II. WOMEN AND CHILD HEALTH

1. BODY COMPOSITION MEASUREMENT BY DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA) IN WOMEN FROM AN URBAN SLUM

Overweight and obesity are rapidly escalating problems in developed as well as developing countries. Excess body fat, in particular abdominal fat, is a harbinger of several adverse metabolic consequences, including impaired glucose tolerance, hyperlipidaemia and other features of metabolic syndrome. Overweight and obesity are commonly defined by the measurement of body mass index (BMI). However, this is an imperfect measure, because it cannot differentiate between fat and fat-free mass (bone, muscle, viscera and body water). A more accurate definition of overweight and obesity should be based on the total amount of body fat. The upper limits of body fat for defining obesity have been set as 25% for males and 30% for females *(Hortobagyi et al. Eur J Clin Nutr 1994; 48:769-75).* In Caucasian men and women a BMI of 30 corresponds to 25% and 30% body fat in males and females respectively.

Numerous methods are available to assess body composition, all with their own advantages and limitations. Since the development of Dual Energy Xray Absorptiometry (DXA) in early 1990s, it has emerged as one of the most widely accepted methods of measuring body composition. It is a safe, convenient and non-invasive method. It can measure % fat with great precision and it correlates well with other methods of measuring body composition. Asian Indians have a characteristic phenotype, consisting of excess body fat, abdominal adiposity and less lean tissue. Excess body fat and less lean tissue complement each other in volume and weight so that the value of BMI does not increase. Further, the risk for co-morbidities such as diabetes, dyslipidaemia and hypertension in Chinese residing in Hong Kong started to increase from a BMI of 22 kg/m^2 onwards. *(Ko et al., Int J Obesity 1999; 23:1136-42).*

Taking these into consideration, the WHO working group has redefined the criteria of obesity in Asian population acknowledging the need for 'different standards that are culturally specific'. The proposed reclassification of overweight for adult Asian is >23 kg/m^2 and for obesity it is >25 kg/m^2. Obesity is an emerging problem in all the socio-economic groups in India, and even the urban slum-dwellers. Many of them are migrants from rural areas and have changed their life-styles during adulthood. This study was therefore carried out to measure the body composition by DXA in women from the low socio-economic group residing in an urban slum.

Hypothesis

Indian women have a characteristic body composition with higher levels of body fat per cent at lower BMI levels than other ethnic groups.

Objectives

1. To assess body composition by DXA in non-pregnant,non-lactating women between the ages of 30-60 years from low socio-eonomic group.
2. To relate body composition parameters such as lean mass, fat mass and % fat to the anthropometric parameters.
3. To explore the relationship of BMI to the body fat per cent.

Methodology

Sample size: All the women were a part of earlier study on bone status of women from low income group. Two hundred and seventy eight perimenopausal women from a large urban slum (Addagutta) in Hyderabad between the ages of 30 to 60 years were recruited for this study. They belonged to a poor socio-economic group and were involved in various occupations in the non-formal sector. Background information including reproductive history such as age at menarche, number of children, duration of breast feeding, menopausal status and age at menopause was collected. Anthropometric indices including height, weight, arm circumference and skin fold thickness at triceps, biceps, sub-scapular and suprailliac regions were measured using standard procedures.

Body composition measurements were carried out using DXA (Hologic 4500W).

Results

1. Characteristics of the study group (Mean $\pm$ SD)

Age	-	41.0 $\pm$ 8.60 years
Height	-	149 $\pm$ 5.49 cm
Weight	-	49.2 $\pm$ 9.85 kg
BMI	-	22.1 $\pm$ 3.99
Parity	-	3.3 $\pm$ 1.38
Number of postmenopausal women	-	120 (41%)
Number of premenopausal women	-	170 (59%)
Age at menopause	-	40.8 $\pm$ 5.86 yrs
WB-Fat mass	-	16.5 $\pm$ 6.24 kg
WB-Lean mass	-	30.3 $\pm$ 4.10 kg
WB-Lean +bone mass	-	31.8 $\pm$ 4.27 kg
WB- %fat	-	33.0 $\pm$ 6.38

2. Women stratified by weight

When the subjects were divided into 5 weight groups (<40, 40-45, 45-50, 50-55 and $\geq$ 55 kg), all the components i.e. WB-Fat mass, Lean mass and % fat increased significantly with increasing weight ($P<0.001$).

3. Women stratified by BMI groups

When the women were divided in 3 BMI groups ie. < 18.5, 18.5-22.9 and $\geq$ 23, it was observed that the BMI increased whole body (WB) fat mass increased disproportionately to the lean mass, thereby increasing the body fat per cent. Even the women with a desirable BMI i.e. 18.5 to 23 had a high level of body fat per cent (32%).

4. Women stratified by height groups

Women were divided into four height groups i.e. <145, 145 - 150, 150-155 and >155cm. As expected, the mean body weight and WB-fat increased with increasing height. But interestingly whole body lean mass also increased significantly with increasing height in all the height groups, thus increase in height was not associated with increase in body fat per cent.

5. Body composition parameters such as WB-Fat mass, WB-Lean mass as well as % body fat were not related to the age or the menopausal status in this group of women.

6. Relationship of BMI and body fat per cent - When body fat per cent was plotted against BMI (Figure.4) it showed a curvilinear relationship as shown by many of the previous studies (*Flegal KM. Et al. Obes.Res.1997; 5:93S).*

To create the prediction model for body fat per cent, inverse of BMI (1/BMI) was used as the mean predictor variable as done by Gallagher et al. *(Am J Clin Nutr 2000; 72:694-701).* This approach improved the linearity of the association between body fat per cent and BMI (Figure 5). It also increased the per cent explained variance and reduced the Standard Error of the Estimate (SEE) thereby improving the accuracy of the prediction.

The relationship between body fat per ent as dependant variable and BMI, age and menopause as independent variables was analysed and a prediction formula for calculating body fat per cent was derived as follows:

Body fat % = 65.468-671.044/BMI
(SEE = 3.32%)

Figure 4. Correlation of BMI & % fat (DXA)

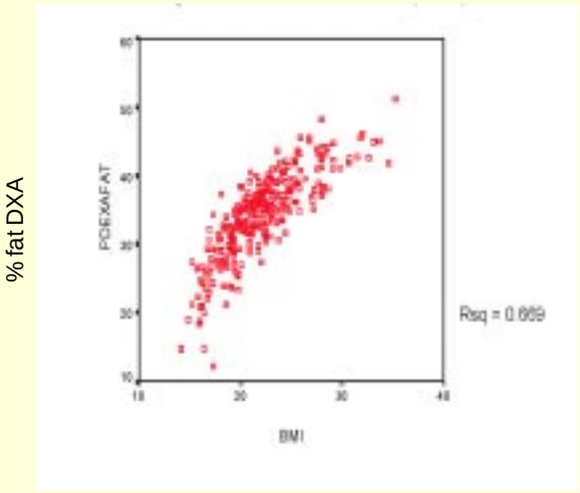
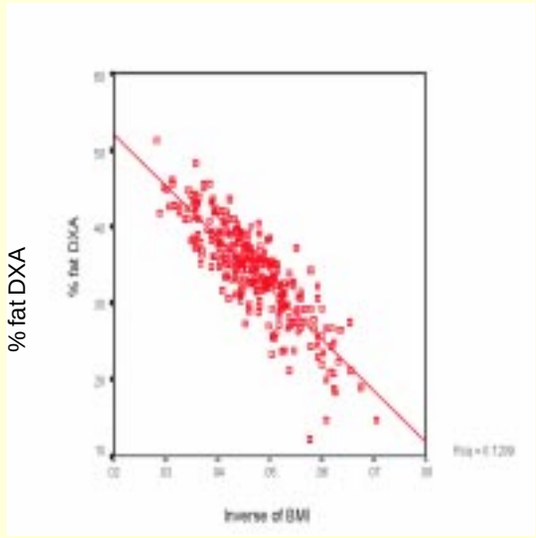


Figure 5. Correlation of inverse of BMI & % fat (DXA)



Conclusions

This estimation of body fat per cent from BMI is less dependant on intra and inter observer errors than the routinely used skinfold method. This study thus confirms that Indian women have high levels of body fat per cent at comparatively lower BMI levels than the values reported for the other ethnic groups. Increasing weight and BMI is associated with increase in body fat per cent levels. Whereas increase in height is associated with increase in lean mass but not body fat per cent. Increase in height during growth phase appears to be an important way to improve the muscle mass without increasing body fat per cent at population level. Role of childhood nutrition is important.

2. PREGNANCY INDUCED HYPERTENSION AND ANTIOXIDANT NUTRITION

Pregnancy induced hypertension is seen in approximately 10-20% of all pregnant women in India. It is associated with increased maternal morbidity and mortality related to intrauterine growth retardation, premature delivery and perinatal asphyxia. Pregnant women with pre-eclampsia are at increased risk for abruptio placenta, intra-cerebral hemorrhage and hepatic and renal failure *(Cunningham FG, 1992).*

Pre-eclampsia is associated with oxidative stress, Imbalance between pro-oxidants and anti-oxidants leads to potential cell or tissue damage *(Hubel CA, 1989).* Vascular endothelial damage is known to play a key role in the pathophysiological mechanism of pre-eclampsia *(Robert JM, 1989).* Free radical mediated lipid peroxidation may be involved in the endothelial damage in pre-eclampsia. Available data suggests increase in lipid peroxidation products and decrease in antioxidant activity in Pre-eclampsia compared with normal pregnancy *(Wang Y, 1992*). Excess free radical disturbances are associated with increased utilization of antioxidants resulting in decrease in their concentration *(Mikhail MS, 1994).* Reduced ascorbic acid is the biological labile form of ascorbic acid. It is a water-soluble antioxidant, which acts as the first antioxidant defense against free radicals present primarily in plasma. Plasma ascorbic acid levels were decreased with mild and severe pre-eclampsia. α -tocoferol and ß carotene are lipid soluble antioxidants, which have capacity to quench free oxygen radicals primarily present in the lipid membrane. α Tocoferol and β carotene were decreased only in severe form of Pre-eclampsia *(Mikhail MS, 1994).* This study was carried out to assess the antioxidant nutritional status in pregnant women with pre-eclampsia belonging to low socio-economic group attending a local government hospital.

Hypothesis

Decreased antioxidant nutrient status in pre-eclampsia may predispose to adverse outcome (course of the disease and outcome of pregnancy).

Objectives

To assess the plasma levels of antioxidants (reduced ascorbic acid, α tocoferol and ßcarotene) in pregnant women with different grades of pre-eclampsia and its relation to pregnancy outcome.

Sample size

Taking 20% as prevalence of pre-eclampsia with 80% power at 5% significance and 15% variation in proportions - 50 pregnant women with pre-eclampsia with matched controls were recruited.

Methods and Material

Subject recruitment

Fifty pregnant women with different grades of pre-eclampsia with matched control were enrolled.

Criteria

Demographic, socioeconomic, nutritional and obstetric data was recorded using a precoded proforma. A detailed clinical nutritional anthropometric and obstetric examination was carried out. Fasting

blood samples were collected to estimate antioxidant nutrients by standard techniques. Pregnant women were followed up till delivery to record pregnancy outcome and birth weight of infants.

Results

Of fifty pregnant women with pre-eclampsia recruited, twenty two had severe toxaemia while 28 had mild to moderate toxemia. The mean antinatal weight and post natal BMI were found significantly (P< 0.05) higher among cases compared to controls (Table 4). Antioxidants (vitamin C, vitamin E and carotene) were found significantly lower in pregnant women with pre-eclamptic toxaemia compared to control group (Table 5). Vitamin C was found significantly (p<0.05) lower in women with severe pre-eclampsia compared to mild to moderate group. The mean gestational age (weeks) at delivery and birth weight (kg) were lower in women with pre-eclampsia compared to control group. The premature delivery and intrauterine growth retardation were higher in women with pre-eclampsia (Table 6).

Table 4. Obstetric profile

	PET (50)	CONTROL (50)
Age (yr)	20.9±2.65	20.4±2.06
ANW (kg)	56.8±8.75	52.9±5.81*
P Natal BMI	22.1±3.41	20.7±2.58*

** P<0.05*

Table 5. Pre-eclampsia and antioxidants

Antioxidants	Pre-eclampsia	Control	Significance
Vit C µmol/L	31.9 ± 18.26	43.1 ± 22.15	P<0.05
Vit E µg/ml	4.3 ± 2.90	6.2 ± 3.88	P<0.02
β-carotene ng/ml	45.8 ± 31.86	68.1 ± 36.88	P<0.01

Table 6. Delivery outcome

	PET (50)	CONTROL (50)
G Age at Del [wk]	36.4±3.01	38.9±1.14*
BW kg	2.38±0.52	2.68±0.31*
PMD	46 %	8 %
Term IUGR	20 %	12 %
Hb g/dl	8.6 ±1.80	9.5±1.76

Conclusions

- Significant differences were observed between pre-eclampsia and controls with respect to obstetric profile and antioxidant levels.
- Serum vitamin C levels showed significant differences between mild to moderate group and severe degree of pre-eclampsia.
- Effect of antioxidant supplementation in primigravida on prevalence of pre-eclampsia has to be planned.

3. VITAMIN A MODULATES IMMUNE RESPONSE IN ACUTE RESPIRATORY INFECTIONS

Micronutrients can influence and modulate immune response and alter the course and outcome of most infectious illnesses. Poor nutritional status, or deficiency of specific nutrients like zinc and vitamin A, has been shown to suppress several facets of immune response. Among the various nutrients, vitamin A, iron and zinc have a significant role in immune response. Animal studies have shown that in addition to generalized effects on immune function, zinc and vitamin A can influence Th1 and Th2 cytokine responses and thus have a profound impact on the outcome of infectious illnesses. Studies have documented low serum retinol and zinc levels during acute infection. Based on these findings clinical trials were conducted on the assumption that infectious disease outcome might improve with zinc or vitamin A supplements.

However, though zinc supplementation studies proved beneficial; vitamin A supplementation studies on children with respiratory infection have been disappointing. Some researchers suggested that vitamin A (VA) should not be used therapeutically in patients with pneumonia unless there is clinical evidence of vitamin A deficiency or concurrent measles infection. Considering the above views, It is hypothesized that vitamin A may modulate the Th1 andTh2 bias and alter the course of respiratory infection. Thus in this study the micronutrient status of children during acute respiratory infection (ARI) and their association with local cytokine (Th1, Th2) response was determined. In addition; to study the impact of large dose vitamin A on Th1 andTh2 modulation, cytokine response was studied after oral administration of 2 lacs IU of vitamin A in normal children.

Materials and Methods

Study design

Children aged 10 months to 3 years, suffering from ARI (pneumonia, bronchiolitis and upper respiratory tract infection), with a history of illness for not more than 5 days, were recruited from Niloufer Hospital, Hyderabad. Clinical diagnosis of pneumonia was confirmed by X-ray chest; and bronchiolitis was diagnosed in infants based on the classical clinical signs of wheezy cough, dyspnoea and irritability, with or without x-ray evidence of hyperinflation of lungs. Children with cough; fever and rhinitis were grouped as upper respiratory tract infection (URTI). Children with congenital heart disease, chronic lung disease or family history of asthma were excluded from the study. Sample size estimate was based on mean and SD of IL2 cytokine in vitamin A deficient ARI children with 90% power and 5% significance. This yielded a sample size of 20 ARI children with low vitamin A (20 µg/dl). Thus, 72 children with ARI were recruited, of whom there were 38 children with vitamin A deficiency for comparison of cytokine response. Thirty apparently normal children of similar age group and socioeconomic status were taken as control group. The control group was taken to compare micronutrient status in the ARI children.

Nutritional status

Anthropometric measurements were taken to assess their weight for age using Gomez classification. After obtaining an informed consent from parents, 2 ml of blood sample was collected from children, and their hemoglobin (Hb) status, serum zinc and vitamin A levels were determined. Serum retinol was measured by HPLC. Serum zinc was measured using atomic absorption spectrophotometry (AAS) after diluting (1 in 5) serum in deionised water. Heamoglobin was determined by cyanmethemoglobin method.

Systemic and local, Th1/Th2 response in children with ARI

Nasopharyngeal aspirates (NPA) were collected aseptically by passing size 5 feeding tube into the nasopharynx and applying gentle suction with a syringe. NPA secretions were rinsed into collecting vials containing 1ml phosphate buffer. After centrifugation of nasopharyngeal secretions to precipitate cells, the supernatant was frozen at -70 C till analyzed for cytokines by ELISA. An aliquote of serum was also preserved at -70 C to analyze cytokines at a later date.ELISA (Diaclone research) was used to determine Cytokines (IL2, IL4 and IFN-γ) from NPA and serum. Recombinant cytokines of known concentrations were used to produce the standard curves. The lower limit of detection for IL2, IL4 and IFN-γ were 5.6, 1.1 and 12.5 pg/ml respectively, with intra and inter assay variability of less than 10% and 5% respectively. The total protein from NPA was determined by modified Lowry's method.

Th1/Th2 response after 2 lacs IU of oral vitamin A in normal children

In ten apparently normal children of same age group and weight for age, blood sample was collected initially and 15 days after 2 lacs IU of vitamin A orally. The blood samples were processed for isolation of peripheral blood mononuclear cells (PBMC). PBMC was isolated on Ficoll hypaque and stimulated with PHA for 18 hours at 37°C and 5% CO_2. . After 18 hours culture, the supernatant was harvested and IL2, IL4 and IFN-γ were analyzed by ELISA. Cytokine production by cultures without PHA was below the limit of detection of ELISA.

Statistical Analysis

Statistical analysis was done with SPSS PC software. A 'P' value of 0.05 was used to determine significance. Student's t test was used to compare nutritional variables (WFA, Hb, zinc and vitamin A) between ARI and control groups. Relationship of cytokine response and vitamin A concentration in ARI children was done using the nonparametric Mann-Whitney test, as the cytokine values were not distributed normally. Correlation coefficient was done after log transforming the cytokine data. Paired t test was used to compare cytokine response from PBMC of normal children before and after 2 lacs IU of vitamin A.

Results

The mean age in months and weight for age (WFA) was comparable between ARI and the control group. Mean serum zinc, vitamin A and Hb were significantly lower in the ARI children compared to the control group (Table 7). Vitamin A was <20µg/dl in 38 of 72 ARI children and 30 children had hemoglobin less than 9 g/dl. Low zinc (<70µg/dl) level was seen in 32 ARI children. Thus, more than 50% of the ARI children had low vitamin A.

Table 7. Correlation coefficient for nutritional status and NPA IL2 response in children with acute respiratory infections and in children with pneumonia alone.

	r value (ARI, n= 50)	r value (Pneumonia, n=14)
WFA	0.069	0.337
Hb	0.175	0.134
VA	-0.324*	-0.563*
Zinc	0.142	0.108

** P<0.05* *WFA: weight for age; Hb: hemoglobin; VA: serum vitamin A*

1. Local cytokine response

NPA IL2 was detectable in 53 (73.6%) of 72 ARI children and ranged from nondetectable to 472.4 pg/mg protein. The mean CI was103 (64.1, 142.9) pg /mg total protein.

NPA IL4 was detectable in 84.7% of ARI children and ranged from nondetectable to 103.4 pg/mg protein. The total mean and CI was 9.3 (-5.2, 23.9) pg/mg protein. On the other hand, IFN-γ was detectable in only 7of 72 ARI children.

2. Serum cytokine response

Of the 72 ARI children, only 8 and 21 showed serum IL2 and IL4 in the detectable range, with mean (CI) of 8.3 (-6.7, 23.5) and 2.3 (0.2,4.4) respectively. Serum IL2 was not correlated with NPA IL2 levels, while IL4 showed a negative correlation between NPA and serum. Serum IFN-γ was below detectable level in all the ARI children.

3. Nutritional status and cytokine response in children with ARI

When the mean values of NPA IL2 was compared between children with Hb of 9 g/dl and <9 g/dl, there was no significant difference. Similarly a cut off value of zinc at 70ug/dl showed no difference in IL2 response, while WFA and vitamin A were inversely related with NPA IL2 concentration.

However, the inverse association of IL2 with WFA disappeared when the data was controlled for vitamin A, while the association with vitamin A ($P<0.05$) remained strong when controlled for other nutritional parameters.

Correlation coefficient on log-transformed data showed a significant ($P<0.05$) inverse association of serum vitamin A and NPA IL2 in children with ARI (Table 7). A similar association was seen in children with pneumonia; that is after excluding cases of URTI and bronchiolitis. Other nutritional parameters (WFA, Hb, zinc) showed no correlation with NPA IL2.

NPA IL4 was not related either with WFA, Hb, and vitamin A or zinc levels. Correlation of IFN-γ with nutritional status was not attempted as very few children had detectable levels. Serum cytokines were not associated with nutritional parameters.

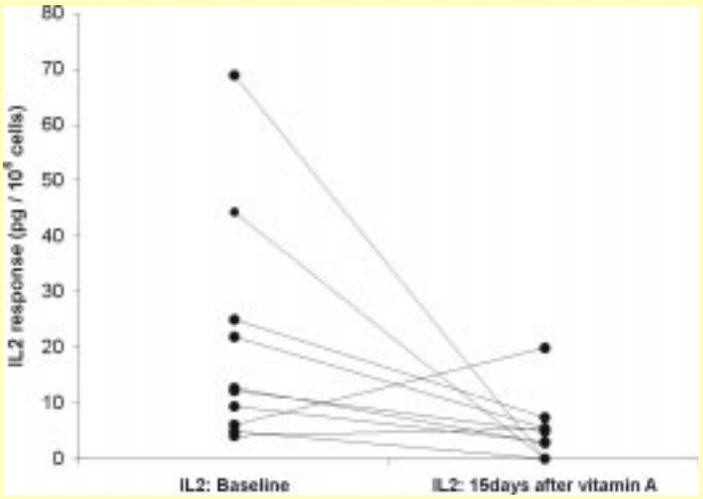
4. Effect of oral vitamin A on IL2, IL4 and IFN-γ response in normal children

To examine the direct effect of vitamin A supplementation and since vitamin A cannot be supplemented to children with ARI the effect of large dose vitamin A was studied in normal children.

The baseline vitamin A status of these children was adequate, however, serum vitamin A increased 15 days after vitamin A (2 lakhs IU) supplementation, while the IL2 concentration in the PBMC culture supernatant reduced significantly ($P<0.05$; paired t test) from the baseline concentration.

The concentration of IL4 and IFN-γ were comparable to the baseline response (Figure 6). Furthermore, increasing concentration of vitamin A added to PHA stimulated cultures showed a progressively decreasing secretion of IL12, in addition to lower IL12 secretion in the presence of vitamin A sufficient serum compared to deficient serum, indicating that vitamin A might regulate Th1 (IL2) response by modulating IL12, which is a Th1 regulatory cytokine.

Figure 6 Effect of Vitamin A on IL2 response from PBMC



Conclusions

1. Vitamin A suppresses Th1 response (IL2), which may be mediated by down regulation of IL12; that is vitamin A could be anti-inflammatory.
2. Vitamin A might alter the course of immune response in acute respiratory infection (URTI, pneumonia and bronchiolitis) and thus influence the outcome of respiratory infection.
3. Weight for age (WFA), hemoglobin (Hb) and zinc did not show any association with Th1 or Th2 cytokines.

Though the present study does not show the effects of vitamin A on respiratory morbidity, it does signify that vitamin A modulates Th1 response and thus might alter the course and outcome of infectious diseases. In depth studies are needed to delineate the role of vitamin A on Th1 and Th2 response and it's effect on IL2 receptors in acute respiratory infection in children.