ASSESSMENT OF VITAMIN A DEFICIENCY AMONG PRE-SCHOOL CHILDREN IN A RURAL AREA OF MIZORAM, NORTH-EAST INDIA, 2004.

By

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(MAE- FETP Scholar 2003-2004)

Dissertation project submitted in partial fulfillment of the requirements for the degree of
Master of Applied Epidemiology (M.A.E) of

Sree Chitra Tirunal Institute for Medical Sciences and Technology,
Thiruvananthapuram Kerala-695 011.

This work has been done as part of the two year Field Epidemiology Training Programme (FETP) conducted at

National Institute of Epidemiology,
(Indian Council of Medical Research),
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April 2005

CERTIFICATION

This is to certify that this dissertation entitled "Assessment of vitamin A deficiency in a rural area of Mizoram, North-East India, 2004" submitted by Dr Pachuau Lalmalsawma in partial fulfillment of the requirements for the degree of Master of Applied Epidemiology is the original work done by him and has not been submitted earlier by him in part or whole for any other (Publication or degree) purpose.

Date 01-05-2005

DIRECTOR

ACKNOWLEDGEMENT

I express my gratitude to the following dignitaries, my colleagues and dear ones for rendering their valuable support during the course of carrying out these projects:

Prof. M.D. Gupte, Director National Institute of Epidemiology (NIE), Chennai for his valuable guidance in spite of his tight schedule.

Prof. K. Ramachandran, Formerly Professor and Head of the Department of Biostatistics, All India Institute of Medical Sciences, New Delhi and presently Adviser to DG ICMR for Field Epidemiology Training Programme at NIE, Chennai for his valuable teaching.

Dr R Ramakrishnan, Assistant Director, NIE, my mentor in the Institute for encouragement, guidance and dedication.

Dr. Vidhya Ramachandran, Assistant Director, NIE, formerly mentor and MAE-FETP course coordinator, and Dr. Manoj Murrhekar, Deputy Director for their close guidance and encouragement.

Dr P Manickam, Research Officer, for valuable advice and assistance.

Dr. T. Longvah, Deputy Director, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, for making it possible analyses of laboratory samples.

Dr Rothangliana, Director of Health Services, and Dr Zoremthangi, Director of Hospiptal and Medical Education, Dr N. Pallai, Joint Director of Health Services, Dr P.Sangzuala, Chief Medical Officer, Aizawl West, Dr Zarzoliana, DIO, Aizawl East District, Health and Family Welfare Department, Government of Mizoram, for their encouragement and facilitation during my training.

My colleagues in the Health and Family Welfare Department, Government of Mizoram especially Dr David and Dr Fela, medical officers of the study area and their staff for assistance in the field.

I express my gratitude Mr. S. Satish librarian and Uma Manoharan Secretary to the FETP for their constant support and guidance.

My thanks to Dr Yvan Hutin, Resident Advisor of WHO to NIE for his guidance and encouragement.

My thanks to the pre-school children and their parents for cooperation and my colleagues in the field study namely, Tanpuii, Zari, Zoteii and Khuma for their dedication and sincerity in the conduct of the study.

My wife Hmangaihzuali for bearing with me in this endeavor of hard work with patience. I remain thankful to my parents Lalchungnunga and Zathuami for encouragement and moral support.

Pachuau Lalmalsawma

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Abstract

Introduction: Vitamin A deficiency (VAD) is a global problem of public health significance in under- privileged communities of the world. There is a high prevalence of VAD in Asia, Latin America and Africa. Very little data is available about Vitamin A status in the state of Mizoram.

Objective: To assess the prevalence of vitamin A deficiency among pre-school children in a rural block area of Mizoram, study their nutritional status and determine the risk factors with respect to the vitamin A deficiency among the study children.

Study design: Cross-sectional survey

Study setting: Thingsulthliah Rural Block area

Study Subjects: 460 pre-school children aged 12-59 months consisting of 233 boys and 227 girls were selected at random by population proportional to size.

Outcome measures: Diet survey with respect to vitamin A intake was undertaken. Blood samples from 160 children were collected at random from consenting parents for estimating serum retinal levels. Anthropometric and measurements and clinical assessment for ocular manifestation of vitamin A deficiency were done on all the subjects

Results: The study indicated that vitamin A deficiency was highly prevalent in the study area among the pre-school children. Bitot's spot was detected in 4% (19/460) children and 10% of the children were reported to have night blindness. Estimation of serum retinol level by HPLC indicated the prevalence of vitamin A deficiency (<20ug/dl) as 80% (95/117) pre-school children. The vitamin A intake of more than half (53%, 83/156) of the pre-school children was less than 400ug/day of vitamin A. The overall prevalence of under-nutrition among the pre-school children was 16% (76/460. Respiratory disease was the most common illness among the morbidity variables studied and 44 % (202/460) of the children suffered from it during the last 30 days, diarrhoeal incidence was 28%. 11% (51/460) of the study population were infested with intestinal worms. History of illness in the last month and quantity of vitamin A consumption in daily food were found to be significantly associated with serum retinal levels in the blood

Conclusion: The prevalence of Vitamin A deficiency was high in the study area and a public health problem requiring immediate intervention.

Keywords: Vitamin A deficiency, pre-school children, nutritional status, morbidity

Introduction

The problem of vitamin A deficiency

Vitamin A deficiency (VAD) is the leading cause of preventable blindness in children and raises the risk of disease and death from severe infections. In pregnant women VAD causes night blindness and may increase the risk of maternal mortality. Vitamin A deficiency is a public health problem in 118 countries, especially in Africa and South-East Asia, once again hitting hardest young children and pregnant women in low-income countries. Approximately 258 million children are vitamin A deficient worldwide (1). An estimated 250 000 to 500 000 vitamin A deficient children become blind every year, half of them dying within 12 months of losing their sight. Nearly 600 000 women die from childbirth-related causes each year, the vast majority of them from complications which could be reduced through better nutrition, including provision of vitamin A (2).

Vitamin A deficiency problem in India

Vitamin A deficiency is a public health problem in India. It has recently been estimated that among the 92 million children aged 1-5 years, 7.4 million have non-corneal and 0.22 million have corneal xerophthalmia at any one time. It is estimated that 52,500 children become blind and between 110,000 and 132,000 become partially blind in India every year (3). VAD is the most common cause of preventable blindness in children and leads to increased morbidity and risk of mortality. Although prevalence of xerophthalmia (clinical eye lesions leading to nutritional blindness) has decreased significantly over the past 15 years, there remains clinical and biochemical evidence of VAD. An estimated 5-7% (6.6-9.2 million) preschool children suffer from xerophthalmia, which causes at least 60,000 children to go blind each year (4).

In 1970, the Government of India as a Centrally Sponsored Scheme initiated the National Prophylaxis Programme against Nutritional Blindness. All children between the ages of 1-3 years were to receive 6-monthly massive dose of vitamin A. In addition, all children with measles and kwashiorkor should also receive massive dose under the scheme. This programme had been implemented in all the states and union territories. However, coverage figures for the massive dose vitamin A remained sub-optimal. In an attempt to improve coverage especially in the vulnerable age group between six to 23 months of age, the Government of India took a decision to link up vitamin A administration to the ongoing immunization programme. Under the revised regimen, a dose of 100,000 IU is to be given to all infants at 9 months along with measles vaccine and a next dose of 200,000 IU is to be administered along with booster dose of DPT and OPV. Subsequently children are to receive three doses of 200,000 IU of vitamin A every 6 months until 36 months of age (5).

The nationwide survey of blindness (6) conducted during 1986-1989 found the prevalence of vitamin A deficiency below six years of age to be 6.01 percent although there was a wide range of variations between the states, between less than 1% to more than 6%. Recent studies have indicated that vitamin A deficiency contributes not only to nutritional blindness but also to increased morbidity and mortality (7).

National Programme for Prophylaxis against Nutritional Blindness has been in operation in Mizoram for a number of years. No information is available about the vitamin A deficiency among pre-school children in this area. We therefore carried out a study to assess the prevalence of vitamin A deficiency among pre-school children in a rural area of Mizoram and to understand important risk factors associated with it.

Review of literature

Vitamin A:

Vitamin A is a fat-soluble substance available in liver, egg yolk and dairy products. The pre-cursor of vitamin A, carotenoids, are present in green leafy vegetables, red palm oil and yellow fruits. They can be converted to retinol in the gut wall. 50 to 90% of the ingested retinol is absorbed in the small intestine and transported to the liver in association with chylomicra where it is stored mainly as retinyl palmitate. In response to the demand of the body, it is released into the blood stream as retinol in combination with retinol-binding protein. Retinol binding protein is a specific carrier protein manufactured by the liver. The retinol is then dissociated from the serum for utilization by the target cells whose metabolism is influenced by retinal. There are specific receptors for vitamin A complex or its active metabolites on the surface and nucleus of target cells. When the intake of vitamin A and pro-vitamin A carotenoids exceeds the body requirement, 180 to 450 ug per day, which depends on the age, sex and physiological status, the excess is stored in the liver increasing the reserve. On the other hand, when the intake of vitamin A is lower than the requirement of the body, it is released from the liver stores to maintain the serum retinol at a normal level, which is above

20ug/dl. As a consequence of prolonged deficient intake of vitamin A, the liver stores of vitamin A are depleted resulting in the lowering of serum retinal level. This has a deleterious effect of impairment of cellular function in the form of abnormal differentiation leading to the development of xeropthalmia. The speed at which the cellular abnormalities develop depends on various factors such as the duration of inadequate intake of vitamin A or its pre-cursors, the extent of pre-existing liver stores and the rate at which the body utilizes vitamin A. Children with marginal intake of vitamin A will have very low level of vitamin A stores in the liver. Further sudden drop in the intake as a result of dietary change or impairment of absorption as in gastro-enteritis, sudden increase in metabolic demand as in the event of fever or growth spurt will cause depletion of the limited reserves with rapidity. These conditions can precipitate blinding xeropthalmia, overwhelming sepsis and even death (10).

Role of vitamin A in the body:

Vitamin A is indispensable for normal vision. It contributes to the production of rhodopsin, retinal pigments, which are necessary for vision in dim light. It plays a vital role in the maintenance of the integrity and the normal functioning of glandular and epithelial tissues lining the intestinal, respiratory and urinary tracts including the skin and the eyes. It also plays an important role in growth especially skeletal growth. There is an increased susceptibility to infection and lowered immune response in deficiency states of vitamin A. Some studies suggested the role of vitamin A in the prevention of epithelial cancers such as bronchial carcinoma although the data are not fully consistent ((11).

Vitamin A deficiency:

Vitamin A deficiency refers to a state in which liver stores of vitamin A and its surrogates are below 20ug/dl (12). Vitamin A deficiency can occur at any age, with consequences ranging from sub-clinical effects that increase the risk of morbidity and mortality to blinding nutrition (Keratomalacia) indicating clinical vitamin A deficiency (13). Vitamin A deficiency occurs when body stores are depleted to the extent that physiological functions are impaired in spite of the apparent absence of clinical eye signs. The level of depletion at which impairment of physiological functions take place is not entirely clear (13).

Classification of clinical vitamin A deficiency:

The signs and symptoms of xeropthalmia are given in Table-1.

Table 1: Classification of xeropthalmia

XN	Night blindness
X1A	Conjunctival xerosis
X1B	Bitot's spot
X2	Corneal xerosis
X3A	Corneal ulceration/ keratomalacia <1/3 corneal surface
X3B	Corneal ulceration/ keratomalacia >/= 1/3 corneal surface
XS	Corneal scar
XF	Xeropthalmia fundus

(Ref: 13)

Indicators for vitamin A deficiency surveillance

The major aim of vitamin A deficiency surveillance is to determine its magnitude, severity and distribution through surveys. The data can be used to highlight the significance and initiate action. Both clinical and sub-clinical biological indicators of vitamin A nutritional status are essential for the purpose. Tables 3 and 4 describe biological indicators of vitamin A status, cut-off levels that define vitamin A deficiency and minimum prevalence levels that define a public health problem (13).

Table 2: Indicators of clinical vitamin A deficiency-xerophthalmia in children 6-71 months of age

Indicator	Minimum prevalence*
Conjunctival xerosis/ with Bitot's spot (X1B)	>0.5%
Conjunctivalxerosis/ ulceration/ keratomalacia (X2, X3A, X3B)	>0.01%
Coneal scar (XS)	>0.05%

**Prevalence of any one or more indicators above the minimum level signifies a public health problem*

Table 3: Biological indicators of sub-clinical vitamin A deficiency in children aged 6-71 months of age

Prevalence below cut-offs to define a public health problem and its level of significance			
Indicator (cut-off)	Mild	Moderate	Severe
<u>Functional</u> Night blindness (present at 24-71 mo)	>0 - <1%	≥ 1%- <5%	>5%
<u>Biochemical</u> Serum Retinol (≤ 0. 70 μmol/l)	≥2 - <10%	>10 - <20%	≥ 20
Breast milk retinol (≤ 0.05μmol/l)	<10%	≥ 10-<25%	≥ 25%
<u>Histological</u> CIC/ICT (abnormal at 24-71 mo of age)	<20%	≥ 20-<40%	≥ 40%

There is a public health problem (the level of public health importance is indicated by the prevalence noted in the table) when:

The prevalence in a population of at least two of the biological indicators of vitamin A status is below the cut-off; or when one biological indicator of deficiency is supported by at least four (two of which are nutritional and diet related as in table-4) of a composite of demographic and ecological risk factors such as:-

- Infant mortality rate >75/1000 live births; under-5 mortality rate >100/1000 live births;
- full immunization coverage or, particularly measles immunization coverage, in< 50% of children at 12-23 months of age;
- < 50% prevalence of breastfeeding in 6-month old infants;
- median dietary intake <50% recommended safe level of intake among 75% of children 1-6 years of age;
- 2-week period prevalence of diarrhea $\geq$ 20%;
- measles case-fatality rate $\geq$ 1%;
- no formal schooling for $\geq$ 50% of women 14-44 years of age, and
- < 50% of house-holds with a safe water source.

Table 4: Nutrition and diet related indicators of Vitamin A deficiency

Indicator	**Prevalence**
Breast feeding pattern	
<6 months of age	<50% receiving breast milk
≥ 6- 18 months of age	<75% receiving vitamin A containing food in addition to breast milk, 3 times/week
Nutritional status (<2SD fro WHO/NCHS reference for children <5 year of age)	
Stunting	≥ 30%
Wasting	≥ 10%
Low birth weight (<2500 g)	≥ 15%
Food availability	
Market	DGLV unavailable ≥ 6 months/year
Household	≤75% households consume vitamin A rich foods at least 3 times/week
Dietary pattern	
6-71 months old children Pregnant and lactating woman	< 75% consume vitamin A rich food at least 3 times/week
Semi-quantitative/qualitative	
Food frequency	Foods of high vit. A content eaten < 3 times/week by ≥ 75% vulnerable groups

Epidemiology of vitamin A deficiency (14):

Vitamin A deficiency as a public health problem occurs within an umbrella of ecological, economical and social deprivations in the macro environment in which the populations live in different regions and countries, and in the microenvironment in which the families live in communities and households. The influence of the causal factors may vary at the regional, national and even at local levels. This necessitates situation analysis to identify the factors leading to the problem of vitamin A deficiency. There are some underlying epidemiological traits that tend to characterize most situations where vitamin A deficiency exists as a public health burden (15)

Ecological factors: Hostile environments such as arid, infertile land, periodicity of excessive rain, and humidity partly determine the amount and variety of foods rich in vitamin A that can be cultivated and the duration of their availability. Countries or parts of countries deprived of water for prolonged period and relatively hot temperatures are more likely to face vitamin A deficiency problem than other countries with relatively consistent water supplies. Vegetable and fruit crops usually occupy a second place and do not compete for land use. Vitamin A rich crops are often provided through horticulture activities at micro-level. Environmental hostility at the community and house-hold levels due to shortage of water limit home and community gardening activities at the micro-level with the result that the people have less access to vitamin A-rich vegetables and fruits which contribute to the occurrence of vitamin A deficiency in the communities. The seasonality of vitamin A deficiency is partially related to ecologic factors influencing food availability. Vitamin A deficiency tends to reach its peak when diarrhoeal and respiratory diseases are also at their peak prevalence. Overcrowding, contamination of environment, poor living conditions also contribute to the problem. Epidemics of measles in these environments worsen the problem and often precipitate vitamin A deficiency leading to night blindness and even deaths for many children.

Social factors: Poor social development in a country limits availability of health and social services including education. Traditional ideas and practice are usually followed by impoverished and illiterate women and are hesitant to engage in social interactions where modern concepts and practices are promoted. The under-education comes in the way of their learning from educational displays at health centers and those used in health related community educational activities including those with appropriate child care and feeding practices. The low standard of living in the backward impoverished environment favors large family size with the resultant overcrowding and its associated undesirable environmental sanitation as well as personal hygiene which are the determining factors to the occurrence of malnutrition and vitamin A deficiency. Crowded conditions in urban areas provide the ideal environment for the culture and spread of old diseases, such as cholera and TB, as well as for many newly emerging diseases, such as HIV (16).

Economic factors: Vitamin A deficiency is usually seen in poor countries, neighborhood and families, which depend largely on less expensive, pro-vitamin A sources. The series of events between consumption and conversion of pro-vitamin A involve several steps that require normal physiological functions. Foods of animal origin contain pre-formed vitamin A but are generally expensive. It is more difficult to meet the vitamin A activity needs of infants and young children from foods of vegetable origin than from food of animal origin. Unemployment and low-paid jobs are also the major factors leading to vitamin A deficiency in communities.

Clustering: There is a tendency for clinical vitamin A deficiency to occur in clusters than to be evenly distributed. Ecological factors are related to clustering within a country at the macro-level exacerbated by poor infrastructure to evenly distribute vitamin A-rich containing foods from areas abundant in vitamin A-rich foods to areas with deficiency of these food items. Vitamin A-rich food items are usually easily perishable and particularly susceptible for uniform intra-country distribution.

Host factors: *Sex*: Differences in the occurrence of vitamin A deficiency have been reported among certain sexes from some cultures. These differences are more likely to be connected to cultural practices, which favor a particular gender in feeding and care rather than to physiological differences.

Feeding practices: Readily absorbable form of retinal is provided by breast milk. Among populations in which breast-feeding prevails, clinically apparent vitamin A deficiency is not common. Dietary fat is very important for the absorption of vegetable sources of pro-vitamin A. The diet of a newly weaned child frequently has little vitamin A and often contains less fat than at any other period at life cycle of man. The post-weaning period is therefore a period of vulnerability to vitamin A deficiency for the child.

Disease patterns: The severity, frequency and duration of infections contribute directly and indirectly to the vulnerability of the child to vitamin A deficiency. Infections reduce appetite of the child and are especially devastating to the child. In addition, infections reduce the efficiency of absorption, conservation and utilization of vitamin A. Repeated bacterial infections cause damage to the mucosal surfaces required for absorption. Further, intestinal worms may directly compete for the intake of vitamin A besides their deleterious impact on health by reducing appetite. The frequency of diarrhoeal and respiratory infections is associated with vitamin A vulnerability (17). Restoration of vitamin A status in diarrhoeal cases decreases the severity of subsequent episodes and lowers the risk of death (18).

A Malaysian study of vitamin A absorption in children with ascariasis indicated that in populations with marginal intake of vitamin A, is an important contributing factor in producing clinical vitamin A deficiency. This study also demonstrated that stool egg counts for *Ascaris lumbricoides* were not related to the degree of vitamin A mal-absorption. Immediately after de-worming and expulsion of the worms, vitamin A absorption improved in 93% (13/14) patients. In addition, eradication of *Ascaris lumbricoides* and *Giardia duodenalis* in infected children promptly led to significant improvement in vitamin A absorption and restoration of its level to normalcy (19). Malnutrition, complicated by parasitic infections, is often found in impoverished societies with inadequate sanitation. Malnourished individuals, particularly children, are seriously affected by parasitic infections because these infections can result in reduction of nutrient availability. In addition to this, the presence of intestinal parasites frequently diminishes appetite and food intake besides increasing the loss of nutrients by causing diarrohea and dysentery. Hookworms can suck as much as 30 milliliter of blood from an infested person each day, gradually weakening the resistance of the individual to other diseases (20). An estimated 5-20% of the daily food intake of an individual is used to offset the effects of parasitic illnesses thus markedly lowering the overall nutritional status of a parasite-infested person over time (21). Acute infection is associated with increased metabolism, micronutrient loss, and protein consumption (22).

A study carried out in Ecuador suggested that weekly low-dose vitamin A supplements prevent acute lower respiratory infections (ALRI, or pneumonia) in underweight and stunted children (23). The beneficial effect of vitamin A supplements on the prevention of severe diarrhoea has been documented (24). Periods of increased physiological need: During growth spurt, vitamin A needs on the basis of body-weight are increased which is one of the major reasons for the vulnerability of younger children. School going-age are growing but at a slower rate than the younger age group. Height seems to be influenced by vitamin A through growth hormone production (25). The only biochemical parameter validated and found practical for routine use is serum retinol concentrations. Since severely depressed retinol concentrations for example, equal to or less than 0.35micromol/ L are difficult to reproducibly and reliably detected in routine laboratory tests, the index has been raised to less than 0.70ugmol/L or 20ug/dl, double the concentration originally adopted in 1980 (3).

As a larger number of children will have these higher levels, the prevalence criterion was raised from more than 5% to more than 15% (26). It is widely accepted that vitamin A deficiency occurs when the liver store of vitamin A falls below 20ug/dl. By convention, serum retinol levels less than 20ug/dl are considered deficient although in most well-nourished populations, average serum retinal levels generally exceed 30 ug/ dl (27).

Serum Vitamin A may not be an accurate measure of an individual's nutritional status. However, the frequency of very low or very high concentration of Vitamin A in a population is a useful measure of the average nutritional status of the community. The study of serum retinol in a sub-sample of children in the 1992 survey conducted by the Government of Indonesia (7) showed that among clinically normal children, about 50% had serum retinol levels less than 20µg/dl with 6.3% under 10µg/dl. For every case of clinical vitamin A deficiency, there could be several cases with sub-optimal serum retinol levels (20 mcg /dl).

There is no information at national level on the extent of sub-clinical vitamin A deficiency among children in India. However, in clinic based studies conducted at the National Institute of Nutrition, Hyderabad, Andhra Pradesh, retinol levels 20mcg / dl were observed in 30-40 % of apparently normal pre-school children having no clinical signs of vitamin A deficiency (36). This indicates that while clinical xerophthalmia prevalence has declined dramatically, sub-clinical vitamin A deficiency remains a significant public health problem.

The cut-off values suggested for ecological and demographic risk factors are arbitrary. A contextual analysis is necessary to determine the likely causes of the public health problem and timely attention is required for allocating resources and taking action. Two biochemical indicators are currently recommended for determining whether vitamin A deficiency (VAD) is a public health problem: serum retinol and serum retinol-binding protein (RBP). After consideration of 40 data sets and the original rationale for previously proposed cut-offs, a cut-off for serum retinol concentration was proposed at <0.70 micro mol/L (20 micro g/dL) in > or =15% of the sampled population. This cut-off should be applied to a representative group of preschool age children who are 6-71 months old. Because measurement of low serum retinol concentrations requires high precision, analysis by High Performance Liquid Chromatography is necessary

(28). High Performance Liquid Chromatography (HPLC) is considered the only laboratory technique sufficiently reliable for routine use and reporting (29).

Nutritional status and vitamin A deficiency:

An association seems to exist between malnutrition and vitamin A deficiency (30). A number of studies have demonstrated the association between increasing severity of anthropometric deficits and mortality, and the substantial contribution to child mortality of all degrees of malnutrition is now widely accepted (31). For assessment of malnutrition at population level, anthropometric measurements of have been internationally recommended. Anthropometric indices are constructed based on these measurements of height and weight (32). In children the three most commonly used anthropometric indices are weight-for-height, height-for-age, and weight-for-age. These indices can be expressed in terms of z-scores, percentiles, or percentage of median, which enable comparison of a child or a group of children with a reference population (33). After selecting an anthropometric indicator and a reference population, it is necessary to determine the limits of "normality". There are three classification systems for comparing a child, or a group of children, to the reference population: *Z*-scores (standard deviation scores), percentiles and percent-of-median (34).

Since the late 1970s, WHO has recommended using the *Z*-score system because of its several advantages .The present practice also quite often recommend the use of a universal cut-off point, for example, -2 *Z*-score, which proved very useful for population-based monitoring. Nutritional status can be assessed using clinical signs of malnutrition, biochemical indicators and anthropometry. Inadequacies in nutritional intake eventually alter functional capacity and result in many adverse health outcomes that are distinct expressions of malnutrition's different levels of severity (35).

Justification for the study

The study area, Thingsulthliah Rural Block, belongs to Aizawl district in the state of Mizoram. Mizoram, lies adjacent to Assam and is the southern-most state in North-East India, sharing international boundaries with Myanmar in the East and Bangladesh in the West. The National Survey of Blindness[36] (1986-1989) showed the prevalence of Vitamin A deficiency as 4.8% for Mizoram among children in the age group 0-6 years. However, the sample size for this survey included only 24 children, which was rather small. Hence, there is a need to conduct a survey covering a larger population to assess the magnitude of the problem of vitamin A deficiency.

In the reviewed literature, no study is reported about the magnitude of Vitamin A deficiency in the state of Mizoram. Among the reported diseases from all the health centres in Aizawl East District of Mizoram, 11% were due to nutritional deficiency (unpublished data). No disaggregated information is available on the type of nutritional deficiency. Hence, there is a need for carrying out a properly planned and well-conducted field studies on micronutrient deficiencies in this setting. This would help in planning nutritional and health education as well as intervention programs.

Goals

Our goal was to assess the magnitude of the problem of vitamin A deficiency among pre-school children (12-59 months) in the study area.

Objectives

1. To estimate the prevalence of vitamin A deficiency among pre-school children of the study area and asses their nutritional status to study its relationship with the level vitamin A deficiency.
2. To identify factors contributing to the prevalence of vitamin A deficiency in the study area.
3. To make appropriate recommendations to the Government of Mizoram based on the results of objectives 1 and 2.

Methodology

Study area

We selected Thingsulthliah rural Development Block by lot out of the five blocks in Aizawl district of Mizoram, India. This rural block has a total population of 32,841 in 24 villages with 6,207 households (37).

Sample size

According to National Survey of Blindness (1986-89), the prevalence of vitamin A deficiency, according to clinical assessment among children less than 6 years is 4.8% (8). The required sample size is calculated as follows:

Hypothesized prevalence: 5%

Absolute precision: 2%

α error was set at : 5% (Z_{α}=1.96)

$$\text{Sample size} = \frac{1.96^2 \times prevalence\ \% (100 - prevalence\ \%)}{precision\ \%^2}$$

Required sample size = 4 x 0.05 x 0.95 / $(0.02)^2$

= 460 (approximately)

Sampling method

The study covered all the 24 villages in Thingsulthliah Rural Block area. We assumed 8.5% of the total population in the area as belonging to 12-59 months old (based on the population break up of the last National Census, 2001). The required number of children (n=460) in the study age group 1-5 years were be selected from each of the 24 villages according to the percentage of children in the age-group 1-5 years to the total population. We obtained a list of all eligible children residing in the 24 villages from the health workers of the local health sub-centers. This was followed by selection of study children from each village using a stratified sampling with proportional allocation. The list of villages with population, number of eligible children, percentage of eligible children in the villages to the total eligible children in the block and the number of children to be selected from each village is appended in Annexure.

Study subjects

We selected 460 pre-school children between the ages of 12 and 59 months living in Thingsulthliah Rural Block constituted the study population.

Data collection

The field investigators first informed the local leaders about the study and procedures for creating awareness and requested their co-operation and support. These field investigators along with local volunteers conducted the survey. We collected data through interview of the mothers of the pre-school children or adults taking care of the index children when the mothers were not available. We used pre-tested, pre-structured questionnaire, which was developed by National Institute of Nutrition (ICMR), Hyderabad. In addition, we interviewed 14 health workers of the sub-centers using pre-tested questionnaire in the study area to collect information on immunization and events that might contribute to the occurrence of vitamin A deficiency in the area.

Ascertainment of age: The age of the child was ascertained by examination of birth certificates issued by the government through Health Department of the state and in the absence of birth certificates, we used baptismal certificates issued by the church for ascertainment of age.

Birth-weight: We faced no difficulty in obtaining birth weight as it was remembered by all the mothers or guardians interviewed. By international agreement, low birth has been defined as- birth weight of less than 2.500(up to and including 2499grams), the measurement taken preferably within the first hour of life, before significant post-natal weight loss has taken place (39).

Anthropometry:

Height: Height in centimeters was measured with the help of wooden flat vertical rod specially constructed with a wooden flat platform surface. The instrument was standardized using the steel anthropometer at the office of the chief medical officer, Aizawl East, Health and Family Welfare Department, Government of Mizoram. Infantometer was used for children below two years. The instrument was placed against the wall during measurements of height of the children. The mothers were asked to remove the foot wear of the children, and let them stand with heels together and head positioned so that the line of vision was perpendicular to the body. A glass scale was brought down to the topmost point on the head. Height was recorded to the nearest 1 cm.

Weight: An electronic digital personal weighing scale imported from Germany was used to weigh the pre-school children. This scale was standardized at the Legal Metrology Department of the Government of Mizoram prior to use in the field. It was calibrated against known weights with regularity. The children were weighed with minimum clothings. Weight was recorded as the reading given by the scale. Mild wasting was defined in children with a weight-for- height for age below -2 Z-score (35)

Clinical examination for Vit. A deficiency signs: We clinically assessed the presence or absence of ocular manifestations of vitamin A deficiency in all the study subjects(13). We collected data on dietary intake of vitamin A by 24-hour recall method in one–third of the children whose blood were collected for estimation of serum retinol levels.

Assessment of serum retinol levels: We collected venous blood samples from 160 pre-school children of the study area by random sampling. Out of this, only 117 serum samples were found fit and the remaining 43 samples were not included in the analysis either due to haemolysis or small quantity. Serum retinol levels were estimated by High Performance Liquid Chromatography method at Department of Analytical Chemistry at the National Institute of Nutrition, Hyderabad carried out the serum retinol analyses (38).

Data management and analysis

We entered, processed and analyzed the data in Epi Info 3.3 version.

Results

The sample consisted of 460 pre-school children between the ages of 12 and 59 months of which 50.7% (233/460) were males. The mean age of the children was 27.32 months (Standard deviation: 13.32 months). The mean birth-weight was 3.14 kilograms (SD= 0.45). Twenty-two of the 460 (4.8%) children were with birth-weight less than 2500 gms. Among the 460 pre-schoolchildren studied, only three (0.7%) were never breastfed. Average length of breastfeeding among breastfed children was18 months with a range of 10 to 36 months.

Among the pre-school children studied, 89% (411/460) were fully immunized against six vaccine preventable diseases while 11% (49/460) children did not complete the immunization schedule as reported by the mothers. 87.8% (404/460) of the children were administered vitamin A prophylaxis as scheduled for ages between nine months and 36 months. Two of the pre-school children (0.4%) never received vitamin A from the prophylaxis programme. Among the 460 study subjects, 82% (376/460) attended Anganwadi center run by the Government in the implementation of Integrated Child Development Services.

Table 5: Characteristics of pre-school children in Thingsulthliah Rural Block, Mizoram, 2004

Characteristics	Number	%
Gender		
Male	233	50.7
Female	227	49.3
Age group (months)		
12-35	205	44.6
36-59	255	55.4
Birth weight (Kg)		
Mean (Standard deviation)	3.14 (0.45)	
Number with birth weight <2.5 Kg	22	4.8
Number of children who were breast fed	457	99.3
Immunization status		
Complete vaccination	411	89.3
Incomplete vaccination	49	10.7
Vitamin A prophylaxis		
Received as per schedule	404	87.8
Not completed as per schedule	54	11.7
Did not receive at all	2	0.4

Household characteristics:

Household characteristics of the pre-school children included in the study are presented in **Table 6**. The mean size of family was 5.2 (SD=2.0) with a range of 3 to 7 persons. All the parents were literate and attended at least primary school. Majority of the mothers (366/460, 79.6%) were housewives while 18% (83/460) of were farmers. Among the 460 families, 350 (76%) were living in their own houses. The remaining 110 families (24%) were staying in rented houses. Only 4.6% (n=21) had monthly income below one thousand rupees. Majority of the (98%) had access to safe water with no shortage and 85% had the system for proper disposal of human wastes in the form of pit-latrine.

Prevalence of malnutrition among the pre-school children

The results of anthropometric examination indicated that 5% of the pre-school children (25/460) were having moderate or severe wasting and 11% (51/460) mild wasting (**Table 7**). The overall prevalence of under-nutrition among the pre-school children was 16% (76/460).

Table 6: Household characteristics of the studied sample, Thingsulthliah Rural Block, Mizoram, 2004

Characteristics	**No (%)**
Mean family size (Standard deviation)	5.2 (2.0)
Educational status of mother	
Matriculation and above	27 (0.4)
Below matriculation	
Higher Secondary	
High school	171 (37.2)
Middle school	180 (39.1)
Primary school	82 (17.8)
Educational status of father	
Postgraduate	3 (0.7)
Graduate	25 (5.4)
Higher Secondary	26 (5.7)
High school	187 (40.7)
Middle school	160 (34.8)
Primary school	59 (12.8)
Occupation of mothers	
Housewife	366 (79.6)
Farmer	83 (18.0)
Others	11 (2.4)
Mother attending Anganwadi centre	426 (92.6)
Mother's knowledge about source of Vit. A	
Good	3 (0.7)
Fair	46 (10.0)
Poor	323 (70.2)
Nil	88 (19.1)
Availability of safe and adequate water for the family	451 (98.0)
Availability of system for waste disposal	391 (85.0)

Table 7: Nutritional status of the studied pre-school children, Thingsulthliah Rural Block, Mizoram, 2004

Z-score of (weight- for- height)	Frequency	(%)
< -3*	25	5.4
-3 to<-2 **	51	11.1
-2 to<-1***	83	18.0
-1 to< 0	155	33.7
0 to <1	107	23.3
1 to <2	29	6.3
>2	10	2.2

(*Moderate and severe wasting, ** mild wasting, ***Normal nutritional status)

Dietary intake of vitamin A

There was a wide variation in the intake of vitamin A among 156 preschool children whose intake of vitamin A was assessed (Table 8). The interview of the mothers or guardians of the children using 24-hour dietary recall method for the previous three days indicated that the diet of pre-school children in the study area contained low vitamin A (beta-carotene). The vitamin A intake of 53% (83/156) of the pre-school children was less than 400ug/day of vitamin A.

Table 8: Dietary vitamin A intake of pre-school children (n=156), Thingsulthliah, 2004

Vitamin A intake in μg	Number (%)
<99	21 (13.5)
100-199	13 (8.3)
200-299	13 (8.3)
300-399	36 (23.1)
400-499	13 (8.3)
500-599	6 (3.8)
600-699	6 (3.8)
700-799	10 (6.4)
800-2100	38 (24.4)

Prevalence of vitamin A deficiency among the study subjects

Of the 460 children examined for clinical signs of vitamin A deficiency, 4.1% (n=19, 95% Confidence interval: 2.6-6.5) had Bitot's spots. Interview of the mothers indicated that 9.8% (n=45, 95%CI= 7.3-13.0) of the pre-school children as having night- blindness.

Of the 117 sera samples from pre-school children tested, 81.2% (n=95, 95% CI=72.0-87.1) were having for serum retinol levels below 20ug/dl, indicating severe to moderate Vitamin A deficiency; 2.6% being severe. The serum retinal levels in different age groups and sexes are given in Table 9. The prevalence of vitamin A deficiency was not significantly different in different age groups (χ^2=1.5, p=0.69) and sexes (χ^2=0.57, p=0.45).

Table 9: Prevalence of vitamin A deficiency (serum retinol level<20ug/dl) among preschool children (n=117) by age and sex, Thingsulthliah, 2004

	No. of under-five children	Vitamin A deficiency		
		Severe (serum retinol level <10 ug/dl)	Moderate (serum retinol level between 10 and 20 ug/dl	Total
Age in months				
12-23	19	1 (5.3)	16 (84.2)	17 (89.5)
24-35	24	0 (0.0)	18 (75.0)	18 (75.0)
36-47	26	1 (3.8)	20 (76.9)	21 (80.8)
48-59	48	1 (2.1)	38 (79.2)	39 (81.3)
Sex				
Male	58	2 (3.4)	43 (74.1)	45 (77.6)
Female	59	1 (1.7)	49 (83.1)	50 (84.7)
Total	117	3 (2.6)	92 (78.6)	95 (81.2)

Morbidity pattern among pre-school children over last one month

Respiratory tract infection was the most common illness among the pre-school children with an incidence of 43.9% (202/460, 95% CI= 39.4-48.5) over last 30 days before the day of investigation. 20% of the children with night blindness (9/45), 42% of the children with bitot's spots (8/19) and 46% of the children with low serum retinol levels (44/95) suffered from respiratory infections over last one month.

The second most commonly reported morbidity among the pre-school children was diarrhoea and its incidence among the study pre-school children during the last 30 days was 27.6% (127/460). 31% of the children with retinol level less than 20ug/dl (29/95) and 13% of children with night blindness (6/45) suffered from diarrhoeal diseases over last one month.

A total of 62 children suffered from measles over last one month (14%, 95% CI= 5.1 - 26.8). Measles was reported from 26% of the cases with Bitot's spots, 4% of the cases of night blindness and 17.9% of the cases with low serum retinol levels.

Fifty one (11%) of the study subjects were infested with intestinal parasites. The intestinal parasitic infestation was reported from 20% of children with low serum retinol levels, 15.8% of the children with Bitot's spot and 8.9% of the children with night blindness.

Coverage of vitamin A in different sub-centers in the study area

There were 14 health sub-centers in the study area. The coverage of vitamin A prophylaxis ranged between 46% and 99% with a mean of 62%. The coverage for measles vaccination was 73% with a range of 55% to 98%.

Factors associated with Vitamin A deficiency

Factors associated with Vitamin A deficiency are presented as univariate analysis (Table 10) and multivariable analysis (Table 11). History of morbidity and intake of Vitamin A rich food during the last month were the only variables associated significantly with serum retinal level.

Table 10: Univariate analysis of the factors associated with Vitamin A deficiency in pre-school children Thingsulthliah, 2004

Variables	N	No. of children with Vit. A deficiency (%)	Prevalence ratio	95% CI
Mothers education				
Under-Matriculation	107	87 (81.3)	1.0	0.7-1.4
Matriculation and above	10	8 (80.0)		
Father's education				
Under-Matriculation	103	83 (80.6)	0.9	0.7-1.2
Matriculation and above	14	12 (85.7)		
Monthly income				
<3000	50	42 (84.0)	1.1	0.9-1.3
>=3000	67	53 (79.1)		
No. of family members				
<=4	44	33 (75.0)	1.1	0.9-1.4
>5	73	62 (84.9)		
Place of living				
Own house	82	64 (78.0)	1.1	0.9-1.3
Rented house	35	31 (88.6)		
Z score				
<-2	19	14 (73.7)	0. 9	0.7-1.2
-2 and above	98	81 (82.7)		
Immunization status				
Vaccinated completely	106	85 (80.2)	1.1	0.9-1.4
Vaccinated partially	11	10 (90.9)		
Intake of Vit. A				
< 400ug/day	37	35 (94.6)	1.3	1.1-1.5
>= 400ug/day	80	60 (75.0)		

History of diarrhoea/ARI/measles in last one month				
Yes	67	59 (88.1)	1.2	1.0-1.5
No	50	36 (72.0)		
Mother's knowledge about vit. A rich foods				
Fair/good	16	12 (75.0)	1.1	0.8-1.5
Nil/poor	101	83 (82.2)		

Table 11: Multivariate analysis of the factors associated with Vitamin A deficiency in pre-school children Thingsulthliah, 2004

	B	S.E.	OR	95% C.I.		P
				Lower	Upper	
History of any morbidity in last month*	1.540	0.561	4.662	1.553	14.000	0.006
Vitamin A intake <400 ug	2.194	0.808	8.971	1.841	43.720	0.007
Education of parents	-0.254	0.693	0.776	0.200	3.016	0.714
Nutritional status	-0.118	0.556	0.888	0.299	2.644	0.831
Place of living	-0.212	0.595	0.809	0.252	2.595	0.721
Immunization status	0.992	1.179	2.697	0.268	27.188	0.400
Family size	0.424	0.587	1.528	0.483	4.833	0.470
Mother's knowledge about vit. A rich foods	0.289	0.720	1.335	0.325	5.476	0.688
Constant	-0.034	0.958	0.967			0.972

Discussion

The results of the study indicated that vitamin A deficiency was an important health problem among the pre-school children in Thingsulthliah Rural Block area of Mizoram. More than 80% of the of children were having the sub-clinical deficiency of Vitamin A symptoms.

The prevalence rate of vitamin A deficiency (80%) in the study area using the cut off level below 20ug/dl of serum retinol was higher than the prevalence rate reported in other parts of India - Madurai, Tamil Nadu (70.8%), Bombay (77.0%), but similar to that of Hyderabad, Andhra Pradesh (80%) among the same age group(43).

Clinical examination of the eyes for Bitot's spots in 0-6 years old rural children (n=21) indicated vitamin A prevalence rate as 4.7%. This supports the existence of vitamin A deficiency as a public health problem in the area(44). The prevalence rate was higher than that obtained from Andhra Pradesh (1%), Karnataka (1.1%) and Orissa (1.1%).The prevalence rates of vitamin A deficiency on clinical examination of all age groups were7% among males and 8% among females in Aizawl, 3% in Lunglei and 4% in Chhimtuipui districts of Mizoram(45). However, literatures on prevalence study for vitamin A deficiency in the state of Mizoram using serum retinol level as a parameter is not available.

Night blindness (XN), although a symptom, is used as a parameter for measuring vitamin A deficiency as a public health problem. The World Health Organization had set the prevalence of night blindness more than 1.0% in children 24 to 71 months of age as an indication of vitamin A deficiency to become a public health problem (46). The present study indicated the presence of vitamin A deficiency as a serious public health problem because the prevalence rate of night blindness (XN) was 9.8% (45/460). This prevalence rate is higher than that found in other five states of India (47) – Assam (3.06%), Bihar (1.78%), Orissa (3.95%), West Bengal (1.15%) and Tripura (3.03%).

Weight –for –height is the index of choice for determining current severe malnutrition or disease The overall prevalence of malnutrition (16%) by Z-score of weight- for- height indicated the presence of malnutrition in the form of wasting as a public health problem (48) among the pre-school children in the study area. Our finding is similar to the prevalence of wasting (10-15%) determined by weight-for-height (<-2 Standard Deviation) among children below five years of age in Mizoram (49) Wasting reflects body proportions, and is easily affected by acute growth disturbances (50). Repeated infections and deficient energy intake are primarily responsible for the development of wasting (51).

Despite the co-existence of malnutrition and vitamin A deficiency among the study population, our study did not suggest statistical association between vitamin A deficiency and malnutrition (Risk ratio =0.89, 95% Confidence limits: 0.67-1.18, P=0.26) It has been recognized that vitamin A deficiency is invariably accompanied by protein- energy malnutrition(52). But not all children with protein-energy malnutrition develop keratomalacia. The association of protein-energy malnutrition and the development of keratomalacia would depend on vitamin A stores of the child. If a child with adequate stores of vitamin A develops kwashiorkar, he may not manifest any ocular signs. However, the risk of developing keratomalacia is high in a child with poor vitamin A stores(53). In some parts of Africa, habitual diets contain red palm oil which is a rich source of beta-carotene. In such populations, though protein-energy malnutrition is widespread among children, ocular signs of vitamin A deficiency are extremely rare (54). In Java, among urban school children whose protein intake was adequate but low in vitamin A, nearly 60 % had xeropthalmia. However, Javanese rural children who had low protein in their diet but a good amount of vitamin A, Kwashiorkor was frequently seen while xeropthalmia was very rare (55). In Singapore where infants were fed with skim milk and sweetened condensed milk, severe eye lesions were reported without any sign of protein-energy malnutrition (56). A study conducted by the National Institute of Nutrition, Hyderabad, India, indicated that in malnourished children with corneal lesions, in spite of the serum retinol level considerably lowered, the concentration of retinal binding protein did not fall to the same extent (57). While deficiency of protein may cause impaired synthesis of retinal binding protein, vitamin A deficiency actually interferes with the release of retinal binding protein from the liver. This indicates that vitamin A, rather than protein, is the limiting factor for change in vitamin A status in malnutrition (57).

Among our study subjects, pre-school children with low retinol levels did not correspond with night blindness or Bitot's spots judged by clinical examination. Studies reported from Egypt suggested that clinical manifestations depend on the duration over which low levels of vitamin A exists (58).

In our study, the intake of vitamin A in the diet of the pre-school children was assessed for every third child and the analysis indicated that the intake of vitamin A (beta-carotene) by more than 50% of the pre-school children studied was below the recommended dietary intake (400ug/day) for pre-school children of 12-59 months old. Surveys among pre-school children in India indicated that the daily intake of vitamin A was about 100ug per day (59) against the recommended vitamin A intake of 400ug of retinol equivalents per day for pr-school children (60).

During acute infections, besides reduction in food intake, metabolic alterations are known to occur. Fall in circulating vitamin A has been reported in subjects suffering from various diseases (61, 62).Studies in Indian children indicated that absorption of vitamin A was considerably impaired in acute respiratory infections and diarrheoa (63). However, the present study did not suggest statistical association between respiratory infections (Risk ratio=2.11, 95% Confidence interval: 0.58-9.05, P = 0.91).However, children with respiratory diseases were two times more at risk of developing vitamin A deficiency. There was no statistically significant association between diarrhoea (Risk ratio=1.03, 95% Confidence interval: 0.93-1.55, P=0.55) and vitamin A deficiency by serum retinol level less than 20microgram per deciliter. The proportions of pre-school children with these diseases were 28% and 44% respectively. There were studies documenting the impairment of vitamin A absorption in children with ascariasis (64) and in those suffering from giardiasis (65). Vitamin A absorption was also impaired in severe hookworm infestation (66), salmonellosis and schistosomiasis (67). Cases of helminthiasis (intestinal worm infestations) was reported from 11% (51/460) of the children, although not statistically associated, these children were two times more at risk of developing vitamin A deficiency (Prevalence ratio=2.29, 95% Confidence limits: 0.58-9.05, P=0.16). In spite of the availability of several studies reporting a fall in the level of plasma vitamin A concentration during febrile illnesses of various kinds and a return towards normal level during convalescence (68), we did not get similar result in our study with respect to low retinol level and fever as an independent risk variable (Risk ratio= 1.03, 95% Confidence interval: 0.86-1.22, P = 0.96).

An Indonesian study indicated the existence of an association between measles and a high proportion of blindness due to corneal damage (69). Seventy cases of measles – related corneal destruction were reported to have occurred from Northern Nigeria (70). A study in India indicated an increased risk of developing corneal disease among children suffering from measles (71). We did not get a significant association between vitamin A deficiency (Retinol level less than 20ug/dl) and measles (Risk ratio: 1.00, 95% Confidence interval: 0.79-1.25, P=0.59). The association between measles and night blindness was not established (Risk ratio: 1.04, 95% Confidence interval: 0.97-1.3, P=0.18).

The prevalence of vitamin A deficiency (70%) was very high among the mothers with poor or no knowledge about the rich sources of vitamin A (Confidence interval 65.8-74.3). In addition, there were 88/460 (19%) mothers who had no opinion about the rich sources of vitamin A. Mizoram holds the second highest status in literacy rate (88.49%) among all the states and union territories of the Union of India (72). However, our study indicated positive impact of level of education, as the proportion of vitamin A deficiency was lower among parents with higher levels of education.

Under the Child Survival and Safe Motherhood Programme of the Government of India under the Ministry of Health and Family Welfare[3], vitamin A prophylaxis coverage is given to cover children between the ages of 9-36 months old.

Although the coverage of vitamin A prophylaxis ranged between 46% and 99% with a mean coverage of 62%, the prevalence of vitamin A deficiency among the preschool children was very high by serum retinal below 20ug/dl.Our study indicated that prevalence of vitamin A (51%, 60/117) was higher among children belonging to higher age groups (36-47and 48-59 months) than that (29%, 34/117) among the lower age groups of 12-23 months and 24-35 months old pre-school children. However, there was no significant difference in the prevalence of vitamin A deficiency by serum retinol level below 20ug/dl among males and females

The prevalence of childhood illnesses of all types among the children under the 14 sub-centers of our study area was reported as 48% during the last 30 days (November, 2004) before the commencement of the study. Cases of measles cases constituted 22% of the illnesses in spite of the mean coverage of measles vaccination for the children reported as 73% with a range of 55% to 98%. This suggests that either the coverage for measles was over-reported or the efficacy of the vaccine questionable. The Country Health Profile for India stated that in 1998-99, the eligible population (i.e. infants reaching their first birthday) that has been fully immunized according to national immunization policies was 34.5% and the proportion of infants reaching their first birthday that have been fully immunized against measles 41.7% during the same period.

The proportion of infants reaching their first birthday that have been fully immunized against diphtheria, tetanus, and whooping cough was 52.1% and for full immunization against tuberculosis, the coverage was 61.1%. (Country Health profile – India, Country Reported Data on Health Indicators, WHO Regional Office for South East Asia, 1998)

Limitations of the study

Biases and confounders

Information on economic status: The monthly family income was not constant or fixed for the villagers. It differs from season to season and their income was higher following harvest. The fluctuation in income was likely to have direct effect on the quality or variety of diet items.

Diet recall: Being a rural area, some families could not recall the actual dietary intake of the child. The dietary intake of vitamin A could have been influenced by recall bias where the parents or guardians could not recall the approximate intake thus increasing or reducing the total intake. The 24-hour diet recall while having the advantage of quick dietary assessment do not give an account of the dietary habit as it gives information of the intake during the last twenty four hours only. Change in the diet can take place from season to season and there could be a drastic diversion of diet for the particular day for which the interview is conducted.

Night blindness: Similarly a few number of the mothers could not give details of circumstantial evidence of night blindness.

Although we collected possible risk factors that might lead to the occurrence of vitamin A deficiency for each child, there could be overlapping in the tabulation of diseases as it is possible for a child to present with fever and - measles, diarrhoea, respiratory infections. We admit the missing analysis on this lapse.

Information on proportion of monthly income spent on food could not be presented by majority of the families although it was feasible during pre-testing of the questionnaire. This problem prompted us to drop the item from the interview schedule ultimately.

Collection of blood samples: We received tremendous support and co-operation from all the families. However, the volume of blood to be drawn (5 ml.) was rather high and some of the children did not cooperate and the prescribed quantity could not be collected resulting in the laboratory rejecting two serum samples.

Results of vitamin A analyses of a population are likely to be influenced by potential confounders like infection during the study. This confounder can be identified by analysis of alpha acid glycoprotein levels, which was not done due to technical problem.

In families where there were a number of children, only available child or children were included in the study. Equal chance was not given to all due to limitation of time which restricted the study team to have repeat visits.

The schedule for children indicated enquiry of morbidity such as diarrhoea during the past thirty days. During this period, many of the children who had suffered from the illness might have recovered fully. This could affect the resultant findings and interpretation contradictory to many studies concerned with the association of vitamin A deficiency and diseases.

Recommendations

We make the following recommendations for improvement of the nutritional status, particularly that of vitamin A in view of the results emerging from our study.

Nutrition and Health Education: The assessment of knowledge of the mothers, whose children were vitamin A deficient, about the rich sources of vitamin A indicated that 89% had poor knowledge about the rich sources of vitamin A. In addition, 99% of the mothers were poor in their knowledge about the functions of vitamin A in the body. Out of this, 55% had no idea at all. These indicate the need to undertake nutrition and health education among the population especially among the women. The information, education and communication (IEC) activities can be taken up by the Department of Health and Family Welfare through the medical officers, Health educators and the health workers at the Community Health Center at Saitual and the Primary Health Center at Thingsulthliah and their as a separate campaign or the topics on malnutrition incorporated in campaigns for other health programmes. These activities should, ideally, be preceded by workshop on nutrition education. As micronutrient deficiencies, including vitamin A deficiency, are known to occur as a result of childhood illnesses, the advantages of prevention and prompt treatment of the associated diseases should be highlighted in these activities. The Department of Social Welfare has an effective network of trained staff and volunteers in the implementation of Integrated Child Development Services throughout the state of Mizoram, including our study area. These service of these staff may be utilized to intensify the campaign against vitamin A malnutrition as the staff and volunteers are already engaged in the integrated Child Development Services scheme which has promotion of children's nutrition as one of the major components in the programme.

The general public in the area can also be reached through regional and local newspapers and special programme on the promotion of nutrition may also be broadcasted through local radio and cable television network in Saitual and Thingsulthliah towns. Mizo Hmeichhe Insuihkhawm Pawl (MHIP), a non-governmental organization of Mizo women, has its units in every village. The organization can play a vital role in the promotion of nutrition and health in coordination with either the Department of Health and Family Welfare or Social Welfare Department or both.

The campaigns should ideally emphasize on the functions of vitamin A in the body, the locally available rich sources of vitamin A, the need to take advantage of local seasonal fruits, the hazards of its deficiency including the symptom (Night blindness) and ocular manifestations as far as possible.

Topics in the campaign may also include the contributing or known risk factors in the development of vitamin A deficiency including dietary habits and diseases. Household and personal hygiene may be promoted by education on the importance of habits of proper hand washing before eating and after defaecation.

We recommend giving special attention to parents of severely vitamin A deficient children for administration of vitamin A solution supplied to the sub-centers even if they belong to per-school children of older group, 36-59 months to break the cycle of infestation with intestinal parasites.

Revised Vitamin A prophylaxis: Awareness may be created with the renewed emphasis on the importance of giving vitamin A prophylaxis to the target groups of the programme. The health staff may be encouraged to ensure that all target children are covered with the right dose and at the right time according to schedule. It is our recommendation for the government to initiate the coverage of pre-school children above three years of age in the programme of vitamin A prophylaxis with the knowledge that these groups are particularly vulnerable to vitamin A deficiency.

Evaluation and monitoring: Every national health programme needs monitoring and evaluation. Likewise, we recommended vitamin A prophylaxis programme be reviewed, evaluated and closely monitored by the Department of Health and Family Welfare in the state with particular attention to the study area.

Surveillance for nutritional deficiency: In our study, the reported overall prevalence of nutritional deficiency of all types from the 14 sub-centers was only 1.7%. Passive surveillance for nutritional deficiency, particularly that of vitamin A may be encouraged to collect maximum number of cases after imparting a separate training on nutrition to the public health staff involved in the surveillance.

Conduct of similar research at other areas of the state: As the prevalence of vitamin A deficiency in the present study indicated the existence of vitamin A as a public health problem in the study area which is not reasonable to extrapolate neither for the district nor the state. We recommended that similar study be conducted to cover all the districts of the state to generate state level data for future course of action in the control or elimination of vitamin A deficiency in the state.

Conclusion

Our present study indicates that vitamin A deficiency is a public health problem in Thingsulthliah Rural Block area. Low dietary intake was the associated with the deficiency even as environmental factors like availability of proper and safe disposal of human wastes, mother's education, age of the child, mother's knowledge about vitamin A sources and role of vitamin A in the body and childhood diseases especially worm infestation suggested the background for the occurrence of the problem of vitamin A deficiency.

Because vitamin A is affected by many factors, integrated strategies for the improvement of the current problem of vitamin A deficiency in the area was necessary as a joint effort of the departments of Health and Family Welfare, and Social Welfare with the active involvement of local non-governmental organizations.

APPENDIX I

EXTRACTION, SEPARATION AND QUANTITATION OF RETINOL IN PLASMA/SERUM

1.Retinol: One drop of standard retinol (all trans, pure, sigma) was dissolved in absolute ethanol (5ml) and designated as stock solution. 1ml of stock solution was taken and made up to 5ml with ethanol. The absorption spectrum was scanned between 280 and 400mm in a Gilford response spectrophotometer to test the purity. Retinol concentration was calculated from the peak absorbance at 325mm using extinction co-efficient $E^{1cm}{}_{1\%}$= 1845. A range of standards from 2 to 20ng in 20μl were prepared from this stock solution to construct a standard curve.

$$1)\text{Retinol concentration (ng/μl)} = \frac{\text{Absorbance at 325mm}}{\text{Extinction co-efficient (1845)}} \times 10{,}000$$

2.Retinyl acetate:Standard retinyl acetate (for internal standard) was prepared as described for retinol, except for the use of extinction colffinent $E^{1\%}{}_{1cm}$= 1560 with absorption maximum at 325nm.

$$\text{Concentration of retinyl acetate (ng/μl)} = \frac{\text{Absorbance at 325nm}}{\text{Extinction co-efficient (1560)}} \times 10{,}000$$

Extraction of Retinol: 500 μl plasma/serum was collected in a tube. To this, 20μl of internal standard (retinyl acetate 20 mg) and 500 μl of absolute ethanol was added and thoroughly vortexed for one minute. Then 1ml of hexane was added to this aqueous phase and again vortexed for one minute to extract the lipids from plasma/serum, into hexane layer. The tubes were centrifuged at 3000 rpm for 5 minutes. The upper solvent layer was quantitatively transferred to micro test tubes.

The extraction process was repeated two more times with 1ml of hexane and the pooled hexane layer was evoporated under a stream of nitrogen and the residue dissolved in 25μl of ethanol for immediate injection on to the HPLC system.

HPLC separation of Retinol: The HPLC system (Shimadzu 6 A model) was equipped with UV/viscible detector. The isocratic solvent system of methanol and water (95: 5) was used at a flow rate of 1.5 ml per minute. The detector response was set up at 0.001 (AUFS). Internal standard retinyl acetate (20μl) was injected on to the column, (shodex c18, 15 x 0.45cm, 5mm) the retention and peak area were recorded. The external standard retinol was injected and the retention time was noted. Later a mixture of internal and external standards was injected. Both the peaks resolved well with the retention times 2.68 minutes and 3.01 minutes for retinol and retinyl acetate respectively. Linearity of; detector response, as well as retention times for retinol was confirmed by injecting various concentration (2 to 20 ng) on to the column. Standard curve was plotted for area under the curve against retinol concentrations. Samples of plasma/serum in ethanol were injected on to the column and calculated concentration of retinol using standard curve.

(John G. Bieri, Teresa T. Tolliver B.S. 7 George catignani:(1979) Simultaneous determination of alpha tocopherol and retinol in plasma or red cells by HPLC. American Journal of Clinical Nutrition, 32, 2143 – 2149)

APPENDIX II

QUALITY CONTROL

1.The principal investigator was sent to the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad for standardization in the diagnosis of ocular manifestations of vitamin A deficiency prior to the commencement of the research. Standardization for clinical examination was conducted by the medical officer in charge of the Nutrition Ward of Nilloufer Hospital in the hospital during August, 2004.

2. The interview schedules were pre-tested in the area prior to actual conduct of the research.

3. The principal investigator cross-checked every 10th schedule applied and ensured all the entries were correctly done.

4. The weighing scale used was standardized and its accuracy checked, prior to its use in the field, by the Department of legal metrology, Government of Mizoram, Aizawl. The

accuracy was checked after every 15th measure of children.

5. The research was supervised by the Faculty members of National Institute of

Epidemiology, Chennai, throughout the period of study.

6. The laboratory of the National Institute of Nutrition, where the samples were analyzed for serum retinol, is a leading and premiere institute in the field of nutrition and with

international repute, governed by the Indian Council of Medical Research and as such, some of samples were rejected by the laboratory owing to hemolysis and deficiency in quantity.

6. The data were entered and analyzed under the supervision of faculty mentor and we used Epi info 2000 version for the analysis.

APPENDIX III

NATIONAL INSTITUTE OF EPIDEMIOLOGY

CONSENT FORM

Individual No: | | |

Date:__________ Time:_______

Village: ______________________

Purpose of study:

This study is being conducted by the TEPHINET, Atlanta, USA, in collaboration with the National Institute of Epidemiology, Indian Council of Medical Research, Chennai, to assess the micronutrient deficiency in the community. The aim is to estimate the prevalence of vitamin A deficiency among the pre-school children aged 12-59 months.

Procedure:

If you agree to let your child to participate in this study, we will ask you some questions concerned with dietary habits of the child for calculation of vitamin A intake. We will have to draw five milliliter of blood from the child which will be tested for the level of vitamin A. If you need any more clarifications or more information, please fell free to contact the interviewer or the principal investigator. We are staying at ____________ during this study in your village.

Voluntary:

Your participation in the study is completely voluntary. You may refuse to participate, choose to end your participation at any time or skip any particular question, you do not wish to answer, you are free to do so and the information you have provided will be kept confidential.

Risk: There will be no physical risk to you by taking part in this study.

Benefits: Your participation in this study may help us to estimate the prevalence of vitamin A deficiency among the 12-59 months old children The laboratory reports which calls for special care of the child will be followed at the earliest opportunity.

If you agree to participate in this study please sign or put your thumb impression in the place mentioned.

I have read the above information/the above information has been read to me in my own language. I have had an opportunity to ask questions and the questions that I have asked, have been answered to my satisfaction. I consent voluntarily to participate in this study. I also agree to give my blood sample for estimation of hemoglobin for this study.

(*Investigatorin a thil zir chhan leh kalphung , thisen lak a tul chenin min hrilh fiah a, he thil zirna ah hian kan nau leh chhungkaw duh hun hunah a chhan leh vang sawi kher lovin tin san thei kan nih pawh hriain he zirna atan hian kan in phal e*).

Date : Signature :

Address :___________________

Hming(Name):_________

Date : Witness's signature

:

Address : ___________________ Hming (Name)

:_________

Inlaichin dan

:___________

(Relationship)

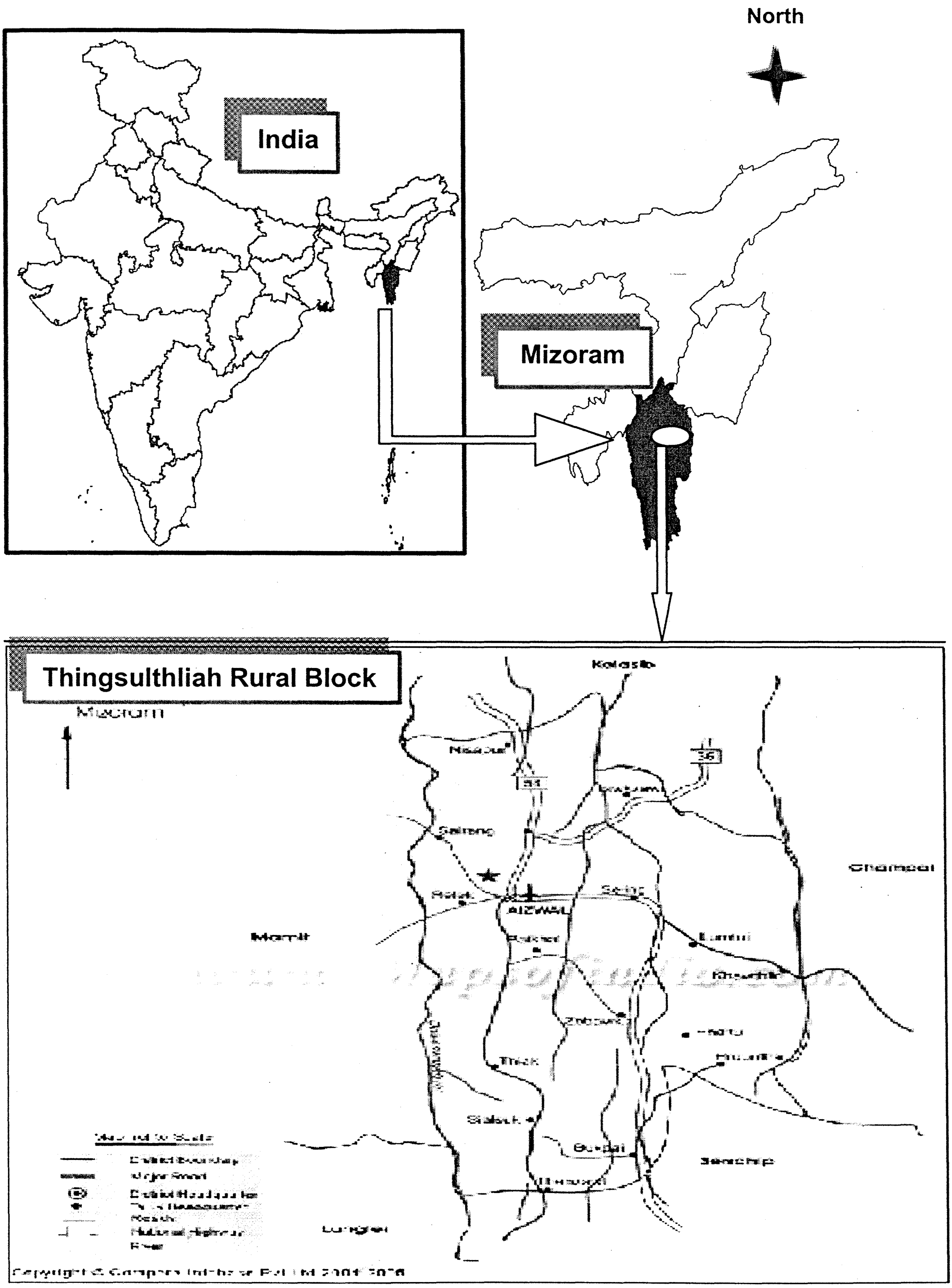
(Hriatthiam theih tur ang berin he zirna hi nu leh pa/ guardian hnenah hrilhfiah ani a, patient information sheet pawh pek ani).

Date _______________ Signature of the

interviewer

Hming (Name) :

Figure 1. Geographical location of Thingsulthliah Rural Block, Mizoram, North East I



APPENDIX IV

Table 1. Prevalence of night-blindness in children and pregnant women in five states of India

	No.of children examined	No.of cases	Preva lence (%)	No.of pregnant examined	No.of cases	Preva-lence (%)
Assam	685	21	3.06	195	23	15.86
Bihar	1011	18	1.78	268	36	13.43
Orissa	985	39	3.95	262	87	3.21
West Bengal	751	9	1.15	355	37	10.42
Tripura	66	2	3.03	50	6	12.00

Source : Journal of Indian Medical Association, Vol.98, No 9, September, 2000, page 541.

ASSESSMENT OF VITAMIN A DEFICIENCY IN A RURAL AREA OF MIZORAM

SCHEDULE FOR CHILDREN

Name of the respondent : ____________________

Relationship with the child : ____________________

Name of the child : ________________________ Village : ____________________

Father's / Guardian's name: ____________________ Locality : ____________________

A. Demographic information :

1. ID No. ☐☐☐

2. Age of the child : ☐☐

3. Date of birth: ☐☐ (Date) ☐☐ (Month) ☐☐☐☐ (Year)
   (*Cross-check with birth certificate or baptismal certificate*)

4. Community : ☐ — **1** (Scheduled caste) **2** (Scheduled tribe)

5. Religion : ☐ — **1** (Christianity) **2** (Hinduism) **3** (Muslim) **4** (Others)

6. Education of the father : ☐

7. Education of the mother : ☐

1 (Primary school) **2** (Middle school) **3** (High school) **4** (Plus two) **5** (College) **6** (Graduate) **7** (Post-graduate)

8. No. of family members: ☐☐

9. Adults: Male : ☐

Female: ☐

10. Children: Male: ☐

Female: ☐

B. Socio-economic status

1. Place of living : ☐ **1** (Rented) **2** (Own)

2. Type of house ☐ **1** (Kutcha) **3** (Mixed) **2** (Assam type) **4** (RCC)

3. No. of regularly employed members in the family :

4. Monthly income (In rupees) : ☐
   - **1** (<1000)
   - **2** (>1000- 3000)
   - **3** (3000 to< 5000)
   - **4** (5000 to < 10,000)
   - **5** (10,000 and above)

5. Percentage of income spent on food : ☐☐ %

C. Water supply and sanitation :

1. Availability of adequate and safe water ☐ **1**(Yes) **2** (No)
2. Availability of adequate human disposa ☐ **1** (Yes) **2** (No)

D. History of the child :

1. Birth-weight (in kgs) ☐
2. Whether breastfed : ☐ **1** (Yes) **2** (No)
3. Immunization status : ☐ **1** (Fully immunized)
   (Cross-check with **2** (Partially
   immunized)
   immunization card) **3** (No immunization)
4. Status of vitamin A prophylaxis : ☐ **1** (Completed as
   schedule)
   2 (Incomplete)
   3 (Not given)
5. Recent illness ☐ Measle ☐ Diarrhoea ☐ Respiratory
   diseases
   ☐ Fever ☐ Helminthi ☐ Others________
   (specify)
6. Whether attending Anganwadi Center: ☐ **1**(Yes) **2** (No)

E. Physical measurements:

	1st measurement	2nd measurement	Average
1. Height (in cms):	____________	____________	________
2. Weight (in kgs):	____________	____________	________

F. Ocular examination :	**Right eye**	**Left eye**
1. Conjunctival xerosis / with Bitot's spot (X1A, X1B)		
2. Corneal xerosis (X2)		
3. Corneal ulceration/ Keratomalacia (X3A, X3B)		
4. Scars (XS)		

DIETARY INTAKE (for every third child)

Cereals	Frequency of intake	Last 24 hours	Quantity
1. Rice	________	________	________
2. Dal	________	________	________
3. Chana	________	________	________
4. Wheat (atta/maida)	________	________	________
Green leafy vegetables			
5. Cabbage	________	________	________
6. Mustard leaves	________	________	________
7. Pumpkin leaves	________	________	________
8. Bean leaves	________	________	________
9. Others (specify)	________	________	________
Roots and tubers			
10. Carrot	________	________	________
11. 0nion	________	________	________
12. Potato	________	________	________
13. Raddish	________	________	________
14. Others (specify)	________	________	________
Other vegetables			
15. Beans	________	________	________
16. Cluster beans	________	________	________
17. Brinjal	________	________	________
18. Cowliflower	________	________	________
19. Bitter gourd	________	________	________
20. Ladies finger	________	________	________
21. Knol khol	________	________	________
22. Tomato (green)	________	________	________
23. Tomato (ripe)	________	________	________

Frequency :
1 (daily)
2 (3- 5 days/ week)
3 (1- 2 days /week)
4 (once in 2 weeks)
5 (rarely)

Last 24 hours
1 (consumed yesterday)
2 (not consumed yesterday

Quantity
Raw food (in gms) usually used in cooking each day.

	Condiments and spices	Frequency of intake	Last 24 hours	Quantity
24.	Dry chillies	______	______	______
25.	Green chillies	______	______	______
26.	Others (specify)	______	______	______
	Fruit			
27.	Banana	______	______	______
28.	Guava	______	______	______
29.	Orange	______	______	______
30.	Lime	______	______	______
31.	Mango	______	______	______
32.	Papaya	______	______	______
33.	Others (specify)	______	______	______
	Non-vegetarian food			
34.	Liver	______	______	______
35.	Fish	______	______	______
36	Beef	______	______	______
37.	Pork	______	______	______
38.	Eggs	______	______	______
	Others			
39.	Milk	______	______	______
40.	Ghee	______	______	______

Blood collected for serum retinol : ☐ **1** (Yes) **2** (No, refused)

Signature of investigator ______________

Date______________

Place______________

ASSESSMENT OF VITAMIN A DEFICIENCY IN A RURAL AREA OF MIZORAM

Schedule for mother

Name :

Village :

Sub-center :

1. ID No. : ☐☐☐

2. Age : ☐☐

3. Occupation : ☐

1 (none/housewife)

2 (Agriculture)

3 (Petty business)

4 (Lower government job/ small business)

5 (Higher government job/ established business)

4. Distance of the PHC from home (in kilometers) ☐☐

5. Distance of the Sub-center from home (in kms. ☐☐

☐

7. Whether breastfed the child/children: **1** (Yes) **2** (No)

8. Average length of breastfeeding (in months): [ ][ ]

9. Food supplements given to the child /childre[ ] **1** (Locally available foods)

2 (1 + seasonal fruits)

3 (Formulated products)

4 (3 + seasonal fruits)

10. Immunization status of the child/children: [ ] **1** (all fully immunized)

(*Cross-check with immunization cards*) **2** (only some fully immunized)

3 (none immunized)

11. Distance of the nearest Anganwadi Center (in k[ ][ ]

12. Whether the mother attends the Anganwadi: [ ] **1** (Yes) **2** (No)

13. Whether the child/children attends Anganwadi: [ ] **1** (Yes) **2** (No)

14. According to your belief, what fruit and food items rich in vitamin A?

1 ________ 2________ 3________

4________ 5________ 6________

15. What roles are played by vitamin A in our body?

____

____

16. Does your child have difficulty in seeing in dim light? ☐ **1**(Yes)

(mitmalh a nei em ?) **2** (No)

Signature of investigator

Place____________________ Date______________

ASSESSMENT OF VITAMIN A DEEFICIENCY IN A RURAL AREA OF MIZORAM

Schedule for Health Workers

Name of the Health Worker:____________________

Name of Sub-Center:__________________________

Name of PHC/ CHC:___________________________

1. Population of children (0-5 years) during 2003-2004 [ ]

2. Number of target beneficiaries for vitamin A prophylaxis during 2003-2004: [ ]

3. Number of children covered for vitamin A prophylaxis: [ ]

4. Status of supply of vitamin A to the Sub- Center: [ ] **1**(Adequate) **2**(Inadequate)

5. Coverage of measles vaccine: [ ]

6. Number of illness among 0-5 years: [ ]

7. Number of cases of measles: [ ]

8. Number of cases of diarrhoeal disease [ ]

9. Number of Respiratory diseases: [ ]

10. Number of Helminthic diseases: [ ]

11. Number of live-births: [ ]

12. Number of children born with low- birth weight: [ ]

13. Number of children with signs of nutritional deficiencie [ ]

14. Whether nutrition education is given by the staff of the sub-center in the area concerned : [ ]

1 (Yes)
2 (No)

Signature of Investigator

Date: ________________

Place: _______________