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INDIAN COUNCIL OF MEDICAL RESEARCH

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Annual Report

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RESEARCH HIGHLIGHTS

1. Assessment of current nutritional status of below five year children and performance of ABM project in the districts of Madhya Pradesh

The study was undertaken to assess the changes in the nutritional status as well as IYCF indicators among <5 year children after launching of Atal Bal Arogya Evam Poshan Mission (ABM) implemented during the past 5 years (2011 v/s 2016). It was a community based cross-sectional study carried out in all the districts (51) of Madhya Pradesh by adopting systematic random sampling procedure. A total of 37924 <5 year children from 30585 households were covered. About 79% fathers and 68% mothers were literate as against 71% and 48% covered in previous survey. Proportion of HHs living in pucca house has increased from 10% to 24% during the same periods. Use of sanitary latrine was 33% as against 12% in 2011. Antenatal check ups has increased from 78% in 2011 to 98% in 2015 and registration in first trimester has increased to 76% from 28% in last survey. Consumption of ≥90 IFA tablets during pregnancy has improved from 20% in 2011 to 59% in 2015. There was change in prevalence of low birth weight (19% vs 16%). The initiation of breastfeeding within 1 hr of birth has increased to 58% from 26% in 2011. About 93% children were exclusively breastfeeding in current survey as against 71% in 2011among 0-5 mo. children, while exclusive breastfeeding up to 6 months has increased from 43% to 64% among 6-11 mo. children. The ICDS supplementation has also improved among 6 to 59 months children (81-84% in 2015 vs 68-72% in 2011). The prevalence of underweight, stunting and wasting has declined to 41%, 43% to 50% in 45 districts while 5 districts showed increase in prevalence.

2. Introduction of vitamin C rich fruit in supplementary nutrition programme for improving micronutrient status, gut health, growth and development: A randomized trial among ICDS pre-school beneficiaries

Iron deficiency anemia (IDA) is a significant public health issue in India affecting nearly all the vulnerable segments of the population. IDA is caused due to low consumption of iron-rich foods combined with its poor bioavailability. To date, interventions aimed at inclusion of vitamin C rich fruits in the regular diet showed to improve iron absorption, but the effect on iron status is inconclusive. Considering the ongoing national program for preschoolers in India, a cluster randomized controlled trial (RCT) was designed to test the hypothesis that inclusion of vitamin C rich fruit in a regular meal would improve iron absorption and lead to better child iron and micronutrient status, cognitive development and gut health while reducing morbidity. A 3-arm, open-labeled, assessor-blinded, cluster randomized control trial among 2-5yr old ICDS beneficiaries was adopted (CTRI/2014/09/004983) in sixteen villages of Alair sub-district, Nalgonda, Telangana State. Villages were randomized to experimental guava group (GG), passive control banana group (BG) and active control cucumber group (CG). Participants (n=407) received 25g of fruit for 140 (137-144) days along with the supplementary meal catered through Supplementary Nutrition Programme (SNP). Cognitive development, using Mullen's scale, anthropometrics, blood and stool samples were assessed both at baseline and endline. Morbidity was monitored bi-monthly using caregiver's recall for last 15 days. A linear mixed model accounting for adjusting clustering and confounder effect was applied. Demography, socio-economic status and child's dietary diversity at home were comparable across the groups. The inclusion of guava improved iron:vitamin-C molar ratio from 1:1 to 1:12. Group difference between endline and baseline measures were significantly higher in GG for Hb (0.8g/dL; p=0.002), SF (5.5µg/L; p<0.001), vitamin-C (0.7mg/dL, p=0.047) and lower for sTfR (-0.6mg/L; p<0.001) over the controls. Though, there was no significant group difference in cognitive development and growth, morbidity on acute lower respiratory infection (ALRI) was significantly lower in GG (42%) compared to BG (62%, p=0.01) and CG (55%, p=0.08). These results suggest that diversifying meals of preschoolers with guava significantly improves iron status and can be adopted as a strategy to address ID among children.

3. Monitoring of nutritional status and catch up growth of boarders of Andhra Pradesh Social Welfare Residential Educational Institutions (APSWREI)

Good nutrition during growing phase is an investment that has many long-term benefits. Andhra Pradesh Social Welfare Residential Educational Institutions (APSWREI) which has been providing residential facilities that includes nutrition at no cost for children from deprived settings provides an opportunity to assess the benefits of good nutrition during adolescent phase. This study was conducted in APSWREI in the state of Andhra Pradesh. Seventeen APSWREI schools were randomly chosen from seven districts. Quantitative and qualitative methods were used in data collection. A total of 7325 children were surveyed and 48.4% of them were boys. The median age of the children was 14 years (8-19 years Range). The overall thinness was 15.8% and was higher among boys (22.5%) compared to girls (9.5%). The overall stunting was 28.5% and was similar in boys (28.3%) and girls (28.6%). Severe thinness and severe stunting were less than or about 5% for both boys and girls. Milk was the commonest food skipped by APSWREIS due to rising costs. The monotonous nature of the diet was a hindrance for regular food consumption among the students. Based on the findings, a new model menu to APSWREIS and inflation adjusted food rates have been recommended.

4. Effect of *Yoga Nidra* on blood pressure and mental health status of hypertensive subjects - A pilot study

Yoga Nidra is a "systematic" method of inducing complete physical, mental and emotional relaxation. This study was conducted to assess the effect of "Yoga Nidra" on blood pressure and mental health among hypertensive subjects. Both known and newly diagnosed subjects (n=74) with HTN aged between 35-70 years were included in this study. Subjects with critical illness and any psychological ailments were excluded. The Experimental group (n=32) included subjects who showed interest in Yoga Nidra to practice daily (45 min/day) for 3 months. Others were included in control group (n=42) and were asked to continue with their regular diet and physical activity along with medications prescribed by their Physicians. After adjusting baseline data, highly significant reduction of Systolic Blood Pressure (mean 16 mm/hg) and Diastolic Blood Pressure (mean 8.6 mm/hg) was observed among experimental group ($p<0.001$**). A significant reduction of mean Hs-CRP ($p<0.001$**) was observed among experimental group. Similarly a significant reduction of Depression, Anxiety and Stress were observed among experimental group. In this pilot study, a significant reduction of blood pressure and level of Stress, depression and anxiety were significantly reduced among Yoga Nidra group as compared with control group were observed. There were no side effects observed during this study.

5. Modeling developmental origins of health and disease in the mouse embryonic stem cells/ mESCs and BMMSCs - with Folate deficiency – Cellular, Molecular / Epigenetics

Nutritional perturbations *in utero* have shown to have a direct bearing in the health of offspring being complemented with several altered metabolic indices which co-precipitate as NCDs adults. Using adult bone marrow derived stem cells (BM-MSCs) which possess multipotent trilineage differentiation functions (osteo, adipo and chondro), have been able to show for the first time *in vitro*, for an altered cellular milieu of BM-MSCs with 70 % folate deficiency akin to the published data using model system. These include upregulation of pro-inflammatory cytokines, altered: leptin to adiponectin ratio, epigenetic changes and increased commitment towards visceral adipogenesis. The findings advocate for the promises of stem cells as an *in vitro* model system to recapitulate the developmental changes and as an alternative to minimize the usuage of large number of animals required for nutritional based studies for better understanding of nutrient and gene interactions.

6. Exploring the beneficial effects of endothelial cells generated from human derived mesenchymal stem cells in the management of lymphodema - *In vivo* approach

The field of lymphoangiogenesis has made rapid and exciting developments over the last few years more so than any other field in vascular biology. Lymphoedema of the limbs and other parts of the body due to lymphatic blockage secondary to parasitic infection due to wuchereria bancrafti or due to

mastectomy. The importance and therapeutic value of stem cells in lymphangiogenesis are poorly understood due to the lack of specific lymphatic molecular markers and the unavailability of optimal experimental models. *In vitro*, characterization of lymphoangiogenic markers which were more predominantly expressed in omental adipose tissue as compared with subcutaneous depots were demonstrated. The upregulation of VWF and Cd31, specific for lymphoangiogenesis in omental tissues have been depicted in the study. *In vivo* experiments were could not carried out to transplant the omental lymphoendothelial cells in rat model system due to technical difficulties of inducing lymphaedema in rats.

7. Modulation of adipose tissue inflammation by dietary n-3 polyunsaturated fatty acids - Potential role in metabolic syndrome

It is well established that the increase in visceral adiposity is the central component of metabolic syndrome. Increased adiposity leads to a chronic low grade inflammation in the adipose tissue, resulting in increased production of pro-inflammatory cytokines and decreased production of anti-inflammatory cytokines which in turn decrease insulin sensitivity. An animal experiment was carried out to investigate the impact of substitution of dietary n-6 PUFA (linoleic acid) with n-3 PUFA (α-linolenic acid present in vegetable oil or long chain n-3 PUFA present in fish oil) on adipose tissue inflammation in fructose induced model of metabolic syndrome and to understand the molecular mechanism by which n-3 PUFA increases insulin sensitivity. The results showed that rats fed high fructose diet increased visceral adiposity, dyslipidemia and insulin resistance compared with starch fed control rats. Fructose feeding also induced adipose tissue inflammation, infiltration of macrophages and increased oxidative stress. Substitution of linoleic acid with α-linolenic acid (n-6:n-3 ratio of 2) or LC n-3 PUFA (n-6:n-3 ratio of 5) reduced visceral adiposity, increased insulin sensitivity and corrected dyslipidemia. The improvement in insulin sensitivity by n-3 PUFA supplementation was associated with marked reduction in adipose tissue inflammation, decreased macrophage infiltration and decreased oxidative stress. The results of the present study provide insights into the therapeutic implication of n-3 PUFA supplementation in obesity related metabolic sequelae.

8. Assessment of nutritional and morbidity status and utilization of health care facilities in the elderly population aged 60 years and above

The prevalence of chronic energy deficiency (CED) was low (9.6%) and overweight, obesity (43.3%) and central obesity (67.6%) was high among urban elderly population. According to the Mini Nutritional Assessment (MNA) 62.3% of urban elderly are at the risk of malnutrition. The mean consumption of all the food groups, (except cereals & millets and Fats & oils) is below the RDI. Similarly, the mean intakes of all nutrients (except total fat) are below the RDA (Dietary Guidelines, 2010). The overall prevalence of anemia, diabetes and hypertension in urban elderly was 46.44%, 32.4% and 74.6% respectively. The overall self-reported prevalence of general morbidities like poor vision, joint pains, forgetfulness, diminished hearing and chewing problems in urban elderly subjects are 73.3%, 62.6%, 28.3%, 27.1% and 24.8% respectively. Based on Barthel ADL Index, the overall prevalence of functional disability in urban subjects is 23.3%. In overall, majority (63.7%) of urban elderly utilized private hospital services, 14.2% utilized public hospital services. Similarly, majority (80%) of urban elderly utilized private doctor services, 17% utilized medical doctor (government doctor) services.

9. Impact of hyperglycaemia on invasion properties of first trimester trophoblastic cells: Implication on preeclampsia

During early gestation, a hypoxic condition is critically maintained by optimal glucose metabolism and transporter activities. Glucose is readily available energy nutrient required for placentation. However, limited data are available on glucose uptake and its transporters during first trimester placentation processes. To this end, effects of glucose and the roles of glucose transporters (GLUTs) were investigated during hypoxia on trophoblast migration and placental angiogenesis processes using early gestation-derived trophoblast cells, HTR8/SVneo, and first trimester human placental explant tissues. The study suggests that putative roles of GLUT1 in the glucose uptake and tube formation of the first trimester placental trophoblast cells, HTR8/SVneo. Increased glucose uptake and GLUT1 activities favor HIF1α

activation in first trimester trophoblast cells that may stimulate glycolytic and lipid metabolic activities with stimulation of angiogenesis due to increased tube formation and expression of several pro-angiogenic mediators. such as VEGF, MMP9, GLUT1, and FABP4. Increased lactate production in response to glucose could induce VEGF pathway of placental angiogenesis in first trimester trophoblast cells. This work reported that GLUT1 plays an important role in both basal and glucose-stimulated glucose uptakes, glucose-stimulated tube formation, and insulin-stimulated glucose uptake of the first trimester HTR8/SVneo cells. GLUT1protein expression and glucose transporter activity are decreased in pre-eclampsia, which indicates that optimum GLUT1 function may be required to prevent development of IUGR. However, further work is required to underpin the mechanisms of GLUT1 action in first trimester placental trophoblast cells.

10. Prevalence of vitamin deficiencies in the apparently healthy urban adult population: Assessed by sub-clinical status and dietary intakes

In this exploratory study, sub-clinical vitamin status of apparently healthy adults is evaluated. A total of 270 apparently healthy urban adults aged 30-70 years from Hyderabad city, forms the study subjects. Blood levels of vitamins (A, B1, B2, B6, total and active B12, D & folate) and homocysteine were assessed. Anthropometric parameters were measured, and dietary intake was obtained by food frequency questionnaire and probability of adequacy (PA) was calculated by estimated average intake. Among the study population, the overall prevalence of deficiency of vitamin B2 was strikingly high (50%) followed by vitamins: B6 (46%), active B12 (46%), total B12 (37%), folate (32%), D (29%), B1 (11%) and A (6%). Hyperhomocysteinaemia (HHcys) was widely prevalent (52%) in the study subjects. In conclusion, the study demonstrated that a high prevalence of multiple sub-clinical vitamin deficiencies, dietary inadequacies along with HHcys among apparently healthy adults which are possible risk factors for disease burden.

11. Factors associated with adequacy of micronutrient intakes among the urban adult population of Hyderabad city

Though the Indian urban population is assumed to have better access to diversified foods, data on micronutrient adequacies are meager in urban adults. This study assessed the adequacy of micronutrient intakes, dietary patterns and the associated factors among the apparently healthy urban adults. The community-based cross-sectional study involved 300 urban adults (distributed into age groups: 21-40, 41-60 and >60 years) residing in Hyderabad city. Dietary intakes were assessed by a three-day 24-h dietary recall and calculated the probability of adequacy (PA) using estimated average requirement. The PA of folate, B12, and zinc (1-11%) were noticeably low. The mean probability of adequacy (MPA) across the ten micronutrients was 38% and was comparable among the age groups and genders. Cereals & millets contributed to thiamine, niacin, zinc, and iron, green leafy vegetables and fruits for vitamins A, C, folate, and iron, animal foods for B12, milk & milk products for calcium, vitamin A, riboflavin and B12. The overall prevalence of anemia, iron deficiency (ID) and iron deficiency anemia (IDA), were 30%, 23% and 14.3%, respectively. The unadjusted and adjusted (age and gender) logistic regression models revealed that the risk of micronutrient inadequacy is associated with higher odds of lower educational status, IDA, and folate deficiency. These results indicate a higher micronutrient inadequacy among the healthy urban adults.

12. Circulating levels of Hsp27 in microvascular complications of diabetes: Prospects as a biomarker of diabetic nephropathy

Heat shock protein 27 (Hsp27) is a small heat shock protein known to protect the cells from apoptosis under stress. In the present study, the plasma Hsp27 levels in type 2 diabetes subjects without and with microvascular complications- diabetic retinopathy (DRe), diabetic nephropathy (DNe), and diabetic neuropathy (DNu) were estimated to understand if it could serve as a marker for these complications. This is a hospital-based case-control study with 754 subjects including 247 controls, 195 subjects with diabetes, 123 with DRe, 80 with DNe and 109 with DNu. Plasma Hsp27 levels were measured by ELISA. The mean plasma Hsp27 was higher in the DNe group (631.5±355.2) compared to

the control (496.55±308.54), diabetes (523.41±371.01), DRe (494.60±391.48) and DNu (455.21±319.74) groups with a p-value of 0.018. Receiver operating characteristic (ROC) curve analysis of Hsp27 in DNe group showed an area under the curve (AUC) of 0.617. Spearman correlation analysis shows a positive correlation of plasma Hsp27 with serum creatinine (p=0.053, r-value 0.083). Gender, age and BMI did not affect the plasma Hsp27 levels. The plasma Hsp27 levels in the DNe group are higher compared to the control and other complications, thereby it could be explored to be used as a potential biomarker of DNe.

13. Implication of altered ubiquitin-proteasome system and ER stress in the muscle atrophy of diabetic rats

Skeletal muscle is adversely affected in type-1 diabetes, and excessively stimulated ubiquitin-proteasome system (UPS) was found to be a leading cause of muscle wasting or atrophy. This study investigated the role of UPS and ER stress in the muscle atrophy of chronic diabetes rat model. Diabetes was induced with streptozotocin (STZ) in male rats and were sacrificed after 2nd and 4th months. In another experiment, 2-months post-STZ-injection diabetic rats were treated with MG132, a proteasome inhibitor, for the next 2-months. The muscle fiber cross-sectional area was diminished in diabetic rats. The expression of UPS components: E1, MURF1, TRIM72, UCHL1, UCHL5, ubiquitinated proteins, and proteasome activity were elevated in the diabetic rats indicating activated UPS. Altered expression of ER-associated degradation (ERAD) components and increased ER stress markers were detected in 4-months diabetic rats. Proteasome inhibition by MG132 alleviated alterations in the UPS and ER stress in diabetic rat muscle. Increased UPS activity and ER stress were implicated in the muscle atrophy of diabetic rats and proteasome inhibition exhibited beneficiary outcome.

14. Development and validation of a comprehensive index for assessing food safety at household level

Food safety is an essential pillar along with nutrition and food security to ensure peoples' health and its sustainable development. In India, majority of foods are prepared at household level. Stored cooked foods (>2 hours) and uncooked foods are susceptible to microbiological contamination due to unsafe handling practices. The study aimed to develop and validate a household food safety index (HFSI) that can predict household food safety status. In this cross-sectional study, for development of HFSI, primary food prepares (N=400) were selected @200 each from rural (Ranga Reddy district) and urban (Hyderabad) homes of Telangana. An 87-item pre-tested questionnaire covering knowledge (43), practices (36) and enabling assets (8) was administered on subjects. Weightages were assigned for responses and maximum possible score was 205. Simultaneously, at consumption point @400 each of stored cooked-food, drinking water samples and hand rinses were collected from participants for microbial analysis. Each of the 87 variables were associated with high-risk food-borne pathogen *(Salmonella* spp.) risk value (1.55logCFU/g) in order to identify the key risk variables that would form the HFSI. Eleven out of 87 parameters were significantly associated ($p<0.05$) with pathogen risk in foods. The final HFSI therefore was a 11-item questionnaire. The optimal cut-off value for validated 11-item HFSI score estimated 9 and it was found to have a sensitivity (77%), specificity (74%) and AUC-0.808 which are acceptable. Rural households showed significantly lesser HFSI score than the urban. The HFSI was then administered on 200 rural and urban (@100each) subjects for assessing the food safety status. To address the identified critical food safety issues at home, key messages were developed and implemented as an educational intervention on 120 households from Rural, Urban and Slum (@40each). There is a significant ($p<0.05$) improvement in HFSI score after educational intervention. HFSI is simple tool to rapidly assess food safety status at household level. Identified critical issues of food safety can be addressed through focused food safety education.

I. PUBLIC HEALTH NUTRITION

1. Assessment of current nutritional status of <5yr children and performance of ABM project in the districts of Madhya Pradesh

ICMR-National Institute of Nutrition has carried out district level nutrition surveys in the year 2009-10, with the objective to assess the nutritional status of under 5 year children and infant and child feeding practices of mothers of under 3 year children in rural areas of Madhya Pradesh. Based on the results, the Government of Madhya Pradesh has launched an nutrition intervention project titled "Atal Bal Arogya Evam Poshan Mission (ABM)" in April 2010 to reduce child malnutrition 10% per centage points from the baseline including child morbidity and mortality at significant extent. In order to assess its impact, the present study was undertaken to assess change in the nutritional status <5 year children and performance of ABM nutrition intervention taken up in each of the district in the state of Madhya Pradesh, with the following objectives:

OBJECTIVES

1. To assess the nutritional status of <5 year children in terms of anthropometry such as heights and weights and prevalence of clinical signs of nutritional deficiency.
2. To assess the nutritional status of mothers by BMI and prevalence of anaemia by haemoglobin estimation, on all the pregnant and lactating mothers of the index children covered for the survey.
3. To assess the prevalence of morbidities among <5 year children during the preceding fortnight.
4. To assess the infant and young child feeding (IYCF) practices of mothers of under 3 years children,
5. To assess district wise performance of the functionaries of the ABM / ICDS projects.
6. To assess the changes, if any, in the prevalence of under-nutrition among <5 year children over a period of time by comparing with the results of the earlier study (2009-10) carried out by the NIN and Annual Health Survey.

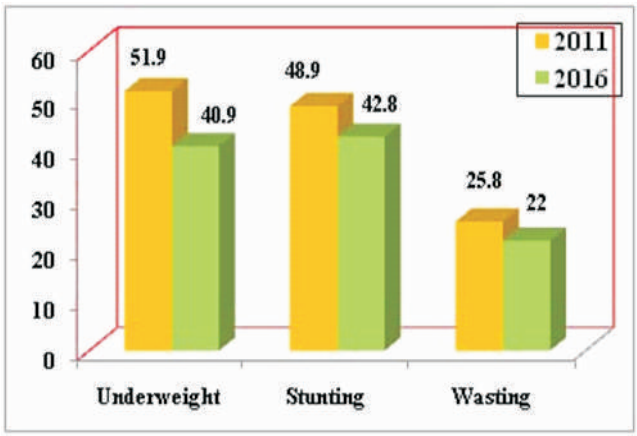
METHODOLOGY

Sampling Design: It was a community based cross sectional operational research study carried out by adopting systematic random sampling procedure in each of the 51 districts in the state of Madhya Pradesh.

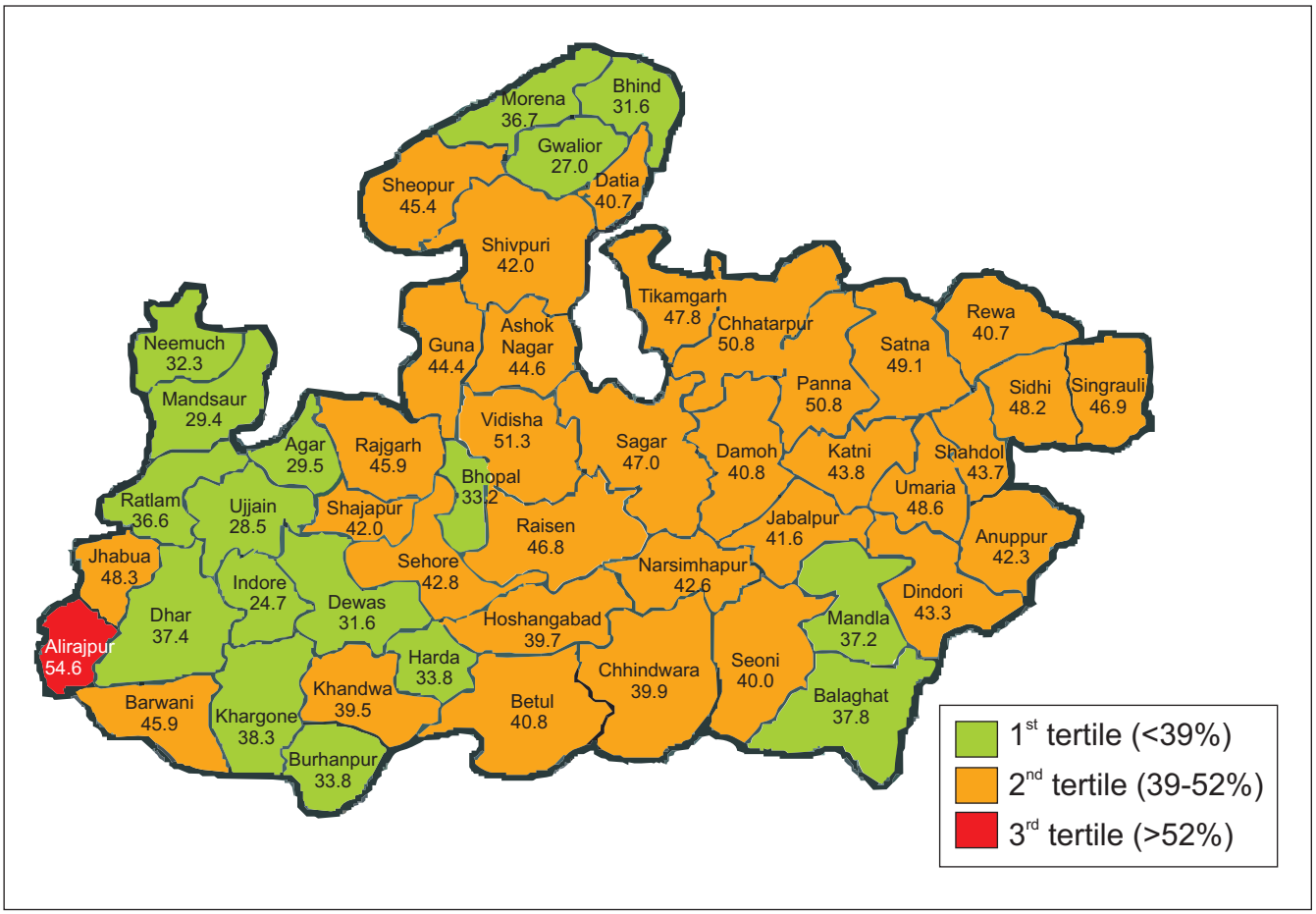
The salient observations of the study

- A total of 30,585 HHs were covered from 51 districts and 37,924 children were covered for anthropometric measurement and IYCF practices of mothers of <3 years children.
- Majority (93.2%) were Hindus, 28% belonged to scheduled tribe, 50% were living in nuclear families, about 79% fathers and 68% mothers were literate
- About 55% families were residing in kutcha houses, 22% had access to tap water for drinking purpose and 33% HHs had sanitary latrine facility and were using it.
- There was an improvement in the IYCF practices over the periods.
- Utilization of Antenatal care (ANC) during last

Nutritional status of 0-5 year children by period



Cluster map based on prevalence of underweight by tertiles



pregnancy has improved from 79% during 2010 to 98% in current survey, utilization of at least 3 ANCs has increased from 36% to 57% in current study,

- About 76% of pregnant women registered for ANC before 12 weeks of gestation as against 28% in the previous survey.
- Consumption of >=90 IFA tablets during pregnancy has improved from 20% to 59% in present survey, also the % of women who consumed any IFA tablets has improved from 70% during previous survey to 95% in current survey.
- About 81% women had attended Mangal Diwas at AWC and 52% received hot cooked food on that day.
- Institutional deliveries has also improved from 79% during previous survey to 83% in the survey.
- The prevalence of low birth weight (<2.5kg) is 14% in current survey as against 19% in the previous survey.
- Initiation of breastfeeding within 1 hour has improved from 26% during previous survey to 58% in current survey.
- About 93% of 0-5 month children were solely breast fed in current survey as compared to 71% in the previous survey, while 43% were exclusively breast fed up to 6 months during previous survey and is 64% in the current survey.
- About 90% children (6-35 months) received THR from AWC in current survey as against 72% in 2010-11.
- About 93% children (12-23 months) were fully immunized in present survey as against 84% during 2010-11.
- Hand washing practices with soap among mothers before feeding the child has improved from 24% during previous survey to 43% in the current survey.

- The prevalence of undernutrition (<Median -2SD) among <5 year children such as underweight, stunting and wasting had declined from 52%, 49% and 26% during 2010-11 to 42%, 43% and 22% respectively in the present survey.
- The prevalence of chronic energy deficiency among NPNL women was 36%in the current survey..

2. Introduction of vitamin C rich fruit in supplementary nutrition programme for improving micronutrient status, gut health, growth and development: A randomized trial among ICDS pre-school beneficiaries

The trial (CTRI/2014/09/004983) aimed to provide evidence base for functional benefit of long term intake of vitamin C rich fruit and its inclusion as part of Supplementary Nutrition Programme.

Objectives were to assess the impact of daily consumption of 25 g of guava with SNP meal among 2-5 years old ICDS beneficiaries on:

i) Biomarkers of iron, vitamin-C, B12 and folate status
ii) Cognitive development
iii) Growth
iv) Intestinal gut flora
v) Morbidity

The trial was conducted in Alair Mandal, Nalgonda District, Telangana State. A total of 642 ICDS pre-school beneficiaries (2-5yr) of 28 AWCs from 16 villages were enrolled. Written consent was collected from 419 care-givers and 402 pre-schoolers were recruited (estimated sample size was 390 for 3 groups) in the study. After baseline sample collection and deworming, 16 villages were randomized into 3 arms: i) experimental: 25g guava (GG), ii) passive control: 25g banana (BG), iii) active control: 25g cucumber (CG). Fruits were supplied for 140 days from Oct, 2015 to Mar, 2016.

Baseline Characteristics

At baseline all the background characteristics including age, gender, community, maternal education, etc were comparable across the group.

Intervention effect on Biomarkers of Iron, Vitamin-C, B12 and Folate status:

Inclusion of guava in the meal of the preschoolers in the Anganwadis improved iron: vitamin-C molar ratio from 1:1 to 1:12.Group difference for end line to baseline measures were significantly higher in GG for hemoglobin(0.8g/dL; p=0.002), serum ferritin (5.5µg/L; p<0.001), and lower for sTfR (-0.6mg/L; p<0.001) compared to the controls (Figure 2). sTfR/SF ratio (p<0.001) were significantly lower (p<0.001)in GG compared to the controls.

At endpoint, 0.7mg/dL higher plasma vitamin-C concentration was observed in GG compared to the controls(p=0.047). Folate and vitamin B12 levels were found to increase within the groups at end line but significant group difference was not observed. Iron deficiency was also lower in the GG compared to the BG and CG as shown in figure- 3.

Intervention effect on Cognitive Development: No significant group difference was observed for visual reception (p=0.518, 0.796), fine motor (p=0.436, 0.997), receptive (p=0.793, 0.771) and expressive language (p=0.667, 936) development assessed by Mullen's Scale of Early Learning at either baseline or in the mean changes from baseline to end line.

Figure 1: Consort Diagram

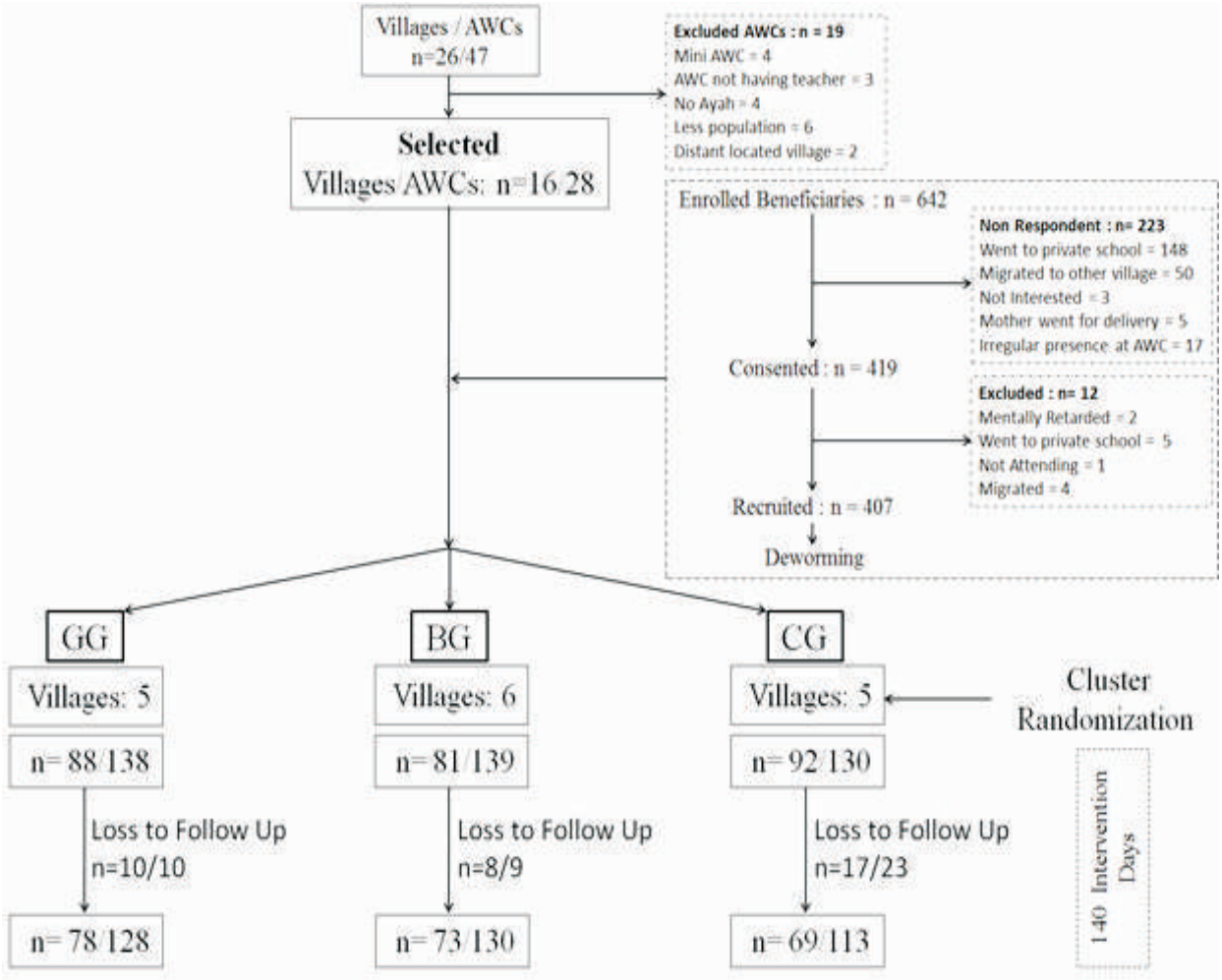
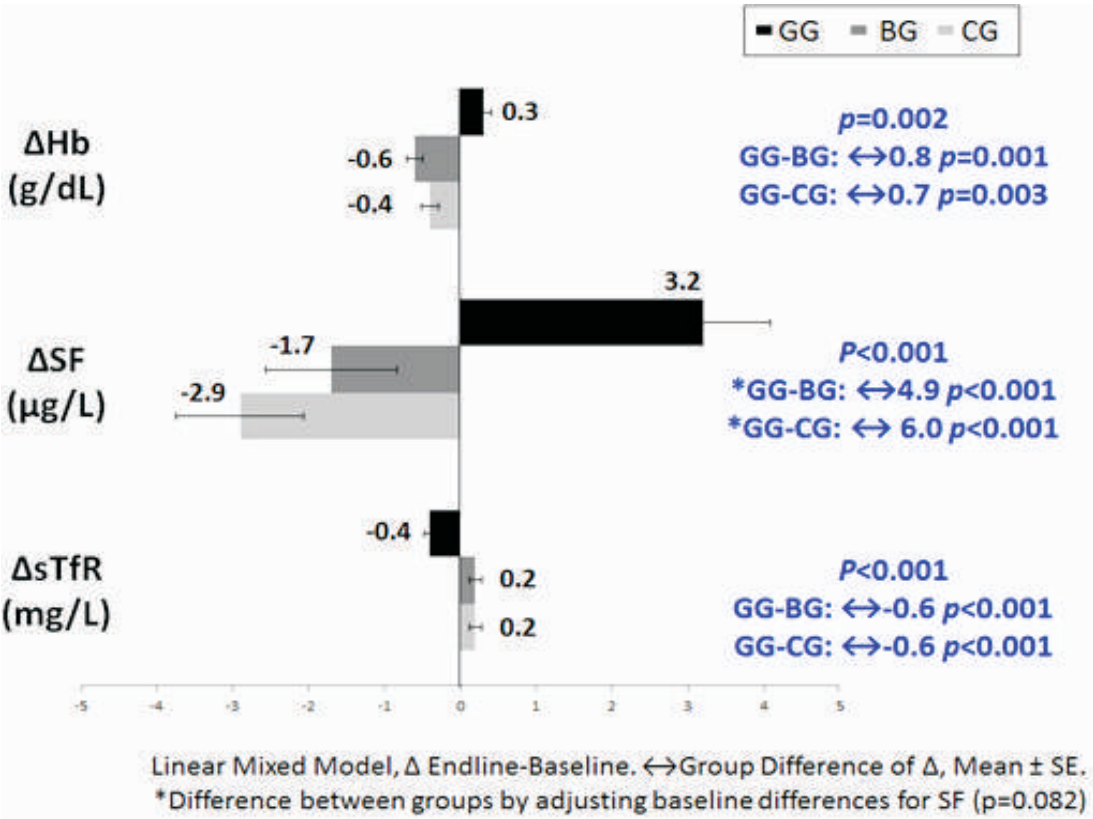


Table 1. Group comparisons of demographic and other variables at baseline

Parameters	GG	BG	CG	p
[1]Age (mo)	36.7 ± 1.13	36.3 ± 1.07	36.4 ± 1.15	0.975
[2]Gender	56.5% G	52.5% G	48.5% G	0.478
[2]Marginal Community (%)	37.0	38.7	27.3	0.640
Mother's Age (years)	25.3 ± 0.37	25.9 ± 0.37	25.8 ± 0.38	0.477
[2]Mother's Education (%) Didn't Attend School Secondary Higher Secondary onward	 16.1 60.6 23.4	 20.9 58.2 20.9	 7.9 67.5 24.6	 0.069
[1]SES	29.3 ± 0.70	28.0 ± 0.70	29.2 ± 0.73	0.394
[1]Household Food Security	4.3 ± 1.23	4.8 ± 1.13	4.5 ± 1.23	0.948
[1]Dietary Diversity Score	4.1 ± 0.14	4.2 ± 0.13	4.1 ± 0.14	0.824
[1]BMI (Kg/M^2)	14.4 ± 0.18	14.7 ± 0.17	14.8 ± 0.23	0.211

Figure 2: Intervention effect on Biomarkers of Iron



Intervention effect on Growth: There was no group difference both at baseline and baseline to endline change of z-scores of weight for height (*p*=0.244, 0.331), height for age (*p*=0.513, 0.103), weight for height (*p*=0.243, 0.383), BMI for age (p=0.244, 0.298) and MUAC for age (p=0.464, 0.817).

Intervention effect on Intestinal Gut Flora: Stool DNA isolation followed by RT-PCR analysis of targeted gut flora have been completed and data analysis is expected to be completed by August 2018.

Intervention effect on Morbidity: Gastrointestinal morbidity (GI) was low in all the 3 groups of 2.0%, 1.9% and 3.0% in GG, BG, and CG respectively with no group difference. After adjusting the cluster effect, seasonality and time variation, there was significant group difference with a lower prevalence of ALRI of 42.4% in GG compared to 61.8% in BG (p=0.011) and 55.2% in CG (p=0.083) at endpoint (Figure 4).

Figure 3: Iron deficiency at baseline and end line in the three study groups

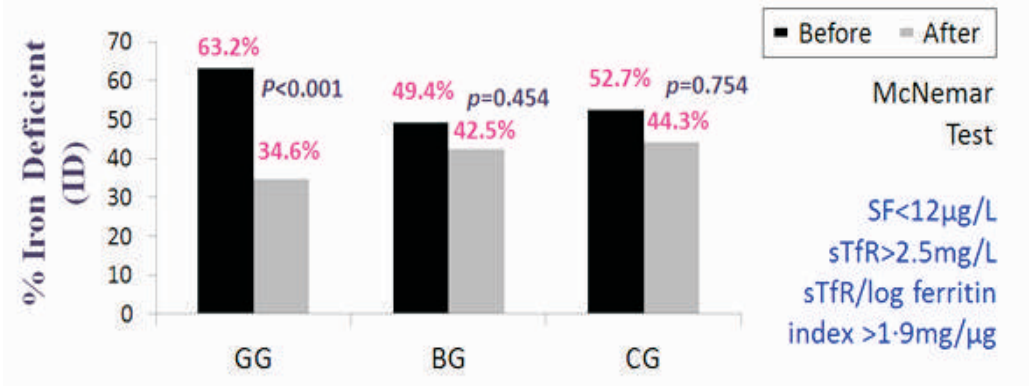
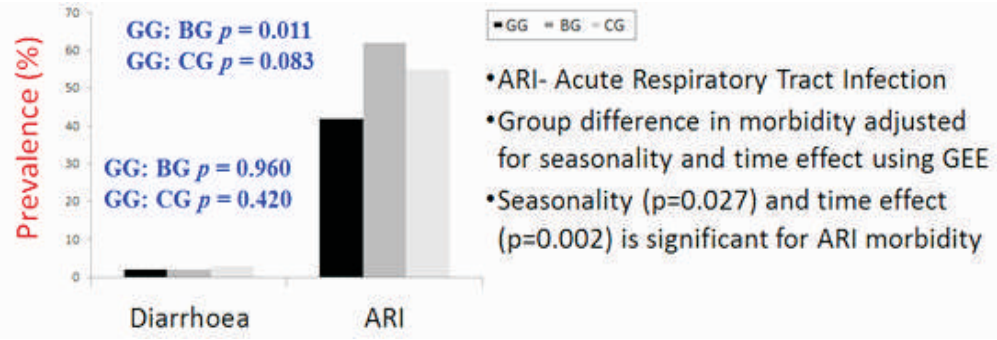


Figure 4: Effect of Intervention on Prevalence of Morbidity



RECOMMENDATIONS

Introduction of Vitamin-C rich fruit in the SNP meal was accepted by all stakeholders including the pre-school ICDS beneficiaries, their caregivers and the ICDS staff. The benefits of inclusion of the fruit were seen in the increased hemoglobin and iron stores of the pre-schoolers. In addition to improved iron status, the acute lower respiratory infection-related morbidity was lower in the Guava group compared to the control group(s), indicating further benefits of the Vitamin-C rich fruit. Given the additional cost of Rs.0.80 per child/ day, adding the fruit to the meal is still a beneficial long-term strategy as this is a natural source with multiple benefits. It can also be beneficial to the community by including local vendors and is also environment friendly.

II. CLINICAL EPIDEMIOLOGY

1. Monitoring of nutritional status and catch up growth of boarders of Andhra Pradesh Social Welfare Residential Educational Institutions (APSWREI)

Good Nutrition during growing phase (first two decades of life) is an investment that has many long-term benefits. The first 1000 days is undoubtedly the most important phase for the long term growth of children due to peak velocity of growth, however various intervention studies have only shown modest improvements in nutritional parameters. Adolescent phase is another phase of peak velocity of growth and there is a growing interest in adolescent health as an entry point to improve the health of women and children, especially because an estimated 10 million girls younger than 18 years globally are married each year. It has been suggested that evidence-based interventions must be introduced in the pre-conception period and in adolescents in countries with a high burden of under nutrition and young age at first pregnancies; however, targeting and reaching a sufficient number of those in need may be a challenge.

Government School going adolescent children may be the ideal age for nutrition interventions to improve nutritional status for following reasons. 1. Enrolment rates are high compared to higher secondary school (HSC) education, 2. When secular trends in height happen, maximum increments in heights are seen at 12 years for girls and 15 years for boys, 3. About 20% of bone mass found in adult, is accreted during this age group (ICMR Expert Committee 2010), 4. The requirements of Iron in girls are increased during this age due to the onset of menarche (ICMR Expert Committee 2010), 5. As the food is delivered at school, food is not shared by other family members; benefits are directly reaching the target population unlike take home rations.

APSWREI which has been providing residential facilities that includes nutrition at no cost for children from deprived settings provides us a unique opportunity to assess the benefits of good nutrition during adolescent phase. The cost of diet given to the children has recently been upgraded to increase the ration of milk and eggs to the children. Inclusion of protective foods such as milk and eggs are likely to improve the nutritional status of children of low socio-economic group. However there exists no data on the nutritional status of children from deprived settings who are given “better diet”. We therefore planned a baseline study to assess nutritional status of boarders of APSWREI from 17 centres across Andhra Pradesh.

METHODOLOGY

This study was be conducted in APSWREI in the state of Andhra Pradesh. Seventeen APSWREI schools were randomly chosen from seven districts were from two regions (Coastal Andhra, Rayalaseema). A pretested questionnaire on socio-demographics was administered. Anthropometry (height to nearest 0.1 cm and weight to nearest 100 grams) was measured using standard equipment. Implementation of model menu was assessed by interviews with principals, wardens and children of APSWREI. Assessment of sanitation and hygiene was done after careful examination of premises of APSWREI. Data was entered in CSPro software (version 6.0) for Windows. Data analysis was carried out using R programming language (version 3.0.1). WHO growth standards were used for calculating thinness and stunting.

RESULTS

A total of 7325 children were surveyed and 48.4% of them were boys. The median age of the children was 14 years (8-19 years Range). The socio demographic and nutritional status of boys and girls are shown in Table 1. Majority of the children belonged to Scheduled Caste Category (86.5%), followed by Scheduled Tribe Category (6.1%). About 61% of the children belonged to Hindu Religion, followed by Christians (39.1%).

Table 1. Socio-demographic and Nutritional status of girls and boys

n	Overall	Female	Male
	7325	3764	3531
Age in months (mean (sd))	165.80 (27.25)	163.02 (27.77)	168.75 (26.37)
Category (%)			
General	75 (1.1)	39 (1.1)	36 (1.1)
OBC	431 (6.3)	201 (5.8)	230 (6.8)
SC	5932 (86.5)	3005 (87.0)	2927 (86.0)
ST	418 (6.1)	209 (6.0)	209 (6.1)
Religion (%)			
Christian	2684 (39.1)	1320 (38.1)	1364 (40.1)
Hindu	4158 (60.6)	2132 (61.6)	2026 (59.5)
Muslim	14 (0.2)	8 (0.2)	6 (0.2)
Others	9 (0.1)	2 (0.1)	7 (0.2)
Height (mean (sd))	147.61 (11.45)	144.72 (9.20)	150.67 (12.73)
Weight (mean (sd))	38.28 (9.94)	37.37 (9.04)	39.24 (10.74)
Thinness (%)			
Normal	6124 (84.2)	3395 (90.5)	2729 (77.5)
Moderate	930 (12.8)	311 (8.3)	619 (17.6)
Severe	218 (3.0)	44 (1.2)	174 (4.9)
Stunting (%)			
Normal	5204 (71.5)	2677 (71.4)	2527 (71.7)
Moderate	1706 (23.4)	893 (23.8)	813 (23.1)
Severe	366 (5.0)	181 (4.8)	185 (5.2)
Thinness = Yes (%)	1148 (15.8)	355 (9.5)	793 (22.5)
Stunting = Yes (%)	2072 (28.5)	1074 (28.6)	998 (28.3)

Table 2. Nutritional status of boys and girls of various classes

	5th	6th	7th	8th	9th	10th	11th	12th
Boys *Thinness (%)*								
Normal	278(78.3)	375 (80.3)	289 (68.3)	400 (76.6)	399(74.4)	352 (78.7)	302 (82.3)	330 (82.3)
Moderate	67 (18.9)	76 (16.3)	94 (22.2)	104 (19.9)	98 (18.3)	77 (17.2)	48 (13.1)	55 (13.7)
Severe	10(2.8)	16 (3.4)	40 (9.5)	18(3.4)	39 (7.3)	18 (4.0)	17 (4.6)	16 (4.0)
Stunting(%)								
Normal	276 (77.7)	340 (72.8)	293 (69.3)	343 (65.6)	346 (64.4)	323 (72.3)	295 (80.2)	307 (76.6)
Moderate	65 (18.3)	109 (23.3)	106 (25.1)	135 (25.8)	146 (27.2)	102 (22.8)	65 (17.7)	85(21.2)
Severe	14 (3.9)	18 (3.9)	24 (5.7)	45 (8.6)	45 (8.4)	22 (4.9)	8 (2.2)	9 (2.2)
Girls *Thinness (%)*								
Normal	433 (93.3)	417 (90.5)	421 (88.8)	433 (90.2)	515 (89.6)	404 (91.0)	409 (91.7)	363 (89.4)
Moderate	26 (5.6)	35 (7.6)	48 (10.1)	42 (8.8)	55 (9.6)	36 (8.1)	32 (7.2)	37 (9.1)
Severe	5 (1.1)	9 (2.0)	5 (1.1)	5(1.0)	5 (0.9)	4 (0.9)	5(1.1)	6(1.5)
Stunting (%)								
Normal	368 (79.3)	334 (72.5)	320 (67.5)	360 (75.0)	403 (70.1)	319 (71.8)	301 (67.5)	272 (66.8)
Moderate	79 (17.0)	105 (22.8)	113 (23.8)	105 (21.9)	135 (23.5)	107 (24.1)	127 (28.5)	122 (30.0)
Severe	17 (3.7)	22 (4.8)	41 (8.6)	15 (3.1)	37 (6.4)	18 (4.1)	18 (4.0)	13 (3.2)

Mean (SD) height for girls and boys respectively was 144.7 (9.2) cm and 150.6 (12.7) cm. Similarly, mean (SD) weight for girls and boys respectively was 37.4 (9.0) cm and 39.2 (10.7) cm. The overall thinness was 15.8% and was higher among boys (22.5%) compared to girls (9.5%). The overall stunting was 28.5% and was similar in boys (28.3%) and girls (28.6%). Severe thinness and severe stunting was less than or about 5% for both boys and girls.

Table 2 shows nutritional status of boys and girls from 5th to 12th Class. In boys, thinness was highest in those who were studying 7th class (31.7%) and lowest in those studying 11th and 12th class (17.7%). Stunting for boys was also lowest among 11th Class students (19.8%) and was highest in 9th Class students (35.6%). In girls, thinness was lowest in 5th Class (6.7%) and was highest in 7th Class (11.2%). Stunting in girls was lowest in 5th Class (20.7%) and was highest in 12th Class (33.2%).

With respect to qualitative findings, it was observed that the model menu was not being implemented properly and milk was the commonest food that was skipped due to inflation. The monotonous nature of the diet was also a hindrance for regular food consumption among the students.

Based on our findings, we have recommended a new model menu to AP social welfare residential schools. The diet charges of the revised model menu is higher by 3 Rupees 77 Paisa compared to the existing model menu based on current rates W.E.F 01-01-2016. As the prices of food items are constantly changing every year, inflation adjusted rates be provided each year to compensate for the price rise.

2. Effect of Yoga Nidra on blood pressure and mental health status of hypertensive subjects - A pilot study

Hypertension (HTN) exerts a substantial public health burden on cardiovascular health status and healthcare systems in India. HTN is directly responsible for Public health surveys and Statistics show that, in India, HTN is a direct cause in 57% of deaths due to stroke and 24% of those related to coronary heart disease (CHD). Recent NNMB report on prevalence of hypertension among urban men and women in India was reported to be 31% and 26%, respectively. However, only about 25.6% of treated patients had their Blood Pressure under control. According to World Health Organization (W.H.O) blood pressure of 160/95 mm of Hg or higher should always be considered as hypertension. It has been found that, nutrition transition, stress, high risk behaviours, sedentary and unhealthy lifestyle are major causes for hypertension.

Dyslipedemia is recognized as a prominent risk factor for Cardio Vascular Diseases (CVD). Lipid abnormalities, including high levels of Low Density Lipoprotein Cholesterol (LDL-C), elevated Triglycerides and low levels of High Density Lipoprotein Cholesterol (HDL-C), are associated with an increased risk of cardio vascular events. There are multiple scientific publications to show that stress can cause an increased level of cholesterol in humans. It is postulated that stress increases blood Lipids through increasing hepatic Lipoprotein Lipase activity caused by a heightened sympathetic neural response.

Hypertension and its complications are a leading cause of death in modern societies globally. Today anti-hypertensive drug therapy is the only effective form of management of Hypertension. However, it has been associated with major side effects in many patients that may include impacting the quality of life and also reduction in life span in some cases.

Patients with hypertension may experience many negative emotions which increase their risk for the development of mental health disorders particularly anxiety, depression and stress. Stress and strain of day to day life has been shown to have an adverse effect on vital body organs through several psycho-physical mechanisms.Several studies have been conducted that use diet and physical activity to reduce blood pressure in hypertension subjects. Although, these have been beneficial to a large extent, they tend

to be effective as long as a given regime is followed, and often people find it difficult to incorporate certain lifestyle changes. So, as we see that stress, is a major cause of hypertension (and subsequently the related CHD or CVDs) in the modern developing societies, we decided to initiate an effort to study and show the positive effect of alleviation of stress on hypertension and related biochemical parameters. While Yoga and meditation have been used as simple stress busting methods for centuries, there are very few studies that have been conducted under the aegis of a reputed scientific and medical organization that can authentically point towards the beneficial effects of yoga and mindful meditation.

It is observed that Yoga Nidra (that incorporates both yoga and mindful meditation techniques), when used along with a standard therapy, is a safe, inexpensive and very effective method of management of hypertension. Yoga Nidra has emerged as a "systematic" method of inducing complete physical, mental and emotional relaxation. Yoga Nidra has been used in patients of many chronic diseases such as menstrual abnormalities, post-traumatic stress disorder, diabetes, anxiety and depression but little is known about its effect on hypertension and sleep or sleep disorders.

Yoga Nidra has been used earlier as a therapeutic option with no documented side effects. There is no scientific report of its application in reducing blood pressure among subjects with hypertension. The objective of the study was to develop Yoga Nidra as a powerful complementary model of management of hypertension.

AIMS AND OBJECTIVES

- To assess the effect of "Yoga Nidra" on blood pressure among hypertensive subjects.
- To assess effect of Yoga Nidra on Blood Lipid profile, Insulin, ICAM, VCAM, Homocysteineand Adiponectin parameters.
- To assess effect of Yoga Nidra on Depression, Anxiety and Stress among subjects with Hypertension.

METHODOLOGY

***Sample Size and Study Subjects*:** Since this study was planned to be a pilot, we planned to recruit a total of 72 subjects in both groups (Test and Control) taking into consideration 20% dropout rate. Following standard inclusion and exclusion criteria were used. **N**on probability convenient sampling technique was adopted to select the subjects for this study.

Recruitment: Subject with HTN, both known and newly diagnosed cases, were enrolled and invited for supervised Yoga Nidra intervention at National Institute of Nutrition. Subjects with blood pressure ≥140/90 mm Hg between age 35-70 and those who could comprehend the relaxation techniques were included in this study. Subjects with critical illness, pregnant and lactating women and those having hearing impairment or on sedatives or any psychological treatments were excluded from this study. The Experimental (or Test) group was formed with those subjects who showed interest in Yoga Nidra and who could commit to attend and practice regularly per our recommendation. Others were included in control group and were asked to continue with their regular diet and physical activity along with medications prescribed by their Physicians. Total 74 subjects with HTN were recruited after obtaining NIN-IEC approval, and their consent for the study was recorded.

Intervention group*:* Thirty two hypertensive subjects of both treated and untreated HTN were included in intervention for Yoga Nidra, that was practiced daily (45 min/day) for 3 months at NIN premises under the guidance of a trained Yoga instructor. During the study, Blood Pressure was monitored weekly by the Physician before Yoga Nidra session - using both Manual as well as Digital B.P apparatus.

Selection of the Tool and Delivery Method of Yoga Nidra for Intervention Group: For a study of this type, it is important to keep the interest and commitment of the subjects sustained throughout the study period, in order to obtain a good response and arrive at a meaningful conclusion. While researching for the best way to deliver Yoga Nidra sessions to the subjects in this study, we found the Yoga Nidra method developed by Anandmurti Gurumaa of the NGO named Rishi Chaitanya Trust to be the most promising, due to the powerful and resonating voice of the narrator and an equally effective background music score which is especially composed with all notes and scales selected very thoughtfully to support the Yoga Nidra process in the most desirable way and elicit the maximum positive result. We felt this Yoga Nidra method would be most appropriate to undertake such a study.

Control group: Forty two hypertensive subjects with or without treatment were included in control group without any lifestyle modification whatsoever.

Blood Pressure Data collection : Blood pressure was taken in baseline as well as endpoint for control group whereas blood pressure was measured every week for a period of 12 weeks for all subjects in treatment group using digital blood pressure monitor (oscillatory - OMRON)and mercury sphygmomanometer (auscultatory) in sitting position before starting Yoga Nidra practice. Each subject was asked to relax in a sitting posture for 5 minutes. Three readings of digital blood pressure monitor and one reading of mercury sphygmomanometer was taken in order to ensure accuracy. The first reading of blood pressure was measured using a digital blood pressure monitor and with an interval of 1 minute in between other two readings were recorded. Fourth reading was taken using a mercury sphygmo-manometer. All readings were duly recorded.

Biochemical Parameters: 5ml blood samples were collected (twice) for all recruited subjects to assess effect of Yoga Nidra on Blood Lipid profile, Fasting glucose, Insulin, ICAM, VCAM, Adiponectin, Homocysteine and thyroid profile. Standard techniques and protocols were used to estimate above parameters per manufacturer instructions.

Mental health status assessment by DASS-21 score in baseline and endpoint: Depression Anxiety Stress Scales (DASS)-21 questionnaire is a very well-known tool that consists of 21 symptoms divided into 3 subscales (depression, anxiety, and stress) of each 7 items and has an excellent reliability estimates. DASS21 symptom based on 4-point severity scale ranging from 0 to 3 were measured and the scores were categorized into normal, mild-moderate and severe with the scale depressive (0-9, 10-20, and >20), anxiety (0-7, 8-14, and >14), and stress (0-14, 15-25, and >25). DASS Score was assessed for both group during baseline as well as endpoint.

***Statistical Analysis*:** SSPS version 20 was used to analyze data.

RESULTS

Baseline and Endpoint Parameters (mean, SD and p value) of this study are as shown in Table 1 and 2.

Table 1: Baseline parameters (anthropometry, blood pressure and biochemical among Experimental as well as Control group subjects

	N+	Experimental N=32		N+	Control n=42		P value
		Mean	±SD		Mean	±SD	
BMI	32	28.59	3.8	42	28.08	5.61	0.661
SBP	32	143.63	16.69	42	135.24	14.93	0.026 **
DBP	32	90.28	10.55	42	87.57	11.43	0.3
PR	32	80.41	11.36	42	80.52	10.81	0.964
TC	32	184.97	31.66	42	173.43	34.18	0.142
HDL	32	50.75	11.82	42	49.9	10.82	0.75
LDL	32	97.63	29.58	42	92.76	26.36	0.458
VLDL	32	31.21	38.47	42	27	14.84	0.517
Trigl	32	156.09	192.38	42	135	74.203	0.517
FBS	32	108.59	48.84	42	105.93	45.94	0.811
HbA1C	32	5.92	1.49	42	5.48	1.54	0.222
Insulin	31	14.39	8.66	40	15.25	9.75	0.731
HOMA-IR	31	3.87	2.92	40	4.13	3.71	0.748
Hs-CRP	32	2.2	1.48	42	1.44	1.08	0.0132**
VCAM	32	735.1	138.47	40	841.18	259.65	0.040*
ICAM	32	1025.4	321.1	40	1121.8	275.03	0.175
Adiponecti	32	26.8	14.7	40	26.51	13.52	0.93
Homocyst	31	151.4	78.23	42	199.7	368.79	0.477
Cortisol	30	122.2	61.13	37	109.8	39.43	0.317
TSH	32	2.83	1.72	41	2.52	1.27	0.373
T3	32	1.53	0.53	42	1.45	0.62	0.552
T4	32	7.41	1.32	42	7.15	1	0.321

N= total number of subjects enrolled, N+ Number subjects assessed SD- Standard deviation. *- Significant, ** Highly significant (p value <0.005)

Table 2: Endpoint parameters (anthropometry, blood pressure and biochemical) among Experimental as well as Control group subjects

	N	Experimental		N	Control		P value
		mean	SD		mean	SD	
BMI	31	28.27	3.62	31	27.6	4.95	0.526
SBP	31	118.7	9.2	31	130.71	16.21	0.001**
DBP	31	77.03	6.5	31	84.16	9.82	0.001**
PR	31	78.94	9.05	31	81.71	10.5	0.27
TC	31	186	33.65	25	181	34.86	0.589
HDL	31	54	14.03	25	51.73	12.5	0.536
LDL	30	89.63	28.4	25	88	31.902	0.842
VLDL	31	31.74	28.15	25	26.17	10.92	0.355
Trigl	31	158.71	140.73	25	130.84	54.63	0.355
FBS	30	95.87	39.2	24	94	30.6	0.849
HbA1C	30	4.96	0.51	25	5.06	0.707	0.552
Insulin	31	11.44	5.82	26	11.88	5.72	0.772
HOMA-IR	30	2.86	2.15	24	2.8	1.5	0.914
Hs-CRP	32	1.06	0.821	25	2.6	1.71	0.001**
VCAM	31	1048.75	317.26	26	1085.48	451.86	0.721
ICAM	31	771.79	285.23	26	797.19	212.19	0.709
Adiponecti	31	80.22	25.55	26	76.89	25.54	0.627
Homocyst	31	351.14	73.43	26	338.56	8.2	0.389
Cortisol	31	103.98	43.9	26	112.48	57.78	0.531
TSH	31	1.91	1.1	26	1.64	0.61	0.266
T3	31	2.48	0.63	26	2.6	0.69	0.528
T4	31	11.17	1.35	26	11.05	1.47	0.746

N= total number of subjects enrolled, N+ Number subjects assessed
SD- Standard deviation, *- Significant, ** Highly significant (p value <0.005)

Table 3. Comparison of DASS Score among experimental and control groups

Domains	Test	N	Experimental Group	P	Control Group	P
			Mean ± SD		Mean ± SD	
Depression	Pre	31	1.58±1.36	0.005	2.39±1.70	0.296
	Post	31	1.26±0.97		2.58±1.50	
Anxiety	Pre	31	3.32±2.21	0.000	4.00±2.48	0.856
	Post	31	2.52±1.36		4.06±2.48	
Stress	Pre	31	6.16±3.04	0.000	6.61±2.81	0.854
	Post	31	4.94±2.56		6.55±2.80	
DASS Total	Pre	31	11.06 ± 5.54	0.000	13.00±5.50	0.272
	Post	31	08.71 ± 4.18		13.39±6.62	

Key Findings

The present study concluded the following important evidences

1. After adjusting baseline data, highly **significant reduction of SB**P (mean 16 mm/hg) and DBP (mean 8.6 mm/hg) observed among experimental group ($p<0.001^{**}$).
2. A *significant reduction of mean Hs-CRP (2.2 vs 1.06 $p<0.001^{**}$)* was observed among experimental group after 12 weeks regular practice of Yoga Nidra.
3. No significant changes in lipid profile, blood glucose, homocysteine, adiponectin, ICAM, VCAM and thyroid profile after Yoga Nidra practice.
4. Reduction of Sr.Cortisol was observed among experimental group but it was not statistically significant.
5. A significant reduction of Depression, Anxiety and Stress were observed among experimental group.

CONCLUSIONS

In this pilot study, it was observed that a significant reduction of blood pressure among Yoga Nidra intervention group as compared with control group. Level of Stress, depression and anxiety were significantly reduced among Yoga Nidra group as compared with control group. There were no side effects observed during this study.

RECOMMENDATION

Keeping in view the significant findings of the present study and the potential beneficial impact of Yoga Nidra in management of HTN and related health issues, it is highly recommended to continue the effort with larger and better designed studies with larger subject groups using various inclusion and exclusion criteria and advanced statistical analyses.

III. BASIC STUDIES

1. Modeling the developmental origins of health and disease in the Mouse Embryonic Stem Cells (MESCS) – cellular, molecular/ epigenetics approaches

Maternal nutrient deficiencies are known to predispose the offspring to a number of metabolic disturbances and studies have shown that maternal vitamin B12 and/or folate restriction is associated with oxidative stress, altered energy homeostasis and lipid profile, insulin resistance etc. Stem cells as a tool to study effects of maternal under nutrition in fetal programming for adult diseases appear as a promising. Embryonic stem cells (Es) represent as a potential valuable and renewable source of cells which could be used for transplantation therapy. Embryonic stem cells are pluripotent cells derived from the inner cell mass of pre-implantation embryos and have the ability to differentiate into cells comprising all three embryonic germ layers. Apart from the various growth factors and signaling molecules, cells have to rely on a variety of potent antioxidant defense mechanisms and a close interaction of antioxidant molecules to scavenge the ROS generated in actively cells can be altered by growth factor signaling depending on the nature of the signaling, growing and proliferating cells. The degree of cellular oxidation/reduction in progenitor molecules, it can render the progenitors more reduced or oxidized. Antioxidant compounds thus were shown to determine the stem cell's fate in different tissues and stem cell types.

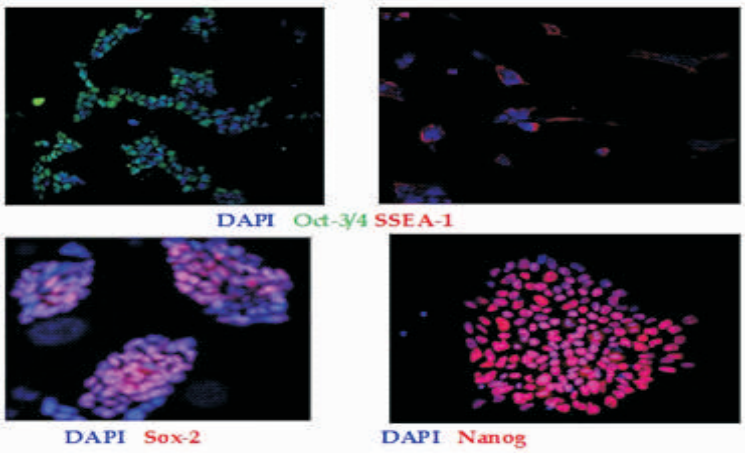
The present study was carried out to explore the feasibility of using the mouse Es cell line and differentiate them into lineages. Essentially to recapitulate the in utero developmental process using the mEBSc with V it B12, folate and Vit B6 deficiency.

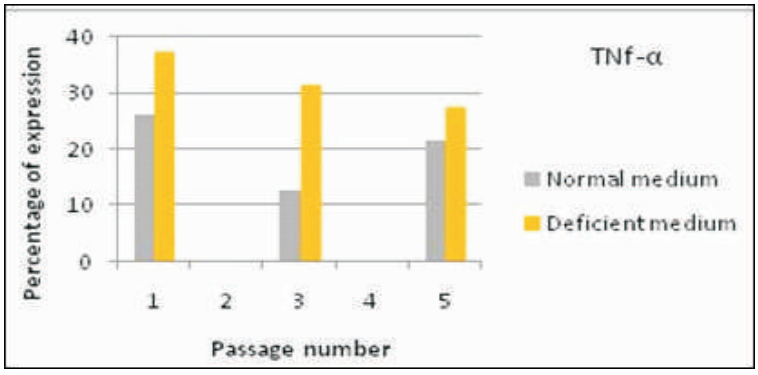
METHODOLOGY AND RESULTS

I) mESCS were characterised for pluripotent markers viz Oct-4, Sox-2, Nanog and SSEA-1 under the Control and deficient conditions(Folate –ve/ Folate –ve+Vit B6 –ve).

Levels of inflammatory markers

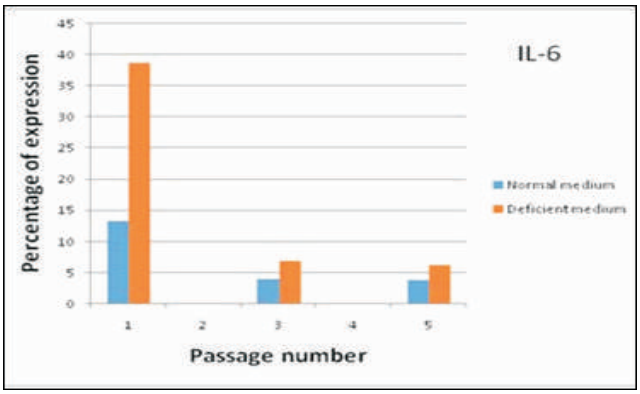
- Expression of inflammatory markers such as TNf-α and IL-6 were examined under the Control and experimental conditions.Cells at P-1,P-3,P-5 were used for the study and the parameters were measured in Flowcytometry (BD FACS Aria-II).
- The levels of TNf-α and IL-6 were high in deficient cells compared to their normal counterparts and was significant in first passage as compared with 3rd /& 5th. With IL-6 the magnitutde of decrease was higher in deficient conditions as compared to TNf-α.



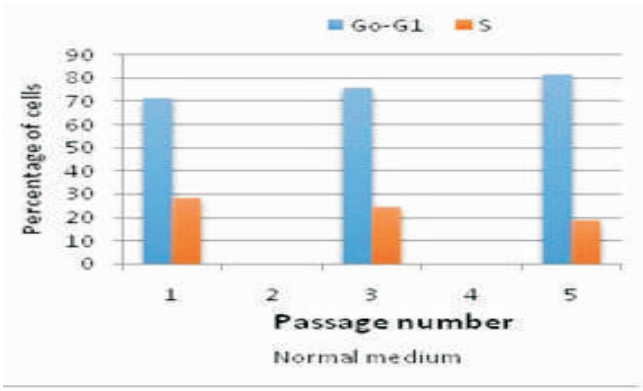


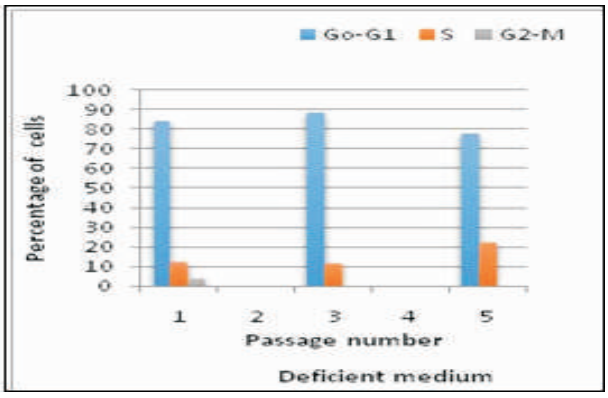
ii) Cell cycle distribution: mES cells were assessed for cell cycle analysis by FACS.

Cells	Go-G1	S	G2-M
Normal P-1	71.5	28.5	0
Normal P-3	75.79	24.21	0
Normal P-5	81.57	18.43	0
Deficient P-1	84.19	11.98	3.83
Deficient P-3	88.29	11.71	0
Deficient P-5	77.95	22.05	0



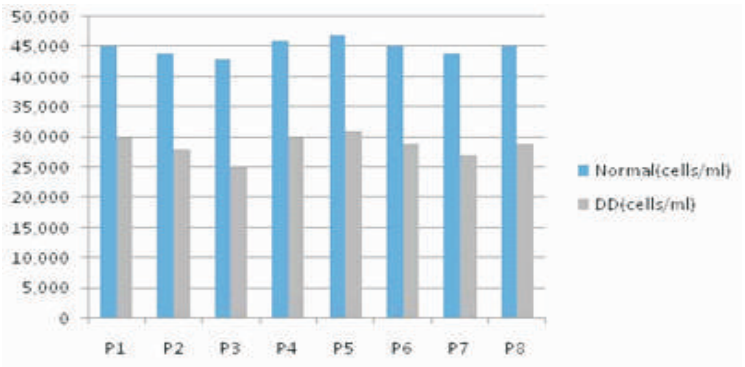
With increase in passage no the cells subjected to deficient conditions demonstrated an increase in percentage of cells in S-phase, unlike the normal (control) cells which were maintained in optimal conditions showed an decrease with increase in passage. This may suggest for the possible arrest of cells in S-phase owing to Folic acid deficiency.



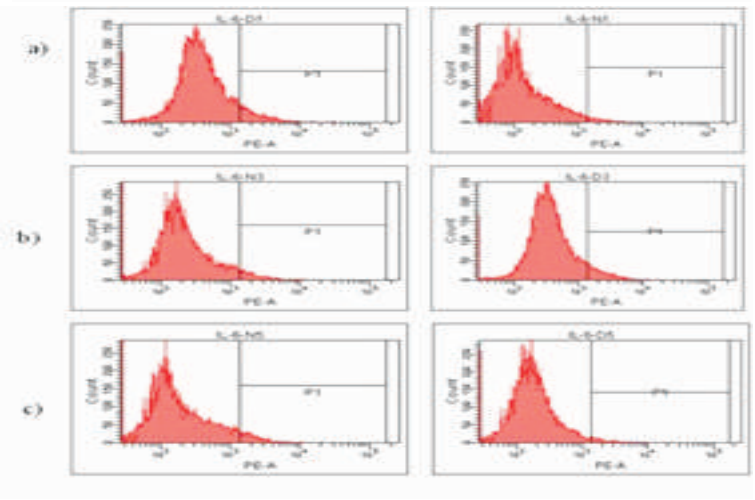


iii) Population doubling Assay

Over the passages in double deficient condition, the number of cells were counted in each passage by using a hemocytometer and the growth was monitored for 24 hrs. Bar graph represented by blue colour shows the number of cells at each passage under normal condition whereas the cell number variation under double deficient condition (Folate-ve + Vit B6-ve) as shown below.



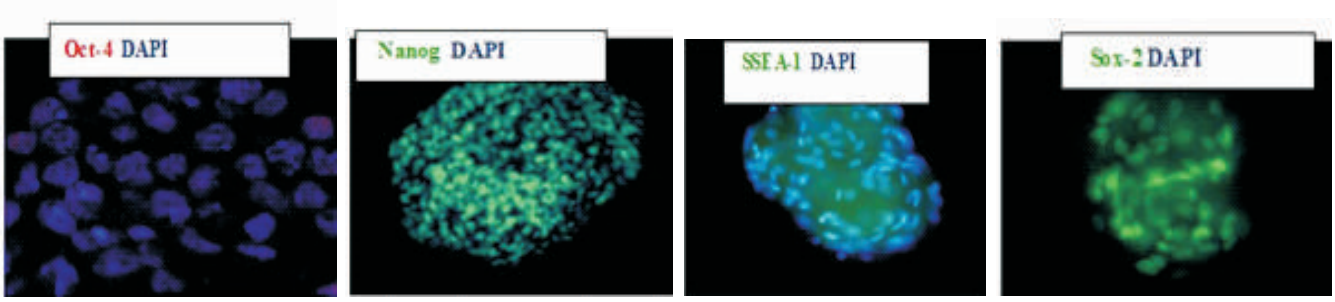
To study the inflammatory reactions in alternative passages we collected the cells and did a check on inflammatory markers like TNF-α and IL-6 by using FACS. The data obtained clearly shows that there is a variation in the double deficient condition when compared to normal.



iv) Embryoid Bodies (EBs) / characterization

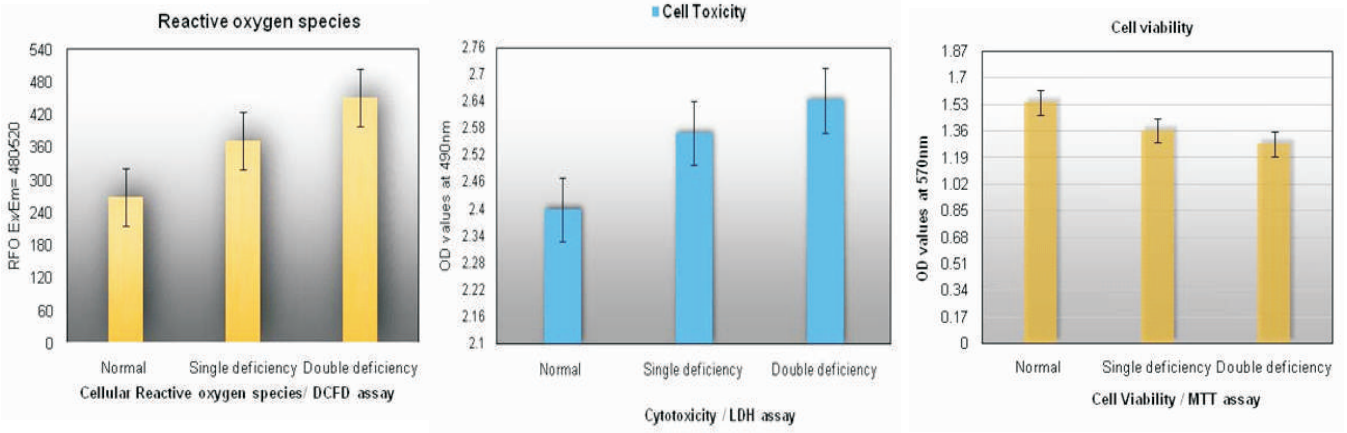
Embryoid bodies are the three dimensional cellular aggregates formed

from mESCs when grown under LIF (-) conditions. The embryoid bodies were generated by suspension method(earlier report). EBs generated from the normal and deficient conditions were characterised for pluripotent markers as given below Oct-4, Sox-2, Nanog and SSEA-1.



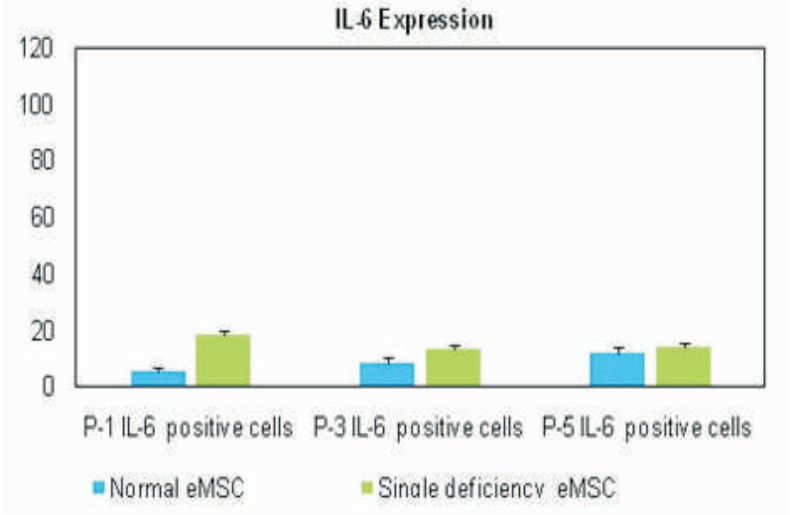
Experiments carried out under folate and B6 deficiency

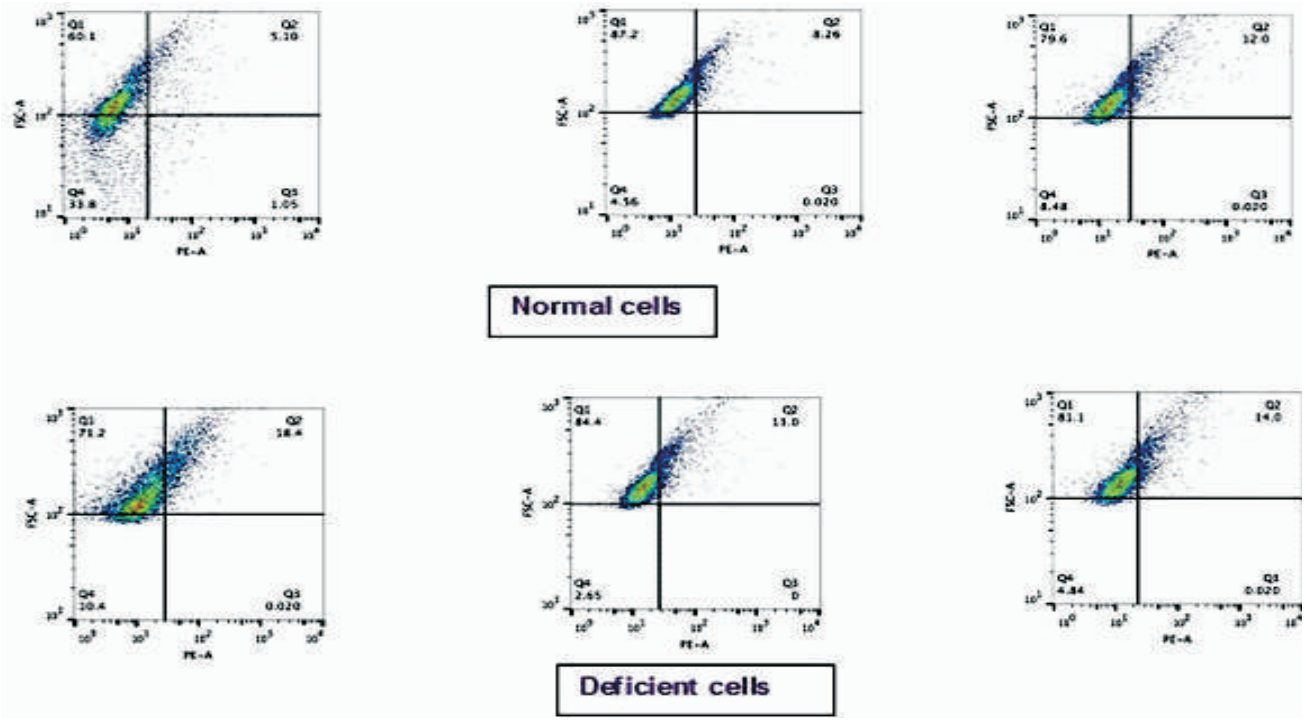
The viability and the levels of Reactive Oxygen species and cytotoxicity induced under the deficient (both SD and DD) were measured by MTT, DCFDA and LDH assays respectively. Also PCR was done to check the expression levels of inflammatory markers under the same conditions. As found from the results of the above said assays, viability was not found to be effected but there was an increase in the levels of Reactive Oxygen Species (a fold change of 1.4 in SD and 1.68 in DD) reflecting the oxidative stress induced in these cells by growth under Vitamin deficient conditions. Compared to their normal counterparts mESCs grown under both Folate as well as Folate and B6 deficient conditions have been found to experience more cyotoxicity (a fold change of 1.07 in SD and 1.10 in DD), though their viability was not found to be effected under such conditions to a significant extent.



Experiments carried out under Folate deficiency

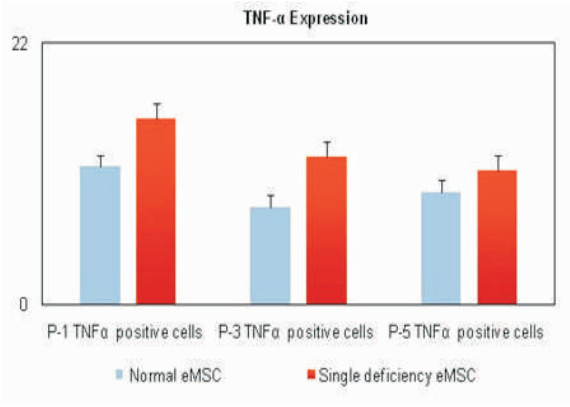
Percentage of cells expressing the pro inflammatory marker IL-6 was measured by Flow Cytometry upto 5 passages for cells in both normal and Folate deficient medium. Briefly, mESCs at 70% confluency were trypsinised and fixed in 70% Ethanol and processed for Flow Cytometry following previously published and established protocol. Cells are stained with a primary anti IL-6 antibody (abcam; ab6672) and have used the secondary Goat anti Rabbit antibody conjugated to Phycoerythrin (PE).

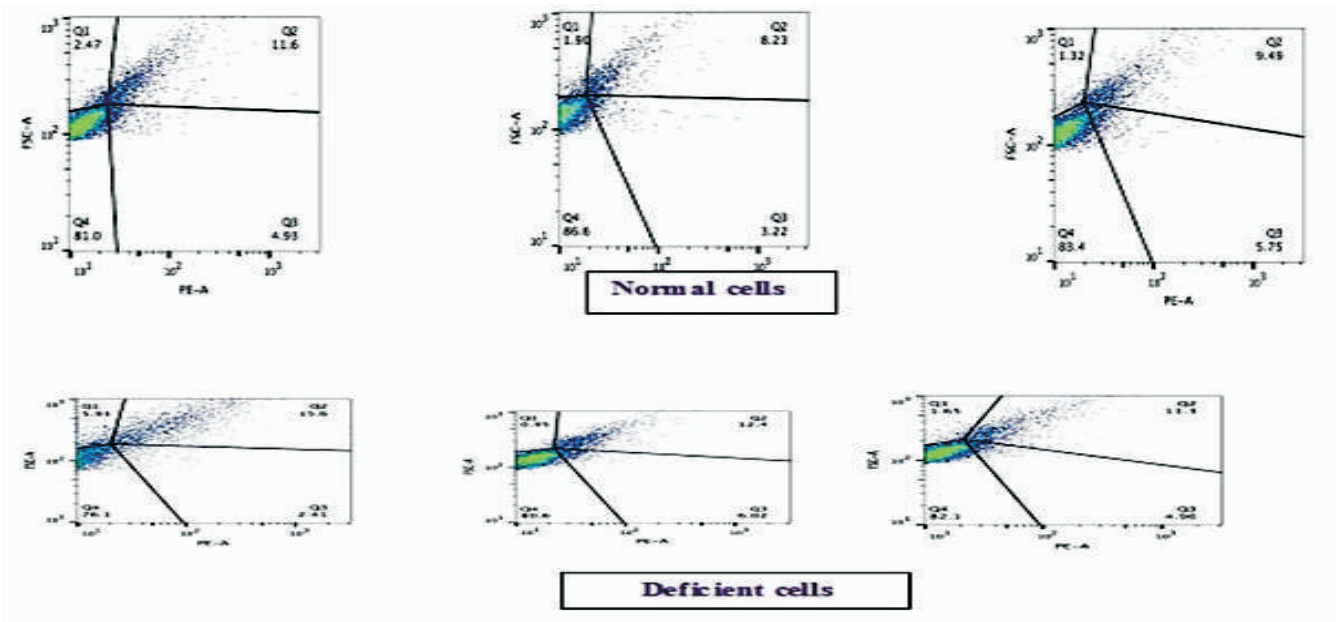




The percentage of cells positive for IL-6 deficiency were more in Folate deficient cells and more so in the first passage cells compared to their normal counterparts (3.6 fold). Although the percentage of cells positive for the inflammatory marker have decreased with passage in Folate deficient cells, it was more compared to cells grown in normal medium in any passage.

In another set of experiment, another pro inflammatory marker TNF alpha expression was studied by the same method using anti mouse TNF alpha PE/1;200 dilution (BD Biosciences, USA).

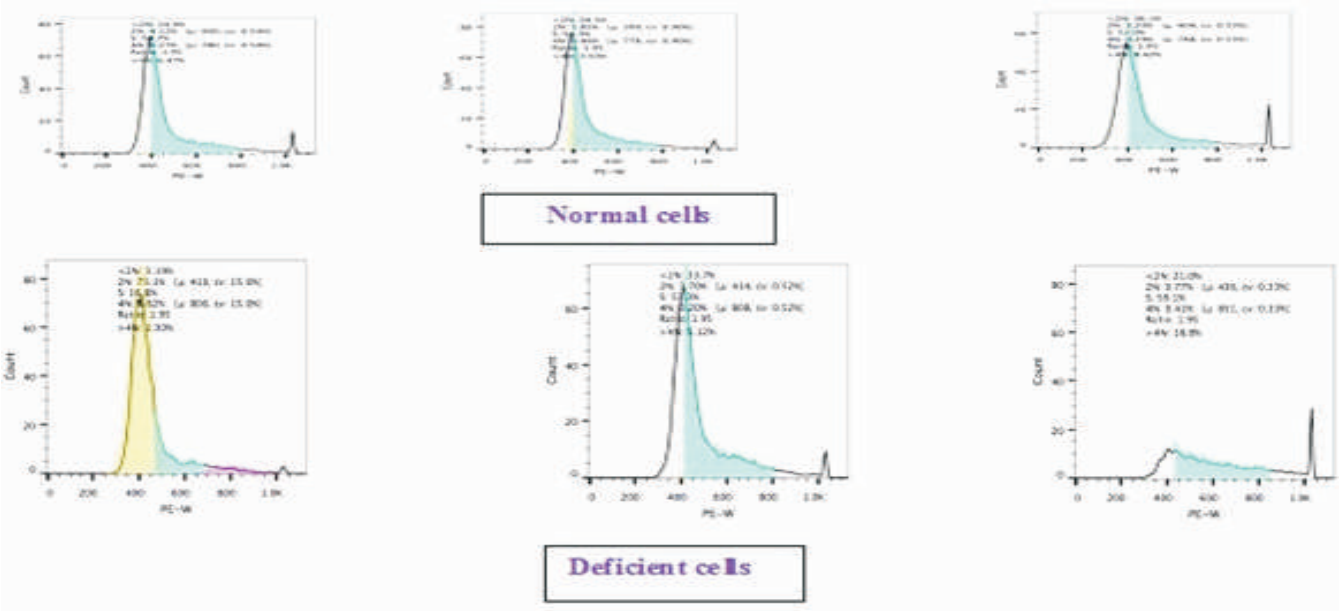


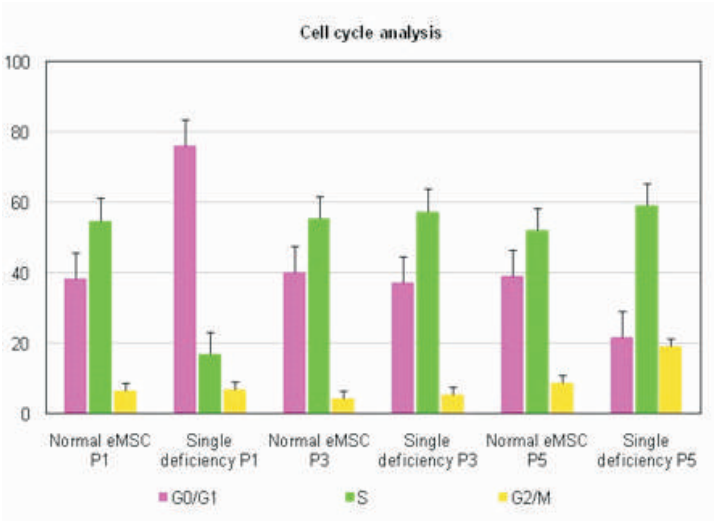


TNF alpha expression is 1.6 fold more in folate deficient cells than the cells grown in normal medium, indicating that cells deprived of folic acid deficiency have experienced profound inflammatory stress.

Cell cycle distribution

Folic acid, being a key methyl donor in the one carbon metabolism influences the nucleotide biosynthesis and hence DNA replication and other cell cycle events. Hence, the distribution of ES cells into different cell cycle phases under Folic acid deficiency and sufficiency was studied by Flow Cytometry (BD FACS Aria-II).





mESCs show a reduced G1 phase, an absence of DNA-damage checkpoint in G1, the absence of cell cycle– dependent expression of cyclin E, and lack of dependence on persistent serum stimulation and active MEK signaling during cell cycle progression. They are characterized by an extremely rapid transit through the G1 phase, which accounts for 15% of the total cell cycle duration and newly formed cells can enter a new phase of DNA replication very shortly after exit from mitosis. Nearly 50-60% of mESCs under normal conditions are found in S-phase of cell cycle. Cell cycle analysis by PI staining of fixed mESCs and Flow Cytometric measurement of percentage of cells was performed in each phase of cell cycle using Flowjo 10.3 software. From the analysis of the Flow Cytometric data, it appears that the classical cell cycle distribution pattern of mESCs is greatly altered under Folate deficient conditions as evidenced by a greatly reduced percentage of cells in S-phase of cell cycle I passage one. However, over passaging the percentage started coming closer to the ideal distribution. However, such alteration was not observed in normal cells. Similar kind of experiments need to be carried out under folate and B6 dual deficient conditions to arrive at a conclusion. However, apoptotic rate, senescence (If any) has to be measured in these cells to understand the exact ways by which the insult is acting upon the cells.

CONCLUSIONS

- Nutritional perturbation in utero have shown to have a direct bearing in the health of post natal life of the offspring, complementing with several altered metabolic indices. These could be mediated either by epigenetic - gene regulation or through gene- nutrient interaction.
- Embryoid bodies by hanging drop method was generated from both Control (MNC) and Folate deficiency (MNR/70%). All the three Germ layers were characterized: Nestin for Ectoderm, GATA-4 for Endoderm and Brachyury for Mesoderm. Interestingly, both mESCs and BM-MSCs under MNR showed up regulated expression in TNF α with mEBCs>BM-MSCs.
- Interestingly supplementation of 70% folic acid to the deficient mEBs caused reversal of the cell cycle to normalcy. The data showed comparison between control, deficient (70%) and supplementation (70%) with folic acid.
- Based on the above findings, the application of mEBs as important *in vitro* tool to study the developmental vs nutritional programming vis a vis for nutritional interventional studies was observed.

2. Exploring the beneficial effects of endothelial cells generated from human derived mesenchymal stem cells in the management of lymphedema - *in vivo* approach

Lymphedema describes a progressive pathologic condition of the lymphatic system in which there is a chronic swelling of the extremity. It occurs when the capacity of lymphatic system drains the protein-rich fluid excessively and subsequently causing inflammation, adipose tissue hypertrophy, and fibrosis. The lymphatic system plays a central role in the immune response through trafficking of immune cells and represents a key route for tumor metastasis. The underlying etiology in lymphedema is one of lymphatic transport dysfunction.

However, the molecular markers of lymphatic endothelium unlike the endothelium are not fully understood. It was discovered that VEGF-R3 and VEGF-C/VEGF-D were the key growth factors controlling lymphatic endothelial proliferation. Recent studies have demonstrated that transfer of healthy tissues can be used as a means of bypassing damaged lymphatics and ameliorating lymphedema. Although various conservative therapy and surgical therapies are available, the cellular and molecular crosstalk's between the lymphatic drainage and the regeneration is not fully understood. Furthermore, the mechanism that regulates lymphatic regeneration was also only partially understood. Besides, the role of stem cell transplantation in lymphatic regeneration has not been widely studied well.

Hence, the origin of the proposal lies in regeneration of the damaged lymphatic system by a stem cell based approach in the *in-vivo* animal model system. This is derived from the efficacy of stem cells that has already been clinically proven for the treatment of certain diseases. Besides, the effect of the mesenchymal stem cells in endothelial differentiation ad maturation has also been studied. However, there exist difference between the lymphendothelial and endothelial cell differentiation, maturation and functioning. A lymph capillary endothelium is different from other endothelium in which collagen fibers are directly attached to its plasma membrane. It is a specialized form of epithelium, distinct from but similar to vascular endothelium. Although the endothelial differentiation and maturation has widely been studied to target inflammation and wound healing in several disorders, the potential of the effect of lymphatic regeneration in a lymphatic drainage system is not fully understood, with the intricacies of its cross-talks of the underlying cellular and molecular mechanisms, thus justifying its significance.

To date, the role of stem cells in physiological and pathological lymphangiogenic processes remains unclear. The cell type that contributes to lymphatic regeneration may depend to a degree on the tissue environment. The potential role of mesenchymal stem cells (MSCs) in lymph angiogenesis has not been investigated to date. However, the source of the cells that contribute to lymphangiogenesis may mirror to a degree the description for haematic endothelial precursors.

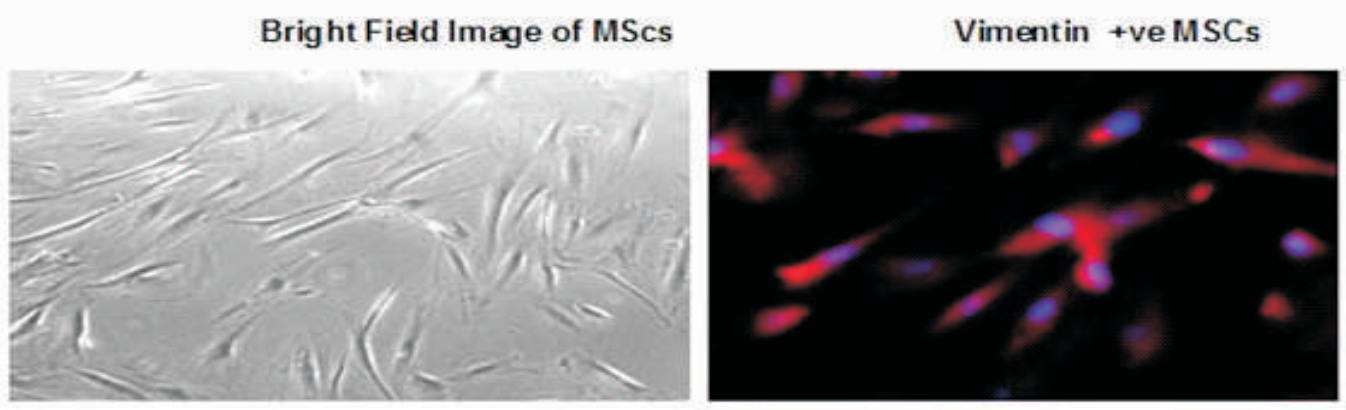
OBJECTIVES

- To induce the lymphatic dysfunction in rabbit animal model (Inguinal region) by surgical method b) Isolation and characterization of PD-MSCs (Allogenic) and to differentiate PD-MSCs into lymphendothelial cells.
- To inject (as an option device was also tried) the MSCs/ endothelial cells to the lymphedema rabbit model system. Isolation and characterization of PD-MSCs (Allogenic) and to differentiate PD-MSCs into lymphendothelial cells.
- To identify the necessary factors and to monitor the cross talk underlying differentiation of MSCs into Lymphendothelial cells and *in vivo* efficacy of the Tx cells.

METHODOLOGY AND RESULTS

A) Isolation of human placetal derived from mesenchymal stem cells (hPMSCs)

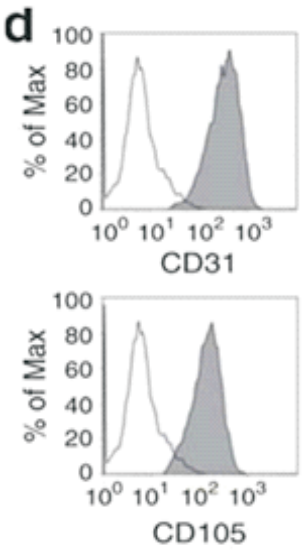
The methodology was similar to the tissue was digested with collagenase 1, and subjected to washings and centrifugations. The mononuclear cells obtained in the pellet was seeded, and put for culture supplemented with DMEM knock out medium with 10% FCS and Antibiotics. The MSC phenotype was visible by 5th day and by 6-7 days attained confluence (70-80%). The cells were trypsinized, passaged (4-5) and characterized for MSCs phenotype.



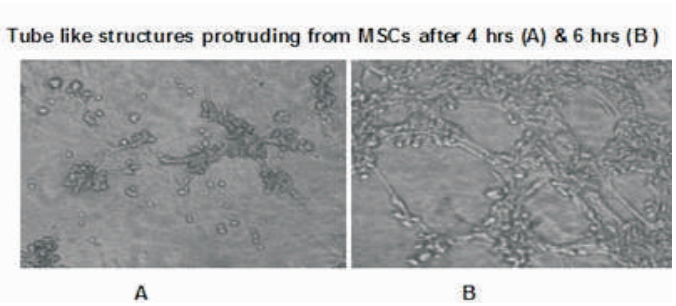
B) Viability measurements

The viability of the cells were determined (P5) in both Control and Experimental groups by MTT assay (3 - (4, 5- dimethylthiazol-2-yl) - 2, 5-diphenyltetrazolium bromide, a yellow tetrazole), based on the formazan formation. In addition, Trypan Blue exclusion (TBE) was also used for the quatititative measurements.

Surface Marker Characterisation: Single cell suspension (1X10^6 / ml) from both Control and Experimental groups in Phosphate Buffered Saline (Ca^{+2}/Mg^{+2} free), were incubated with 1^{0}Ab dye conjugates (1:100 Dilution) for CD29-FITC (HA2/5), CD90-PE(Ox-7),CD31-PE(TLD-3A12) (BD Biosciences, USA) in 1:100 dilution at 4^{0}C for 30 minutes. A total of 10,000 events were acquired in FACS Aria and data were analyzed by using FACS Diva software (BD, San Jose, CA).Values represent Mean ± 6 SE, and median fluorescence intensity (MFI) (CD90, CD29 and CD31)/percentage positivity. The data was computed from three independent experiments carried out in duplicates.



C) Tube formation assay



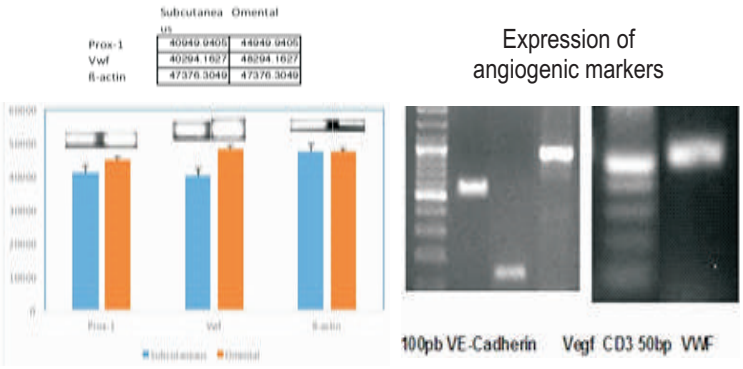
The wells were coated with Matrigel under cool room temperatures, as the gel solidifies above 25^{0}C. The 12 well plates were coated with 350µl Matrigel and incubated at 37^{0}C for 1 hour. PDMSC's cultured in normal growth media containing 10%FBS and in restricted media with 10%FBS were seeded at a density of 50,000 cells/ well and incubated with 50 ng. of vascular endothelial growth factor. Ring formation was observed and qualitatively analyzed using a bright field phase contrast microscope (Nikon eclipse TE 2000-s) and photographs were taken at every 2 hours. For the wound scratch assay a 100% confluent plate of PDMSC's were wounded by a scratch on the monolayer by using a 1000 µl pipette tip. To determine the endothelialization potential of PDMSC's secrete pro-angiogenic fators, wound scratch assay was performed by PDMSCcondition media of cells cultured in growth media with 10% FBS for 72 hours. Conditioned media were prepared in each cell type by growing the cells in 70% confluent plates in growth media with 10% FBS for 48 h. The respective media were collected and centrifuged at 1800 rpm for 10 min to remove cellular debris.

d) Semi-quantitative PCR

Total RNA from PDMSCs differentiated from Matrigel were taken and separated. The cell pellet was obtained by centrifuging at 1800 rpm for 15 min. Further, the cells were taken out and added 0.5 ml of TRI reagent according to the manufactures instructions (Sigma Aldrich, St. Louis, MO, USA). Reverse transcription reactions were performed in a 20 ml volume with 1-5 mg of total RNA using the AMV-Reverse transcriptase (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol. Using the cDNA, expression of mRNA related to angiogenesis was investigated using GAPDH as a positive control. Specific primers from the cDNA were amplified by using RT-PCR master mix (Platinum PCR Super Mix, Invitrogen, US), DEPC water and the respective primers (IDT). VEGF, VE-cadherin, CD-31, VWF were checked for confirming the angiogenic property loring the differential expression of prox-1 and vwf. As given below Semiquantitative PCR of PROX-1 and Vwf, shows that PROX-1 and Vwf were upregulated in both subcutaneous and omental adipose tissue. These findings are in par with the reported data towards the lymphoendothelial markers present in the adipose.

Primer sequence Tm(˚c) product size (bp)
Vegf F- 5'ctacctccaccatgccaagt-3'
R- 5'gcagtagctgcgctgataga-3' 52 109
VWF F- 5'cggcttgcaccattcagcta-3'
R- 5'tgcagaagtgagtatcacagccatc-3' 53 90
CD 31 F- 5'attgcagtggttatcggagtg-3'
R- 5'ctcgttgttggagttcagaagtgg-3' 52 965
VE-cadherin F- 5'acgggatgaccaagtacagc-3'
R- 5'acacactttgggctggtagg-3' 52 596
Tie-2 F- 5'agaccagcacgttgatgtga-3'
R- 5'tgggttgcttgaccctatgt-3' 60 282
Ang-1 F- 5'agcagcctgatcttacac-3'
R- 5'atgatgatggtcgacggc-3' 50 11

e) Expression of Angiogenic markers in omental vs subcutaneous



CONCLUSIONS

- It is evident that MSCs, which have inherent immunomodulatory functions vis a vis potent anti-inflammatory would form the target of choice for the tx programmes.
- Based on our *in vitro* studies, omental adipose tissue was more promising for lymphoangiogenesis, which was assessed based on the gene expression of VWF and CD31.
- The in vitro experiments were successfully undertaken, and *in vivo* experiment was not undertaken due to technical difficulties.

3. Modulation of adipose tissue inflammation by dietary n-3 polyunsaturated fatty acids (PUFA) - Potential role in metabolic syndrome

Metabolic syndrome is characterized by obesity, insulin resistance, dyslipidemia and hypertension. It is associated with increased risk of type 2 diabetes and cardiovascular diseases. The incidence of metabolic syndrome is increasing exponentially as a consequence of escalating rise in obesity. The public health strategy to prevent obesity has been largely unsuccessful. Hence, in recent years, studies are focused on dietary interventions that attenuate the severity of the metabolic syndrome. Dietary fatty acids plays a major role in the development of insulin resistance, obesity and metabolic syndrome. Earlier studies in rats have shown that substitution of starch with sucrose induces insulin resistance by decreasing peripheral insulin sensitivity. Supplementation of either α-linolenic acid (2.3en%, n-6/n-3 ratio 2) or long chain (n-3) PUFA (0.56 en%, n-6/n-3 ratio 5) prevented sucrose induced decrease in peripheral insulin sensitivity. However, the molecular mechanisms by which n-3 PUFA improve the insulin sensitivity is not known.

Adipose tissue was earlier considered as an energy storage organ. However, recent studies have demonstrated that adipose tissue produces and secretes a variety of biologically active molecules collectively known as adipocytokines which plays major role in modulating insulin sensitivity. Dysregulated production of adipocytokine plays major role in the pathogenesis of metabolic syndrome. Increased oxidative stress in accumulated fat has been suggested to be cause of dysregulation of adipocytokines in metabolic syndrome. More recent studies have demonstrated that increased adiposity is associated with increased macrophage infiltration into adipose tissue and these macrophages are the major contributing factors to local inflammatory cytokine production. Although the molecular mechanism involved in macrophage infiltration is not known, oxidative stress could be the cause. In this context, a recent study has shown that, increased adiposity caused over-expression of glucose 6 phosphate dehydrogenase in adipose tissue which induces oxidative stress, macrophage infiltration and subsequent adipocytokines dysregulation and insulin resistance. It is well established that n-3 PUFA has potent anti-inflammatory effect and the insulin sensitizing effects of n-3 PUFA could be mediated through its effects on adipose tissue inflammation and function.

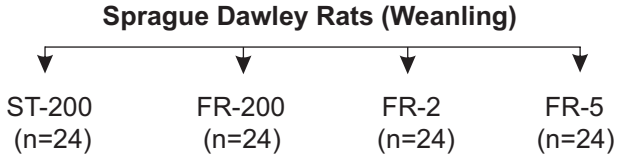
AIMS AND OBJECTIVES

- To study the effects of dietary n-3 PUFA on body composition.
- To investigate the effects of dietary n-3 polyunsaturated fatty acids (PUFA) on inflammatory alterations and secretory function of adipose tissue.
- To study the molecular mechanism by which n-3 PUFA attenuate the adipose tissue inflammation

METHODOLOGY

Weanling male WNIN rats (n=96) were divided into four groups and fed starch/ fructose - casein based synthetic diet as per AIN-93 requirements. Metabolic syndrome was induced by replacing starch with fructose. The n-6:n-3 fatty acid ratio was altered by blending of groundnut oil, palm oil, linseed oil (source of α-linolenic acid) to get the ratio of 200 and 2 or by blending of groundnut oil, palm oil and fish oil (source of DHA & EPA) to get the ratio of 5. The total fat content was 10%. Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA were kept similar in all the groups and n-6:n-3 fatty acid ratio was altered by substituting n-6 PUFA with n-3 PUFA.

STUDY DESIGN



In the ST-200 group, starch was used as the source of carbohydrate and the ratio of n-6:n-3 was formulated to 200 using blends of groundnut oil, palm oil and linseed oil. In the FR-200 group, fructose was used as the source of carbohydrate and the ratio of n-6:n-3 was formulated to 200 using blends of groundnut oil, palm oil and linseed oil. In the FR-2 group, fructose was used as the source of carbohydrate and the ratio of n-6:n-3 was formulated to 2 using blends of groundnut oil, palm oil and linseed oil. In the FR-5 group, fructose was used as the source of carbohydrate and the ratio of n-6:n-3 was formulated to 5 using blends of groundnut oil, palm oil and fish oil. All the animals were fed the respective experimental diets. The animal experiment was terminated at two time points (3 month & 6 month). At each time point, 12 animals from each group were sacrificed.

RESULTS

- Rats fed high fructose diet increased visceral adiposity and fat mass without altering lean body mass and fat free mass and substitution of n-6 PUFA with n-3 PUFA normalized visceral adiposity and fat mass only at 6th month.
- Substitution of n-6 PUFA with n-3 PUFA prevented the fructose induced dyslipidemia at both the time points.
- Fructose feeding induced insulin resistance as evidenced by increased fasting plasma insulin, HOMA-IR and AUC of insulin and substitution of n-6 PUFA with n-3 PUFA prevented insulin resistance only at 6^{th} month.
- Fructose feeding increased plasma leptin level and decreased adiponectin level and substitution of n-6 PUFA with n-3 PUFA normalized the leptin level at both the time points whereas adiponectin level was normalized only at 6^{th} month.
- Among the plasma adipocytokines, MCP-1 was increased whereas IL-10 was decreased by fructose feeding and substitution of n-6 PUFA with n-3 PUFA normalized MCP-1 at both the time points while IL-10 was normalized only at 6^{th} month.
- Fructose feeding induced adipose tissue oxidative stress as evidenced by increased MDA level and substitution of n-6 PUFA with n-3 PUFA prevented the oxidative stress at both the time points.
- Fructose feeding upregulated the expression of adipose tissue proinflammatory cytokines such as TNFα and MCP-1. Substitution of n-6 PUFA with n-3 PUFA normalized the levels at both the time points.
- Fructose feeding induced endoplasmic reticulum stress as evidenced by upregulation of GPR-78 and substitution of n-6 PUFA with n-3 PUFA prevented the fructose induced endoplasmic reticulum stress at both the time points.
- Fructose feeding induced the macrophage infiltration into the adipocytes and substitution of n-6 PUFA with n-3 PUFA ameliorated the macrophage infiltration at both the time points.

CONCLUSIONS

- The present study provides evidence to support the optimal balance of n-6 and n-3 PUFA for the prevention of metabolic syndrome.
- It also reinforce the current dietary recommendations of moderating the intake of n-6 PFA and increase the intake of n-3 PUFA for the prevention of diet related chronic diseases including metabolic syndrome.

4. Assessment of nutritional and morbidity status and utilization of health care facilities in the elderly population aged 60 years and above

There has been a steady increase in the proportion of the elderly population in both developed and developing countries during the last few decades due to increased longevity. In India the proportion of elderly people were increased from 7.7% in 2001 to 8.6% by 2011. This increased longevity is associated with burden of several chronic non-communicable age-related diseases. Nutrition is an important aspect of health in the older population and affects the aging process. It has been found to be a valuable determinant of quality of life as well as morbidity and mortality. Though there are studies on nutritional status of elderly, majority of them are rural and/or hospital based. Though food intake assessments play a crucial role in detecting dietary exposure and disease causation, such assessments are rarely conducted among the elderly persons. Hence, the evaluation of nutritional status of geriatric population is very important to assess both the under nutrition as well as overweight/obesity, to enable to undertake appropriate interventions on the health and nutritional needs of the elderly. Thus, the main aim of the study is to assess the health and nutritional status of geriatric population in a comprehensive manner.

OBJECTIVES

a) To assess the nutritional status of elderly population

b) To assess the nutrition related morbidity status in the elderly population

c) To assess the utilization of health care facility in the elderly population

METHODOLOGY

Sample design: A community based cross sectional study was adapted using 30 cluster sampling procedure and a total of 900 (450 males and 450 females) subjects were recruited from Hyderabad.

A pre-tested questionnaire was administered to all the subjects to collect the information on socio-demographic particulars, lifestyle habits, general morbidity and history of non-communicable diseases (NCDs). Anthropometric measurements were taken from all subjects using standard equipment and methods. Dietary intake was assessed in one fourth of the subjects using 24hr/one day dietary recall method and nutrient intake was calculated.

Statistical analysis: Data were entered into Epi info software version 7. The final data were transported and the analysis was performed using IBM SPSS 21 version. Chi-square and t-test were used to compare the means between two groups. One way analysis of variance with Post Hoc test of LSD was used to compare more than two means. P value less than 0.05($p<0.05$) was considered to be level of significance.

RESULTS

- The prevalence of chronic energy deficiency (CED), overweight, obesity and central obesity was 9.6%, 43.3% and 67.6% respectively among the urban elderly population.
- According to the Mini Nutritional Assessment (MNA), only 33.8% of urban elderly were well nourished and 62.3% of them were at the risk of malnutrition, but only 3.0% of the elderly subjects were malnourished.
- The mean consumption of all the food groups, (except cereals & millets and fats & oils) was below the RDI. Similarly, the mean intakes of all nutrients (except total fat) are below the RDA (ICMR Dietary Guidelines 2010).
- The prevalence of anemia, diabetes and hypertension in urban elderly was 46.44%, 32.4% and 74.6% respectively, whereas, the prevalence of hypertriglyceridemia and hypercholesterolemia was 13.9% and 20.3% respectively.
- The self-reported prevalence of general morbidities like poor vision, joint pains, forgetfulness, diminished hearing and chewing problems in urban elderly subjects were 73.3%, 62.6%, 28.3%, 27.1% and 24.8% respectively. The prevalence of other general morbidities are; loss of appetite

(19.7%), constipation (14.2%), difficulty in walking (12.7%), urinary incontinence (6.0%), depression (5.9%), chronic cough (5.3%) and skin problems (5.0%).

- The proportion of urban elderly subjects those who are taking medication for their general morbidities were very low (13.5%). Majority (72.6%) of medicines were prescribed by the private doctors followed by government doctors (20.2%).
- The prevalence of chronic morbidities (based on medical records) like hyperacidity, hypertension, diabetes, muscular pain, back pain, fracture, glaucoma and cataract were 22.3%, 51.7%, 26.8%, 13.3%, 19.7%, 9.6%, 10.4% and 41.1% respectively. The prevalence of other chronic morbidities are; Coronary artery disease (6.6%), asthma (6.3%), dyslipidemia (4.4%), stroke (3.6%), kyphosis (2.3%), osteoporosis (1.7%), tuberculosis (0.6%) and cancer (0.2%).
- The proportion of subjects with un-healthy personal habits like, tobacco and alcohol consumption (in any form) was 34% and 41.3% respectively. Nearly 32.7% of urban elderly have healthy habits like slow walking and walking.
- Based on Barthel Activities of Daily Living (ADL) Index, the overall prevalence of functional disability in urban subjects was 23.3%.
- All urban elderly participants received health care whenever they needed.
- Majority (63.7%) of urban elderly utilized private hospital services, 14.2% utilized public hospital services and only 4.3% utilized private clinic services.
- Majority (80%) of urban elderly utilized private doctor services, 17% utilized medical doctor (government doctor) services and only 0.7% of urban elderly utilized ayurvedic doctor services.

CONCLUSION

Though all urban elderly population received health care facilities whenever they needed, but still the prevalence of above nutritional related health problems are high which need to be addressed at regular intervals.

5. Impact of hyperglycaemia on invasion properties of first trimester trophoblastic cells: Implication on preeclampsia

Invasive trophoblasts are exposed to low oxygen tension in endometrium until twelve week of gestation where sustained hypoxia up-regulates the expression of genes associated with glucose metabolism, glucose transport, angiogenesis and invasion in order to adapt to anaerobic conditions. Hypoxic balance is critically essential to maintain angiogenesis of the placentation. Placentation that mimics confined regulated invasion, may involve interplay of glucose transporters and hypoxia-inducible factor 1 alpha (HIF1α) since positive interplay of the duo drives the development of carcinoma cells. Hypoxia alters the expression of GLUT1 and GLUT3 in human placenta in order to adjust the metabolic needs of the cells. Reduction in placental glucose transport is the key factor for reduced fetal growth under condition of severe hypoxia. Induced hypoxia conditions up regulated the activation of GLUT1 via HIF1α in placental BeWo cells. In vitro, basal membrane GLUT1 is positively regulated by insulin-like growth factor 1 (IGF1) and hypoxia while it is also up regulated in gestation with diabetic pregnancies *in vivo*. Few reports postulate that human placenta is insulin resistant while others demonstrated that glucose uptake can be stimulated by insulin in placental cytotrophoblast. IGF1 increases the basal membrane content of GLUT1 and thereby up regulates glucose transport in BeWo cells. Little is known on the expression of endogenous regulator of glucose metabolism such as HIF1α in response to glucose-stimulated tube formation (*in vitro* angiogenesis) and their regulation by insulin in the first trimester human placental cells. We hypothesize that GLUT1 may be involved in the glucose uptake and tube formation of the first trimester placental trophoblast cells.

OBJECTIVES

To investigate the effects of endocrine, environmental and nutrient factors such as insulin, hypoxia and glucose on early placentation using explant culture of the first trimester placental villous tissue and extra villous trophoblast cells, HTR8/SVneo.

METHODOLOGY

Ethical approval was obtained from the Osmania Medical College, Hyderabad, India to collect 8-12 weeks of first trimester human placentas after written consent from the subjects (OMC/Hyd/12/12-6-2014). Placental tissues were collected by scrapping under aseptic condition. Tissues were rinsed in sterile ice cold (4ºC) Dulbecco`s Phosphate Buffered Saline (DPBS) containing penicillin and streptomycin until blood was washed out completely. Placental tissues were further minced aseptically and transferred into ice cold HBSS (Hank's Buffered Salt Solution). Floating villous tissues (30-40 mg/tube) equilibrated in 1 ml of HBSS for 5 min at 37°C in an orbital shaker. Subsequently, villous explants were conditioned at different concentrations of $CoCl_2$ (0-250 µM) in Dulbecco's Modified Eagle Medium-Ham's F12 (DMEM-F12) medium for 24 h to establish hypoxia condition. HTR8/SVneo cells (2-8 passages) were cultured in glucose free RPMI and D-glucose was added exogenously as described before. Cytochalasin-B (Cyt-B) was used as GLUT inhibitor and GLUT1 siRNA were used to study GLUT1 activity. Tube formation was performed to study angiogenesis. Glucose uptake was measured by radiolabelled deoxy-D-[^{3}H] glucose. Migration rate was determined by wound healing assay. Real-time PCR and Immunoblotting were performed to determine expression of mRNAs and proteins. Data were analysed using Graphpad prism and SPSS16.

RESULTS

Salient findings

GLUT1 expression was stimulated by insulin and hypoxia but not with glucose. Cyt-B inhibited insulin- and glucose-stimulated tube formation but not with hypoxia stimulated one. Hypoxia-stimulated migration was increased to greater extent in response exogenous glucose (25mM) as compared to 11mM glucose suggests a requirement of glucose to stimulate tube formation and migration of the first trimester trophoblast cells. Insulin, close to the physiological concentration (1ng/ml) stimulated tube formation with concomitant expression of KDR/VEGFR2 which is required for vessel formation to promote angio-genesis in placenta. Silencing of *GLUT1* inhibited the glucose and insulin-stimulated tube formation as well as glucose uptake. Exogenous glucose stimulated the activation of AKT and protected PI3K inhibition indicates that glucose uptake and GLUT1 expression stimulated by insulin is associated with AKT activation. Based on the data new roles of GLUT1 in the glucose uptake and tube formation of the first trimester placental trophoblast cells, HTR8/SVneo was hypothesized (Fig.1).

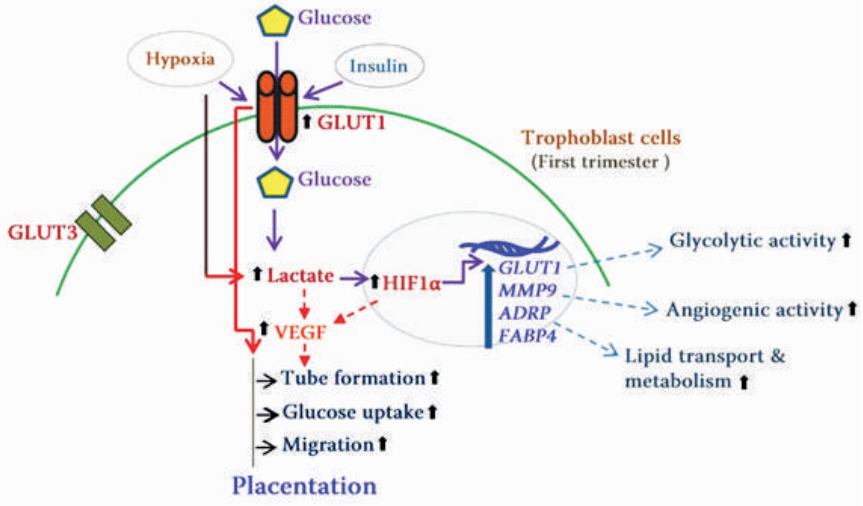


Fig.1: Schematic diagram of the putative roles of GLUT1 in the glucose uptake and tube formation of the first trimester placental trophoblast cells, HTR8/SVneo. Uptake of glucose and expression of GLUT1 is stimulated by glucose, hypoxia as well as insulin. Silencing of *GLUT1* abrogates the glucose and insulin stimulated glucose uptake & tube formation indicating that GLUT1 may be involved in these processes. Lactate production by glucose or in hypoxia also may contribute in glucose stimulated tube formation by activating pro-angiogenic factor, VEGF. (Basak et.al 2018, Mol.Cell Biochem., article in press)

Increased glucose uptake and GLUT1 activities favor HIF1α activation in first trimester trophoblast cells that may stimulate glycolytic and lipid metabolic activities with stimulation of angiogenesis due to increased tube formation and expression of pro-angiogenic mediators such as VEGF, MMP9, GLUT1, FABP4 etc. Increased lactate production in response to glucose could induce VEGF-stimulated placental angiogenesis in first trimester trophoblast cells.

CONCLUSIONS

GLUT1 plays an important role in both basal and glucose-stimulated glucose uptake, glucose stimulated tube formation and insulin stimulated glucose uptake of the first trimester HTR8/SVneo cells. GLUT1 protein expression and glucose transporter activity were decreased in pre-eclampsia indicates that optimum GLUT1 function may be required to prevent development of IUGR. However, further work is required to underpin the mechanisms of GLUT1 action in first trimester placental trophoblast cells. This is the first report that demonstrates glucose uptake and tube formation of the first trimester placental trophoblast cells is mediated in part by GLUT1.

6. Understanding the role of t cells in obesity and diabetes

The adipose tissue, the central component of obesity, was initially thought to be an inert tissue meant only for energy storage in the form of triglyceride, but later on recognized to be an active endocrine and paracrine organ which plays an important role in the development of metabolic syndrome. Various studies in the past decade indicated that obesity is associated with chronic inflammatory response characterized by production of various proinflammatory cytokines and activation of inflammatory signaling pathways. An interesting feature of the inflammatory response in obesity is that it appears to be triggered and reside predominantly inside the adipose tissue TNF-α was identified as the first molecular link between obesity and inflammation and was implicated in the development of obesity associated insulin resistance. Subsequently, other proinflammatory cytokines like IL6 and MCP-1 have been implicated with the development of obesity associated insulin resistance. MCP-1, an essential chemokine or macrophage activation and infiltration, recruits macrophages in the inflamed adipose tissue which get further activated leading to production of proinflammatory cytokines in a positive feedback paracrine loop leading to development of insulin resistance. For the first time reported earlier that, in addition to the macrophages, T cells also migrate into the adipose tissue, recruited by the RANTES produced by the stromal-vascular fraction upon some inflammatory cues. We have shown that, RANTES, though a T cell specific cytokine, is also an adipokine. An increased expression of its receptor CCR5 was observed during obesity indicating that there could be a functional consequence of increased T cell migration into the adipose tissue. Activated T cells inhibited adipocyte differentiation, similar to the macrophages. However, the functional consequences of T cell migration into the adipose tissue yet to be understood. It was hypothesize that, T cells and secreted cytokines may modulate adipocyte function leading to development insulin resistance. In this proposed project, it was aimed to understand the pathophysiological significance T cell infiltration and their relationship with insulin resistance.

OBJECTIVES

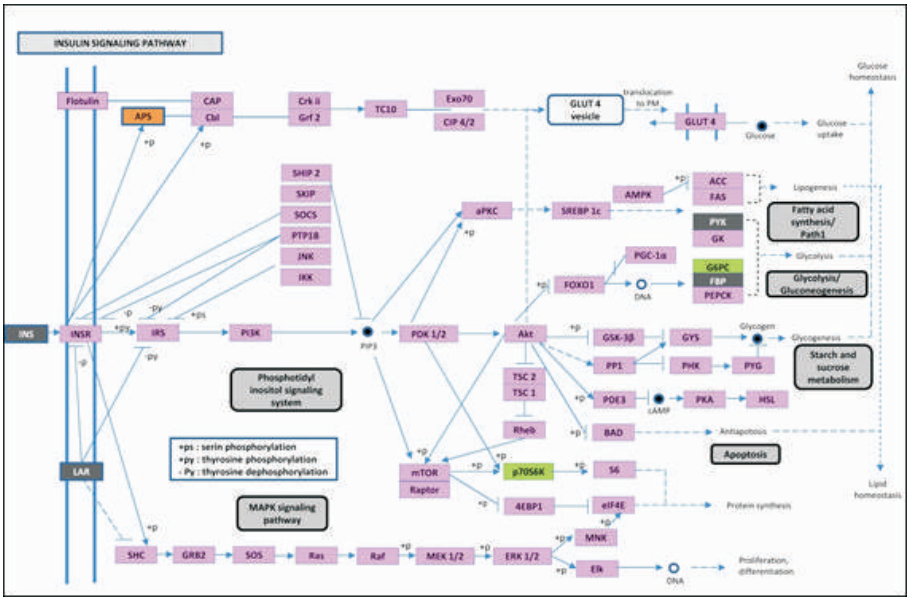
1. To identify the subsets of T cells infiltrating into the adipose tissue in diet induced obese mice.
2. To establish the role of T cells and secreted cytokines like RANTES in modulating adipocyte functions.
3. To identify and characterize the genes regulated in preadipocytes and adipocytes by T cells.

METHODOLOGY

The mice strain C57BL/6 was used for all the experiments. All procedures were performed in accordance to the guidelines approved by the Institutional Animal Ethics Committee of National Institute of Nutrition, Hyderabad. For differentiation experiments, 3T3-L1 murine preadipocytes were cultured to confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum and differentiated using DMI cocktail containing 1µM dexamethasone, 0.5 mM isobutylmethylxanthine, and 1µM bovine insulin and at day 8-9 post differentiated adipocytes were used for all experiments. For coculture experiments splenic T cells were isolate using nylon wool columns or in some experiments pan T cells were isolated using MACS. For real time PCR, total RNA was isolated from treated cells using trizol and after DNAse-1 treatment and reverse transcribed to cDNA using commercially available c-DNA synthesis kit. Glucose uptake assays were performed on fully differentiated 3T3-L1 adipocytes (*days 7* and *8*). Lipolysis is the hydrolysis of triglycerides within the cell into glycerol and free fatty acids. The degree of lipolysis was measured by measuring the amount of free glycerol released in media by using Free Glycerol Determination kit (Sigma) as per manufacturer's instruction. Transcripome analyses were carried out using Ion Proton NGS. Standard protocol followed for Western Blotting experiments using appropriate combinations of primary and secondary antibody.

RESULTS

Fig. Activated t cells modulate several genes of insulin signaling cascade



The major aim of the work was to investigate whether the infiltrated t cells play any direct role in dysregulating critical adipose tissue functions. It was found that conA-activated t cells could significantly inhibit insulin-stimulated glucose uptake in 3T3-L1 adipocytes. Though, insulin-stimulated glucose uptake in adipocytes constitute only about 10% of the total body glucose uptake, it appears to be very critical in regulating glucose metabolism in muscle and liver and overall energy homeostasis. Activated t cells also induced increased lipolysis in 3T3-L1 adipocytes, which can increase the concentration of circulating FFA that can induce hepatic insulin resistance as well as increased hepatic glucose output resulting in higher fasting blood sugar. Nonetheless, FFA can also induce inflammation through activation of endoplasmic reticulum (ER) stress and IKKβ pathways and activation of NF-κB transcription factor which can induce transcription of several proinflammatory cytokines.

Most interestingly, it was found that the activated t cells require direct cell to cell interaction with the adipocytes to affect adipocyte functions. Though the exact nature of the cell surface ligands and receptors involved is not understood it appears that CD40-CD40L dyad is involved at least to some extent. T cells were found to induce expression CD40 in adipocytes and there was concurrent upregulation of its cognate ligand (CD40L) in the interacting t cells themselves. Blocking CD40-CD40L interaction by using anti-CD40 antibody on adipocytes was found to partially ameliorate t cell mediated insulin resistance. Activated t cells were also found to induce more robust activation of ERK1/2 and p38MAPK in adipocytes when cocultured in direct contact with adipocytes. In addition, activated t cells were found to inhibit the adipogenic program by inhibiting C/EBP and PPARγ and the downstream genes lipid synthesis/storage were also affected. It

was found that activated t cells could inhibit several genes like fatty FAS, CD36, SREBP1c and SCD1. Interestingly, RANTES a major t cell chemokine was not found affect adipocyte functions significantly. RNA sequencing was performed in an Ion Proton Semiconductor Next Generation sequencer expression of about 5078 genes were found to be either significantly ($p<0.05$) upregulated or down regulated including some genes that are known to play some role in obesity, inflammation and insulin resistance.

Therefore, the data presented herein, clearly established a direct role of t cells in modulating adipocyte functions through its direct cell-to-cell cross talks in adipocytes by upregulating inflammatory gene network in adipocytes. Therefore, targeting t cell recruitment to the adipose tissue in obesity may constitute an attractive option to develop suitable therapeutics against obesity-associated disorders.

7. Prevalence of vitamin deficiencies in the apparently healthy urban adult population: Assessed by sub-clinical status and dietary intakes

Micronutrient deficiencies referred to as hidden hunger comprise vitamin and mineral deficiencies, propagated mostly through dietary inadequacies, are not apparent but ubiquitous affecting more than two billion people globally, and one-third of them are residing in India. Despite the vital physiological significance of all the vitamins, globally the major human epidemiological and intervention studies have focused on a select group comprising folate, vitamin B12 and vitamin A owing to their widespread prevalence and distinct roles in health and disease. Deficiency of other vitamins has hitherto received relatively less attention. However, it is noteworthy that deficiency of other vitamins both independently or in combination results in deleterious consequences. A classic example is hyperhomo-cysteinaemia (HHcys), a proven risk factor for cerebrovascular and cardiovascular diseases (CVD), dementia-type disorders, involves the deficiency of other B-vitamins in addition to vitamin B12 and folate. Similarly, persistent deficiency of various vitamins is incriminated in chronic degenerative diseases such as coronary artery disease, cancer, osteoporosis, cataract, arthritis, and disorders of the nervous system.

They continue to be the most critical risk factor for disease burden, especially in the developing nations. Knowledge of the prevalence of vitamin deficiencies and associated factors will be crucial in the development of appropriate intervention strategies for their control and treatment. However, previous investigations assessing the vitamin status have primarily focussed on the vulnerable groups such as pregnant women and children. Nevertheless, sub-clinical vitamin deficiencies concealed in the apparently healthy adult population which go unrecognised are widespread and pose a potential threat. The recent studies indicated that, alarmingly high prevalence of deficiency of vitamin B12 (41%) and vitamin D (45%) among apparently healthy adults. These studies emphasized that the apparently healthy adults who rarely get their vitamin levels screened may be silent victims of possible sub-clinical deficiencies of vitamins. Since they constitute the primary workforce, they can have direct implications on the economic productivity of the nation. Considering the contribution of vitamin deficiencies towards many age-related non-communicable diseases (NCDs), assessing the magnitude of vitamin deficiencies among healthy adults is vital.

OBJECTIVES

The main objective of this exploratory study was to assess the sub-clinical status of vitamins [A, D, B1, B2, B6, B12 and folate] along with their dietary intakes among apparently healthy adults.

METHODOLOGY

A community-based cross-sectional exploratory study was conducted in an urban setup on 270 (147 men and 123 women) apparently healthy adults aged 30-70 years of Hyderabad city. The study was approved by the Institutional Ethics Committee. Following the written consent, 6 ml of fasting blood and 5 ml urine was collected. Demographic and socio-economic data of the subjects participating in diet survey was collected using validated questionnaires, and *anthropometric measurements* were recorded. *Biochemical estimations such as* fasting blood glucose (FBG), HbA1c, lipid profile were carried out. Renal function was assessed by creatinine and microalbuminuria was assessed as the urinary albumin-to-creatinine ratio (UACR).

Estimation of vitamins and total homocysteine: Vitamin A and total homocysteine (tHcys) were measured in plasma by reverse phase HPLC columns. The active biological forms of vitamins B1, B2, and B6: thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), and pyridoxal-5′-phosphate (PLP) respectively, were measured in whole blood (TPP and FAD) or in plasma (PLP). ELISA was used to quantify 25-hydroxyvitamin D. Simultaneous analysis of plasma levels of total B12 and folate were carried out by radio immuno assay. Vitamin B12 fraction, bound to transcobalamin II is considered biologically active hence called active B12, was estimated in plasma by enzyme immunoassay.

Dietary assessment: Individual dietary intake was assessed in a subset of the samples (n=111, 55 men, 56 women). A validated raw food-based food frequency questionnaire (RFFQ) of one-year duration was used for dietary assessment. The nutritive value of raw foods was calculated using the new Indian Food Composition Tables, and for the ones that were missing, United States Department of Agriculture food and the nutrient database was used. Food and nutrient intake from the RFFQ was calculated based on individual consumption unit (CU) and was computed using the in-house software. Considering the adult male doing sedentary work as one (unit), arbitrary calorie coefficient values have been assigned for people of different age, sex and activity groups. Finally, the food intake was calculated by converting the different frequencies of food consumption to per day intake. Assessment of dietary adequacy of nutrients using 'probability approach method' was employed which relates usual individual intake to the distribution of requirements for a particular life stage and gender group using estimated average requirement (EAR) and its standard deviation (SD). The probability of adequacy (PA) of six vitamins was calculated: vitamins A, B1, B2, B6, folate, and B12. The mean probability of adequacy (MPA) is the average of PAs for the six vitamins.

Statistical Analyses: As most of the data were skewed, the population characteristics were reported using medians, 25th (P_{25}) and 75th (P_{75}) percentiles, and comparisons for the same were carried out by the Mann Whitney U test. The chi-square test was used for comparison of the prevalence of vitamin deficiencies. Student t-test was used to compare the PA and MPA between the genders and food habits. A Spearman rank correlation analysis was carried out to evaluate the correlation coefficients among the anthropometric, clinical and biochemical parameters on a scale of -1 to +1. Logistic regression analysis was applied to examine the association of age, gender, food habits and vitamin status with HHcys. The level of significance was considered at $p<0.05$.

RESULTS

The median values, 25th and 75th percentiles of anthropometric, clinical and biochemical characteristics of the subjects by gender are shown in Table 1. Among the biochemical parameters, median values of vitamins A ($p=0.002$), B1 ($p=0.034$) and tHcys ($p<0.001$) are considerably higher in men than in women whereas other vitamins are not significantly different between the genders. About 4.5% of the subjects have low BMI and suffer from chronic energy deficiency (CED) whereas 45% are overweight and 28% are obese. Around 68% of the subjects (62% men and 73% women) have abdominal obesity as assessed by waist circumference. The overall prevalence of anemia is 20% (16% men and 26% women), and hypertension is 33% in the study subjects. The prevalence of hypercholesterolemia is 22%, hypertriglyceridemia is 21%, high LDL levels are 25%, and low HDL levels are 67%. Microalbuminuria is observed in 10% of the subjects. Prevalence of vitamin deficiencies between the genders is depicted in Fig 1. The overall prevalence of deficiency of vitamins A, D, B1, B2, B6, and folate are 6%, 29%, 11%,

Table 1: Anthropometric, clinical and biochemical profile of the study subjects by gender

Parameters	Men (n =147)	Women (n =123)	Pooled (n =270)	p value
	Median (P_{25}-P_{75})	Median (P_{25}-P_{75})	Median (P_{25}-P_{75})	
Age, years	60.0[a] (48.0-65.0)	56.0[a] (45.0-62.0)	59.0 (45.0-64.0)	0.347
Height, m	1.60[a] (1.6-1.7)	1.54[b] (1.50-1.57)	1.6 (1.53-1.67)	<0.001**
Weight, kg	68.9[a] (60.8-70.8)	61.2[b] (51.9-70.2)	65.0 (56.7-73.1)	<0.001**
BMI, kg/m^2	24.6[a] (22.5-27.1)	25.7[a] (22.7-29.2)	24.9 (22.7-27.8)	0.258
WC, cm	94.0[a] (86.4-99.1)	86.9[b] (79.8-94.0)	89.9 (83.8-97.8)	0.004**
SBP, mmHg	132.0[a] (120.0-153.0)	130.0[a] (118.0-147.0)	132.0 (118.5-150.5)	0.897
DBP, mmHg	80.0[a] (71.0-91.0)	79.0[a] (74.0-90.0)	80.0 (73.0-90.5)	0.980
FBG, mg/dl	96.0[a] (89.0-108.0)	98.0[a] (92.0-105.0)	98.0 (91.0-106.0)	0.955
TC, mg/dl	163.2[a] (142.0-194.0)	176.1[b] (149.1-196.8)	169.7 (144.6-195.8)	0.030**
HDL, mg/dl	35.8[a] (29.6-42.5)	41.0[b] (34.4-51.4)	38.2 (31.3-45.8)	0.002**
LDL, mg/dl	101.0[a] (85.0-125.1)	112.8[a] (86.6-132.4)	104.1 (85.5-130.0)	0.074
TG, mg/dl	113.4[a] (71.4-155.6)	83.9[b] (66.4-111.8)	92.1 (68.0-140.0)	<0.001**
Hb, g/dl	14.6[a] (13.4-15.7)	12.4[b] (11.5-13.5)	13.5 (12.0-15.0)	<0.001**
HbA1c, %	5.7[a] (5.5-5.9)	5.8[a] (5.5-5.9)	5.8 (5.5-5.9)	0.246
Creatinine, mg/dl	1.1[a] (1.0-1.15)	0.9[b] (0.8-0.9)	1.0 (0.8-1.1)	<0.001**
UACR, mg/g	9.8[a] (6.0-17.7)	13.7[a] (6.8-25.1)	10.9 (6.1-22.5)	0.149
Vit A, µmol/l	1.4[a] (1.1-1.7)	1.1[b] (0.9-1.4)	1.3 (1.0-1.6)	0.002**
Vit D, nmol/l	35.4[a] (26.7-49.3)	35.8[a] (22.6-58.6)	35.6 (24.7-54.1)	0.903
Vit B1, nmol/l	132.8[a] (94.2-158.8)	112.1[b](93.2-134.0)	119.6 (93.5-147.1)	0.034*
Vit B2, nmol/l	217.5[a] (182.0-287.7)	241.9[a] (191.0-300.0)	222.0 (184.1-292.0)	0.220
Vit B6, nmol/l	21.9[a] (13.0-31.0)	21.5[a] (12.7-32.8)	21.7 (13.0-32.0)	0.771
Folate, nmol/l	12.3[a] (7.9-20.4)	13.1[a] (8.9-21.2)	12.7 (8.4-20.7)	0.762
Total B12, pmol/l	169.7[a] (118.1-258.3)	204.4[a] (135.0-310.2)	184.5 (125.4-280.4)	0.051
Active B12, pmol/l	41.0[a] (20.1-59.3)	38.8[a] (28.3-70.4)	40.0 (22.6-68.3)	0.210
tHcys, µmol/l	20.4[a] (12.9-33.6)	12.3[b] (9.0-21.3)	15.6 (10.2-29.3)	<0.001**

P_{25}, 25th percentile; P_{75}, 75th percentile; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TC, total cholesterol; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TG, triglycerides; Hb, haemoglobin; HbA1c, glycosylated haemoglobin; UACR, urinary albumin to creatinine ratio; Vit A, vitamin A; Vit D, vitamin D; Vit B1, vitamin B1; Vit B2, vitamin B2; Vit B6, vitamin B6; tHcys, total homocysteine.

***Significantly different at p<0.01. *Significantly different at p<0.05.*

Values represent medians, 25th and 75th percentiles. Significant differences (p<0.05, p<0.01) of median values between the genders are indicated by different superscript letters (a, b).

50%, 46%, 32% respectively. The prevalence of deficiency of vitamin B6 is 30% in the mild form and 16% in the severe form. The prevalence of deficiency of total B12 is 37% whereas that of active B12 is 46%. Interestingly, HHcys was observed in 52% of the subjects and is significantly higher in men (66.7%) than in women (36.4%). The median values of vitamin B6 (p=0.026) and total B12 (p<0.001) are significantly different between the vegetarians and mixed-diet group. Prevalence of deficiency of vitamin B6 (p=0.009) and folate (p=0.004) is significantly higher in subjects consuming mixed-diet, while that of total B12 (p<0.001) is higher in vegetarians.

The median dietary intakes and probability of adequacy (PA) of vitamins between the genders and food habits are shown in Table 2. Except for vitamin B12, the median dietary intakes of all other vitamins are significantly different between the genders and were higher in men compared to women. Among all the B-vitamins tested, the PA is lowest for vitamin B12 (4%) and folate (9%). The adequacy of vitamin B1 is significantly higher in men compared to women (p=0.003**)** whereas the adequacy of vitamin B6, B12 and folate is higher in men but not significant. Noticeably, the mean PA (MPA) was only 28% and was

Table 2: Medians, 25th and 75th percentiles of dietary intake and probability of adequacy (%) of vitamins by gender and food habits

Parameter	Gender			Food Habits			Pooled (n =111)
	Men (n =55)	Women (n =56)	p value	Vegetarian (n=34)	Mixed-diet (n=77)	p value	
	Median (P_{25}-P_{75}) PA (%)	Median (P_{25}-P_{75}) PA (%)		Median (P_{25}-P_{75}) PA (%)	Median (P_{25}-P_{75}) PA (%)		Median (P_{25}-P_{75}) PA (%)
Vit A, µg/day	405.6[a] (282-565)	293.6[b] (213-465)	0.006**	303.7[a] (258-420)	343.4[a] (234-510)	0.579	336.0 (251-499)
	23.0[a]	21.0[a]	0.799	19.0[a]	23.0[a]	0.638	22.0
Vit B1, mg/day	1.2[a] (1.0-1.3)	0.8[b] (0.6-1.1)	<0.001**	1.1[a] (0.9-1.2)	1.0[a] (0.7-1.2)	0.132	1.0 (0.8-1.2)
	72.0[a]	44.0[b]	0.003**	72.0[a]	52.0[b]	0.049*	58.0
Vit B2, mg/day	0.9[a] (0.7-1.1)	0.7[b] (0.5-0.9)	<0.001**	0.8[a] (0.7-1.0)	0.8[a] (0.5-1.0)	0.987	0.8 (0.6-1.0)
	25.0[a]	21.0[a]	0.617	21.0[a]	24.0[a]	0.729	23.0
Vit B6, mg/day	1.2[a] (1.0-1.4)	1.0[b] (0.8-1.15)	<0.001**	1.1[a] (1.0-1.3)	1.07[a] (0.87-1.33)	0.839	1.1 (0.9-1.3)
	36.0[a]	23.0[a]	0.133	32[a]	29[a]	0.750	30.0
Folate, µg/day	219.1[a] (185-270)	173.0[b] (143-230)	0.006**	221.6[a] (185-264)	188.1[b] (154-245)	0.020*	198.4 (156-258)
	14.0[a]	5.0[a]	0.105	10.0[a]	9.0[a]	0.867	9.0
Vit B12, µg/day	0.4[a] (0.3-0.9)	0.5[a] (0.3-0.9)	0.928	0.3[a] (0.2-0.3)	0.6[b] (0.4-1.1)	<0.001**	0.5 (0.3-0.9)
	6.0[a]	2.0[a]	0.281	0.0[a]	5.0[a]	0.184	4.0
MPA	34.0[a]	22.0[b]	0.003**	28.0[a]	28.0[a]	1.000	28.0

P_{25}, 25th percentile; P_{75}, 75th percentile; PA, probability of adequacy; MPA, mean probability of adequacy.
***Significantly different at p<0.01. *Significantly different at p<0.05.*

Values represent medians, 25th and 75th percentiles and percentages. The mean probability of adequacy (MPA) is defined as the mean of the PA across the six vitamins. Significant differences of median values and frequencies between the genders (p<0.01) and the food habits (p<0.05, p<0.01) are indicated by different superscript letters (a, b).

significantly higher (p=0.003) in men (34%) compared to women (22%). By food habits, the median intake of folate (p=0.020) is significantly higher whereas vitamin B12 (p**<**0.001**)** intake is lower in vegetarians compared to the mixed-diet group. The PA of vitamin B1 (p=0.049) is significantly higher among vegetarians compared to the mixed-diet group. The MPA was similar in both the food habits.

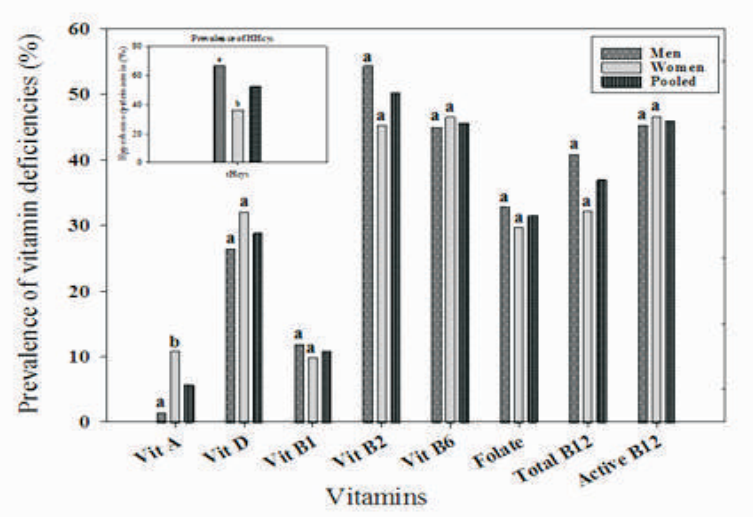
The risk of HHcys and its association with different determinants such as gender, food habits and vitamin status are compared by calculating the odds ratios with logistic regression analysis before and after adjusting for age, gender and food habits. In the unadjusted group, the risk of HHcys is higher in men (OR: 3.5; 95% CI: 1.7, 7.0) and in subjects with deficiencies of folate (OR: 2.9; 95% CI: 1.3, 6.5), total B12 (OR: 2.4; 95% CI: 1.1, 5.2), and active B12 (OR: 2.8; 95% CI: 1.1, 7.0). Model 1 (age and gender adjusted)

and Model 2 (adjusted for age, gender and food habits) did not result in any change in the existing associations. However, the risk of active B12 deficiency almost doubled in Model 1 (OR: 5.8; 95% CI: 1.7, 19.7) and 2 (OR: 6.0; 95% CI: 1.7, 20.8) compared to the unadjusted one.

CONCLUSIONS

The findings of this study demonstrated the widespread prevalence of multiple sub-clinical vitamin deficiencies and inadequate intakes in the apparently healthy adult population, particularly women being at higher risk. Additionally, a high degree of HHcys was observed which amplifies the risk of NCDs. Interestingly, a very high burden of B2 and B6 deficiencies was noticed in addition to the commonly reported vitamin deficiencies such as folate, vitamin B12, D and A. Altogether, the findings of the study highlight the imminent threat of disease burden and suggest a need for screening the apparently healthy adults for sub-clinical vitamin deficiencies. The results also suggest the necessity for nationally representative data required to direct the improvement of nutrition interventions and public health programs, such as multiple micronutrient fortifications, dietary diversification, and supplementation to achieve the sustainable developmental goals.

Figure 1: Prevalence of vitamin deficiencies in apparently healthy adults. tHcys, total homocysteine; HHcys, hyperhomocysteinaemia.



Pooled data represents the total number of samples (n =270). Data represent % deficiency, and significant differences ($p<0.05$) of mean values between the genders are indicated by letters (a, b) above the bars. The inset shows the prevalence of HHcys between the genders along with the pooled levels.

8. Factors associated with adequacy of micronutrient intakes among the urban adult population of Hyderabad city

Undernutrition and obesity continue to exist as dual burden which is hypothesized to be an integral part of the nutritional and socio-economic transition in developing countries like India. The upsurge of micronutrient deficiency (hidden hunger) further adds to the current conundrum, thereby contributing to non-communicable diseases (NCDs). With the rapid increase in economic development and urbanization, the intake of refined foods has increased drastically because of easy accessibility even in the low-income countries contributing significantly to the nutritional transition. The National Sample Survey Organization (NSSO) observed a decrease in the intake of energy and protein and a simultaneous increase in the fat intake. Studies from the National Nutrition Monitoring Bureau have also reported a similar trend with a decline in the prevalence of chronic energy deficiency, and a simultaneous increase in the overweight/ obese population with high prevalence of micronutrient deficiencies among rural populations. Deficiency of micronutrients can lead to a plethora of chronic degenerative diseases and are

linked to a wide range of adverse health outcomes, such as birth defects, growth retardation, impaired cognition & mental development and increased morbidities and mortalities. Micronutrient deficiency is primarily due to inadequate dietary intakes, poor food quality, and minimal dietary diversit, but the extent of deficiencies varies depending on the economic and social factors, varied cultural habits and values, ignorance due to high female illiteracy, isolation and stress.

The micronutrient status among populations can be measured indirectly through dietary assessment, or it can be directly measured by determining the nutrient content in body fluids or tissues. The dietary assessment would be a simple non-invasive tool for assessing the risk of low micronutrient status both at the individual and population level. In a previous study, the plasma concentrations and the dietary intakes of vitamin B12 and folate in the adult population and found that only 40% of the adult population was meeting 70% of the RDA of B12 wee assessed, which is consistent with high prevalence of biochemical deficiency. These findings prompted to extend the study to other micronutrients of public health interest which includes thiamine, riboflavin, niacin, vitamin A, vitamin C, zinc, calcium, and iron in the apparently healthy adult groups. Further, the probability of adequacy (PA) estimated using average requirement (EAR) is considered more relevant than the ratios of intakes to the recommended dietary allowances (RDA), which exceeds the requirements of 97.5% of the population.

OBJECTIVES

The main aim of the study was to assess the dietary adequacy of micronutrients by PA and determine the factors associated with micronutrient status among the urban south Indian adult population.

METHODOLOGY

A community-based cross-sectional study was conducted in an urban setup in Hyderabad city. Apparently healthy subjects in the age group of 21-85 years were included in the study. The sample size was estimated considering *4*0% of the adult population consuming adequate (>70% RDA) of B12. Assuming 95% confidence interval (CI), a relative precision of 20%, and with a design effect of 2, the required sample size was 288. Thus a total of 300 subjects: men (n=144) and women (n=156) were randomly selected for the study and were arbitrarily categorized into three age groups viz., 21-40, 41-60 and >60 years. The subjects were interviewed to obtain information on socio-demographic and anthropometric parameters.

Dietary assessment: A three-day 24 h recall method was conducted to carry out the diet survey (n=300). The nutritive values of raw foods were taken from the Nutritive Value of Indian Foods, while United States Department of Agriculture (USDA) food and the nutrient database was used for those foods that did not have a nutrient value in NVIF. Daily individual consumption of nutrients was calculated by taking the average of 3 days of diet survey. The deficit in intakes of different food groups were compared with recommended dietary intakes (RDIs) for Indians.

Probability of adequacy: The adequacy of micronutrients was assessed using the probability approach which relates an individual's usual intake of nutrients to the distribution of requirements for a particular life stage and gender group by using EAR values and its standard deviation (SD). Thus, the micronutrient adequacy was evaluated by calculating the PA for ten micronutrients: calcium, vitamin A, thiamine, riboflavin, niacin, vitamin C, B12, zinc, folate, and iron. According to sex and age group, the recommended EAR as set by the Institute of Medicine (IOM) was followed. The mean probability of adequacy (MPA) is the average of PA across the ten nutrients. The prevalence of inadequacy was defined considering MPA <0.5.

Biochemical estimations: Fasting venous blood (6ml) was collected from the subjects. Hemoglobin (Hb) by cyanomethemoglobin method, ferritin and C-reactive protein (CRP) by ELISA method was determined. Subjects having ferritin concentrations <15µg/L were considered as iron deficient (ID), and ferritin concentrations <15µg/L along with anemia (<13.0 g/dL males, <12.0 g/dL females) were considered as iron deficiency anemia (IDA). Plasma concentrations of B12 and folate were determined by RIA method.

Statistical analyses: Medians, *25th (P_{25}) and 75th (P_{75}) percentiles* of food groups and nutrients among the age groups were calculated, and comparisons for the same were carried out by Kruskal–Wallis test.

Comparison of mean values of the variables across the age groups was done by one-way ANOVA with a least significant difference (LSD) multiple comparisons and between the genders by student's t-test. The Chi-square test was used for comparison of prevalence of MPA (<0.5) across the age groups and associated factors. Both univariate and multivariate logistic regression analysis was performed to identify factors associated with MPA inadequacy. The level of significance was considered at $p < 0.05$.

RESULTS

The socio-demographic characteristics indicate that the gender distribution was almost similar in all the age groups. Majority of the subjects were bachelor graduates and above (57%) and were consuming mixed diets (75.7%). About 6% of the subjects had low BMI and were chronic energy deficient, 31.3% had a normal BMI, 42.7% were overweight, and 20% were obese. The median intake of vegetables, roots & tubers, spices & condiments, animal foods, milk & milk products, and sugars are significantly different among the age groups (Table 1). The dietary intake of calcium is high in the 21-40 age group whereas riboflavin is significantly high in the >60 age group. The 41-60 age group had significantly low intakes of B12 (Table 1). The median intake of food groups such as cereals & millets, roots & tubers, spices & condiments, and animal foods, and the nutrient intakes of vitamin A, thiamine, riboflavin, niacin, B12, zinc, folate, and iron were significantly lower in women than men ($p<0.05$).

The PA is lowest for folate (1%) followed by B12 (6%), zinc (11%), riboflavin (37%), niacin (40%), calcium (42%), vitamin A (43%), vitamin C (52%), thiamine (58%), and iron (89%). The overall MPA across the ten micronutrients is 38% (Table 2).

Table 1: Median (P_{25}-P_{75}) intake of food groups and nutrients among different age groups

Food groups / Nutrients	21-40 yrs (n=101) Median (P_{25}-P_{75})	41-60 yrs (n=104) Median (P_{25}-P_{75})	>60 yrs (n=95) Median (P_{25}-P_{75})	Pooled (n=300) Median (P_{25}-P_{75})	p value
Food groups					
Cereals & Millets (g)	270.1^{a} (222-328)	253.5^{a} (219-316)	249.6^{a} (209-290)	261.5 (219-320)	0.190
Pulses & Legumes (g)	26.5^{a} (12.0-52.9)	38.5^{a} (21.6-56.4)	36.0^{a} (17.4-56.0)	34.7 (16.7-55.7)	0.078
Green leafy vegetables (g)	12.7^{a} (4.3-28.0)	15.9^{a} (4.9-29.3)	15.5^{a} (3.5-37.0)	15.2 (4.3-30.3)	0.541
Other vegetables (g)	41.0^{a} (17.1-71.9)	60.6^{a} (24.0-94.1)	78.0^{b} (46.3-114)	56.5 (25.1-94.0)	<0.001**
Roots & Tubers (g)	81.2^{a} (55.4-119)	74.2^{a} (40-101)	50.1^{b} (23.5-87.0)	69.5 (37.1-102.8)	<0.001**
Nuts & Oil seeds (g)	6.1^{a} (1.9-13.6)	7.9^{a} (2.8-16.5)	4.8^{a} (0.87-17.2)	6.4 (1.6-16.4)	0.120
Spices & Condiments (g)	11.3^{a} (8.9-15.2)	12.4^{a} (9.2-16.9)	9.4^{b} (6.2-11.8)	11.1 (8.4-14.5)	<0.001**
Fruits (g)	111.1^{a} (65.7 -200.1)	114.3^{a} (67.4-174.)	102.7^{a} (51.1-167)	112.3 (60.6-177)	0.585
Animal foods (g)	34.6^{a} (12.6-69.3)	10.5^{b} (0.0-50.7)	0^{b} (0.0-30.9)	16.3 (0.0-50.1)	<0.001**
Milk & Milk products (g/mL)	203.1^{a} (119-300)	245^{b} (171-365)	346.9^{c} (284-405)	271.1 (176-368)	<0.001**
Fats & Oils (g)	17.7^{a} (10.0-27.0)	18.5^{a} (8.9-29.2)	16.4^{a} (7.7-27.3)	17.6 (8.9-27.9)	0.819
Sugars (g)	11.3^{a} (7.5-16.0)	11.4^{a} (7.5-16.2)	9.3^{b} (4-12.8)	10.5 (6.2-15.0)	0.003**
Nutrients					
Calcium (mg)	669.4^{a} (554-919)	809.8^{b} (626-1054)	873.3^{b} (764-1007)	797.0 (629-991)	<0.001**
Vitamin A (µg)	508.4^{a} (367-689)	509.7^{a} (342-655)	465.6^{a} (298-715)	503.3 (342-688)	0.731
Thiamine (mg)	1.03^{a} (0.77-1.17)	1.03^{a} (0.8-1.3)	1.0^{a} (0.77-1.3)	1.03 (0.77-1.3)	0.788
Riboflavin (mg)	0.83^{a} (0.67-1.03)	0.87^{a} (0.7-1.1)	1.02^{b} (0.83-1.2)	0.90 (0.73-1.07)	<0.001**
Niacin (mg)	10.8^{a} (8.0-13.2)	10.3^{a} (8.9-12.9)	10.1^{a} (8.3-12.6)	10.4 (8.5-12.8)	0.608
Vitamin C (mg)	70.7^{a} (43.3-102)	72.7^{a} (45.9-105)	58.7^{a} (41.8 -103)	66.4 (42.9-104)	0.452
B12 (µg)	0.77^{a} (0.43-1.13)	0.5^{b} (0.33-0.77)	0.57^{ab} (0.43-0.83)	0.57 (0.4-0.95)	0.019*
Zinc (mg)	6.1^{a} (4.7-7.1)	5.7^{a} (4.9-6.8)	5.6^{a} (4.8-6.6)	5.7 (4.9-6.8)	0.305
Folate (µg)	151.9^{a} (116-199)	162.3^{a} (130-198)	167.4^{a} (128-216)	157.7 (123-201)	0.171
Iron (mg)	11.2^{a} (8.7-15.0)	11.7^{a} (9.3-14.4)	10.2^{a} (7.8-14.1)	11.2 (8.3-14.4)	0.142

*Values represent medians and 25th and 75th percentiles (P_{25}-P_{75}); ** Significantly different at p<0.01. * Significantly different at p<0.05. Significant differences (p< 0.01, p<0.05) of median values between the groups are indicated by different superscript letters a, b, c.*

Table 2: Mean PA and MPA among different age groups and between the genders

Nutrients	21-40yrs (n=101)	41-60yrs (n=104)	>60yrs (n=95)	p value	Men (n=144)	Women (n=156)	p value	Pooled (n=300)
PA (%)								
Calcium (mg)	36[a]	43[a]	46[a]	0.261	50[a]	34[b]	0.002**	42
Vitamin A (µg)	44[a]	42[a]	41[a]	0.881	39[a]	46[a]	0.155	43
Thiamine (mg)	55 [a]	62[a]	57[a]	0.482	62[a]	54[a]	0.134	58
Riboflavin (mg)	29[a]	35[a]	49[b]	0.004**	30[a]	44[b]	0.004**	37
Niacin (mg)	42[a]	40[a]	36[a]	0.505	46[a]	34[b]	0.005**	40
Vitamin C (mg)	53[a]	57[a]	44[a]	0.178	48[a]	55[a]	0.204	52
B12 (µg)	9[a]	5[a]	4[a]	0.260	9[a]	4[a]	0.115	6
Zinc (mg)	14[a]	13[a]	6[b]	0.049*	6[a]	16[b]	<0.001**	11
Folate (µg)	2[a]	1[a]	2[a]	0.813	3[a]	1[b]	0.019*	1
Iron (mg)	85[a]	90[a]	92[a]	0.193	95[a]	83[b]	<0.001**	89
MPA (%)								
	37[a]	39[a]	38[a]	0.790	39[a]	37[a]	0.484	38

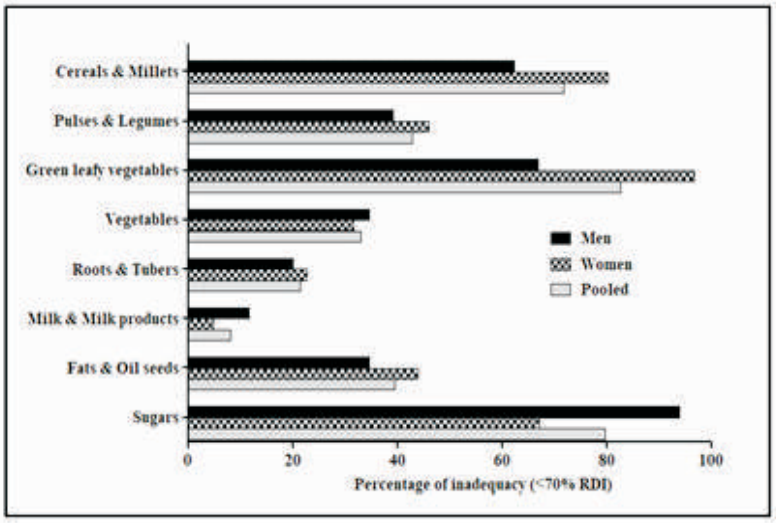
PA – Probability of adequacy. MPA – Mean probability of adequacy.
*Values represent percentages. Mean values across the age groups were compared by one-way ANOVA with LSD multiple comparisons. Mean values between the genders were compared by student's t-test. ** Significantly different at p<0.01. * Significantly different at p<0.05. Significant differences (p< 0.01, p<0.05) of mean values between the groups are indicated by different superscript letters a, b.*

The PA of riboflavin is significantly lower in the 21-40 age group, and that of zinc is significantly lower in the >60 age group compared to the other two respective groups, while the MPA are comparable among the age groups (Table 2). In women, PA of calcium, niacin, folate, and iron is significantly lower, whereas that of riboflavin and zinc is higher compared to men (Table 2). Between the genders, the MPA is similar. The risk of micronutrient inadequacy (MPA <0.5) was about 68% in the study subjects and is not associated with age groups (p>0.05).

The contribution of different food groups to micronutrient intakes are as follows: cereals & millets contribute to 39.2% of thiamine, 25.1% of riboflavin, 62.1% of niacin, 40.4% of zinc, 20.8% of folate, and 35.3% of iron. Pulses & legumes contributed 16.2% of thiamine, and 19.3% of folate. Green leafy vegetables contributed 27.2% of vitamin A, 15.1% of vitamin C, 20.0% of folate, and 12.2% of iron. Fruits contributed 21.0% of vitamin A, and 41.7% of vitamin C. Animal foods contributed 22.3% of B12. Milk & milk products contributed to calcium (59.6%), vitamin A (32%), riboflavin (40.5%), B12 (75.7%), and zinc (18.3%). The inadequacy (<70% RDI) of food groups, when compared with their respective RDIs indicated that pulses & legumes, vegetables, roots & tubers, milk & milk products, and sugars are significantly different among the age groups. The inadequacy of consumption of cereals & millets, and green leafy vegetables is significantly higher in women, while that of milk & milk products, and sugars are higher in men when compared to women. The inadequacy of other food groups is similar between both the genders (Fig 1).

Figure 1: Prevalence of inadequacy (<70% RDI) of food groups between genders.

Pooled data represent the total number of samples (N=300). p<0.05 was considered to be significant



Mean Hb levels are significantly different among the age groups (Table 3A). Ferritin levels among the age groups are comparable, whereas the CRP levels are significantly different between the age groups and were found to be higher in the 41–60 and >60 age groups compared to 21–40 age group (Table 3A). The overall prevalence of anemia is found to be 30% and is relatively higher in women (47.8%) than in men (10.4%). Further, the prevalence of anemia is relatively higher (37.6%) in the 41–60 age group compared to the 21–40 (27.1%) and >60 (24.3%) age groups (Table 3B). The overall prevalence of ID is 23%, and it is higher in 21–40 (30.4%) age group compared to 41-60 (20%) and >60 (18.5%) age groups (Table 3B). The prevalence of IDA is 14.3%, and is higher in the 21–40 (20.3%) age group compared to 41-60 (12.9%) and >60 (9.4%) age groups (Table 3B). Mean plasma concentrations of folate and B12 are significantly different between the age groups (Table 3A). The overall prevalence of folate deficiency is found to be 32.2%, and is significantly higher in the >60 (36.4%) and 21–40 (32%) age groups compared to the 41–60 age group (29.3%) (Table 3B). The overall prevalence of B12 deficiency is 35.5% and is higher in the 21–40 (41.2%) age group when compared to the 41–60 (39.4%) and >60 (23.4%) age groups (Table 3B).

Table 3A: Plasma concentrations of clinical parameters and vitamins among different age groups

Parameter	21-40 yrs (n=101) Mean (SE) Median (P_{25}-P_{75})	41-60 yrs (n=104) Mean (SE) Median (P_{25}-P_{75})	>60 yrs (n=95) Mean (SE) Median (P_{25}-P_{75})	Pooled (n=300) Mean (SE) Median (P_{25}-P_{75})	p value
Hb (g/dL)	13.1^{a} (0.2) 13.4 (11.7-14.7)	12.6^{a} (0.1) 12.5 (11.8-13.6)	13.6^{ab} (0.2) 13.5 (12.1-15.0)	13.1 (0.1) 13 (11.8-14.4)	0.004**
Ferritin (µg/L)	45.6^{a} (5.2) 33.2 (8.5-69.5)	68.1^{b} (8.2) 50.2 (17.5-88.4)	59.2^{ab} (9.1) 34.9 (16.5-74.5)	57.6 (4.4) 36.4 (15.8-77.8)	0.109
CRP (mg/L)	0.76^{a} (0.07) 0.6 (0.34-1.2)	1.8^{b} (0.18) 1.0 (0.7-3.0)	1.48^{b} (0.2) 0.84 (0.54-1.7)	1.36 (0.1) 0.83 (0.5-1.58)	<0.001**
Folate (nmol/L)	13.4^{a} (0.8) 12.2 (9.3-15.0)	16.1^{b} (0.8) 14.0 (9.5-20.4)	13.6^{a} (1.0) 10.9 (7.8-17.2)	14.4 (0.5) 12.7 (9.1-17.4)	0.047*
B12 (pmol/L)	206.6^{a} (14.8) 169.7 (118.1-236.2)	218.2^{a} (14.9) 177.1 (120.3-273.1)	290.8^{b} (22.1) 247.2 (147.6-357.9)	235.2 (10.0) 188.2 (125.5-298.9)	0.002**

Hb – Hemoglobin. CRP - C-reactive protein

*Values are means (SE) and medians (P_{25}, 25th percentile; P_{75}, 75th percentile). Mean values across groups were compared by one-way ANOVA with LSD multiple comparisons. ** Significantly different at p<0.01. * Significantly different at p<0.05. Significant differences (p< 0.01, p<0.05) of mean values between the groups are indicated by different superscript letters a, b.*

Table 3B: Prevalence of anemia, and deficiency of iron, folate and B12 among different age groups

Parameter	21-40 yrs (n=101)	41-60 yrs (n=104)	>60 yrs (n=95)	Pooled (n=300)	p value
Anemia	27.1^{a}	37.6^{a}	24.3^{a}	30.0	0.129
ID	30.4^{a}	20.0^{a}	18.5^{a}	23.0	0.196
IDA	20.3^{a}	12.9^{a}	9.4^{a}	14.3	0.182
Folate deficiency	32.0^{a}	29.3^{a}	36.4^{a}	32.2	0.608
B12 deficiency	41.2^{a}	39.4^{a}	23.4^{b}	35.5	0.030*

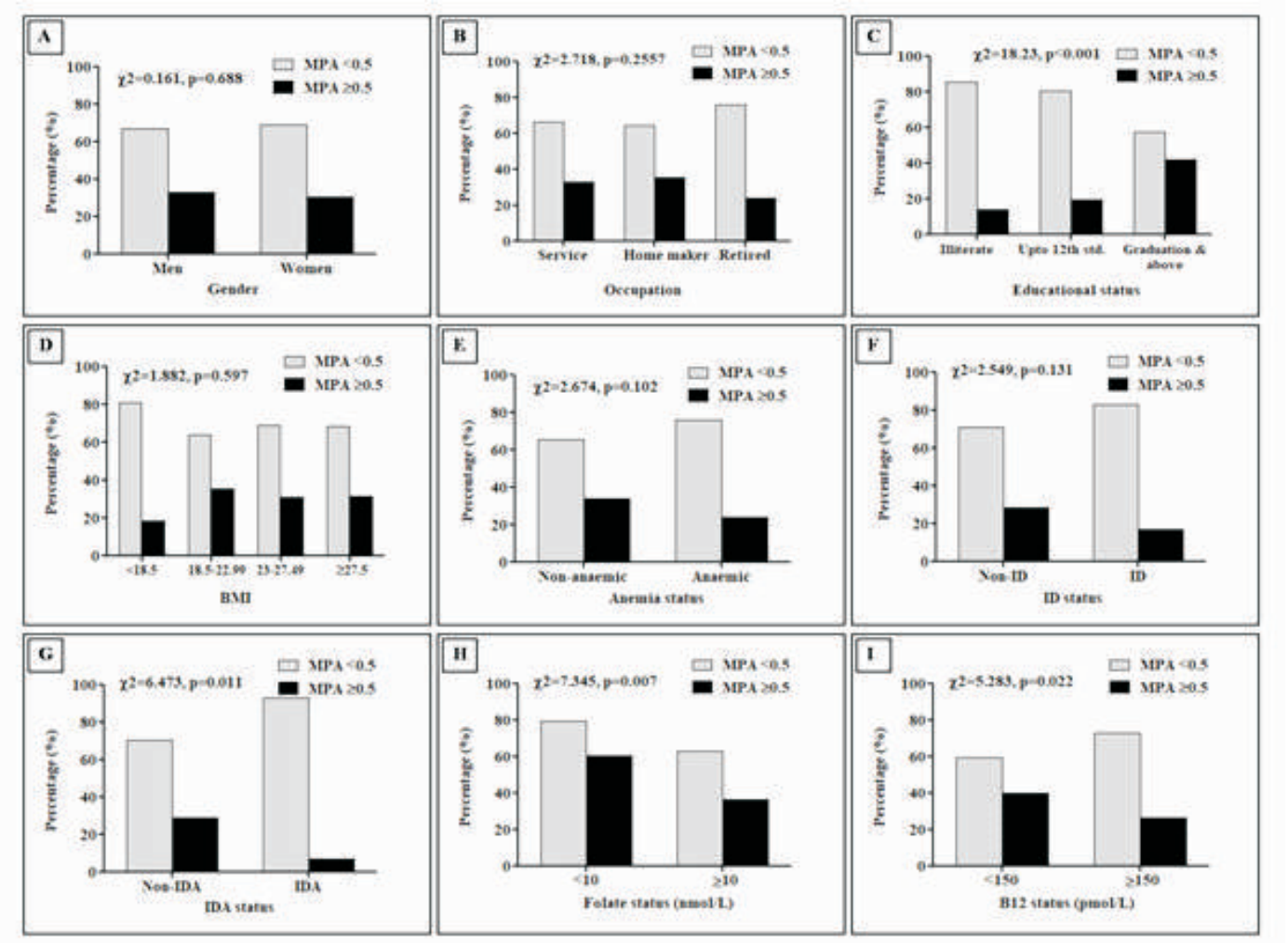
ID – Iron deficiency anemia. IDA – Iron deficiency anemia

*Data represent % deficiency. * Significantly different at p<0.05. Significant differences (p<0.05) of mean values between the groups are indicated by different superscript letters a, b.*

Univariate analysis identified the association of educational status, IDA, and folate status with MPA. The micronutrient inadequacy (MPA <0.5) was higher in illiterates, and decreased with higher educational status (Fig 2). The risk of micronutrient inadequacy and its association with different factors were compared by calculating the odds ratios with unadjusted and adjusted for age (as a continuous variable) and gender logistic regression models. The odds of micronutrient inadequacy is higher in illiterates (OR=4.40; 95%CI: 1.46, 13.3) and the high school group (OR=3.01; 95%CI: 1.65, 5.52) compared to graduation and above. Similarly, subjects having IDA (OR=5.60; 95%CI: 1.28, 24.4), and with low concentrations of folate (OR=2.26; 95%CI: 1.24, 4.11) have a higher risk of micronutrient inadequacy. These associations remained statistically significant after adjusting for age and gender.

Figure 2: Univariate analysis of factors associated with MPA

Gender (A), Occupation (B), Educational status (C), BMI (D), Anemia status (E), ID status (F), IDA status (G), Folate status (H), B12 status (I). Data represents % inadequacy (<0.5) and % adequacy (⩾0.5). p<0.05 was considered to be significant.



CONCLUSIONS

Micronutrient inadequacy was observed in about 62% of the study population which is consolidated by the plasma levels of some micronutrients. The PA of folate, B12, and zinc were noticeably low, probably explained by the low intakes of micronutrient-rich foods. The risk of micronutrient inadequacy is associated with lower educational status, IDA, and folate deficiency. However, nationwide representative data are needed for focused dietary and non-dietary interventions like education programs which are required to improve peoples' decision-making capability regarding food choices, which in turn improve the diet quality.

9. Circulating levels of hsp27 in microvascular complications of diabetes: Prospects as a biomarker of diabetic nephropathy

The number of people with diabetes rose to 422 million in 2014 from 108 million in 1980. Diabetes can lead to macrovascular complications like stroke and cardiovascular diseases and microvascular complications like retinopathy, nephropathy, and neuropathy. The high blood glucose levels in diabetes can damage the blood vessels in retina leading to diabetic retinopathy (DRe). Diabetes can also damage nerves leading to diabetic neuropathy (DNu). Diabetic nephropathy (DNe) includes damage to the capillaries in the glomerulus and is characterized by macroalbuminuria. Hyperglycemia can cause the production of free radicals or reactive oxygen species (ROS). In the absence of an appropriate compensatory response from the endogenous antioxidant network, there is a redox imbalance leading to the activation of stress-sensitive intracellular signaling pathways.

Heat shock proteins (Hsps) are multigene families that range in molecular size from 10 to 150 kDa and are present in all the major cellular compartments. Some of these proteins are constitutively expressed in the cell and are responsible for quality control of protein folding, whereas expression of other proteins of this group increases in response to stressful conditions. Hsp27 is a small heat shock protein (sHsp) and its expression is altered in several disease conditions in various tissues. Recent reports demonstrate that Hsp27 is not restricted to the tissues but is also secreted into the circulation . A study conducted by Jose et al. has concluded that plasma Hsp27 could be a potential index of atherosclerosis. Reduced plasma Hsp27 in patients with atherosclerosis, abdominal aortic aneurysm and peripheral artery disease is reported. An association between the presence of polyneuropathy and circulating Hsp27 has been identified in a large cohort of human subjects with type 1 diabetes mellitus. Thus, serum Hsp27 levels are associated with various diseases like atherosclerosis, abdominal aortic aneurysm, peripheral artery disease, polyneuropathy, type 1 diabetes mellitus, and CKD. However, plasma Hsp27 in microvascular complications of type 2 diabetes such as DRe, DNe, and DNu has not been evaluated.

OBJECTIVES

To determine plasma Hsp27 in individuals with type 2 diabetes mellitus and associated microvascular complications- DRe, DNe and DNu to understand if it could serve as a marker for these complications and enable their detection earlier than the presently used markers.

METHODOLOGY

This is a hospital based case-control study consisting of 754 subjects of which 247 were controls, 195 with type 2 diabetes, 123 with DRe, 80 with DNe and 109 with DNu. Diabetic retinopathy was confirmed based on an ophthalmic examination that includes best-corrected visual acuity, slit-lamp biomicroscopy, indirect ophthalmoscopy, and fundus fluorescein angiography. Diabetic nephropathy was defined by measuring albuminuria followed by estimated glomerular filtration rate (eGFR) estimation by CKD-EPI creatinine 2009 equation method for staging. Diabetic neuropathy was evaluated both clinically and by electroneuromyography. Fasting venous blood samples were collected and plasma was separated.

Estimation of Hsp27 by enzyme-linked immunosorbent assay: The plasma levels of Hsp27 were measured by Duo Set ELISA development system. Briefly, the capture antibody was diluted to the working concentration in phosphate buffered saline and coated on a96-well plate. After blocking was done, standards and samples in reagent diluent were added. Detection antibody was added. Streptavidin-HRP was added followed by substrate solution and the reaction was stopped by adding stop solution. The absorbance was measured at 450 nm with wavelength correction set to 540 nm. A standard curve was constructed by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. The concentration of Hsp27 in the samples was determined from the standard curve and multiplied with the dilution factor.

Statistical analysis: Statistical analysis was performed using SPSS for Windows version 17.0 and Graph Pad Prism 5. Mean and SD were calculated for age, BMI, duration of diabetes, FBG, HbA1c, serum creatinine, total cholesterol, HDL, LDL, triglycerides (TG), and Hsp27. Median and interquartile range (IQR) were calculated. Comparison of mean values of all these variables across the groups was made by one-way analysis of variance (ANOVA) F-test with post hoc test of Fisher's Least Significant Difference (LSD). Correlation between plasma concentration of HSP27 and other clinical parameters was analyzed using the Spearman rank correlation. In order to find out whether Hsp27 could serve as a potential marker of DNe, we performed Receiver's operating characteristic (ROC) curve analysis of plasma Hsp27.

RESULTS

The demographic and clinical profile of subjects across the groups is shown in Table 1. The number of males was 43.3% in control group, 53.4% in the diabetes group, 75.60% in DRe group, 55% in DNe group, 65.13% in DNu group. The mean age of subjects ranged from 54 to 58 years in the groups. The duration of diabetes in subjects of DRe, DNe, and DNu groups was significantly more than the diabetes group but comparable between the complication groups. The mean FBG in the complications was significantly higher than in diabetes group. The HbA1c was higher in the DNu group compared to diabetes, DRe, DNe groups, the latter two groups being comparable with respect to HbA1c. The TC levels were comparable across diabetes and complications groups. The TG levels were high in DNu compared to the control and DNe groups while being comparable to the other groups. The HDL levels were significantly higher in control group than the diabetic and DRe groups while being comparable to DNe and DNu groups. The LDL levels were comparable among control and DRe groups and were significantly higher than diabetes, DNe and DNu groups. The serum creatinine levels were higher in DNe group compared to control, diabetes, DRe and DNu groups.

Table 1: Clinical and demographic profile of study subjects

Parameters	Control (mean±SD)	Diabetes (mean±SD)	Diabetic Retinopathy (DRe) (mean±SD)	Diabetic Nephropathy (DNe) (mean±SD)	Diabetic Neuropathy (DNu) (mean±SD)	F value	p-value
N	**247**	**195**	**123**	**80**	**109**		
Age (years)	54.32^{a}±15.85	57.19^{b}±11.59	54.28^{a}±9.47	55.87^{ab}±9.69	58.19^{b}±8.36	2.93	0.0201
Duration of diabetes(yrs)		7.24^{a}±7.19	11.70^{b}±7.32	11.20^{b}±7.63	9.88^{b}±7.23	8.93	0.0001
Males (%)	43.3	53.4	75.6	55	65.13		
BMI (Kg/m^2)	25.01^{a}±7.93	27.89^{bc}±5.22	25.01^{a}±3.58	27.08^{b}±5.72	29.09^{c}±4.81	7.97	0.0001
FBG (mg/dL)	98.04^{a}±11.35	153.8^{b}±64.51	192.3^{c}±102.7	172.0^{d}±82.19	181.8^{cd}±75.47	57.62	0.0001
HbA1c (%) (mmol/mol)	6.06^{a}±0.78 (43±8.5)	8.08^{b}±1.80 (65±19.7)	7.75^{bc}±1.99 (61±21.8)	7.36^{c}±1.72 (57±18.8)	8.60^{d}±1.92 (70±21.0)	66.12	0.0001
TC(mg/dL)	176.5^{a}- 42.51	166.2^{b}±40.10	176.6^{ab}±50.40	162.5^{b}±53.66	170.3^{ab}±42.37	2.43	0.0466
HDL(mg/dL)	42.18^{a}- 12.45	39.38^{b}±11.77	36.16^{b}±14.46	39.74^{ab}±11.21	39.67^{ab}±15.81	3.11	0.0151
LDL(mg/dL)	113.3^{a}- 35.87	100.7^{b}±33.82	117.4^{a}±47.86	98.38^{b}±42.74	104.5^{b}±40.59	5.07	0.0005
TG(mg/dL)	122.5^{a}- 73.47	136.9^{ab}±96.24	135.2^{ab}±109.4	129.2^{a}±85.85	155.5^{b}±94.72	2.67	0.0309
Serum creatinine (mg/dL)	1.007^{a}±0.31	1.096^{a}±0.91	1.19^{a}±0.54	3.00^{b}±2.02	1.06^{a}±0.48	88.5	0.0001
Hsp27 (pg/mL)	496.55^{a}± 308.54	523.41^{a}±371.00	494.6^{a}±391.48	631.5^{b}±355.2	455.21^{a}± 319.74	3.366	0.018

Values are presented as mean±SD. Mean values across the groups are compared by one-way ANOVA 'F' test with post hoc test of Fisher's Least Significant Difference (LSD). Significant differences ($p < 0.05$) of mean values among the groups are indicated by different superscript letters (a, b, c, d). Mean values with the same superscript are comparable.

The plasma Hsp27 levels across the groups are shown in Figure 1. The plot demonstrates higher levels of Hsp27 in DNe compared to control, diabetes, DRe and DNu groups (p=0.018). The mean plasma Hsp27 was 496.55±308.54 (pg/mL) in control, 523.41±371.00 (pg/mL) in diabetes, 494.6±391.48 (pg/mL) in DRe, 631.5±355.2 (pg/mL) in DNe and 455.21±319.74 (pg/mL) in DNu groups, respectively. The median values were 404 (IQR: 271.1-604.8), 416.8 (IQR: 260.9-652.0), 390.5 (IQR: 220.9-568.0), 529.3 (IQR: 375.9-858.2) and 359.5 (IQR: 247.8-563.0) for control, diabetes, DRe, DNe and DNu groups, respectively. The plasma Hsp27 levels were not affected by gender, age and BMI.

Table 2: Correlation between plasma Hsp27 and clinical characteristics of all participants

Variable	r-value	p-value
Age	-0.027	0.465
BMI	-0.015	0.708
FBG	0.011	0.770
Total Cholesterol	-0.005	0.889
HDL	0.043	0.266
LDL	0.020	0.614
Triglycerides	-0.052	0.178
HbA1c	-0.044	0.274
Serum creatinine	0.083	0.053

Correlation of plasma Hsp27 of all participants with their clinical characteristics is depicted in Table 2. Excepting the serum creatinine, plasma Hsp27 was not correlated with other clinical characteristics of the study population. ROC curve analysis of Hsp27 in DNe group revealed an area under the curve of 0.617 while serum creatinine showed an area under the curve of 0.918 (Figure 2).

Correlations (r-value) were assessed by Spearman rank correlation. A positive r-value indicates a direct correlation while a negative r-value indicates an inverse relation between variables. p-value <0.05 is considered significant.

Figure 1: Plasma levels of Hsp27. Dot plot demonstrates higher plasma levels of Hsp27 in DNe group compared to control, diabetes, DRe and DNu group

(p-value 0.018). The horizontal line represents mean±SEM. p-value <0.05 was considered as significant. The groups are: control, diabetes, diabetic retinopathy (DRe), diabetic nephropathy (DNe) and diabetic neuropathy (DNu).

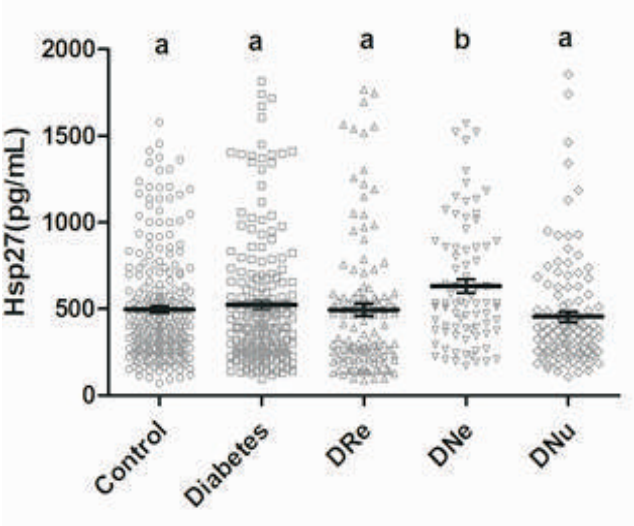
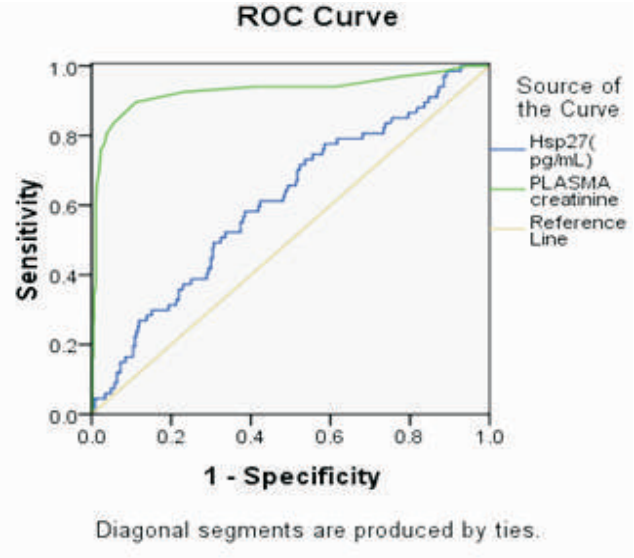


Figure 2: ROC curve analysis of Hsp27 and serum creatinine levels in DNe group.

The closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate the test. The area under the curve (AUC) of serum creatinine is 0.929 and for Hsp27 is 0.612



CONCLUSION

The plasma levels of Hsp27 are elevated in DNe compared to the diabetes and other diabetes complication groups. Hsp27 levels in the DNe group positively correlated with serum creatinine. Studying the levels of Hsp27 at different stages of DNe could help to understand if it could serve as an early marker.

10. Implication of altered ubiquitin-proteasome system and ER stress in the muscle atrophy of diabetic rats

Individuals with prolonged diabetes will develop long-term complications such as cataract, retinopathy, nephropathy and cardiovascular diseases. Though often overlooked, skeletal muscle is also negatively affected by type 1 diabetes (T1D). T1DM is a highly catabolic state characterized by an increased protein degradation rate that produces an accelerated loss of muscle mass. Impairment of skeletal muscle in T1DM, a major metabolic organ in the body, would impact basal metabolic rate affecting the ability of persons to manage their disease. Studies to identify specific proteolytic pathways that are stimulated in experimental animal models of muscle atrophy have repeatedly demonstrated an involvement of the ubiquitin-proteasome system (UPS). In addition to the protein clearance, the UPS regulates various vital cellular signaling pathways that affect cell cycle, growth, apoptosis, immune response, etc. Earlier studies reported changes in muscle E3 ligases and ubiquitinated proteins in skeletal muscle under acute diabetes. However, the status of muscle UPS components under chronic diabetes is unexplored.

UPS is crucial for the degradation of misfolded and unfolded proteins that are accumulated in the endoplasmic reticulum (ER) by a mechanism called as ER-associated degradation (ERAD). Disturbances in UPS or ERAD may lead to protein accumulation in the ER lumen causing ER stress. ER stress has been extensively studied in the liver, pancreas, and adipose tissue where it has been proposed to be involved in the pathogenesis of diabetes. The pathophysiological role of ER stress was also revealed in the development of diabetic complications including neuropathy, retinopathy, nephropathy and cardiac diseases. Even though skeletal muscle is largely responsible for glucose disposal and is related intimately to diabetes, there are no studies on ER stress in the skeletal muscle, particularly in type-1 diabetes. Hence we investigated the status and the role of UPS, ER stress (unfolded protein response), ERAD, and their interrelation in the muscle atrophy of chronic T1DM rat model.

METHODOLOGY

A group of 2-month-old male S.D rats received a single i.p.injection of streptozotocin (STZ) in citrate buffer for inducing diabetes while another group of rats received buffer as a vehicle and served as control. Two and four months after the STZ injection, animals were sacrificed, and gastrocnemius muscle was collected for analysis. In another experiment, a group of diabetic rats was treated with a well-known proteasome inhibitor, MG132 (i.p.; 50 µg/kg body weight/day) for two months starting from two months after the induction of diabetes, after which they were sacrificed to collect gastrocnemius muscle for analysis.

At the end of the experimental period, gastrocnemius muscle of rats was collected, fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The muscle fiber cross-sectional area was measured in transverse paraffinized muscle sections, stained with H&E. visualized under microscope and images were obtained. The mean of muscle fiber cross-sectional areas was determined. Total RNA was extracted from gastrocnemius muscle and reverse transcribed to cDNA. Quantitative RTPCR was performed with cDNA template using gene-specific primers. Data was compared between samples according to a comparative threshold cycle (2-ΔΔct) method and expressed as fold change over control. An equal amount of protein from the muscle homogenates of all the experimental groups was subjected to immunoblotting with specific antibodies. The proteasomal activity in the muscle was assayed using Biovision Proteasome Activity Assay Kit. To determine apoptosis in the muscle, we performed TUNEL assay using a Kit.

RESULTS

The mean cross-sectional area of the gastrocnemius muscle fiber was significantly decreased in diabetic rats relative to the area in the control group (Figure 1). Expression level of muscle-specific E3

ligases: muscle RING-finger protein-1 (MuRF1) and TRIM72 were significantly up-regulated in 2nd and 4th months diabetic rats, when compared with their controls (Figure 2). The ubiquitin-activating enzyme E1, UCHL1 and UCHL5 protein levels increased significantly in the 2nd and 4th month diabetic rats. TRIM72 was significantly increased in 4th month diabetic rats when compared with their respective controls (Figure 2). Increased proteasomal activity and accumulation of ubiquitinated proteins were observed in diabetes. Altered expression of ERAD components and prevalence of ER stress were observed in the muscle of diabetic rats. TUNEL assay indicated very few TUNEL-positive cells at 2 months of diabetes with no significant change when compared to control. However, at 4 months of diabetes, there was a significant increase in the number of apoptotic cells in the diabetic rat muscle when compared with its respective control.

To confirm the role of UPS and its attendant mechanisms in the atrophy of diabetic rat muscle, a group of diabetic rats was treated with MG132, a known inhibitor of the proteasome. The MG132 intervention showed no effect on fasting blood glucose, food intake, and body weight.

Figure 1: Muscle histology of rats

Representative photomicrograph of H&E stained cross-sections of the gastrocnemius muscle and quantitative estimation of myofibers' cross-sectional area.

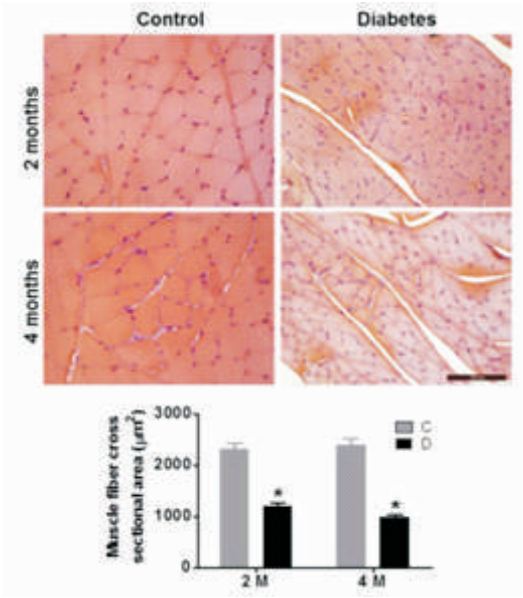
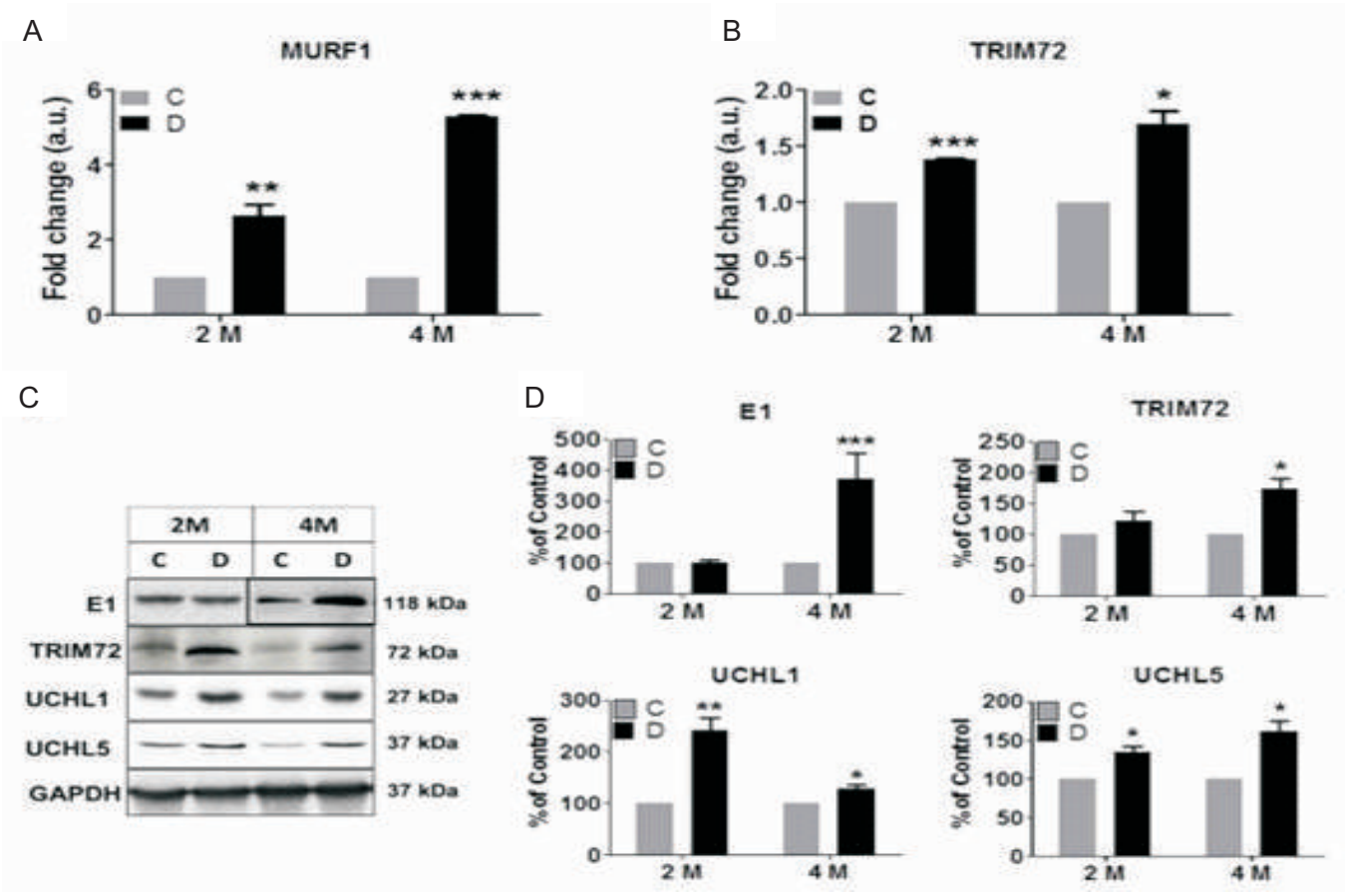


Figure 2: Expression of UPS components (E1, E3 ligases, and UCHs) in the gastrocnemius muscle

Transcript levels of MuRF1 (A) and TRIM72 (B) in 2- and 4-monthdiabetic Representative immunoblots of E1, TRIM72 and UCHs (ubiquitin C-terminal hydrolases: UCHL1 and UCHL5) in 2 and 4-month diabetic rat muscle (C). Quantification of immunoblots; expression was normalized to GAPDH and are represented as percent of control (D).



As anticipated, it inhibited the proteasome activity in the diabetic rat muscle (Figure 3). Further, MG132 treatment suppressed the expression of UCHL1 as well as ubiquitinated protein accumulation (Figure 4). Interestingly, inhibition of proteasome attenuated ER stress as indicated by reduced expression of GRP78, ATF6α, and CHOP. MG132 partially prevented alterations in HRD1 and Derlin1 expression in the muscle of diabetic rats. MG132 treatment resulted in diminished active form (cleaved fragments) of caspase-3 (Figure 4) indicative of lowered apoptosis. Importantly, it restored the muscle fiber cross-sectional area of diabetic rats (Figure 3).

Figure 3: Effect of MG132 intervention on diabetic rat muscle.

Representative photomicrograph of H&E stained cross-sections of the gastrocnemius muscle. Quantitative estimation of myofibers' cross-sectional area (B). Chymotrypsin-like activity of the proteasome in the rat muscle (C).

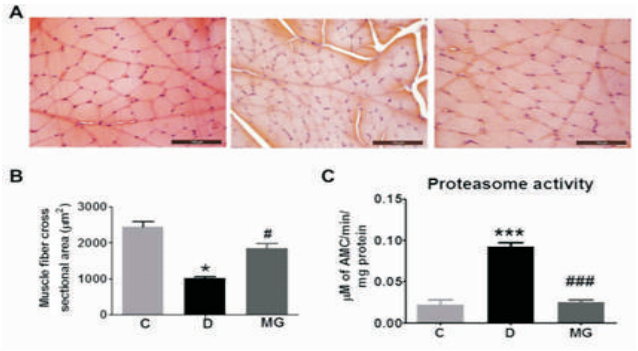
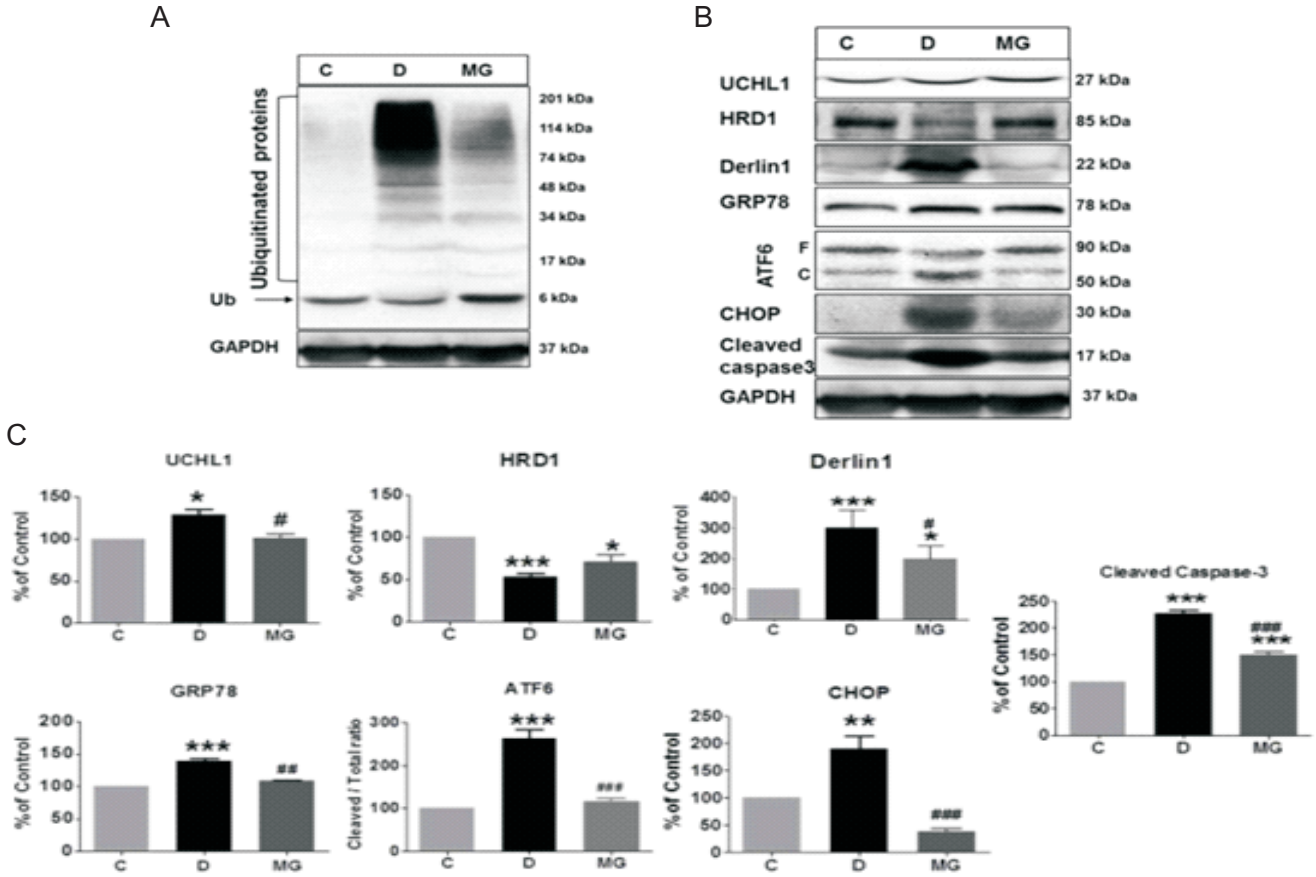


Figure 4: Effect of MG132 intervention on UPS and ER stress markers in diabetic rat muscle

A: Representative immunoblot for ubiquitin (Ub). B: Representative immunoblots for UCH-L1, HRD1 and Derlin1 (ERAD), GRP78, ATF6, CHOP, and cleaved caspase-3 in the rat muscle. C: Quantification of immunoblots; expression was normalized to GAPDH and are represented as percent of control. C-control; Ddiabetes; MG-Diabetes with MG132 treatment.



CONCLUSION

The experimental outcomes indicated that excessive activation of UPS in the skeletal muscle of diabetic rats could lead to muscle atrophy. The altered UPS elicit ER stress probably through ERAD leading to apoptosis. Further UCHL1 mediated p53 stabilization might be contributing to muscle cell apoptosis. The intervention of diabetic rats with MG132, a proteasome inhibitor could alleviate these changes and avert muscle cell apoptosis.

IV. FOOD TOXICOLOGY

1. Evaluation of bioavailability of β-carotene in biofortified food crops

The research efforts to combat subclinical nutritional disorders due to micronutrient /vitamin deficiency are gaining lot of importance. Among many vitamins we have considered to select vitamin A supplementation through biofortified maize hybrid lines developed at ICAR-IARI, New Delhi. After screening 32 hybrid maize varieties, we have identified two potential linesBAT-118 and BAT-122 which when consumed at 200 g / day will meet 60% of RDA for vitamin A. However, establishing the bioavailability of beta carotene is essential to promote it as nutritionally superior.

The objectives of the present investigation are to

i. estimate the carotenoid profile of selected biofortified food crops.
ii. assess *in vitro* bioavailability of carotenoids in raw selected biofortified food crops.
iii. determine *in silico* bioavailability of carotenoids in selected biofortified food crops.

METHODOLOGY

Sample and Study Design: The samples to identify hybrid maize varieties developed with an intention to biofortified vitamin A from ICAR institutes and local market. The selection includes normal, claimed provitamin A biofortified and Quality Protein Maize (QPM).

Study Parameters: All the maize samples have been subjected for proximate analysis which includes crude protein, crude lipids, moisture and carbohydrate by AOAC methods. The total carotenoids have been determined in powdered maize samples by modified method of Kurilich and Juvik, 1999 using HPLC to detect with a sensitivity of 1 ng/µL.

In-vitro bioavailability: This estimation has been done by the artificial stimulation similar to human digestive system. The following estimations have been undertaken in the substance which after digestion.

Isolation of micellar fraction: At the end of small intestinal digestion, samples centrifuged and post-centrifugation, the aqueous fraction containing mixed micelle was collected and filtered through 0.22 µM membrane to remove microcrystalline carotenoid aggregates and microbial contamination, if any.

In-silico bioavailability: The cellularuptake of carotenoids from micellar fraction, generated during in-vitro digestion was studied in differentiated caco-2 cells. micellar fraction was diluted in DMEM and fed to caco-2 cells for a period of 4hrs. At the end of incubation, the spent medium was removed, cell monolayer was washed with PBS to remove residual carotenoids adhering to the cell surface.

Extraction of carotenoids from of digesta, micellar fraction and cell pellets: The carotenoids from of digesta and micellar fraction were extracted by using petroleum ether:acetone (2:1, v/v). The extraction procedure was repeated three times and organic phases were pooled, dried under nitrogen and resolubilized in acetonitrile:dichloromethane (85:15, v/v) to be analyzed by HPLC as described below.

HPLC analysis: Carotenoids were analysed from total extract, micelles and digesta fractionated on a reverse phase column (C-18, 4.6 X 250 mm) with mobile phase acetonitrile:dichloromethane (85:15, v/v) at flow rate 1 mL/min and monitored at 450 nm.

Recommended dietary allowances and computation of retinol equivalence: RDA of vitamin A for adult men is 600µg RE/day. The RE of pro vitamin A provided by each maize hybrid after adjusting for cooking losses, with average consumption of 200g was calculated. As per ICMR, 1RE was considered equivalent to 1, 16 and 16µg of BC, AC and BCX respectively. The % RDA provided by maize was computed considering actual RDA of vitamin A as 100%.

RESULTS

Proximate composition analysis: The proximates viz., crude protein, fat, fiber, moisture, ash and carbohydrate in normal and biofortified maize were estimated. Our data suggests a higher amount of moisture content in biofortified maize samples compared to the normal seed samples.

Caroteneoid content of maize geontypes: Carotenoid content of maize genotypes: Lutein (LUT), zeaxanthin (ZEA), β-cryptoxanthin (BCX), α-carotene (AC) and β-carotene (BC) are the major carotenoids in all the maize hybrids tested (i.e normal, QPM, proVA and QPM + proVA maize hybrids). The highest concentration of BC (1036±13.91 µg/100g) was found in proVA biofortified Pusa-PV3 maize hybrid. Whereas the concentration of BCX and AC, were highest in normal hybrid BML7 (1145.99±21.35 µg/100g) and double biofortified hybrid HM8Q-PV (2046±23.07µg/100g), respectively. The concentration of xanthophylls (LUT and ZEA) were maximum in Madhuri sweet corn (1200.40±19.91 µg/100g) and BML7 (2143.17±47.00 µg/100g), respectively. In general, the proVA carotenoids (BCX, AC and BC) content of biofortified maize were 2-10 fold higher compared to normal maize. The average ratio of non-proVA (LUT and ZEA) to proVA carotenoids was higher in normal maize (2.01) compared to biofortified maize (0.3).

Table 1: **Total carotenoid content and Retinol Equivalents (RE) of pro-vitamin A carotenoids in various regular and biofortified hybrids of maize developed in India, along with their individual percent contribution to Indian RDA of vitamin A.**

Hybrid	Trait	Total content of non-proVA carotenoids (µg/100g)		Total content of proVA carotenoids (µg/100g)			Ratio of non-proVA to proVA	Total RE from proVA/ 200g	% RDA of VA/ 200g
		Lutein	Zeaxanthin	ß-Cryptoxanthin	α-carotene	ß-carotene			
MS	Normal	1200.40± 19.91	766.94± 16.58	285.87± 24.06	61.99± 6.23	83.96± 7.37	4.56	119.00	20
DHM 121		329.74± 23.76	868.89± 14.44	667.54± 29.26	27.40± 12.65	89.53± 4.95	1.53	109.24	18
BML7		504.54± 10.23	2143.17± 47.00	1145.99± 21.35	41.91± 4.69	224.80± 6.46	1.87	204.68	34
HM4Q	QPM	459.19± 21.02	839.00± 23.02	232.00± 13.30	474.00± 10.61	50.50± 17.21	1.72	100.88	17
HQPM1		231.00± 27.52	136.00± 11.36	76.00± 19.21	307.00± 17.90	420.00± 18.87	0.39	152.88	25
Pusa-PV2	ProVA	209.39± 19.73	216.74± 32.18	61.00± 22.26	355.00± 15.72	759.00± 10.68	0.36	241.76	40
Pusa-PV3		349.00± 11.36	166.00± 29.52	60.00± 8.05	1084.00 ±42.46	1036.00 ±13.91	0.24	402.00	67
Pusa-PV4		152.00± 33.12	285.00± 21.95	112.00± 18.11	414.00± 16.92	602.00± 32.44	0.39	216.00	36
HQPM4-PV	QPM+ ProVA	133.63± 38.83	272.00± 13.77	83.00± 23.79	397.00± 34.75	766.00± 27.54	0.33	251.50	42
HM8Q-PV		814.00± 19.64	432.00± 11.57	752.00± 14.43	2046.00 ±23.07	414.00± 19.71	0.39	453.26	76

Recommended dietary allowances and retinol equivalence: The percent contribution of each maize hybrid to the retinol equivalents (RE) and percent contribution to the recommended dietary allowances (RDA) of vitamin A are given in Table 1. The consumption of 200 g per day of HM8Q-PV and Pusa-PV3 biofortified maize hybrids would alone contribute 76% and 67% of RDA of VA, respectively.

Digestive stability and micellarization of carotenoids was found to be enhanced suggesting of benefit of pro vitamin A biofortified maize.

BC uptakeby Caco-2 cells was significantly higher ($P < 0.05$) from biofortifiedmaize compared to that from normal maize hybrids(Fig. 2A). Furthermore, the cellular accumulations of LUT ($r = 0.808$; $p < 0.05$), ZEA ($r = 0.973$; $p < 0.05$), BCX ($r = 0.990$; $p < 0.05$) and BC ($r = 0.959$; $p < 0.05$) (Fig. 2B and C) were significantlycorrelated with their quantity present in micelles generatedduring simulated digestion.

Effect of xanthophyll content on the efficiency of micellarization and uptake of β-carotene: The micellarization of BC is negatively correlated with the content of LUT ($r=-0.590$; $p>0.05$) and ZEA ($r=-0.631$; $p=0.05$) in the digesta.

CONCLUSION

The study revealed that biofortified maize enhances the pro vitamin A carotenoid content and reduces non pro vitamin A xanthophylls.

V. EXTENSION AND TRAINING

1. Development and validation of a comprehensive index for assessing food safety at household level

Foodborne illnesses are a wide spread public health problem globally. Diarrhea is a common symptom of foodborne illness. Diarrheal diseases now cause about 11% of child deaths worldwide. A great proportion of these cases can be attributed to contamination of food and drinking water. In which, a significant proportion of foodborne illness arises from practices in the home kitchen. In order to understand the challenges to food safety at household (HH) level, it is worthwhile to consider the relevant elements that compromise a typical modern day. Food safety practices at household level which are likely to contribute to outbreaks of foodborne diseases are primary control factors i.e., clean, separate, chill, cook and avoid risky food means use safe water and raw material.

In India, ensuring food safety has been recognized as an important component in protecting health of the people. Here, majority outbreaks of foodborne disease go unreported, unrecognized or uninvestigated. At household level various factors will affect the food safety including knowledge on food safety among households, their practices of food safety and enabling environment. These factors will differ between rural and urban community households based on their socio - economic, cultural and environmental conditions. In these contextual factors like location of domestic kitchen, availability of safe water, refrigeration and type of fuel for cooking play an important role in maintenance of food safety. Although few research studies addressing the factors affecting the food safety at household level, incidence of diarrhea and identifying causative bacterial pathogens for diarrhea among children in India, till date there is no validated comprehensive index to assess food safety at household level. Such an index would help in setting goals/ suitable strategies to wherever gaps are present in knowledge, practices and enabling environment of food safety for a specific region and monitoring the progress in measurable terms. Therefore present study attempted to develop and validate a comprehensive index for assessing food safety at household level.

OBJECTIVES

1. To review literature for a comprehensive view on assessing the social, cultural, economic and behavioral factors that affect the food safety at household level.
2. To develop and validate a comprehensive index for assessing food safety at household level.
3. To assess differences, if any, among rural and urban households by using the food safety index in food safety related perception, practices and reasons thereof.
4. To identify critical issues for food safety at household level and identify key messages for promoting food safety advocacy.

METHODOLOGY

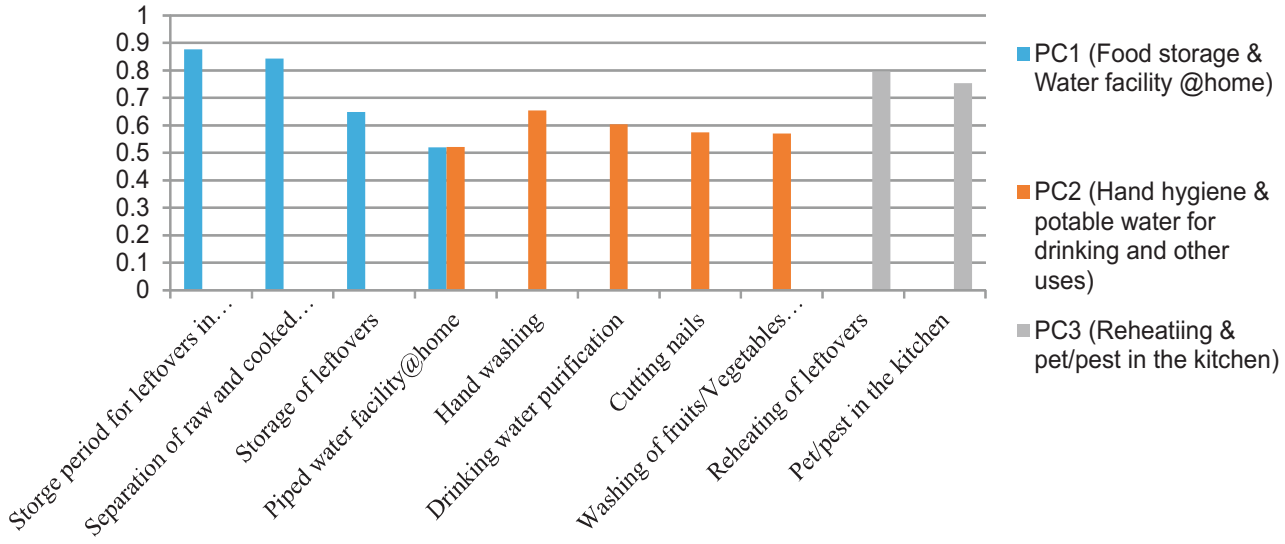
This was a cross-sectional study among primary food preparers in both urban (Hyderabad) and rural (RangaReddy district) homes of Telangana. For the development of Household food safety index (HFSI), subjects (N=400) were selected @200 each from rural and urban areas. An 87-item pre-tested questionnaire covering knowledge(43), practices(36) and enabling assets(8) was administered on subjects. Besides, demographic profile, food safety risk perception, incidence of food/waterborne diseases also collected. Scores were assigned for responses and the maximum possible score was 205. In addition, at consumption point, @400 each of stored cooked food, drinking water samples and hand rinses were collected from all subjects for microbial analysis (USFDA-BAM). Validation of 87 variables'

scores was done against the high-risk foodborne pathogen (Salmonella spp.) risk value (1.55logCFU/g) in homemade foods. Validated HFSI was administered on 200 subjects selected from rural and urban (@100each) areas in Telangana. The Messages were developed to address five key risk behaviours that were affecting safety of foods at household level. Key messages were developed to address these and these messages were tested for efficacy on 120 subjects from slum, urban and rural (@40each) home settings using three types of education materials - power point presentations, pamphlets and videos in three languages (Telugu, Hindi and English).

RESULTS

- The mean score on index questionnaire was 124.9. Overall, 22.2% of incidence of foodborne diseases was reported after consumption of homemade food.
- About 33.7% and 53.2% of drinking water (PoU) and hands of primary food preparers have shown fecal coliforms respectively. Overall, 55.8% of stored cooked foods have contaminated with Salmonella spp. There was a significant association ($p<0.05$) among foods, drinking water and hands with regard to microbial contamination.
- Eleven out of 87 parameters were significantly associated ($p<0.05$) with pathogen risk level in foods (Figure-1). The optimal cut-off value for validated 11-item HFSI score estimated 9 and it was found to have a sensitivity (77%), specificity (74%) and AUC-0.808 which are acceptable.
- Rural households showed significantly lesser HFSI score than the urban. Practice of separating raw and cooked foods in urban, Reheating of stored cooked foods in rural and Hand washing in both rural and urban settings are mostly predicting the food safety risk.
- Eight out of 9 food safety practices evaluated through index have shown significant ($p\leq0.05$) positive change between pre and post intervention using the key messages and educational material. There is a significant improvement in food safety index scores i.e., more than its cut-off value (>9) was observed after intervention in three residential areas (urban, slum and rural) and among subjects who were illiterates, below secondary education and above secondary education.

Figure 1: Principal component Analysis – plotted loadings of 11 index parameters (>0.5)



VI. PRE-CLINICAL TOXICOLOGICAL STUDIES

1. Acute non-clinical toxicity evaluation of bivalent vaccine (TD) for adolescents/ adults in duncan hartley guinea pigs.

Immunization is one of the most well-known and effective methods of preventing childhood diseases. With the implementation of the Universal Immunization Programme (UIP) by the Government of India, significant achievements have been made in preventing and controlling vaccine-preventable diseases (VPDs). Active immunization with Diphtheria and Tetanus vaccine (Adsorbed) is carried out in adults and adolescents, who have not been immunized with DTP, DTaP or Td vaccines or if there is no evidence of their immunization with three doses of any of the stated combination vaccines against Diphtheria & Tetanus infections. As per the regulatory requirements it is mandatory to undertake Preclinical toxicity evaluation of Bivalent vaccine (Td) as per Schedule 'Y' of Drugs and Cosmetics Act 1940 & Rules 1945 and WHO guidelines.

METHODOLOGY

The study has been conducted in Duccan Hartley guinea pigs (6M+6F), aged about 3 months, weighing 250 – 300gm, obtained from NCLAS, NIN, Hyderabad with approval of IAEC. The guinea pigs have been conditioned for one week in the experimental room for acclimatization followed by randomization. As part of acute toxicity testing a high dose (5x HD) of vaccine has been administered (Diphtheria Toxoid – 20Lf, Tetanus Toxoid – 37.5Lf) with a maximum volume of 0.5ml/animal through intramuscular route.

Study parameters

The animals were observed daily for mortality, live phase, cage side, physical, physiological, neurological activity till end of the experiment. The feed intake, body weights were recorded bi-weekly. All animals were euthanized, subjected for gross necropsy and vital organs were collected including site of injection.

RESULTS

- No pre-terminal deaths or morbidity were recorded in guinea pigs exposed to test vaccine.
- No significant effect on body weight gain was recorded.
- Clinical signs, behavioral activity were normal.
- No gross pathological changes in the vital organs were recorded on necropsy.

CONCLUSION

No pre-terminal deaths were recorded in guinea pigs which received a high dose (5x) of intended human vaccine. There were no abnormalities in live phase, physical activity and neurological activity throughout the study period. There was no significant difference in body weight. All the animals were active throughout the study period. No gross necropsy changes were observed.

2. Pre-clinical toxicity evaluation of inactivated chikungunya vaccine

Chikungunya virus (CHIKV) is an arthropod-borne alpha-virus transmitted through the bite of an infected *Aedes aegypti* or *Aedes albopictus* mosquito. It has been widely documented in over 40 countries. It causes an acute infection associated with severe morbidity lasting several weeks and the acute symptoms include fever, myalgia, arthralgia, headache, rash, nausea and fatigue. Indian Immunological Limited has developed a novel inactivated chikungunya vaccine which elicits immunity against chikungunya disease. The strain was obtained from US Army Medical Research and Materiel Command (USAMRM), the Walter Reed Army Institute of Research (WRAIR) and the US Army Medical Materiel Development Activity (USAMMDA) and inactivated the attenuated virus seed at IIL, Hyderabad.

The non-clinical evaluation of this vaccine was undertaken as per the Schedule 'Y' of Drugs and Cosmetics Act 1940 & Rules 1945 and WHO guidelines.

METHODOLOGY

Acute toxicity test in BALB/c Mice and SD Rats: The study has been conducted in BALB/c mice (18M+18F), aged 4 – 6 weeks, weighing 18 – 20gm and SD Rats (18M+18F), aged 6 – 8 weeks, weighing 180 – 200gm (±20%), obtained from NCLAS, NIN, Hyderabad with approval of IAEC (P6F/II-IAEC/NIN/2015/BD). The animals have been conditioned for 8 days in the experimental room followed by randomization into three groups. The test vaccine has been administered in 5XPC (80µg / 0.5ml) by intramuscular route with a volume of 0.5ml / rat and 1ml/rabbit.

The animals were observed daily for mortality, live phase, cage side, physical, physiological, neurological activity till end of the experiment. The body weights were recorded bi-weekly. All animals were euthanized, subjected for gross necropsy and vital organs were collected including site of injection.

Sub-chronic toxicity study in SD Rats and NZW Rabbits: The rats (75M+75F) aged 4 – 6 weeks, weighing 180 – 200 gm, obtained from NCLAS, NIN Hyderabad with approval of IAEC (P6F/II-IAEC/NIN/2015/BD and NZW rabbits (45M+45F), aged 3 – 4 months, weighing 1.1 - 1.9 kg, obtained from Sainath agencies laboratory animals, Hyderabad. The animals were acclimatized for 7 days in the experimental room followed by randomization into five groups to receive various concentrations of test vaccine by intramuscular route. The dosage schedule was once bi-weekly (viz., 0^{th}, 14^{th}, 28^{th} & 42^{nd} day) with a constant volume of 1ml / animal. The animals were observed daily for mortality and physical activity. Live phase, cage side, physiological and neurological activities were monitored bi-weekly in all animals till end of the experiment. The food intake and body weights were recorded bi-weekly. Urine analysis (qualitatively) was monitored pre and post exposure to vaccination in all groups of animals. Blood samples were collected from all animals on 14^{th}, 28^{th} and 42^{nd} day for assessing immune response. The biochemistry and hematology parameters were undertaken within 24hrs, 16^{th} day & 31^{st} day of the last exposure to the test vaccine representing Immediate Exposure Group (IEG), Recovery Group (RG) and Extended Recovery Group (ERG) respectively. Similarly gross necropsy and histopathology of all vital organs have been undertaken in euthanized animals.

RESULTS

Acute toxicity test in SA Mice and SD Rats

- No pre-terminal deaths or morbidity were recorded in animals exposed to test vaccine.
- No significant effect on body weight gain were recorded
- Clinical signs, behavioral activity were normal.
- No gross pathological changes in the vital organs were recorded on necropsy.

Subchronic toxicity study in SD Rats and NZW Rabbits

- There was no mortality either in rat or rabbit during the study phase, expect one female SD rat received 2XPC died on 36th day of experimental period.
- Pre-terminal death of female rat was not attributed to vaccine exposure as there were no abnormalities in either autopsy or histopathological examination.
- The food intake, body weight gain; clinical signs, behavioral activities etc., were normal in rats and rabbits.
- There were significant changes in clinical chemistry and hematological parameters of the rats and rabbits, but were within normal range.
- Rats and rabbits immunized with test vaccine have elicited immunogenic response as compared to Control and Vehicle Control.
- The organ weights found to be normal with no gross changes.
- The histopathological profile did not show any significant changes in the organs which can be attributed to test vaccine exposure except Micro vacuolation in liver and inflammation at the site of injection were observed in all groups of rats. However the degree of inflammation was found to be reduced with duration of the study period.
- The histopathological observations of all organs studied did not show any abnormalities attributed to the exposure of test vaccine in NZW rabbits, whereas in liver Periportal Inflammation (PPI), Portal Tract Inflammation (PTI) and Micro Vacuolation (MV) was seen in all groups of rabbits. In kidneys Focal Interstitial Nephritis (FIN) were seen in all groups of rabbits. Mild Chronic Inflammation at the site of injection was observed in rabbits.

CONCLUSIONS

Acute toxicity test in SA Mice and SD Rats

No pre-terminal deaths were recorded in animals which received test vaccine. There were no abnormalities in live phase, physical activity and neurological activity throughout the study period. There was no significant difference in body weight. All the animals were active throughout the study period. No gross necropsy changes were observed.

Sub-chronic toxicity study in SD Rats and NZW Rabbits

There was no mortality in either rats or rabbits exposed to test vaccine, except one female rat died during the experimental phase and which could not be attributed to test vaccine exposure. The body weights, food intake were found to be normal. The clinical signs, behavioral activity etc was normal throughout the study period. The clinical chemistry and hematological profile were within normal range. There were no significant changes in organ weights. No gross changes were observed in any of the organs examined during necropsy. The histopathological observations of all organs studied did not show any abnormalities attributed to the exposure of test vaccine. There was local inflammation at the site of injection and it was found to be decreased with duration of the study period. No observable adverse effects were recorded with test vaccine exposure to the level of 2XPC.

Study Impression: The test vaccine has been found safe for the clinical trial. The required regulatory reports will be submitted as and when requested.

3. Pre-clinical testing for safety of synthetic peptide 1 of 80kda hsa for development of antifertility vaccine

An 80kDa HSA was initially identified from human sperm extract as an antigen responsible for inducing immunological infertility and later demonstrated to be the promising candidate for development of anti-fertility vaccine. Protein band of 80kDa from humansperm extract reacted specifically with the serum of an immune infertile woman. The preliminary immunization studies conducted at ICMR-NIRRH, Mumbai with 10µg of purified 80kDa HSA elicited gradual increase in antibody titter and induced infertility in male and female rats. Immunized animals regained fertility with decline in antibody titer demonstrating the reversibility. In view of 80kDa antigen's reversible antifertility property, it was considered to promote as one of the product for clinical use. The present investigation as inter ICMR collaborative program has undertaken to carryout pre-clinical safety evaluation as per the regulatory requirement.

METHODOLOGY

Acute toxicity study in mice and rabbits and sub chronic toxicity study in rats and rabbits has been conducted. Therapeutic dosage schedule for mice, rat and rabbits has been calculated based on the intended clinical dosage - 852µg/70kg man.

Acute Toxicity Study in SA Mice and NZW Rabbits

Mice have been dosed with 1107.5µg/kg (10X), 2215µg/kg (20X) and 5537.5 µg/kg (50X) per 0.1ml once by sub-cutaneous route. Similarly, NZW rabbits have been administered with a single 10X dose of 400µg/kg / 0.5ml. All animals were observed daily for 14 days after exposure to the test compound. This is followed by observation on pre - terminal mortality, body weight gain for 14 days. At the end of the experiment blood sample have been collected for clinical chemistry, hematology parameters and euthanized to undertake gross necropsy of all major organs.

Sub Chronic Toxicity Study in SD Rats and NZW Rabbits

The study has been conducted in SD rats (24M+24F), aged 6-8 weeks, weighing 180g -200g, and NZW rabbits (16M+16F), aged 3-4 months, weighing 1.0-1.5 kg were obtained from NCLAS, NIN, Hyderabad after approval of IAEC. The rats and rabbits were conditioned for 7 days in the experimental room followed by randomization into four groups viz., i. Vehicle Control ii. TD, iii. 2.5XTD and iv. 5XTD. Rats were administered at a dose of 15.3µg/0.1ml (TD), 38.25µg/0.1ml (2.5XTD) and 76.5µg/0.1ml (5XTD) through subcutaneous route with 3 Booster doses at 4 weeks interval. Similarly, rabbits were administered with test compound at a dose of 60µg/0.5ml (TD), 150 µg/0.5ml (2.5XTD) and 300µg/0.5ml (5XTD) as explained above.

Study parameters

The animals were observed daily for mortality and physical activity. Live phase, cage side, physiological and neurological activities were monitored bi-weekly in all animals till end of the experiment. The Food intake and body weights were recorded bi-weekly. Urine analysis (qualitatively) was monitored pre and post exposure to test compound in all group of animals. Blood samples were collected for biochemistry and hematology parameters at midterm and final term euthanization. Similarly gross necropsy and histopathology of all vital organs have been undertaken in euthanized animals.

RESULTS

Acute toxicity in Mice and Rabbits

Mortality was recorded in one male mouse on 10^{th} day in 10X dose group, whereas no mortality was observed in rabbits. However, this death of mice was not due to test material as per the necropsy observations. There were no abnormal changes in physical, physiological, clinical and neurological activities. Clinical chemistry, hematology profile was observed to be normal in all groups of animals which received test material at various concentrations.

Sub-chronic Rat and Rabbit

There was no mortality in the rats exposed to test compound. The clinical signs, behavioral activity etc was normal throughout the study period in all groups. The clinical chemistry and hematological profile were within normal range. There were no significant changes in organ weights. No gross changes were observed in any of the organs examined during necropsy. Histopathology evaluation was also unremarkable.

4. Pre-clinical efficacy and safety evaluation of rinifol (a formulation of probiotics, vitamins and zinc) in various dosage forms

Probiotics (*Lactic Acid Bacillus*) are micro-organisms that are believed to provide health benefits when consumed. The term probiotics currently used to name ingested micro-organisms with potential beneficial effects to humans and animals and it is a formulation of bacterial genera and it enhances the amount of intestinal mucosal cells that secrete immunoglobulins. Also, they adhere to the gut wall and thus compete with pathogens and prevent their binding. Probiotics can be administered as adjuvant, in order to maintain the essential microbial flora and replace harmful microbes. *Elan Pharma (India) Pvt. Ltd., Mumbai* has developed rinifol formulations containing lactic acid bacillus, vitamins and zinc for adult and children to restoration of intestinal flora. The formulation was evaluated for efficacy and safety in animals after generating proof of concept investigations.

Efficacy study in SA Mice

METHODOLOGY

Mice were acclimatized for 7 days and randomized into five groups viz., i). Control ii). LB 1X iii). LB – 2X iv). LB – 4X and v). Vitamin + Zinc. Then, test material (0.2ml/day/mouse) was administered through oral route once daily for 14 days, followed by 14 days recovery period. The animals were observed for live phase, cage side, physical, physiological activity and allergencity parameters bi-weekly in all animals till end of the experiment. The feed intake and body weights were recorded biweekly. The blood samples from each group were collected to estimate the serum zinc levels on 15th and 29thday of test material exposure. Intestinal wash collected to estimate the mucosal IgA levels on 15th and 29th day of test material exposure. The localization of Lactobacillus has been monitored in faecal matter collected on day 7th, 14th, 21st and 28thby RT-PCR using Lactobacillus genus specific primers. All animals were euthanized to conduct gross necropsy.

RESULTS

There was no significant difference in body weights, food intake, and cage side activities in any of the groups. There were no allergenicity symptoms in the animals. The Intestinal IgA levels were found elevated in animals exposed to test material, suggesting enhanced mucosal immunization. The Lactobacillus colonization was significantly higher in animals exposed to test material as compared to controls. In the rinifol formulation lactobacillus colonization was 16 folds higher than the control group of animals. In conclusion, the study results showed an effective colonization of lactobacillus when rinifol formulation was used.

Sub chronic study in SA Mice

METHODOLOGY

Mice were acclimatized for 8 days followed by randomization into six groups viz., i. Control ii. RC (A) iii.RPT(C) iv. RS (C) v.RSZ (C) vi. RC (A) – 4X with test compound once daily for 14 days and another 14 days as recovery period. The animals were observed daily for mortality and physical activity. Live

phase, cage side, physiological, neurological observations were monitored bi-weekly in all animals till the end of the experiment. The feed intake was quantified and recorded biweekly. Body weights were recorded biweekly. The urine analysis (qualitatively) was done pre and post exposure to the test material. Half of the animals from each group were subjected to collection of blood samples to evaluate clinical chemistry on 15th day of study and followed by euthanization to conduct gross necropsy and histopathological observations of all vital organs. Similarly, the remaining animals were euthanized and analysed for the above mentioned parameters on 15th day of recovery period. The data was compiled and analyzed for significant differences between individual groups.

RESULTS

There was no mortality in any group of animals which received various test materials at various concentrations. There was no significant difference in body weights, feed intake, and cage side activities. The clinical chemistry profile was in normal range. There were no allergenicity symptoms in the animals. Histological changes observed in the lungs and liver were present in both control and experimental group of animals and hence do not appear to be caused due to the test material administration.

5. Pre-clinical efficacy evaluation of oryzanol

Rice bran, a co-product of milled rice, and its oil may have cardiovascular health benefits. Human consumption of rice bran has been limited, primarily because of the rapid onset of rancidity in rice bran, but methods to stabilize rice bran and to extract its oil have been developed. Rice bran contains 10–23% oil and negligible amounts of water-soluble-glucans and larger amounts of insoluble dietary fiber. Rice bran has many food applications in prepared foods, nutraceuticals, and functional foods. Oryzanol is a class of non saponifiable lipids of rice bran oil (RBO). More specifically, oryzanol is a group of ferulic acid esters of triterpene alcohol and plant sterols. The quality and quantity of dietary fat is known to play a crucial role of plasma lipid concentration. Hence dietary manipulation is known to play an important role in the management of hyperlipidemia and obesity and coronary heart disease. M/S AP Organics have developed the technology for preparing oryzanol in association with CSIR-IIICT under BIRAC scheme. The preliminary studies on efficacy profile at ICMR- NIN have indicated the potential Hypo-cholestermic activity.

Efficacy study in Golden Hamsters

METHODOLOGY

The therapeutic and preventive effect of powdered Oryzanol was evaluated in golden hamsters (n=36) weighing 100-120 g, obtained from NCLAS, NIN, Hyderabad. Animals were conditioned for 8 days and randomized into Group-I (6M) negative control fed on normal standard NIN (AIN 93-G) diet, Group-II (24M) and Group-III (6M) were fed with high fat and high cholesterol diet (HF-HC) for 60 days. The preventive effect was evaluated by dividing Group-II into 4 sub-groups i.e., Group-IIa : HF-HC diet alone (positive control), Group-IIb: HF-HC along with1X ID oryzanol, Group-IIc: HF-HC diet with 2X ID oryzanol and Group-IId: HF-HC diet along with1 X ID commercial oryzanol administrations. Group-III animals were fed with HF-HC diet along with 1X ID oryzanol administration.

RESULTS

The study shows the beneficial effect of administering the 85-95% oryzanol from 85-95% rice bran oil for its hypercholesterolemic activity. The decreased levels of cholesterol, LDL and triglycerides demonstrate the therapeutic benefit of the formulation. Also, there was a significant increase in the levels of HDL which is beneficial for the reduction of bad cholesterol.

LIBRARY AND DOCUMENTATION SERVICES

Library continued to cater to the documentation and 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 going in for publication in Scientific Journals etc., are being routed through the Library and a data-base of the published papers is also made accessible through on-line services using NIN Website (www.ninindia.org).

The Library services are being further strengthened by continuously receiving support from 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 provided by ICMR for accessing E-journals Online Subscription of 4 Core Journals such as LANCET, NATURE, NEJM, SCIENCE has been renewed by ICMR is also accessible.

The Library has continued to provide an excellent Photostat support to the Scientists, technical as well as to the administrative staff.

The following library services were expanded as detailed below:

1. New additions

Books	. . . .	37
Reports	. . . .	157
Thesis / Dissertations	. . . .	5
CD's	. . . .	7

NEW JOURNALS ADDED

Foreign Journals

1) BBA General Subjects
2) Biochemical Pharmacology
3) British Journal of Sports Medicine
4) Environmental Research
5) Exercise and Sports Sciences Reviews
6) Experimental Eye Research
7) Experimental Animals
8) Food Additives and Contaminants Part A
9) Food Additives and Contaminants Part B
10) Health Education and Behaviour
11) International Journal of Diabetes in Developing Countries
12) Journal of Food Safety
13) Journal of Medicinal Food

14) Journal of the Royal Statistical Society Series A (Statistics in Society)
15) Journal of the Royal Statistical Society Series B (Statistics in Methodology)
16) Journal of the Science Food and Agriculture
17) Laboratory Animal
18) Metabolism
19) Molecular Endocrinology
20) Nutrition & Cancer
21) Nutrition Research
22) Nutrition: International Journal of Applied Basic Nutrition
23) Pedagogy in Health Promotion (Health Education and Behaviour)
24) Scandinavian Journal of Laboratory Animal Science
25) Significance (Suppl. To Journal of Royal Statistical Society)
26) Toxicology Letters

JOURNALS DELETED

Foreign Journals
1) International Journal of Laboratory Animal Science
2) Lancet
3) Nature

2. Other activities

Journals Bound	. . . .	404
Visitors using the Library	. . . .	1496
Circulation of Books/Journals etc	. . . .	566
No. of E-mails sent outside	. . . .	510
No. of E-mails received	. . . .	5800
Photocopying (No. of pages)	. . . . .	1,68,783
No. of INTERNET Searches provided	. . . .	140
No. of Reprints sent	. . . .	45

3. Total library collections

Books	. . . .	18,148
E – Books	……	36
Journals (Bound Volumes)	. . . .	40,606
Journals subscribed for 2017	. . . .	129
E – Journals subscribed for 2017	……	16
Journals received (Gratis/Exchange) for 2017	. .	108
Microforms (Microfiche)	. . . .	1,080
Slides	. . . .	280
Reports	. . . .	13,996
Theses & Dissertations	. . . .	429
MEDLINE CDROMS Discs	. . . .	383
Current Contents on Diskettes with abstracts.	. .	664
Proquest (Full Text E-Journals) on CD ROMS	. .	495
General CD's	. ..	329

SCIENTIFIC PUBLICATIONS

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B. PAPERS PUBLISHED IN PROCEEDIDNGS/ BOOKS/ CONFERENCES/ ABSTRACTS

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