

# Survey Report

## **Biochemical Assessment (Folate, Vitamin B12) & Prevalence of Thalassemia genes in women of reproductive age on a subset of National Nutrition Survey-2011**

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## List of Abbreviations

AJK	Azad Jammu & Kashmir
AKU	Aga Khan University
ANC	Antenatal Care
BMI	Body Mass Index
DMU	Data Management Unit
EB	Enumeration Block
EBF	Exclusive Breastfeeding
EPI	Expanded Program for Immunization
ERC	Ethical Review Committee
FATA	Federally Administered Tribal Areas
FBS	Federal Bureau of Statistics
HH	Household
Hib	Haemophilus Influenzae Type B
IDA	Iron Deficiency Anemia
KAP	Knowledge, Attitude & Practice
KP	Khyber Pakhtoonkhwa
LBW	Low Birth Weight
MDG	Millennium Development Goal
MOH	Ministry of Health
MTHFR	Methylenetetrahydrofolate Reductase
NGO	Non-Governmental Organization
NID	National Immunization Day
NNS	National Nutrition Survey
OPV	Oral Polio Vaccine
PDHS	Pakistan Demographic Health Survey
PMRC	Pakistan Medical Research Council
PPS	Proportion to Population Size
PSU	Primary Sampling Unit
SSU	Secondary Sampling Unit
sTfR	Serum Transferrin Receptor
UNICEF	United Nations International Children Fund
USAID	United States Agency for International Development
VAD	Vitamin A Deficiency
WHO	World Health Organization
WRA	Women Reproductive Age

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## Chapter 1: Background

Nutrition surveys are useful tools that provide estimates of the severity and geographical extent of nutrition indicators and groups most affected or at risk for deficits/deficiencies. These surveys also assess the likely evolution and impact of the problem on health and nutritional status, taking into account secondary information, including food security and food distribution. They also identify the need for nutrition interventions and the most effective measures to prevent or minimize the problem from available coverage and access data. Through such surveys the governments can determine the need to establish or expand existing surveillance, so that the effectiveness of measures taken can be monitored overtime. To assess the magnitude of the problem we also need to understand the population size and, if possible, the demographic characteristics of the population and distribution of cases therein.

To understand the reasons for under-nutrition and to plan and implement appropriate interventions and programs, the usual situation for that population, the evolution of the changes, and the context in which the situation has arisen each needs to be considered. There are many sources of information that are relevant in understanding the context and potential responses that may be appropriate and effective. The effects of these factors on livelihoods and the ability of the affected population to cope at a household level are frequently assessed using food security surveys. Additionally formal nutrition surveys remain the best way to estimate accurately prevalence of malnutrition. Records of cases of malnutrition at health centers or during screening cannot be considered sufficiently representative of the population. They do, however, provide an indication of trends in the number of cases of malnutrition and opportunities for action.

The department of Paediatrics and Child Health, Aga Khan University was charged to conduct the National Nutrition Survey 2011 in Pakistan in collaboration of UNICEF and Nutrition wing, Ministry of Health Pakistan to establish the current benchmark of nutrition and related indicators for gauging progress along the targets set for the Millennium Development Goals (MDGs). Additionally the NNS 2011 aimed to establish a benchmark for missing data/indicators, especially as the recent DHS survey (2007) did not include anthropometric indicators, and to prioritize programs/initiatives at the national and provincial level as well as refine planning and implementation of initiatives on the basis of identified priorities.

The specific objectives of the NNS 2011 included the following targets

- To assess the population nutritional status; especially of women and children using standard international parameters and indicators
- To collect data on height, weight and age of children 6 – 59 months, mothers of these children and elderly.

- To collect blood samples for the assessment of micronutrient status of children mainly iron Vitamin A, Zinc, Vitamin D, calcium, and iron status of women of reproductive age.
- To collect urine samples for the assessment of iodine status of women of reproductive age and children aged between 6-12 years old
- To assess infant and young child feeding and care practices, including exclusive breastfeeding and complementary feeding rate and morbidity of children
- To collect data on food intake, food security, water and sanitation.
- To collect data on demographic, socio economic and cultural variables (Income, education, etc.)

Although the objectives of the NNS were comprehensive it was recognized that there were additional opportunities for improving the evidence base for determinants or actions that may be relevant for affecting nutrition outcomes. To illustrate information on family planning and inter-pregnancy spacing and its impact on child growth and nutrition outcomes would be critical to advocate for family planning. While research has demonstrated that children born too close to a previous birth are at increased risk of adverse outcomes and intrauterine growth retardation, systematic information on the relationship of fertility and birth intervals with maternal nutrition outcomes is deficient [1-5]. It is however well known that pregnancy itself increases energy needs by 13%, protein needs by 54%, and depending on the vitamin or minerals in question, increase requirements by up to 50% [6-7]. Maternal under nutrition is associated with intrauterine growth retardation (IUGR) and associated increased risk of perinatal mortality and neonatal morbidity [8]. Short inter-pregnancy intervals have been associated with adverse perinatal outcomes [9-10]. In recent years the association of maternal under nutrition with micronutrient deficiencies, especially folic acid and B12 has been documented with impacts on both short term and long term outcomes [11-12]. In particular, such deficiencies have been well recognized in India and documented to lead to high concentration of homo cysteine and adverse outcomes [13-17].

Although maternal depletion has been described from Pakistan [18-19], the association of under nutrition with overall fertility rates, birth interval etc. is not well characterized. This is important from the point of view of policy and for efforts to integrate family planning reproductive health and nutrition. The National NNS 2011 hence offered a unique opportunity to obtain this information from a national sampling frame and hence influence policy. This adjunct to the NNS 2011 was deemed to be much more cost-effective than attempting to address these issues de novo in a new survey.

Comprehensive childhood vaccination can change the epidemiological dynamics of infectious diseases. It may result in a limited persistence of natural and vaccine-induced immunity and a

higher mean age of infection, which may lead to a greater risk of complications. There is limited information on effective and true coverage for childhood immunizations and reliable data from recall and record evaluation is lacking. Given the planned blood sampling within the NNS 2011, the assessment in a subset of vaccine sero-conversion of EPI vaccine remains scarce in the context of Pakistan.

The epidemiological situation should be monitored and immune surveillance based on the assessment of specific antibodies against vaccine-preventable diseases in human serum is one of the tools. Given the policy interest in immunization coverage in Pakistan, there was the need for documenting the extent of coverage and antibody testing for measles and tetanus in less than five populations at household level. We complemented the NNS 2011 with an additional module and relevant laboratory parameters.

Considering the importance of these issues the department of Paediatrics and Child health Aga Khan University proposed and conducted a supplementary activity as an add-on to the National Nutrition Survey 2010-2011. The supplementary activity hence utilized some resources from National Nutrition Survey Project. Resources like (but not limited to) Staffing Support, Travel, Transportation, Supplies, Payments to Federal Bureau of Statistics for Technical inputs, Payments to Pakistan Medical and Research Council for collaborative field work in some areas, Training, Monitoring Visits, Data Analysis etc). The Global Alliance for Improved Nutrition (GAIN) was requested to provide funds for the following four additional analyses, the proposal for which was approved

#### **1. Objectives of the additional module of the NNS:**

- a)** Assess the prevalence of folate deficiency in women of reproductive age
- b)** Assess the prevalence of Vitamin B 12 deficiency in women of reproductive age
- c)** Assess the concentration of CRP in women of reproductive age
- d)** Assess the prevalence of Beta Thalassemia genes in in women of reproductive age on a subset

## Chapter 2: Description of Methods for NNS 2011

### 2.1. Study sites

This survey was conducted in all the four provinces (Sindh, Punjab, Balochistan and KPK) plus Azad Jammu & Kashmir, Gilgit Baltistan and FATA as defined by the 1998 population census.

### 2.2. Study design

The study was conducted at national scale through a stratified representative cross-sectional Survey at household level. Cross-sectional surveys are useful in providing an overall estimate of prevalence and coverage in a geographic area. A cross-sectional study is a descriptive study in which disease and exposure status is measured simultaneously in a given population. Cross-sectional studies can be thought of as providing a "snapshot" of the frequency and characteristics of a problem in a population at a particular point in time. This type of data can be used to assess the prevalence of acute or chronic conditions in a population.

### 2.3. Approaches:

Both quantitative and qualitative approaches were incorporated to achieve the objectives of the study. For the quantitative component a cross sectional survey was conducted to elucidate the information regarding households, physical examination, and anthropometry and biochemical indicators. A structured questionnaire was used to accomplish this information. The survey also explored the knowledge attitude and practices (KAP) of the population regarding micronutrient deficiencies. For the qualitative component focus group discussions (FGDs) and in-depth interviews (IDIs) were conducted from different groups.

### 2.4. Study Population

The target populations of the NN Survey are:

- Women of Reproductive Age Group: women age 15 – 49 years,
- Children: Under five years
- Children 6-12 years of age (Urinary Iodine)
- Elderly > 50 years of age

The Quantitative indicators required for the study were achieved through following components

1. Household information
2. Dietary Patterns (Food Intake and Food insecurities etc.)
3. Physical examination with anthropometric measurements
4. Biochemical measurements



## 2.5. Sample size and its allocation

Considering the variability of characteristics for which estimates were prepared, population distribution, and field resources available, a sample size of 30, 000 sample households was considered appropriate to provide reliable estimates of key characteristics at the desired level. An exercise to compute sample size based on the prevalence rate of three key variables namely wasting and stunting in children less than five years , mother iron deficiency and mother iron deficiency anemia was undertaken keeping in view 95% level of 5% margin of error . Further, 5% non-response rate was also considered. The design effect of 1.6 was used to finalize and fix overall sample size.

The entire sample of 30,000 households (SSUs) was fixed comprising 1500 Primary Sampling Units (PSUs) out of which 618 were urban and 882 were rural. As urban population was more heterogeneous therefore, a higher proportion of sample size was allocated to urban domain. Similarly NWFP and Balochistan being the smaller province and to get reliable estimates, a higher proportion of sample was assigned to these provinces. After fixing the sample size at provincial level, further distribution of sample PSUs to different strata in rural and urban domains in each province were made proportionately. The distribution of PSUs and SSUs enumerated in the urban and rural domain of the provinces and regions is as under:

**Table 2.2 Sample Size and Allocation Plan**

Province/Region	Number of sample PSUs			Number of sample SSUs		
	Total	Urban	Rural	Total	Urban	Rural
Punjab*	682	307	375	13640	6140	7500
Sindh	323	157	166	6460	3140	3320
KPK	218	67	151	4360	1340	3020
Balochistan	110	44	66	2200	880	1320
FATA	67	0	67	1340	0	1340
AJK	66	28	38	1320	560	760
GB.	34	15	19	680	300	380
<b>Total</b>	<b>1500</b>	<b>618</b>	<b>882</b>	<b>30,000</b>	<b>12,360</b>	<b>17640</b>

\* Including Islamabad

## 2.6. Sample selection procedure

### a) Selection of Primary Sampling Units (PSUs)

Enumeration blocks in urban domain and mouzas/dehs/villages in rural domain were taken as primary sampling units (PSUs). In the urban domain, sample PSUs from each ultimate stratum/sub-stratum were selected with probability proportional to size (PPS) method of

sampling scheme. In urban domain, the number of households in enumeration block Economic Census 2004 and population of 1998 Census for each village/mouza/deh were considered as measure of size.

#### **b) Selection of Secondary Sampling Units (SSUs)**

Households within sample PSUs were taken as secondary sampling units (SSUs). A specified number of households i.e. 20 from each urban sample each urban and rural sample PSU have been selected with equal probability using systematic sampling technique with a random start.

In general there is a choice of methods for the selection of the household with some basic requirement. In the context of the Pakistan's housing situation the systematic random sampling method with alternate provision is the only appropriate method for the selection of households due to the unavailability of the accurate households and targeted study participants list. An accurate listing of households is critically important for the implementation of any sampling method. In Pakistan there is no reliable information/source about the national housing situation after the census 1998. To get the accurate list of the household a quick listing was done prior to the execution of the field. The fixed method for selection of household in each EBs was adopted, the households to select in each EBs is determined in advance and same number of households being assessed. We are assessing 20 households from each EB.

#### **2.7. Application of systematic selection technique to select required number of HH:**

First an updated list of households having children 6 months – 5 years of age in the enumeration blocks will be collected In addition to the updated list. Moreover, ineligible household such as abandoned/empty households was excluded from the list.

The next step is enlisting the households in sequential order. This can be explained as under

1. Assume a cluster comprises of 240 households having children 6 months- 5 years of age and as per our protocol we need 20 such households to be selected per EBs.
2. Number the households from 1 to 240.
3. Calculate the sampling interval (Number) = Total number of eligible households/number of required households
4.  $240/20= 12$  (Hence every 12th Household was selected from the list of the households)
5. Select the first household as the starting point (12th household in the list)
6. Select every 12th (sampling interval) household in the list of the eligible households (having children 6 months-5 years of age).

## **2.8. Selection of the respondents and subjects**

### **A) Women of reproductive age women (15-49 years of age)**

One women of reproductive age from each selected household was selected for interview. But the blood specimen was collected from the first three households or till the completion of the required minimum number (three women of reproductive age). Physical examination and anthropometric measurement was performed from the first six households or till the completion of the required minimum number (six women of reproductive age). If number of the reproductive age woman is more than one in the household, one of them was selected randomly by draw method using chits. For example a household where there are three women of the 15-49 years of age, three chits having concealed numbers inside (1, 2, and 3) were mixed and one of the members of the household were asked to pick one. Suppose the picked chit has number 3, interview of the 3<sup>rd</sup> women of the reproductive 15- 49 years was done.

### **B) Children 6 months-5 years of age**

One child 6 months-5 years of age was selected for each selected household. The respondent of the questionnaire modules should be mother or caretaker of the selected child. But the physical examination and blood samples were collected from eleven children and urine samples only from the three children of under five years of age. In case there is more than one eligible child in a household, the youngest child was selected for questionnaire modules as well as physical examination and biochemical specimen collection.

### **B) Children 6-12 years of age**

Three 6-12 years of age children were selected from each enumeration blocks for questionnaire modules as well as physical examination and biochemical examination. The respondent for the questionnaire modules were mother or care taker of the child. In case there is more than one child 6 -12 years of age, in a household, the eldest child was selected for questionnaire modules as well as physical examination and biochemical specimen collection.

## **2.9. Operational Study Procedures**

### **2.9.1. Development of Study Protocol:**

Detailed study protocol was developed in the first phase of study. The protocol followed the standard template of TOR provided by UNICEF by keeping in view the previous experience of such surveys in Pakistan.

### **2.9.2. Development of Instruments:**

Data collection instruments were developed in English to obtain the required information. The questionnaire depends upon the indicators selected, but may include one or more of the following: demographic information; socioeconomic information; knowledge, attitudes and practices (KAP); and information related to the maternal and child health, nutrition and micronutrients status. The questionnaire shall be translated into Urdu and other local languages and then back translated into English to assess whether the essence of the questions had been captured. Additionally, a 24-hour dietary recall questionnaire was also administered on a sub-group of children.

### **2.9.3. Identification and Recruitment of Field Staff**

Advertisements (In-house and in the National Dailies) were placed and candidates were shortlisted and interviewed at Karachi, Faisalabad, Rawalpindi, Peshawar and Quetta.

### **2.9.4. Survey Teams**

Initially 15 Survey Teams were established and more teams inducted as the survey progressed to keep the momentum and to meet the time target. At one point in time 22 Teams were operating in the different parts of the country. Each team consists of 1 Field Supervisor, 1 Team Leader, 4-5 Data Collectors, 3 Registered Nurses with 1 phlebotomist, 2 Logistic Assistants and 2 Community Facilitators. Separate teams consist of Moderators/Facilitators; Observers, Note-Takers and Community Recruiters were also established. National Survey Coordinator, Senior Survey Coordinator and Survey Coordinators were senior medical doctor and lead social scientists with years of experience in nutrition related surveys nationally and internationally. Team Supervisors were highly experienced not less than a decade and mix blend of medical and social science background. Similarly, experienced Team Leaders, all females having Masters in Social Sciences with suitable experience were deployed with the view to easy access into the households to ensure the quality and validity of data. All Data Collectors were at least Graduates supported by Logistics Assistants and Local Community Facilitators.

Separate teams consisted of FBS representative and Logistic Assistant supported by local community facilitators visited each EB / village prior to data collection for demarcation of EB/village as per FBS maps, listing of all structures and households, allotted a unique ID (NNS 1, 2, 3 for structures and HH1, 2, 3 for households), basic data on children <5, household head, women of reproductive age, elder person above 50 years, etc. were obtained. From the listed HHs of each EB, 20 HHs were randomly selected through a computerized program in Excel.

### 2.9.5. Training

Training sessions and refreshers were conducted in Karachi, Faisalabad, Lahore, Peshawar, Abbottabad, Quetta and Gawadar. Four days extensive classroom training was imparted to the teams by the senior and experienced staff of the Department of Paediatrics and Child Health; Aga Khan University who have prior experience of similar surveys. Some of the details of training agenda are shown in Table 2.6.

#### Details of Training Agenda

Staff	Training Components
All Staff	Introduction to NNS Research design Survey methodology
Team Leaders	Community rapport building, Counseling techniques, Research basics, Interviewing techniques, Dress code, Consent procedures Interpersonal skills, Ensuring high response, Sampling methodology, Question by question explanation, Mock interviews, Operational procedures, Field procedures, Daily documentation, Log sheet completion, Dealing with refusals, Spot checking, Random checking, Desk editing
Data Collectors	Community rapport building, Research basics, Interviewing techniques Dress code, Consent procedures, Interpersonal skills, Ensuring high response Sampling methodology, Question by question explanation Mock interviews, Operational procedures, Field procedures Daily documentation, Log sheet completion
Nurses	Physical examination, Anthropometry, Field practice, Urine sampling
Phlebotomists	Blood sampling, Safe injection practices, Safe injection practices Labeling and storage, Transportation of samples, Field practice

### 2.9.6. Piloting/Pre-testing

The pretest was done to try-out of the questionnaire and to see how it works and to catch and solve unforeseen problems before the start of the actual data collection. The objectives of pretest were to improve the wording of the questionnaire; order of the questions; responses must fit the question; check the accuracy and adequacy of the questionnaire's instructions such as "skip" and "go to"; adequacy of instructions to interviewers; eliminate unnecessary questions and add necessary ones; endeavor to lessen discomfort, harm, or embarrassment to the respondent; improve translation of technical terms; and estimate the time needed to conduct the interview. Both the "Participating" and "Undeclared" pretests were done. Participating pretests were done in the classroom among the Interviewers themselves while undeclared pretests were done in the field not tell respondents that it is a pretest. About 100 – 150 respondents, whose characteristics reasonably similar to the survey population were

interviewed in different parts of Karachi keeping in view of cultural segmentation of the population. The questionnaire was then revised and finalized on the basis of pretest results and direct observations by the Supervisors. The pre-testing was closely monitored by the Survey Coordinators.

#### **2.9.7. Coding Scheme for Assigning Processing**

Seven digits coding scheme for providing processing codes to primary sampling units i.e. enumeration blocks/villages (PSUs) and secondary sampling units' i.e. households (SSUs) selected in was developed.

#### **2.9.8. Plan of Operation, Training and Monitoring**

Project planning was critical to the development of detailed field work plans, assessment of the timelines and progress, resources and performance schedules during the execution of the project. Effective tools were developed for periodic checking of works progress against the targets laid down in order to ensure timely completion of the NNS project. One step forward strategy was developed instead of the conventional approaches of monitoring; therefore besides internal monitoring Project stakeholders, especially Federal and Provincial Nutrition Wing, Ministry of Health Government of Pakistan, and UNICEF were proactively engaged in the training sessions and in evaluating and monitoring the progress of the project activities. Inclusive of this, independent and experienced monitors were also engaged.

### **2.10. Laboratory Procedures**

#### **2.10.1. Field Survey Samples Collection**

Each team was provided necessary equipment i.e. centrifuge, mini -20 °C freezer (to store approximately 50 ice packs) generator to run the equipment and keep the ice packs continuously frozen and four samples transportation coolers. The phlebotomist used a separate room for blood collection where the mother and the child stayed. This prevented from scaring the other children to be enrolled for the study.

Blood Samples were collected form every third Household from the WRA and child. The blood sample collected from each house hold was carefully labeled and placed in individual zip lock bags with complete subject and site information, each zip lock bag from individual house hold was placed in a separate plastic sample container placed in -20 freezer. At the time of transportation the plastic sample container with samples tightly screw capped was taken out from the -20 freezer and placed in the designated research transportation coolers well packed with ice packs and sent to satellite collection point to be transported to AKU.

Field teams involved in samples collection were provided training to carefully handle 20mm gauge needle and syringes, tubes for blood and urine collection. The butterfly blood collection system was used for collecting blood samples from children under 5 years. For adolescent and women of reproductive age both 20 and 21 mm gauge needle was used in the field survey. The measurement of zinc in blood samples were collected in trace element free tubes. All needles and sharps were disposed in puncture resistant danger bins.

### **2.10.2. Blood Samples Collection Procedure**

Personal hygiene and cleanness of the blood collecting area was ensured to avoid blood contamination. Disposable gloves and hand sensation lotion were used to maintain sterility of the procedure. Before collecting the blood the skin was cleaned properly with an alcohol swab, if it requires a double cleaning should be done with another alcohol swab to ensure complete disinfection of the skin. A tourniquet was used for the both child and adults to make the vein prominent, the needle is gently inserted in the popped up vein the tourniquet is released and the blood is gently drawn in to the blood collection tube. Care was taken to collect the blood in the first attempt to avoid double puncturing of the veins. The blood was transferred gently into the red top gel sero-separator clot tube for serum and sodium heparin green top tube for plasma, the last drop in the syringe is used for the determination of the hemoglobin concentration. The last blood was transferred to the Hemocue cuvettes for the estimation of hemoglobin concentration.

### **2.10.3. Blood Samples Processing**

The blood collection tube was labeled with a cryo markers, the tube was left undisturbed on the work bench in the Mobile Van or in the cooler for 30 minutes, after which it was centrifuged. Centrifuge is set at 3000 rpm for 10 minutes to get clear serum. 0.5 ml of the serum was transferred into a pre labeled trace element free screw cap tube for zinc measurement and the remaining serum was transferred to slowly transfer into another pre labeled Cryovial with the help of a plastic dropper for the analysis of Retinol, Ferritin, 1-alpha glycoprotein, Calcium and Vitamin-D, it was then wrapped in an aluminum foil to protect the sample from light. All consumables used for blood collection and separation were discarded in puncture resistant danger bins. Care was taken not to touch the red cells with the tip of the dropper to avoid breakage of red cells.

The collection of the biochemical samples for micronutrient assays was be performed by trained phlebotomists as this was one of the most important task in the field surveys. The sample collection was done with the active involvement of 15 teams in the field.

### **2.10.5. Specimen Transportation**

The Micronutrient Laboratory at the Aga Khan University has extensive experience of undertaking processing and analysis of blood samples from several large and medium size surveys. These principally include National Nutrition Survey (NNS) 2001-2002, National Nutrition Survey Republic of Maldives in 2007. The World Food Program (WFP) Sindh survey 2005, Micronutrient survey of NWFP province of Pakistan (1998-99), the Baluchistan (a large province of Pakistan) Infant and Child Malnutrition project (1998), as well as the Rural Sindh Maternal Micronutrient study (2000) etc. These are all large surveys with average sample size ranging from 1500-10,000 individuals.

The laboratory analyses for the stated micronutrients (Serum Retinol, Serum Ferritin, Serum Zinc, Vitamin-D, Calcium, Alph-1 Acid Glycoprotein and Urinary Iodine) were a major component of survey. This analysis was undertaken at the Nutrition Research Laboratory facilities of the Aga Khan University Department of Paediatrics & Child Health and Main University Laboratory at preferential rates, and within the challenging time frame. The Nutrition Research Laboratory at AKU has been conducting these analyses as a routine and all the analysis was being done according to standard protocols.

The blood and urine were be collected from all provinces of Pakistan namely Sindh, Punjab (Central and Northern Punjab) NWFP, Azad Jammu and Kashmir, Gilgit & Baltistan and Baluchistan sampling and all the samples were sent to AKU for further analysis.

- The standard transportation procedures were adopted for transportation of serum and urine samples for laboratory analysis of micronutrients.
- The safe deposition of samples according to the agreed protocol, the maintenance of the cold packs with standardized packaging material and rapid transportation through Aga Khan University Hospital (AKUH) collection points.
- Samples reached from the field site to the satellite collection points an hour before the cut off time mentioned for the transportation of the samples.
- If for any reason the samples did not meet the cutoff point they should be brought back and the plastic sample container should be placed in the -20 freezer to be transported the following day.
- AKU Department of Paediatrics and Child Health was responsible for receiving the designated research coolers at AKUH main collection point Karachi.

### **2.10.6. Satellite Laboratory Collection Points of the Aga Khan University Hospital in Pakistan**

As indicated previously, Micronutrient Lab at the Aga Khan University conducted this laboratory analysis of micronutrients and other biochemical indicators; we adopted the extensive facility of AKUH laboratory pick up points all over the country. Following safe deposition of samples at these pick up points according to the agreed protocol, the maintenance of the cold chain and



rapid transportation (by air) of specimens to the Micronutrient Research Lab in Karachi was the responsibility of the AKUH collection points.

All serum and urine samples were transported in study designated research coolers with ice packs maintaining temperature of 2-8 °C from field collection to satellite AKUH collection points from where they transported through AKUH collection point sample transportation system. The samples were transported along with the lab requisition form separately of each subject in a plastic zip lock bag.

### **2.11. Data management, transfer and analysis:**

The data collected from field sites were transferred to the regional office where the data was checked and validated for completeness and major errors by the team leaders. If they found any error or inconsistency, the forms were sent back to field for proper filling. After the proper review the completed data was packed for sending to Karachi' by keeping the records of received and sent data on the specific log sheets. All files were be labeled accordingly.

#### **2.11.1. Data management team**

Data management team consist for data analyst, data supervisor were assisted by data entry operators.

#### **2.11.2. Software**

Visual Fox Pro was used for designing of databases, data entry software and procedures for data quality assurance. Data entry screens were employed range and consistency checks and skips to minimize entry of erroneous data. Special arrangements were made to enforce referential integrity of the database so that all data tables are related to each other without problem. Analysis of data was done through SPSS version 16

#### **2.11.3. Editing, Coding and Data Transferring**

All data collected was cross-checked by field supervisors at field offices on a daily basis and weekly transferred to the Data Management Center at Aga Khan University from the field stations. Prior to data entry, all forms were checked for completeness and consistency as well as coding of open- ended responses and area codes, etc. In case of inconsistency or missing responses, the editors flagged the errors/omissions and consult the interviewers for possible explanations.

#### **2.11.4. Data Entry and Data Quality Assurance**

All data was double entered for purpose of sufficient accuracy. Data entry started simultaneously with data editing. Data quality was assured by performing dual and error checks simultaneously with data entry. A sub sample of data was checked for validation of data entry

#### **2.11.5. Data Analysis**

For data analysis SPSS version 19 was used and data was analyzed using univariate and multivariate methods. Statistical Analysis was performed after the data was cleaned and quality was ensured. Each file was converted from Fox Pro into SPSS files so that they could be read into SPSS for further analysis. Descriptive statistics for the subjects was obtained and Pearson Chi square test was used to establish association between categorical variables. Simple frequency tables were generated to ascertain the information on Socio-economic and demographic data, similar frequency tables were made for the Micronutrient concentration. Bivariate analysis was done to ascertain odds ratio to establish an association with the duration of birth intervals and micronutrient status and growth parameters such as BMI, stunting, wasting and underweight.

Further Multivariate analysis was done to establish a true association between the factors that were found to be significant during the bivariate analysis. To ascertain the adjusted odds ratio controlling for confounding factors we used Backward LR model to establish the association. Birth interval was considered as independent variable while the outcome measures that were found significant on bivariate analysis were considered as dependent variables.

#### **2.12. Confidentiality:**

Confidentiality of all the data collected from the population was a high priority. All the names and personal information regarding any individual was not to be disclosed and all the names present in the forms were de linked and forms were coded accordingly. Only senior level staff had access to the data. Participant privacy and confidentiality in electronic and printed data, publications, and reports during and following completion of the study was ensured all the data files were password protected and hard copies of the data was kept in locked premises for five years.

#### **2.13. Ethical Considerations:**

The study did not predict any major ethical consideration. Blood and urine collection procedures and the time utilized during the interview might cause inconvenience to some participants but, proper reassurance and consent was taken from every participant before any of the study activity. The proposal was submitted to the Ethical Review Committee of the Aga Khan University and National Bioethics committee and was duly approve

## Chapter 3: Results

### 3.1. Highlights of NNS 2011: (For details, please refer to main NNS 2011 report)

This section summarizes key findings from the National Nutrition Survey in Pakistan; the survey was conducted after a decade by the Division of Women and Child Health, Aga Khan University, Pakistan, Ministry of Health, Pakistan and UNICEF, Pakistan. The survey assessed overall nutritional status of target groups based on anthropometric indices and micronutrient status.

An overall sample size of 30,000 households was deemed appropriate to provide the results and was calculated on the basis of major nutrition indicators of stunting in children and anemia in WRA and children from NNS 2001. During the survey 27963 households were interviewed. Overall 24421 blood samples (Women 12282 Children 12139) were collected across Pakistan. The survey teams also collected 2900 urine samples of women (1460) and children 6-12 years (1457) for urinary iodine assessments.

NNS 2011 summarizes that there has been little change over the last decade in terms of core maternal and childhood nutrition indicators. The survey does point towards improvement in iodine status nationally but is counter balanced by substantial deterioration in vitamin A status and little or no improvement in other areas of micronutrient deficiencies.

NNS 2011 revealed 58 percent households were food insecure nationally. Sindh appeared as the most food deprived province where 72 percent families reported facing food insecurity followed by Balochistan 63.5 percent.

Overall 15 percent mothers were underweight. More than half (54.4 percent) of the mothers had normal (18.5-24.99 BMI) across Pakistan. One third of the women had Urinary iodine deficiency (Urban 45.1 percent and 31.5 percent). Of these 3.1 percent (Urban 4.1 percent and rural 2.6) was severely iodine deficient.

There were widespread micronutrient deficiencies in women. Survey revealed that prevalence of various micronutrient deficiencies in pregnant women were as follows; anemia 50.4 percent, Iron deficiency anemia 24.7 percent, vitamin A deficiency 42.5 percent, zinc deficiency 47.6 percent, hypocalcaemia 58.9 percent and vitamin D deficiency 68.9 percent. Similarly in non-pregnant women the prevalence of various micronutrient deficiencies was as follows; anemia 51 percent, iron deficiency anemia 19 percent, vitamin A deficiency 42.1 percent, zinc deficiency 41.3 percent, hypocalcaemia 52.1 percent and vitamin D deficiency 66.8 percent.

Anthropometry status was not much changed over the years. Among under-five children 43.7 percent were stunted (NNS 2001, 41.6 percent), 15.1 percent were wasted (NNS 2001, 14.3 percent), 31.5 percent were underweight (Same as NNS 2001). The anthropometric indices were relatively better in urban areas.

The micronutrient deficiency was also widespread in children. The results of biochemical analysis revealed that prevalence of various micronutrient deficiencies in children under-five years of age were as follows; anemia 61.9 percent, iron deficiency anemia 32.7 percent, vitamin A deficiency 54 percent, zinc deficiency 39.2 percent and vitamin D deficiency 40 percent.

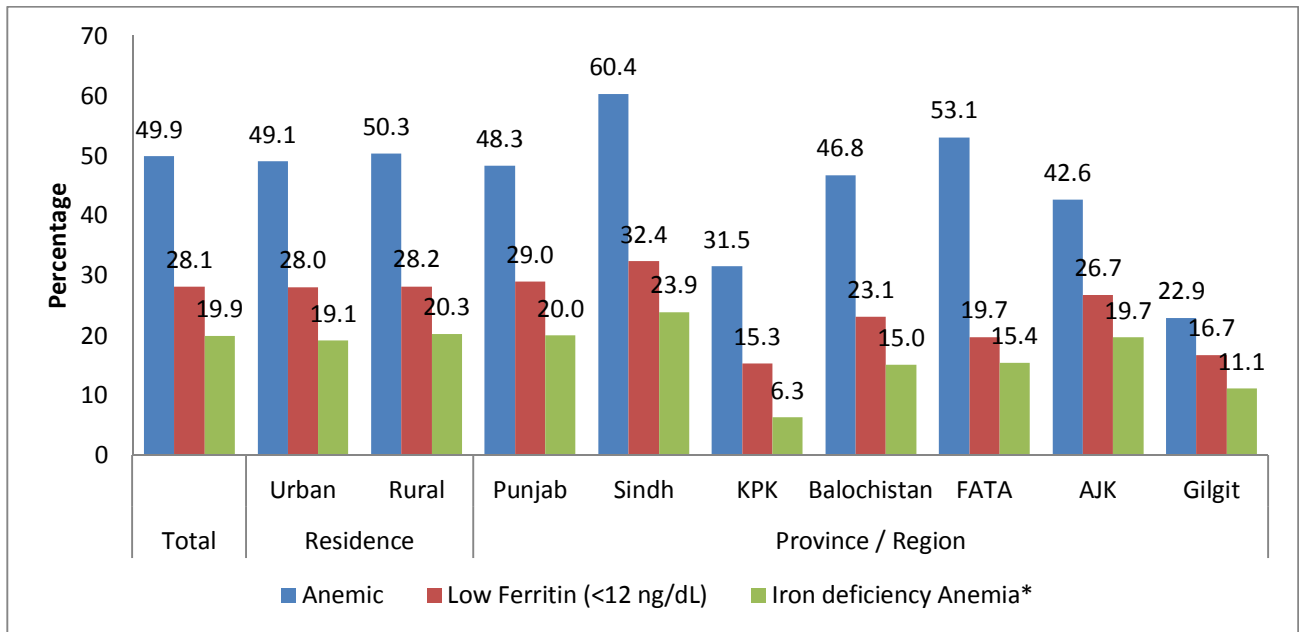
### 3.2. Results of additional Lab Analysis for Gain

Apart from the main laboratory analysis of NNS we conducted some additional lab analysis for the CRP, folic acid, beta Thalassaemia and Vitamin B 12 concentration, these biochemical analyses have not been studied in Pakistan at this scale.

#### 3.2.1: Anemia, ferritin deficiency and iron deficiency

The following figure shows the prevalence of anemia, ferritin deficiency and iron deficiency in the target population. The data showed that overall about 50% women were anemic, 28.1% had low ferritin concentration and about 20% had Iron deficiency anemia. The region wise proportions of these three indicators have been summarized in the below figure

Figure 3.2.1: Anemia, ferritin deficiency and iron deficiency



#### 3.3.2 Findings of Beta Thalassaemia Prevalence Analysis:

Thalassems are the most common autosomal recessive hemoglobin disorders characterized by the reduction or absence of synthesis of the globin chains of the hemoglobin molecule. In Pakistan  $\beta$ -thalassaemia is the most common hemoglobin disorder. In a population of nearly 180 million, and with a  $\beta$ -thalassaemia carrier frequency of almost 5.4%, it is estimated that each year more than 6000 children are born with transfusion dependent  $\beta$ -thalassaemia, but exact

figures are lacking. The affected children become a great source of socioeconomic burden on their families as good quality and screened blood increases its cost. Considering the situation of Thalassemia in Pakistan and the social and economic burden which it is causing on the affected families it is imperative to screen the couples before marriages to avoid this important issue. In this study we attempted to screen about 1800 women of reproductive age group from Pakistan for beta thalassemia screening to estimate the prevalence of Beta thalassemia.

**Table 3.2.1 Results of Hb Electrophoresis for Women of Reproductive Age:**

Types of Thalassemia	#	%
A+A2	1608	87.6
A+ raised A2	64	3.5
A+A2 +F	119	6.5
A+A2+D	16	0.9
A+A2+D+F	8	0.4
A+A2 +S	4	0.2
A+A2+S+F	1	0.1
A+A2+E	5	0.3
A+A2+Q	1	0.1
A+A2+ Unknown	9	0.5
Total	1835	

The analysis revealed that about 87.6 % of women had normal hemoglobin traits while 12.4 % had abnormal hemoglobin trait. The most common abnormal hemoglobin trait was Hemoglobin F as 6.5% of the women were found to have this trait; this was followed by Hemoglobin A2 as 3.5% of the women had the abnormal trait of HbA2, thus the estimated prevalence of Beta thalassemia was found to be 3.5%.

We also found various other hemoglobin abnormalities and the results established that about 0.9% had HbA2D trait, 0.4% had HbA2DF trait, 0.2% had HbA2S trait, 0.1% had HbA2SF trait, 0.3% had HbA2E trait, 0.1% had HbA2Q trait while 0.5% had unknown trait which are relevantly less clinically significant.

We also performed analysis for the hemoglobin, ferritin, Iron deficiency anemia, folic acid and vitamin B 12 on the blood samples of these 1835 women for which hemoglobin electrophoresis was performed. The table below explains this analysis and it was revealed that the prevalence of folic acid deficiency in this subset was found to be about 39.8% which is pretty same as of National estimates. When the folic acid deficiency was checked with abnormal hemoglobin concentration it was found that the folic acid deficiency was slightly raised. Similarly Vitamin B 12 concentration was also assessed for this subset and no major difference observed between the national estimates.

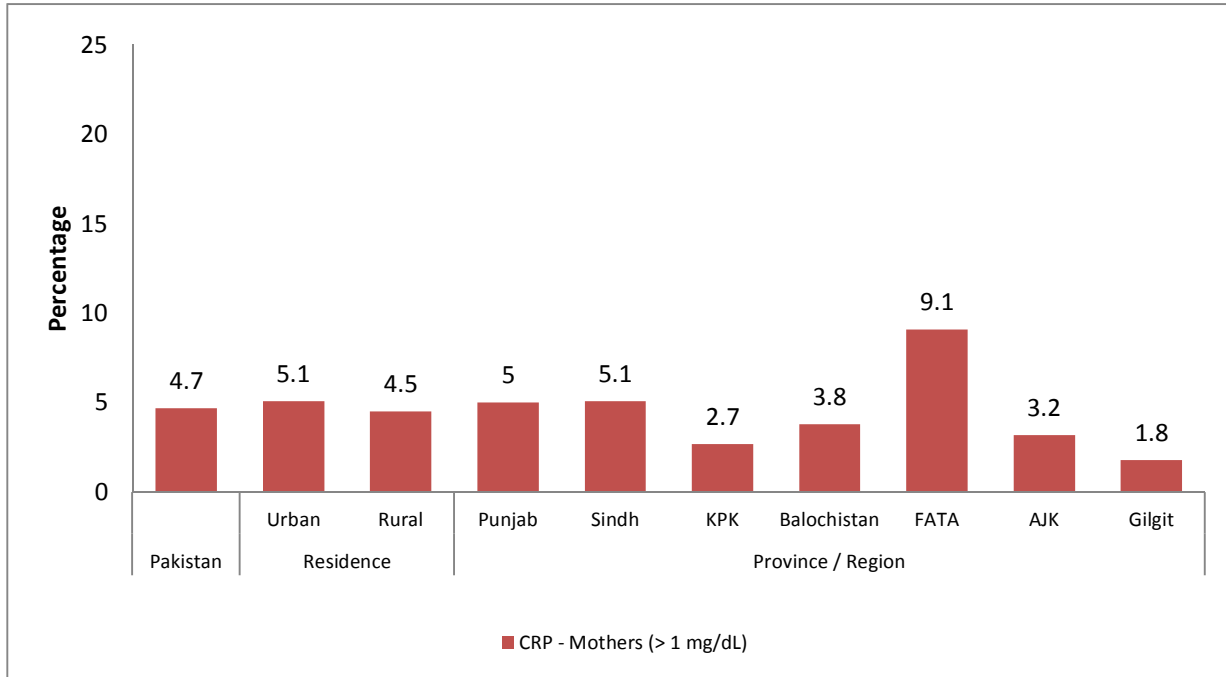
The results were also analyzed for prevalence of serum ferritin level and it was revealed that prevalence of ferritin deficiency was found to be low in both abnormal hemoglobin traits of A2 and F and 19.6% and 18.3% of women in these groups had ferritin deficiency respectively. Similar trends were found for iron deficiency anemia, while the prevalence of anemia deficiency was found to be high in both of the abnormal hemoglobin traits.

Table 3.2.2 Correlation of micronutrient deficiency & anemia with thalassemia:

	Overall	A+A2 (normal A2)	A+A2 (raised A2)	A+A2+F	others
<b>Folic Acid Deficient (&lt;3.0 ng/ml)</b>	39.8	39.6	43.4	44.9	33.9
<b>VB12 Deficient (&lt;191 pg/ml)</b>	49.8	50.3	41.5	50.6	44.6
<b>Ferritin Low Ferritin (&lt;12 ng/dL)</b>	26.1	26.3	19.6	18.3	37.5
<b>Hb gm/dl Anemic (&lt;12 gm/dl)</b>	48.4	46.9	73.5	54.5	51.8
<b>IDA</b>	17.7	17.4	13.6	16	28.6

### 3.2.3: CRP Concentration in women of reproductive age:

Figure 3.2.3: CRP concentration in women

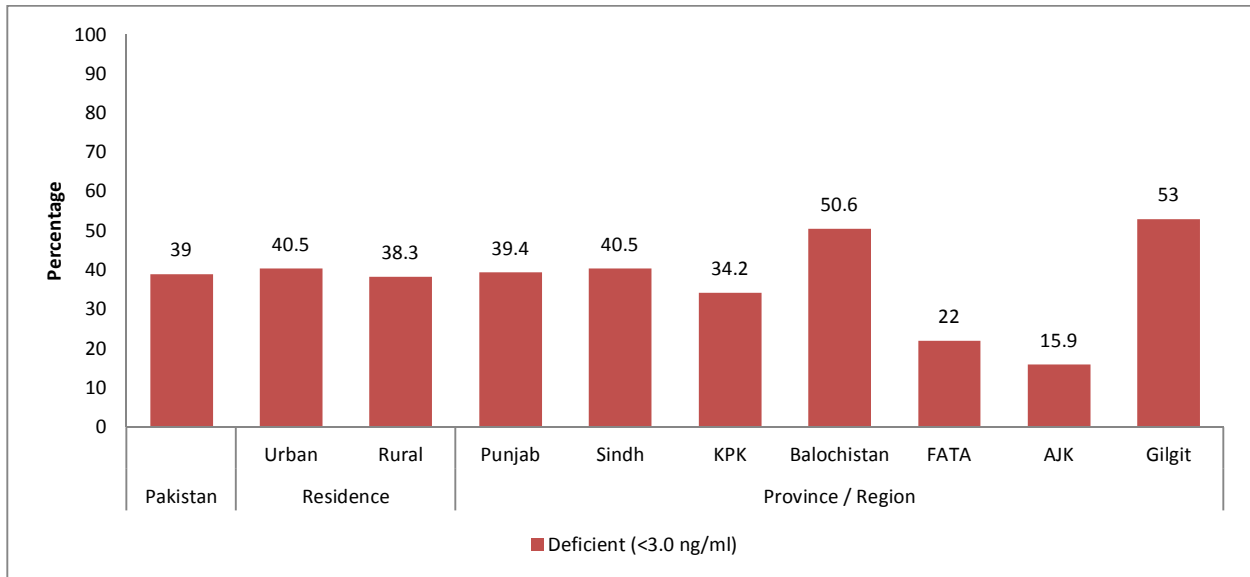


Below figure showing the prevalence of elevated CRP in WRA, it was revealed that in Pakistan about 4.7% mothers were having an elevated CRP, there is a significant difference between the Urban and rural prevalence of elevated CRP as well, when the data was assessed for provincial differences it was found that FATA had the highest prevalence of 9.1 % compared to other

provinces where as Gilgit and KP the prevalence of elevated CRP was found to 1.8 and 2.7 % respectively

### 3.2.4: Serum Folic Acid concentrations in women of reproductive age:

Figure 3.2.4: Folic acid concentrations in women of reproductive age

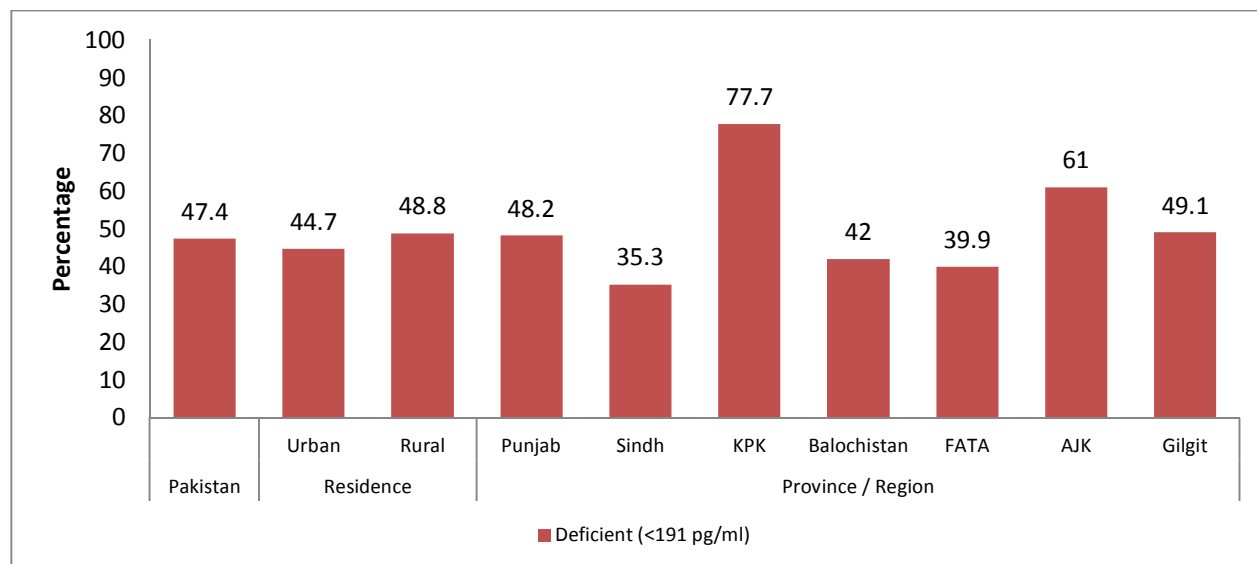


The blood samples were also analyzed for folic acid level, the results showed that in Pakistan about 39% were folic acid deficient, there was no major difference between Urban and Rural residents. When the data was analyzed for cross provincial variance it was revealed that Gilgit had the highest prevalence (53%) of folic acid deficiency followed by Balochistan (50.6%), Sindh (40.5%), Punjab (39.4%), KP (34.2), FATA (22%) and AJK (15.9%).

### 3.2.5: Vitamin B 12 concentrations in women of reproductive age:

The Data for Vitamin B 12 level was also analyzed and it was found that the total prevalence of Vitamin B 12 in Pakistan is quite high reaching to 47.4%, there was no difference between Urban and rural residence. But for provincial variance significant difference in Prevalence of Vitamin B 12 deficiency was observed. KP has the highest prevalence of 77.7% followed by AJK (61%), Gilgit (49.1%), Punjab (48.2%), Balochistan (42%), FATA (39.9%) and Sindh which had the lowest prevalence of 35.3%.

**Figure 3.2.5: Vitamin B 12 Concentrations:**



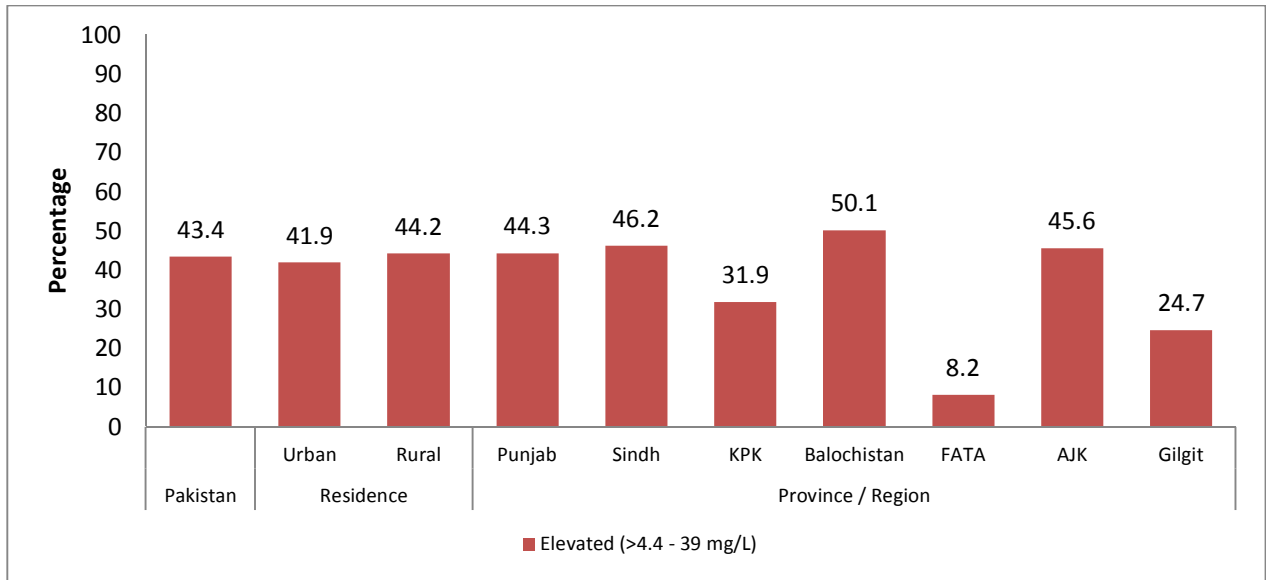
### 3.2.6: Serum Transferrin Receptor Concentrations

Serum Transferrin receptor (sTfR) is a trans-membrane protein that mediates iron delivery to erythroblasts by the interaction of plasma transferrin with cell surface transferrin receptors. Clinically, sTfR measurement is mainly useful for the assessment of total erythropoiesis and diagnosis of functional deficiencies and differentiation of iron deficiency anemia from anemia of chronic disease and. According to some studies sTfR concentration is consistently high in iron deficiency anemia.

Blood Samples were also analyzed for the Serum transferrin receptor level. The serum transferrin receptor (sTfR) blood test may be a better indicator of iron status as it is not affected by inflammation or by advancing age. The analysis revealed that about 43.4% of women had an elevated Serum Transferring receptor level, there was a slight difference between urban and rural residence as the 44.3% of women residing in rural areas had elevated level compared to 41.9% in urban areas. The level were found to be different among the provinces; the highest values were for Balochistan where 50.1% of women had elevated level of Serum Transferring receptor, in Sindh 46.2%, in Punjab 44.3%, in AJK 45.6%, in KP 31.9% in Gilgit 24.7% and in FATA 8.2% women had elevated Serum Transferrin receptor level.



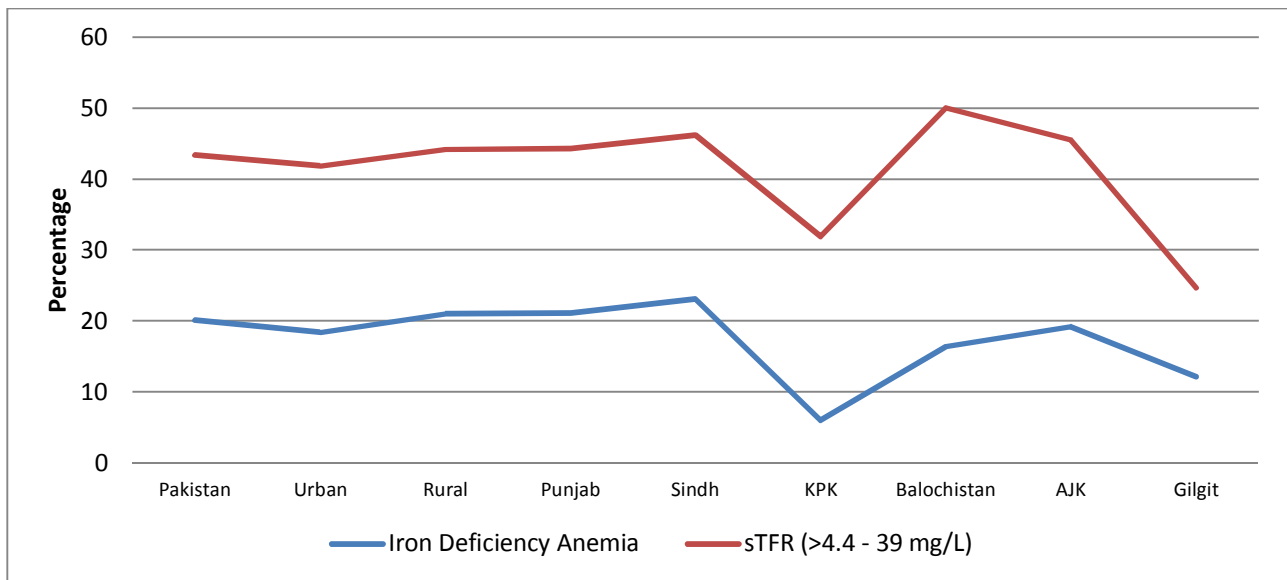
Figure 3.2.6: Serum Transferrin receptor concentrations



### 3.2.7: Relation between sTfR and IDA:

It was further noticed that sTfR concentration was found to be consistently high in iron deficiency anemia. The below figure explains this relationship.

Figure 3.2.7: Relation between sTfR and IDA:



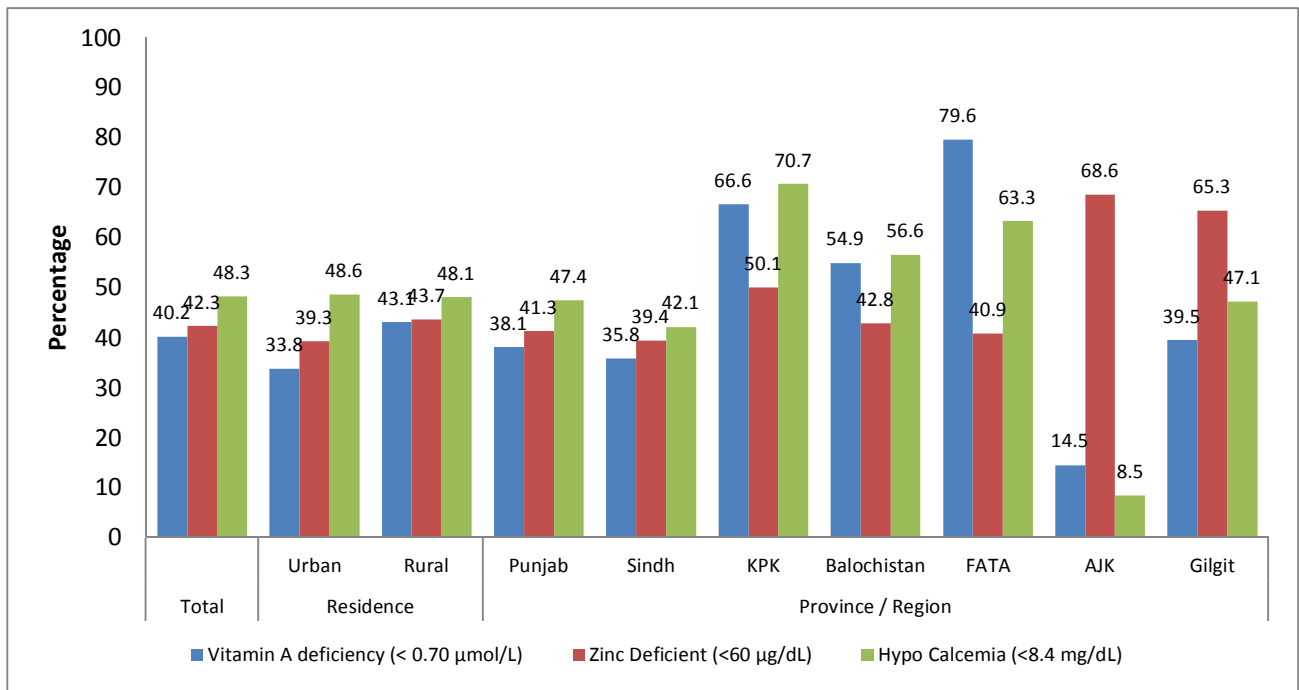
### 3.3 Results Lab Analysis

Blood samples were analyzed for various micronutrients and vitamins apart from the additional level for CRP, Folic acid, vitamin B 12, thalassemia specifically for beta thalassemia and serum transferrin receptors during NNS 2011.

#### 3.3.1: Vitamin A, Zinc and calcium levels

It was observed that overall 40.2% of the women had deficiency of vitamin A, 42.3 % women had zinc deficiency and about 48.3% women had low calcium levels. Similar proportions were found in urban and rural areas where as the regional distribution has been summarized in below figure.

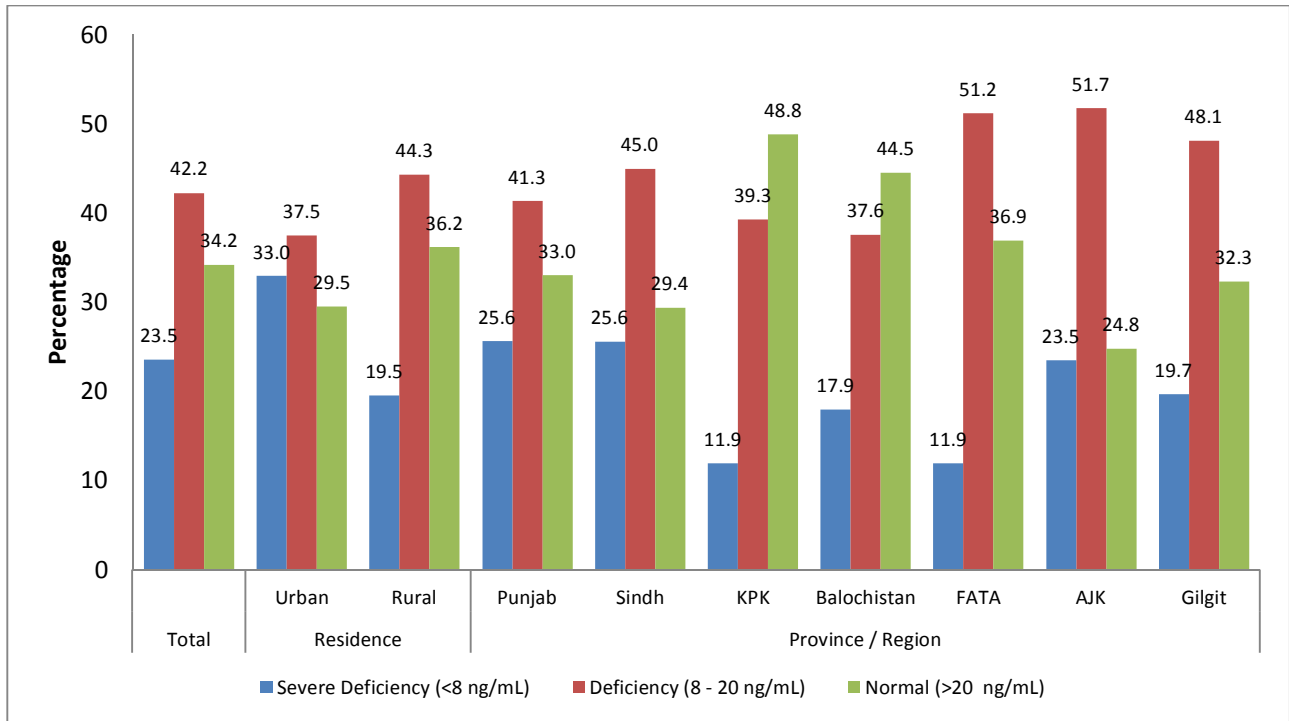
**Fig 3.3.1: Vitamin A, Zinc and calcium levels**



#### 3.3.2: Vitamin D level:

We also analyzed the data for vitamin D level, the results showed that overall 34.2% women had normal vitamin D levels while 23.5% women had severe vitamin D deficiency and 42.2% had deficiency of vitamin D levels. The women residing in urban areas had high proportion of severe vitamin D deficiency. The regional variation in vitamin D has been explained in the figure below.

**Figure 3.3.2: Vitamin D level**



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