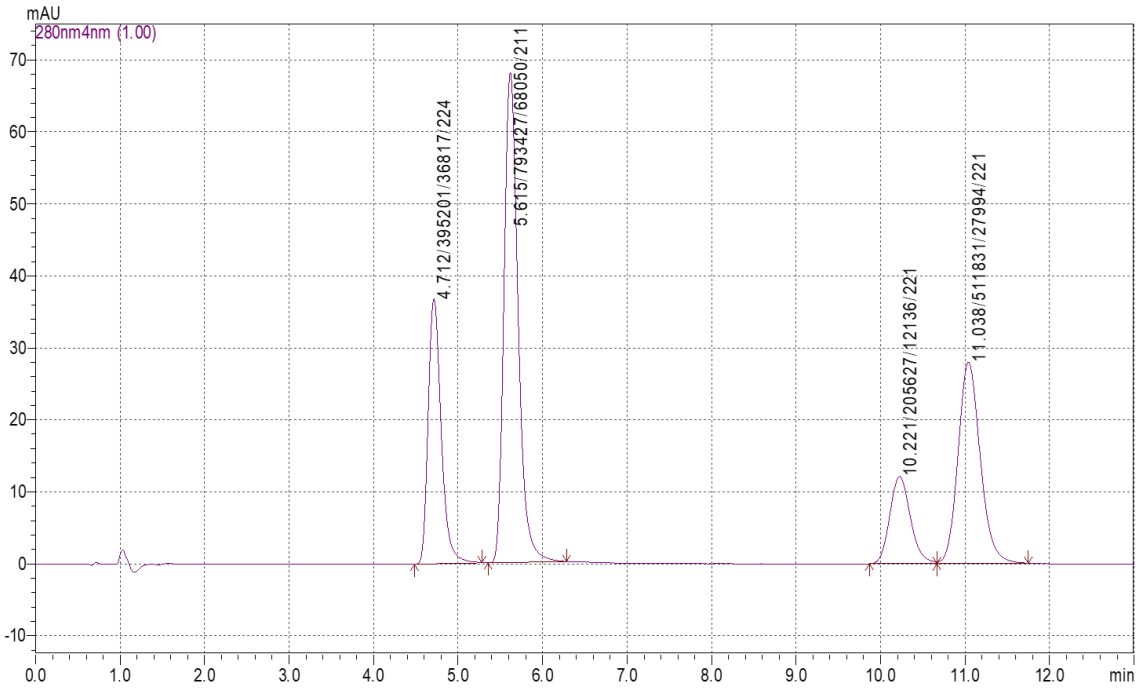
Figure 2: Chlorpyriphos reaction with enzyme



METHOD PARAMETERS

It was found that the separation was easily achieved using acetonitrile–water (40:60, v/v) as mobile phase on C_{18} reversed-phase column with a flow rate of 1 mL/min. Fig. 3 shows the HPLC chromatogram of the standard mixture of 1–napthol (RT: 5.6 minute), 2-napthol (RT: 4.7 minute). 1-naptholacetate (RT: 10.21 minute), and 2-napthol acetate (RT: 11.08 minute). The running time of the chromatograph is 13 minute. The change of retention time (RT) may be ± 0.1 minute for each run but the relative retention time is the same for all compounds throughout the analysis.

Fig.3 HPLC chromatogram of the standard mixture



Validation of methods in op's poisoning cases:

This method was applied to the blood samples to estimates the enzyme activity in unit/ml. The concentration of products (1-napthol, biomarkers of the enzyme, the product concentration was determined using a standard linearity curve of 1, 2, 4, 6, 8, 10, and 12umole of 1-napthol.) divided by time gives enzyme activity. The detail concentration of healthy volunteer as reference as well as OP's poisoning cases has shown in table 2 & 4. In this way, this method could be used for quantification of enzyme activity to the exposed person with pesticides, carbamate, nerve agent and some metals. The merit of this method is that the minimum blood requirement, quick result observed, confirmatory technique and friendly and cheaper methods.

Table 2. Control samples enzyme concentration

S.No	Name of Toxin consumed	Patient Code	Enzyme Conc. Unit/mL
1	Control	RK	25.2161
2	Control	SS	21.3111
3	Control	DL	19.5691
4	Control	MM	18.9350
5	Control	DY	17.2989
6	Control	SNS	28.6175

Table 3. Control samples statistical data

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic					Std. Error	Statistic	
VAR00001	6	11.32	17.30	28.62	21.8246	1.74956	4.28552	18.366
Valid N (list wise)	6							

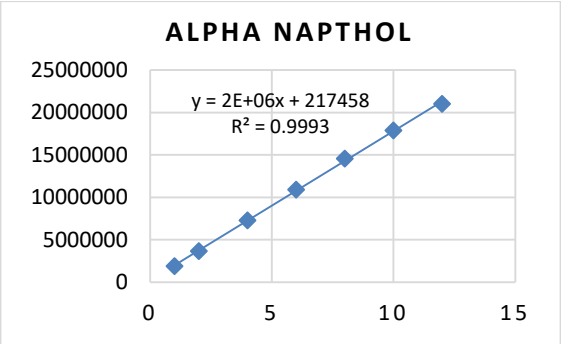
Table 4. Exposed samples enzyme concentration

S.No	Name of Toxin consumed	Patient Code	Enzyme Conc. Unit/mL
1	Monocrotophos	EW 01	1.7990
2		EW 02	1.9988
3		EW 03	3.8615
4		EW 04	8.8193
5		EW 05	7.0084
6		EW 06	10.5181
7	Dimethioate	BH 01	27.9757
8		BH 02	26.4700
9		BH 03	27.1052
10		BH 04	27.5952
11		BH 05	26.8318
12	Unknown	DR 01	6.5470
13		DR 02	5.2668
14		DR 03	4.1069
15		DR 04	3.8363
16	Chloropyrifos	MH 01	19.0173
17		MH 02	18.1965
18		MH 03	20.7442
19		MR 01	15.5453
20	Phorate	MR 02	20.9744
21		MR 03	17.9718
22	Dimethioate	NS 01	8.9033
23		NS 02	11.0806
24		NS 03	12.3375
25	Chloropyrifos	RD 01	12.0677
26		RD 02	10.8213
27	Monocrotophos	RL 01	1.3037
28		RL 02	0.8172
29		RL 03	1.7820
30	Monocrotophos	SR 01	27.6397
31		SR 02	26.8786
32		SR 03	26.3255

S.No	Name of Toxin consumed	Patient Code	Enzyme Conc. Unit/mL
33	Monocrotophos	YD 01	21.5315
34	Monocrotophos	SV 01	5.2227
35		SV 02	4.6352
36		SV 03	6.0929
37		SV 04	6.4711
38		SV 05	8.9368
39		SV 06	7.8883
40	Chloropyrifos	AJ 01	22.0363
41		AJ 02	20.7234
42		AJ 03	20.3081
43		AJ 04	14.3970
44	Asephate	CV 01	11.0774
45		CV 02	15.6615
46		CV 03	13.4813
47		CV 04	18.0146
48	Dichlorovos	FJ 01	15.1301
49		FJ 02	15.6963
50		FJ 03	14.6191
51		FJ 04	18.0238
52		FJ 05	17.8372
53		JT 01	21.5112
54	Monocrotophos	JT 02	0.9958
55		JT 03	1.1231
56	Profanephos	JY 01	7.5747
57		JY 02	7.9158
58		JY 03	7.6856
59		JY 04	10.1101
60		JY 05	11.9697
61		JY 06	11.2194
62	Monocrotophos	NH 01	3.7106
63		NH 02	2.5050
64		NH 03	3.9198
65		NH 04	4.6444
66		NH 05	4.9591
67	Chloropyrifos	RJ 01	8.9363
68		RJ 02	5.6339
69	N/A	RRJ 01	24.0125
70		RRJ 02	26.8413
71	Gulikalu	RRD 01	9.0642
72		RRD 02	10.1638
73		RRD 03	6.6132
74		RRD 04	10.0524
75	Unknown	SA 01	6.7996
76		SA 02	7.7334
77		SA 03	10.2128
78		SA 04	10.8923

79		SA 05	9.6782
80		SA 06	14.1224
81	Deltamethrene & Trizophos	SD 01	13.7066
82		SD 02	14.0255
83		SD 03	14.6443
84	Chloropyrifos	SVR 01	23.7756
85		SVR 02	19.3642

(Fig.4a-b) LOD of alpha napthol and alpha napthol acetate



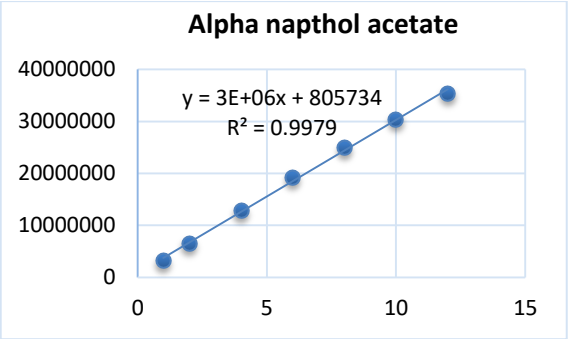
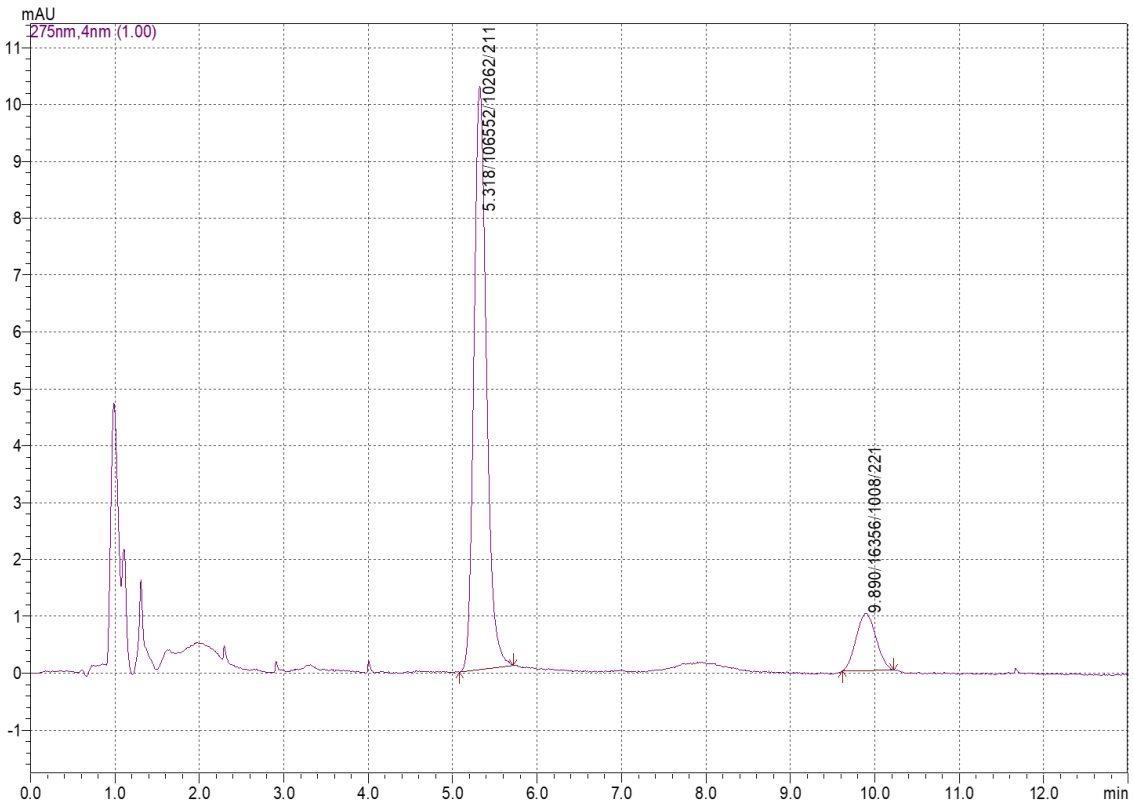


Figure 5. HPLC chromatogram of conversion trail with 10µL blood



Final Trial to reduce the volume of blood used

Final Trial to identify the enzymes has been successfully identified using 10 µL of blood and the complete conversion of the substrate can be seen in the below HPLC chromatogram.

INFERENCE AND CONCLUSION

The developed method fulfils all the objectives of the proposed study and the method is unique to its type as it helps in obtaining results within 10min of extraction of pesticide exposed samples and assesses the achetylcholinestrase enzyme from the samples by using 1-napthol as a marker for identification.

The method could identify the AChE in just 20 uL of blood which is similar to the volume obtained by a prick on the finger. This volume of blood for AChE estimation is the lowest amount of blood that has been utilized whereas all other methods require the least of 1ml of blood.

The time required for identification on the HPLC method is 10min which signifies itself to the developed method which is so far the least time utilized for identification of AChE as compared to the present available methods.

LIST OF PhD STUDENTS

S. No	Name of Student	Name of Guide	Thesis Title
	AWARDED		
1	**N. Himaja**	Dr. Hemalatha R	"Effect of Fructooligosaccharide (FOS) and Probiotics on maternal and fetal immune programming in mice"
2	**K. Narender**	Dr. Hemalatha R	"Prebiotic potential and other beneficial effects of Ocimum, Ginger and Piper nigrum on immune-inflammatory disease conditions"
	ONGOING		
3	**V.Sudharshan**	Dr. Hemaltha R	"Iron homeostasis in adolscent girls with iron defeciency anemia: Role of genetic variants and gut microbiome"
4	**D.Vasundhara**	Dr. Hemalatha R	"Efficacy of probiotic supplementation (*Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14*) in pregnant women with Bacterial Vaginosis"
5	**Mohd. Shujauddin**	Dr. Hemalatha R	"Dynamics of intrauterine inflammation in relation to maternal malnutrition-Foetal outcome and metabolic changes: Effects of fructooligosaccharides in Hamsters"
6	**KB. Chathyushya**	Dr. Hemalatha R	"Isolation and characterisation of human breast milk microbiome"
7	**Mr.H.E.Harshavardhana**	Dr. G. Bhanuprakash Reddy	Profibrotic mechanisms in diabetic complications: Role iof dietary agents
8	**Ms.Richa Pande**	Dr. G. Bhanuprakash Reddy	Development of a Comprehensive symbol scheme for the food labels which can facilate consuemer education
9	**Ms.Paromita Banerjee**	Dr. G. Bhanuprakash Reddy	Promoting nutrition and health of corporate employees with workplace intervention- A study using communication for behavioral impact (COMBI) approach
10	**Mr.S.Udaykanth**	Dr. G. Bhanuprakash Reddy	Role of vitamin B12 in diabetic neurodegerneration
11	**MS.A.Santhoshi Vani**	Dr.G.Bhanuprakash Reddy	Studies on Th2 Cytokines and Micronutrients in Asthma
12	**Mr.Krishna Kalyan**	Dr.G.Bhanuprakash Reddy	Studies on a functional food formulation for diabetes and its complications
13	**James Thomas**	Dr Bharati Kulkarni	Role of Maternal stress and Iron status on cognition in the infants.
14	**Sandip Kumar Kotturu**	Sudip Ghosh	Role of microRNAs in the development of obesity and diabetes

15	**Divya Kumari**	Sudip Ghosh	Understanding molecular cross-talks among functionally contrasting cell lines during zinc deficiency
16	**Arnab Chatterjee**	Sudip Ghosh	Tanscriptomic analyses of functionally contrasting tissues involved in zinc homeostasis
17	**Madhumanti Dhua**	Sudip Ghosh	Amelioration of insulin resistance by TLR2 ligands from Mycobacterium tuberculosis
18	**Summaiya Alam Lari**	Dr. J. Padmaja	Assessment of pesticide residues penetration into the skin using protective gear in field conditions
19	**Arun Pandiyan**	Dr. J. Padmaja	Association between pesticide residues concentration in tissues and with the Lymphoma, Leukemia and Breast cancers
20	**Mehrajuddin Bhat**	Dr. Ayesha Ismail	Vitamin D Deficiency Induced Muscle Wasting and Adiposity Changes: Biochemical and Molecular Mechanisms
21	**Bindu Noolu**	Dr. Ayesha Ismail	Studies on the Anticancer Properties of Murraya *koenigii* Leaves: Role of Proteasome Inhibition
22	**Srividya G**	Dr. Ayesha Ismail	Anticancer potential of Cinnamon and its bioactive component(s) in prostate cancer: *In vitro* & *In vivo* Studies
23	**Ramesh G**	Dr. Ayesha Ismail	Molecular mechanism(s) involved in Vitamin D deficiency induced Muscle Atrophy
24	**Athira AS**	Dr. Ayesha Ismail	Vitamin D deficiency induced cardiomyopathy: Role of ubiquitin proteasome and signal transduction pathways
25	**Hanuma Naik**	Dr. P. Raghu	Mechanism of Iron and Zinc interactions in intestinal cells
26	**Puneeta Singh Yaduvanshi**	Dr. P. Raghu	Studies on the regulation of intestinal iron absorption by iron and Zinc
27	**Konda Venu**	Dr. P. Raghu	Role of Zinc in erythropoeisis
28	**V. Srinivas**	Sanjay Basak	Role of fatty acids and glucose in metabolic and angiogenic function of placenta: implications for fetal growth and development
29	**V. Sai Kanth**	Sanjay Basak	Maternal exposure of endocrine disrupting chemicals during reproductive development: impact on reprodructive and metabolic programing in the offspring
30	**Swetha Boddula**	Dr MS Radhika	Etiology of severe anemia and efficacy of treatment in school children
31	**Sangita Thenarangam**	Dr MS Radhika	To be decided, Broad Area is Child Nutrition
32	**Naga Muralidhar Merugu**	Dr. K. Rajender Rao	Genetic and epigenetic approach towards obresogenesity- using a rat model

33	**Venkatakrishna prasad SM**	Dr. K. Rajender Rao	Biochemical and molecular studies on role of diet in the induction of obesity: rat as a model system
34	**DM Dinesh Yadav**	Dr. K. Rajender Rao	Studies on identification of candidate gene (s) associated with obesity in WNIN/Ob rat
35	**Suresh Kondeti**	Dr. K. Rajender Rao	Studies on the regulation of glucose homeostasis by fibroblast growth factor 21 in a pre-diabetic obese rat model
36	**Srinivas Myadara**	Dr. K. Rajender Rao	Studies on host-genotype impact on the bacterial community in the GI tract
37	**Anuradha R**	Dr. K. Rajender Rao	Effect of paternal calorie restriction of diet induced obese on metabolism of their offspring
38	**Aruna T**	Dr. S. Devindra	Nutritional quality, prebiotic potential and other health benefits of raffinose family oligosaccharides ofpigeopea (*Cajanus cajan, L*).
39	**Deepika T**	Dr. S. Devindra	Studies on resistant starch content of some plant foods and development of low glycemic index food products
40	**Shreyas Elma Mathew**	Dr. S. Devindra	Nutritional quality, prebiotic potential and other health benefits of raffinose family oligosaccharides of Bengal gram (*Cicer arietinum L*).
41	**Sumi MS**	Dr. S. Devindra	Nutritional quality, prebiotic potential and other health benefits of raffinose family oligosaccharides of Green gram (*Vigna radiata*)
42	**SoumyaRanjan Pradhan**	Dr. S. Devindra	Analysis of macronutrients, micronutrients, and glycaemic index of commonly consumed ready-to-eat foods
43	**Pallabika Gogoi**	Dr. Paras Sharma	Nutritional Characterization and Bioaccessibility Studies of Polyphenols and Nutrients from pigmented rice and Maize
44	**Anwesha Mahajan**	Dr. Paras Sharma	Tentative Title: Characterization, encapsulation and bioaccessibility studies of polyphenols extracted from fruits and vegetable waste/by-products

LIBRARY AND DOCUMENTATION SERVICES

Library continued to cater to the documentation and Nutrition information needs of the Institute and other Research Organizations, Home Science and Medical Colleges. The library has played a key role in reference activities by offering information dissemination services like MEDLINE Searches, Proquest Medical Library Full Text Database of journals and other online retrieval activities using the LAN Network of the Institute. Library continued to participate in exchange of data, journals and information using the URL<http://Groups.yahoo.com/group/ICMR Librarians>.

Resource Sharing and User Education Programmes etc are continuously being undertaken by the Library. Institute's scientific papers submitted for publication in Scientific Journals are being routed through the Library and Maintaining data-base of the published papers is also made accessible through on-line services using NIN Website (www.nin.res.in).

The Library services have been continuously strengthening with the support of Indian Council of Medical Research for accessing E-journals from JCCC@ICMR and J-Gate database. The Library is also a member of ERMED Consortia of National Medical Library, New Delhi for accessing E-journals Online Subscription of 4 Core Journals such as LANCET, NATURE, NEJM, SCIENCE being renewed by ICMR regularly.

The Library has continued to provide Photostat facility to the NIN staff.

The following library services are expanded:

1. NEW ADDITIONS

Books	11
Reports	84
Thesis / Dissertations	6

2. OTHER ACTIVITIES

Journals Bound	276
Visitors using the Library	1253
Circulation of Books/Journals etc	181
No. of E-mails sent outside	650
No. of E-mails received	666
Photocopying done (No. of pages)	87,039
No. of INTERNET Searches provided	68
No. of Reprints sent	39

3. TOTAL LIBRARY COLLECTIONS

Books	18,289
E – Books	36
Journals (Bound Volumes)	41,752
Journals received (Gratis/Exchange) for 2020	108
Microforms (Microfiche)	1,080
Slides	280
Reports	14,168
Theses & Dissertations	472
MEDLINE CDROMS Discs	383
Current Contents on Diskettes with abstracts	664
Proquest (Full Text E-Journals) on CD ROMS	495
General CD's	331

SCIENTIFIC PUBLICATIONS

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