CHAPTER 4: MATERIALS AND METHODS

The present study was carried out to assess the availability of supplements, coverage, acceptance, compliance rates of ongoing Calcium and Vitamin D supplementation in three different settings. The detailed methodology followed during the study has been described under the following headings:

4.1 Selection of locale

4.2 Criteria for selection of locale

4.3 Ethical clearance

4.4 Permission to conduct the study

4.5 Study information to-

- 4.5.1 Medical in Charge
- 4.5.2 Child Development Project Officer
- 4.5.3 Anganwadi supervisors and workers
- 4.5.4 Participating subjects

4.6 Study design

- 4.6.1 Supplementation regimen finalization
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  - 4.8.1.1 Enrolment procedure of pregnant women in Group 1
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4.11.4 Haemoglobin estimation

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4.11.5 Diet survey using 24-hour recall

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4.13 Data analysis

4.1 Selection of locale

Currently, in Delhi, antenatal care is being provided both in hospitals and in community settings. Therefore, the study design envisaged that apparently healthy pregnant women with no known systemic or obstetric problems would be enrolled for the study in three settings:

1. Primary health care institution as part of the research study ensuring continuous supply and careful monitoring of the intake along with nutrition education
2. Primary health care institution providing antenatal care under existing service conditions
3. Urban community setting under existing conditions

Figure 4.1: Location of the PHCI at which data of Group 1 collected

Figure 4.2: Location of the PHCI at which data of Group 2 collected

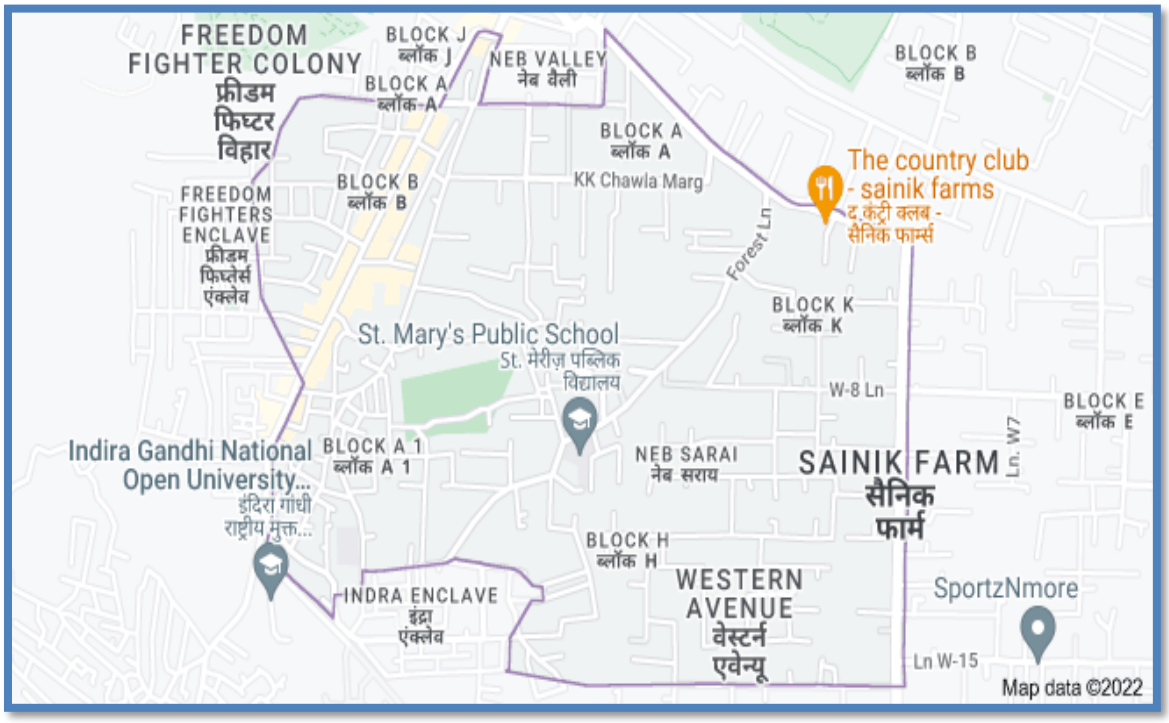

Figure 4.3: Area of Neb Sarai where data for Group 3 was collected

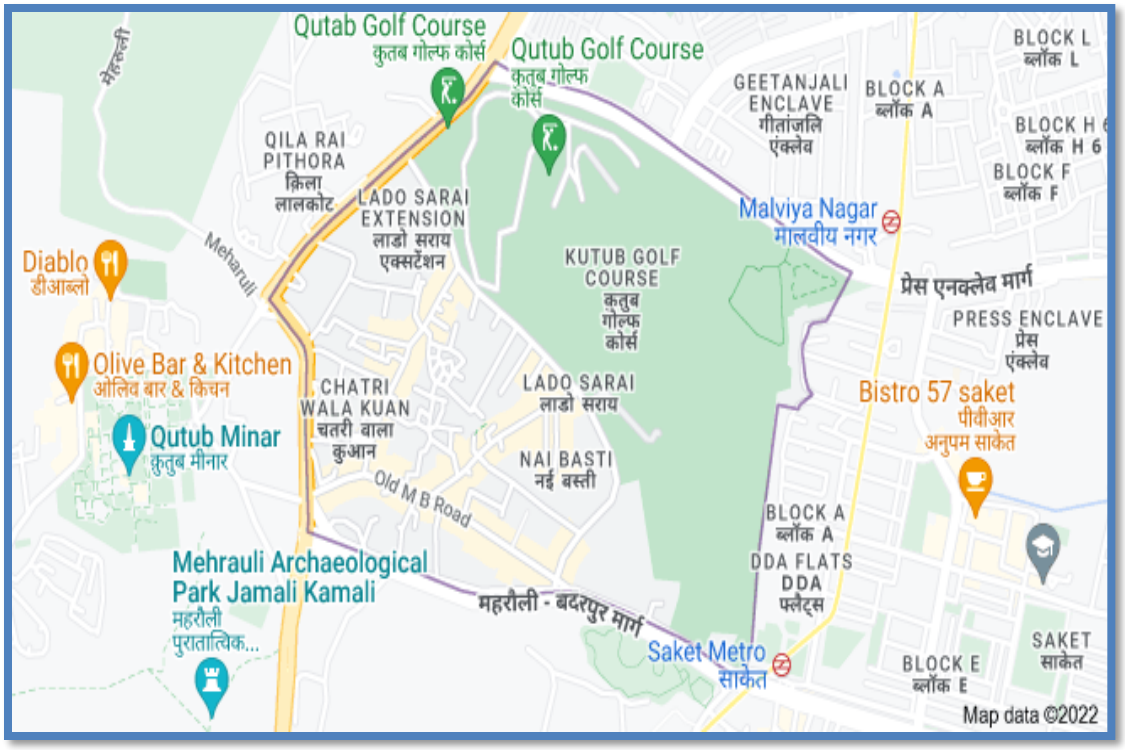

Figure 4.4: Area of Lado Sarai where data for Group 3 was collected

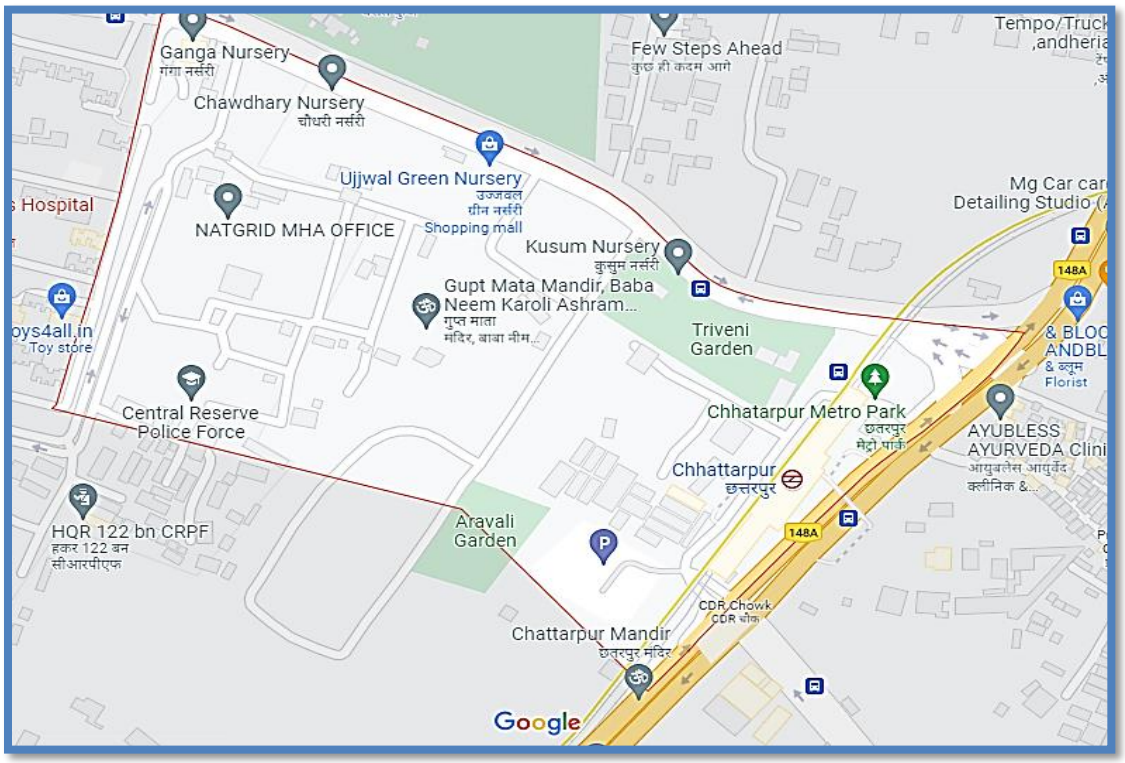

Figure 4.5: Area of Andheria Mor where data for Group 3 was collected

4.2 Criteria for selection of locale

The selected Primary Health Care institutions provided antenatal and delivery services to women mostly from low-income groups. Nutrition Foundation of India (NFI) has been working in these Primary Health Care institutions for years, therefore NFI personnel have developed a good rapport with the staff of the PHCIs.

The population in the community were from the low-middle-income group, many of whom were migrants from nearby states seeking employment opportunities. Nutrition Foundation of India has been working in these areas for over a decade and NFI personnel have developed a good rapport with the population as well as with the ICDS functionaries and health-front line workers.

4.3 Ethical clearance

The Institutional Ethics Committee (IEC) of Nutrition Foundation of India (NFI) has approved the proposal of NFI providing calcium (500mg) and vitamin D (250 IU) supplementation to pregnant women attending a primary health care institution under research conditions and monitoring for the regularity of supplement intake; and observing and collecting data on compliance of calcium and vitamin D in another primary health care institution where antenatal care is provided under existing service conditions and in a community setting where subjects may be attending different ANC clinics (Government and/ or private) and/ or ANM, ASHA are providing antenatal care and calcium and vitamin D supplementation.

The IEC recommended that the study team observe, record and report the availability, acceptance, compliance and continuation rates of calcium and vitamin D supplementation in pregnant women in each of these three settings.

4.4 Permission to conduct the study

The Nutrition Foundation of India had taken permission from Medical Officers and Chief Administrative Medical Officer (CAMO) of the Primary Health Care Institutions to work in the Primary Health Care Institutions; permission was obtained from the Ministry of Women and Child Development (MWCD), State Women and Child Development

(WCD) and Integrated Child Development Scheme (ICDS) coordinator to conduct the study in the urban low-income groups in three ICDS blocks in New Delhi, namely Neb Sarai, Lado Sarai and Andheria mod.

4.5 Study information to

4.5.1 Medical in Charge:

Medical in-charge of the primary health care institutions were given a copy of the permission forms obtained to work in the PHCI, and were briefed about the study.

4.5.2 Child Development Project Officer:

Child Development Project Officer of the selected Anganwadi area was given a copy of the permission forms obtained from DWCD to work in that particular area.

4.5.3 Anganwadi supervisors and workers:

Anganwadi workers and supervisors were briefed about the study. They were given a copy of the permission forms obtained from the DWCD and permission from the area Child Development Project Officer (CDPO).

4.5.4 Participating subjects:

Eligible subjects were briefed about the study and were provided with a study information sheet, written both in the Local Language i.e. Hindi and in English, in which the details about the study, the purpose of the study, the benefits and risks of the study, cost to participants, compensation, how to withdraw from the study, assurance of confidentiality of the data and the number of the contact person were written clearly. They were requested to discuss the study with the other members of the family and then let the NFI team know whether the women were willing to participate in the study. Written informed consent was obtained from those willing to participate in the study and then they were enrolled.

4.6 Study design

The study design envisaged that apparently healthy pregnant women with no known systemic or obstetric problems would be enrolled for the study in three settings:

1. **Primary health care institution** (under SDMC) **as part of the research study ensuring continuous supply and careful monitoring of the intake along with nutrition education** (This setting will be referred as "Group 1" henceforth in the thesis)
2. **Primary health care institution** (under SDMC) **providing antenatal care under existing service conditions (**This setting will be referred as "Group 2" henceforth in the thesis**)**
3. **Urban community setting** (30 Anganwadis of 3 areas in South Delhi namely, Neb Sarai, Lado Sarai and Andheria Mod) **under existing conditions (**This setting will be referred as **"Group 3"** henceforth in the thesis**)**

4.6.1 Supplementation regimen finalization

At the end of 2014, when the study commenced, the Government protocol for IFA supplementation (NIPI) recommended that all the anaemic pregnant women were to be supplied with IFA supplements (containing 100mg elemental iron and 500 mcg Folic acid), twice daily. The adverse consequences of anaemia on mother-child dyad are well known. In the traditional Indian three-meal pattern, there was only one slot left for the calcium and vitamin D supplements, after leaving two slots for IFA supplementation. Therefore, many physicians prescribed 2 IFA and 1 calcium and vitamin D to be taken by pregnant women.

Nutrition Foundation of India carried out a short-term research study on the side effects of IFA (containing 60 mg elemental iron and 500 mcg) and calcium and vitamin D (containing 500mg elemental calcium and 250 IU vitamin D) supplementation to fit two tablets of IFA and two tablets of calcium and vitamin D within the usual three meal pattern and found out that there were no significant increase in the side effects between single tablet of IFA and two tablets of IFA consumed together; whereas there was a statistically significant increase in the side effects between single tablet of calcium and vitamin D and two tablets of calcium and vitamin D consumed together (Ramachandran, Pramanik and Kalaivani, 2019).

Therefore, for the research component of the study, it was decided to provide one tablet of IFA each with breakfast and dinner (provided by the PHCI) and provide 1 tablet of calcium and vitamin D provided by our institution with lunch.

4.6.2 Brief of enrolment and follow up procedure

In view of the known differences in the vitamin D status between different seasons, it was decided to recruit 100 pregnant women attending antenatal clinics in PHCI during each of the four seasons and follow them up till delivery.

In the community setting, efforts were made to detect and enroll pregnant women and follow them up till delivery had been done.

- **Group 1:** Approximately 100 pregnant women in the second trimester willing to participate in the study were enrolled in each of the four seasons. Calcium (500mg) and Vitamin D (250IU) supplementation and nutrition education were given by research staff.
- **Group 2:** Approximately 100 pregnant women attending the ANC in their second trimester and willing to come for follow-up to the same clinic till delivery were enrolled in each of the four seasons. They received calcium and vitamin D supplementation under existing conditions in the hospital. Information on the composition of the tablets supplied in the hospital and on the regularity of supplement supply and intake were recorded. If the subject got the prescription and purchased the supplements then information on the composition of tablets and the number of tablets bought from outside were collected and used for assessing the number of tablets accessed and the number consumed while computing the data on compliance.
- **Group 3:** Pregnant women residing in 30 Anganwadies in 3 areas of South Delhi namely Neb Sarai, Lado Sarai and Andheria Mod were enrolled throughout the year; and information on access to Calcium and Vitamin D supplements and compliance of the same was collected. Monthly follow-up was carried out till delivery and information on the course and outcome of pregnancy was collected.
- **Follow-up Procedure:** In all three settings (Group 1,2 and 3), at the initial visit, all the information on the Sociodemographic profile (SDP), obstetric history, anthropometry (height & weight) and blood pressure was collected.

In PHCI settings (Group 2 and 3) efforts were made to estimate the haemoglobin levels of all the enrolled subjects by cyanmethaemoglobin method.

In a subsample in all three settings information on household food security (amount purchased/ consumption unit) and dietary intake of pregnant women were collected using the food frequency questionnaire and 24-hour recall method.

At each subsequent visit, blood pressure and obstetric examination findings were recorded; information on the number of Calcium and Vitamin D supplements accessed and compliance with the same were also recorded in all three settings. In Group 3, information on antenatal care from all providers, and supplementation prescribed/provided, regularity of intake was recorded.

An attempt was made to follow-up all women till delivery (in PHCI settings, often through mobile telephonic calls) and information on the course and outcome of pregnancy was collected.

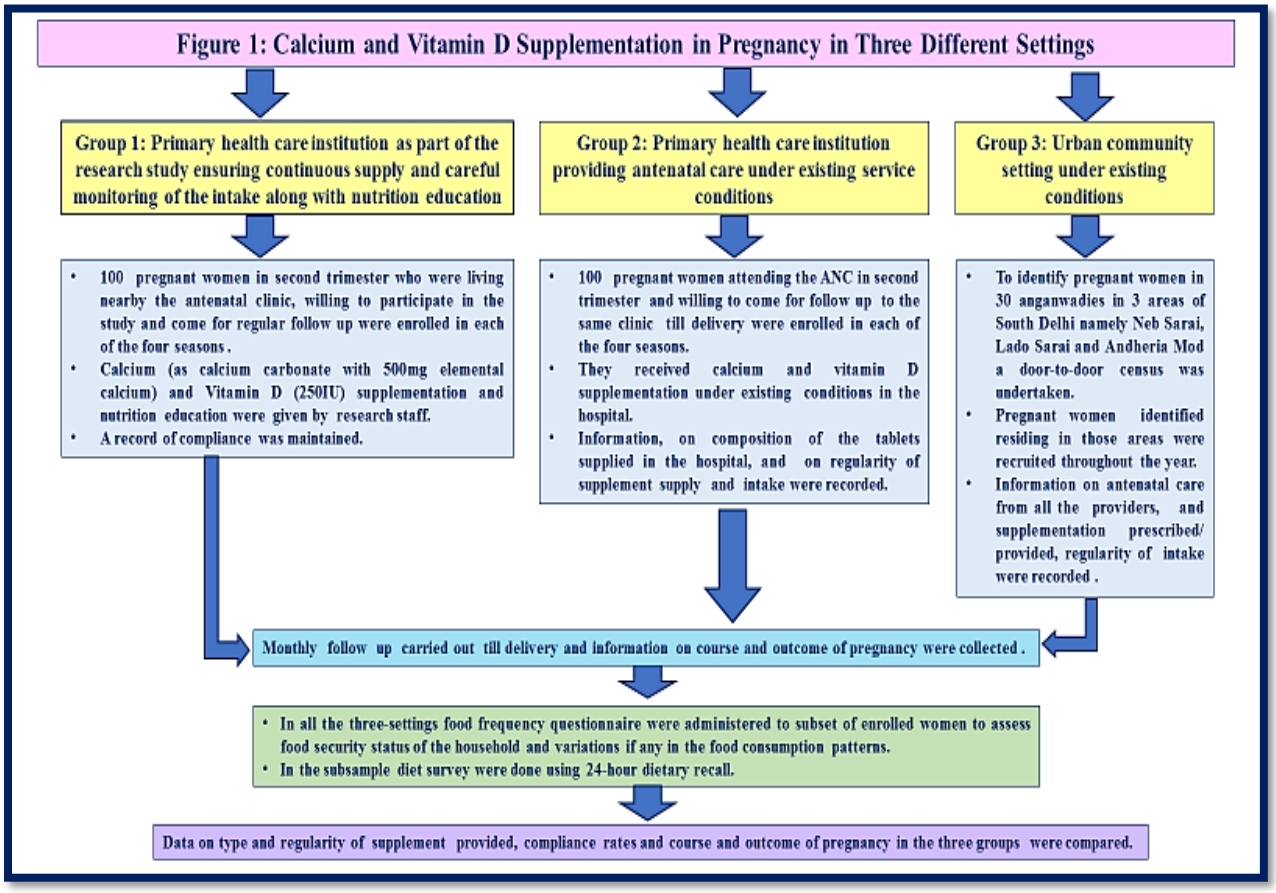

Figure 4.6: Study Design

4.7 Sample size

There have not been any studies documenting the feasibility, acceptance and continuation rates of vitamin D and calcium supplementation in primary health care institutions and community settings. It was therefore difficult to compute the sample size required. Vitamin D levels are known to vary between seasons. It was therefore decided to undertake the study by enrolling 100 women per season for four seasons of three months each and documenting the course and outcome of pregnancy in these women.

4.8 Identification of the subjects, enrolment and follow up

4.8.1 Group 1

Women who were living near the antenatal clinic and willing to come for regular follow-up were enrolled and provided calcium and vitamin D supplementation monthly, till delivery. Vitamin D status is known to vary with season. Therefore, the research study on supplementation envisaged that 100 pregnant women during the second trimester would be identified, enrolled and followed up in each of the four seasons. They were provided with calcium (500mg) and vitamin D (250 IU) supplementation and were monitored for regularity of supplement intake

4.8.1.1 Enrolment procedure of pregnant women in Group 1

Women attending the antenatal clinic during the second trimester were informed about the proposed calcium and vitamin D supplementation study. Those who were willing to take calcium and vitamin D supplements daily and were willing to come once every month for the supply of the tablets were recruited for the study.

4.8.1.2 Inclusion criteria

- Apparently healthy women in the second trimester of pregnancy.
- Women willing to participate in the study, take supplements daily and come for follow-up once a month.
- Willing to allow blood samples to be collected at enrollment and 12 weeks after supplementation.

- Willing to come to hospital for delivery; even when referred to other hospitals continue to take calcium and vitamin D supplements and provide follow-up data on course and outcome of pregnancy.

4.8.1.3 Exclusion criteria

- Women in the first and third trimester of pregnancy.
- Women, who were not willing to take supplements daily.
- Women with systemic diseases.
- Women with bad obstetric history or current obstetric problems.

4.8.2 Group 2

Antenatal care was being provided to the women accessing the antenatal clinic; IFA and calcium and vitamin D supplements were given as and when available.

One hundred pregnant women during the second trimester attending the antenatal clinic in each of the four seasons and receiving calcium and vitamin D supplementation under existing conditions of services from the PHCI were identified and enrolled. Data on the regularity of supplement intake and course and outcome of pregnancy were collected as and when they came to the antenatal clinic. Efforts were made to telephonically contact those who did not come regularly and obtain information on supplement intake, course and outcome of pregnancy.

4.8.2.1 Enrolment procedure of pregnant women in Group 2

Women, attending another Delhi primary health care institution (PHCI), were enrolled for this study. They were receiving the standard antenatal care provided under the Delhi PHCIs and were receiving supplements as provided in the hospital. Women who were from the vicinity and planned to come to antenatal care in the PHCI and who had come to the PHCI for antenatal checkup during the second trimester who were willing to come regularly for antenatal checkups and take the supplements provided were enrolled for the study. Regularity with which calcium and vitamin D supplements were provided and compliance with the supplementation regimen were recorded. Records were maintained to show what proportion of pregnant women who came to the hospital were taking the calcium and vitamin D supplementation, provided by the PHCI or when a prescription for

the same was provided to them, and how many did not receive any calcium and vitamin D supplementation were noted. Information was collected on the composition of the tablets supplied in the PHCI during the period of the study and also when women bought the tablets, prescribed, from outside.

4.8.3 Group 3

The Anganwadi workers and ANMs identify the pregnant women and provide care under existing conditions; women may access dispensaries or private practitioners for antenatal care; they get calcium and vitamin D supplementation and iron-folic acid supplementation according to the availability and practice in the clinic.

4.8.3.1 Census and preparation of area maps for community setting

A complete door-to-door census has been carried out in 30 Anganwadis of 3 areas in south Delhi namely, Neb Sarai, Lado Sarai and Andheria Mod. During census, all the houses in each Anganwadi area were numbered. Numbering was done using small stickers. On the sticker; 'Anganwadi number' was written on the first line and three digits 'House number' in the second line. Anganwadi was given house number 001(three-digit number) and then the numbering was continued on the house to the left side of the Anganwadi. All the households residing in the houses were given consecutive numbers.
Mapping of the area was done and shown in the following figure. The direction of the increasing house numbers was indicated with arrows.

Whenever there were new constructions of buildings between two already numbered houses, those were given a new house number which was alpha-numeric. E.g. If there was a new building construction between house number 77 and house number 78, the new one was numbered as 77A.
After a year all the house numbers were revised and given a new house number and were marked in the photocopy of the original map which contained the originally given house numbers. In the map, the new house numbers were written with a different ink.

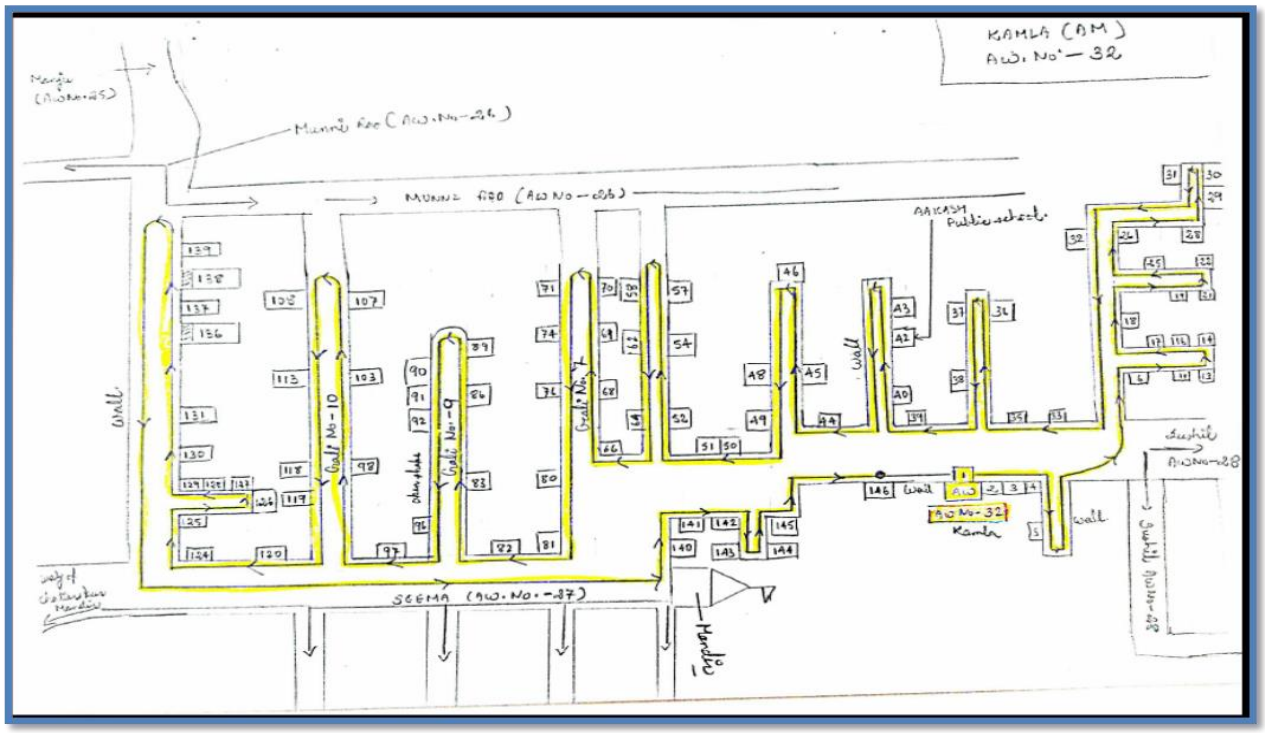

Figure 4.7: Map of Anganwadi showing census

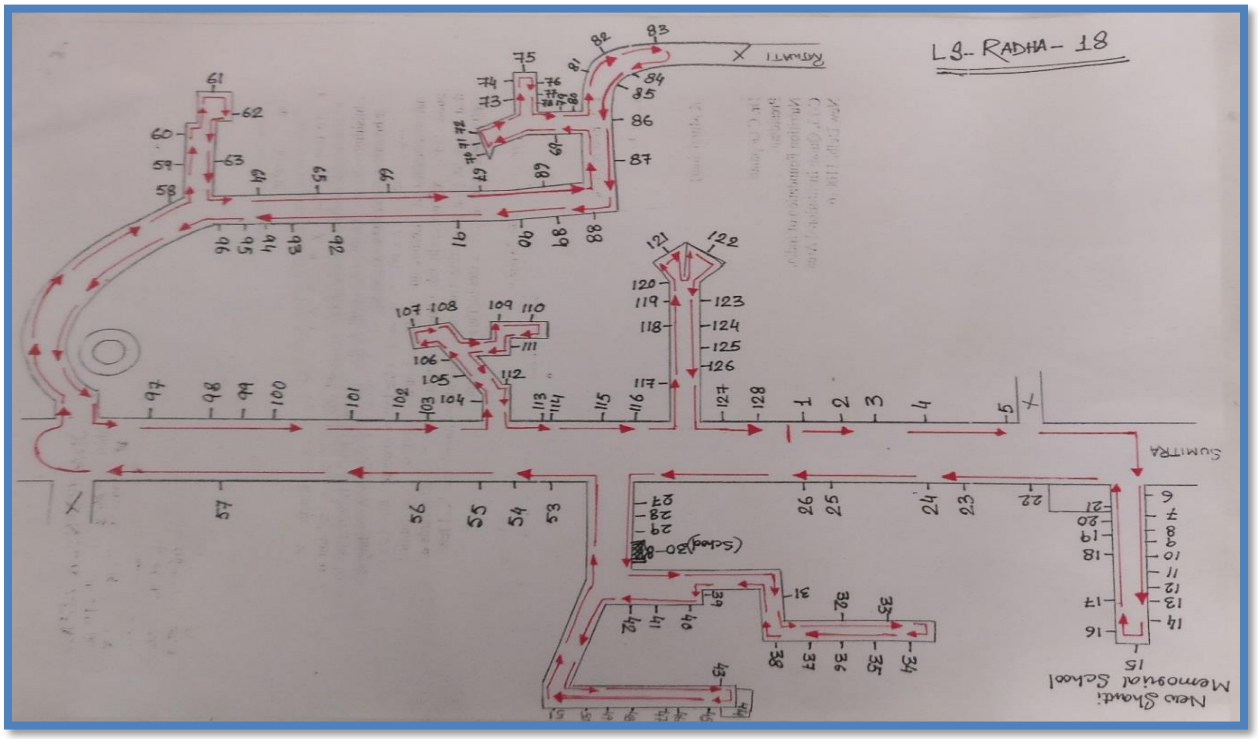

Figure 4.8: Map of Anganwadi showing census

As the area is in the heart of South Delhi, there are a large number of single men and women who are students or employees who are staying as tenants/ paying guests in the residences in these areas. They were excluded. In all these areas, there were substantial numbers of middle-income households residing in flats; they did not come to the Anganwadi to get any care or advice. Most of them informed us they do not want to participate in the follow-up study, even though they were residing in those areas. Households belonging to middle- or high-income groups, those who were likely to move on shortly and those who were not willing to participate in this study were not included. Low or low-middle-income group households who stated that they were likely to stay in the area for a year and were willing to participate in the study were enrolled.

4.8.3.2 Enrolment procedure of pregnant women in Group 3

Inclusion criteria

- Apparently healthy pregnant women, without any systemic or obstetric problems residing in the 30 Anganwadis of 3 areas in south Delhi namely, Neb Sarai, Lado Sarai and Andheria mod, who were likely to stay in the area till delivery. The majority of pregnant women were identified during the second trimester.

Exclusion criteria

- Women with systemic diseases.
- Women with bad obstetric history or current obstetric problems.

An attempt was made to follow-up with these women every month and obtain data on antenatal care given by all providers, and all types of supplementation prescribed/provided, regularity of intake of the supplementation (iron folic acid, calcium and vitamin D) were collected.

4.8.4 Follow-up in all the three settings

The follow-up protocol was similar in all three settings.

- At the time of the initial visit details regarding the sociodemographic profile and obstetric history were recorded.
- In Group 1, all the enrolled women were provided with supplements containing 500mg of elemental calcium (as calcium carbonate) and 250 IU of vitamin D3.

They were advised to take one tablet of calcium and vitamin D daily after lunch. Pregnant women were advised to take the iron and folic acid (IFA) tablets received from the PHCI and the calcium and vitamin D supplements provided by the research staff, separately after breakfast and/ or dinner. A record of compliance was maintained.

- In Groups 2 and 3, information on the type and regularity of the supplements provided and the compliance rates were recorded.
- All three groups were followed up through pregnancy and delivery, to document the course and outcome of pregnancy.
- In a subsample, diet survey was done using the 24-hour dietary recall method and the food security status of the household was assessed using food frequency questionnaire variations if any in the food consumption patterns were recorded.

4.9 Procedure of collecting the information on compliance of Calcium and Vitamin D supplements

4.9.1 Group 1

In Group 1, all the women enrolled for the study were supplemented with calcium 500mg (in calcium carbonate form) and vitamin D3 250 IU from the day of enrolment till delivery by the research team. Women were given the tablets required for the month, and when they came to collect the tablets for the next month, the compliance was checked and recorded.

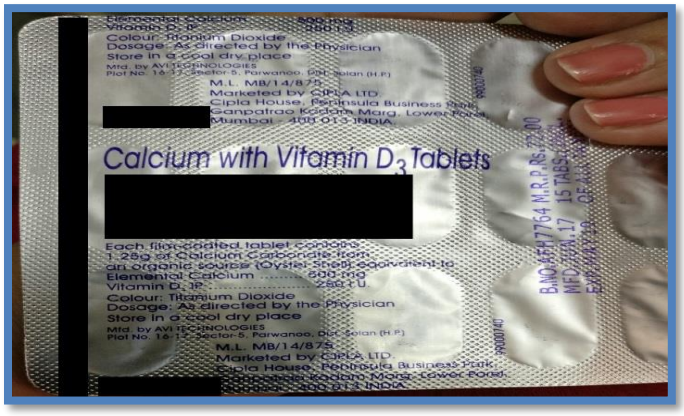

Plate 4.1: Composition of the calcium and vitamin D supplements provided by the research team

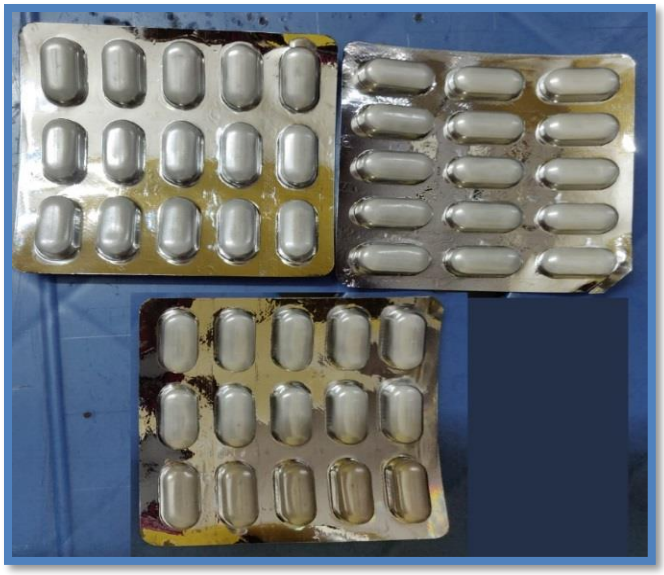

Plate 4.2: Monthly Calcium and Vitamin D supplements provided by the research team

They were requested to bring the completed tablet strips; using this compliance was checked and recorded. In all women the address and mobile phone numbers of the husbands/ neighbour of the women were collected; the mobile phones were used to contact them as and when necessary especially when they did not come for follow-up to the centre on the expected date.

Plate 4.3: Completely empty strips of the supplements brought by pregnant women

The above picture shows that the subject completely consumed the supplements given to her. Some of the subjects also brought the last empty strip of the medicine as they already have discarded the other two empty strips. When they were asked about the reason for not bringing the empty strips, they replied- in some cases the family members had thrown the empty strips out unknowingly; sometimes the subjects had elder children who had taken the empty strips while the subject was away or sometimes the subjects also had thrown it away by mistake. However, they were requested to bring all of the empty strips to confirm compliance with the supplements given and most of them did so.

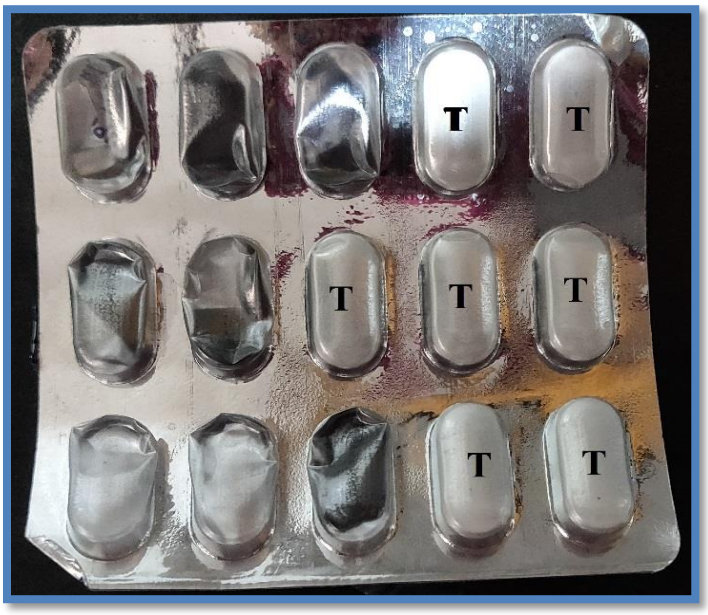

Plate 4.4: Partially empty strips brought by pregnant women

The above figure shows the partially empty strips of the calcium and vitamin D supplements; "T" is marked in the picture to show that in this particular strip, 7 tablets were remaining. These 7 tablets were deducted from the number of tablets available to calculate the number of tablets actually consumed. For example, if the pregnant woman was provided with 30 tablets of calcium and vitamin D by the research team in the previous visit and the available tablets were 30, then deducing the remaining 7 tablets as shown in the picture, the pregnant woman consumed 23 tablets. Now in the current, visit the pregnant woman was provided with 30 more tablets of the same, thus in this particular visit the available tablet count became 37; in the next visit, the remaining

tablets of that particular visit would be deduced from the 37 tablets available from this visit, to calculate the actual tablets consumed between the current date of visit to that subsequent date of visit.

Initial visit/ 1st visit:

- Tablets provided (TP1) = Tablets available (TA1)

2nd visit:

- Tablets available from the previous visit (TA1) – tablets remaining (TR1) = Tablets consumed (TC1)
- Tablets remaining (TR1) + Tablets provided (TP2) = Tablets available (TA2)

3rd Visit/ Subsequent visits:

- Tablets available from the previous visit (TA2) – Tablets remaining (TR2) = Tablets consumed (TC2)
- Tablets remaining (TR2) + Tablets provided (TP3) = Tablets available (TA3)

4.9.2 Group 2

In Group 2, compliance with the calcium and vitamin D supplementation was ascertained from women during each visit. The procedure followed to counter-check the compliance with the supplements was exactly the same as the procedure followed in Group 1. In Group 2, the subjects were provided with the calcium and vitamin D tablets by the PHCI under the routine service condition as and when available; subjects were often provided with a prescription to buy the calcium and vitamin D supplement from outside when there was no supply of the same in the PHCI. All these women brought back the tablet strips and from these, the number of tablets consumed was calculated and counter-checked.

During the follow-up visits, if the supplements were provided by the PHCI the tablets available were recorded on the day it was given but in case the supplements were bought from a pharmacy, the number of tablets bought was confirmed by the pregnant women and the composition were recorded from the empty strips brought by the subjects. The

tablets consumed were calculated and counter-checked from the number of tablets available in the previous visit and the number of tablets remaining in the current follow-up visit.

The PHCI was providing uniform composition of the calcium and vitamin D supplements but the supply of the same was erratic.

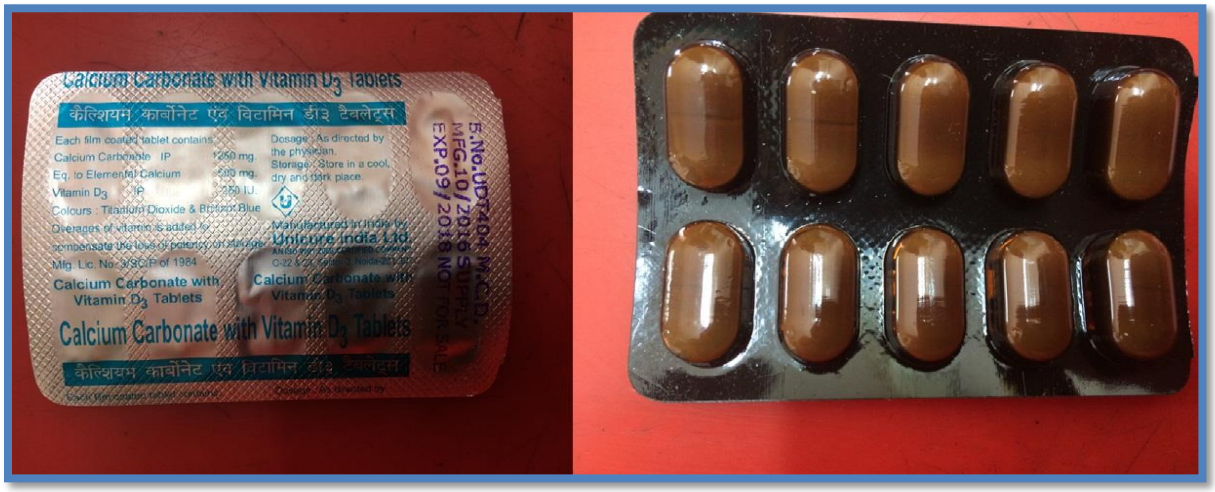

Plate 4.5: The above composition was provided by the PHCI as and when the supply was available

Various compositions of calcium and vitamin D supplements were available in the market. As per the availability of the same in the nearby pharmacy and the affordability of the pregnant women, they bought the supplements. Thus, there was no uniformity of the composition of the calcium and vitamin D supplements, when subjects were provided with the prescription and bought the calcium and vitamin D supplements from outside. During the follow-up visits, therefore, the research team recorded the composition of the supplements bought from outside pharmacies.

Plate 4.6: Pregnant women brought the empty strips of calcium and vitamin D supplements of different composition

4.9.3 Group 3

In Group 3, the procedure to record compliance was similar to that of Group 1 and 2.

Efforts were made by the research team to visit the enrolled pregnant women at least once a month for follow-up visits. Some of these pregnant women attended ANC in Government PHCIs/ hospitals or went to private practitioners for antenatal check-ups. The findings from the OP ticket/ antenatal card given by the doctor were recorded in our forms on every visit starting from the initial till the delivery. Supplements provided by the PHCI/ Government (Govt.) hospital/ ANM or purchased from outside as the private practitioners provided prescriptions for the supplements and the regularity of intake of those supplements were recorded. Pregnant women were requested to keep the empty strips and show them to the research team every month and then the empty strips were discarded after recording. The compositions of the supplements were also recorded by the research team.

Pregnant women, when provided with the prescription by the private practitioners, to buy the supplements from outside pharmacies, bought the calcium and vitamin D supplements of different compositions as per the availability of the same as per their affordability.

Thus, there was no uniformity in the composition of the calcium and vitamin D supplements bought by pregnant women from outside pharmacies.

A number of various compositions of calcium and vitamin D supplements, even when it was provided by the Government ANCs, were recorded by our research team. All the subjects (pregnant women) attended different ANC setups by the Government and did not attend any single Government ANC setup. Different ANCs procured calcium and vitamin D supplements of different compositions, and provided the pregnant women with the same as per the supply.

Not all of the calcium and vitamin D supplements provided by the Government were of the same composition recommended by the "National Guidelines for calcium supplementation during pregnancy and Lactation". Some ANCs provided supplements only with elemental calcium of 500mg without vitamin D along with it and some provided supplements only with elemental calcium of 250mg instead of supplements containing elemental calcium 500mg and vitamin D 250 IU as per the National guidelines.

Plate 4.7: Some of the compositions of Calcium and Vitamin D supplements provided in the Government ANCs

Plate 4.8: Some of the compositions of the Calcium and Vitamin D supplements that the pregnant women bought from outside pharmacy

Plate 4.9: Some of the ANC cards/ prescriptions from where the OP findings and recommended supplements were recorded

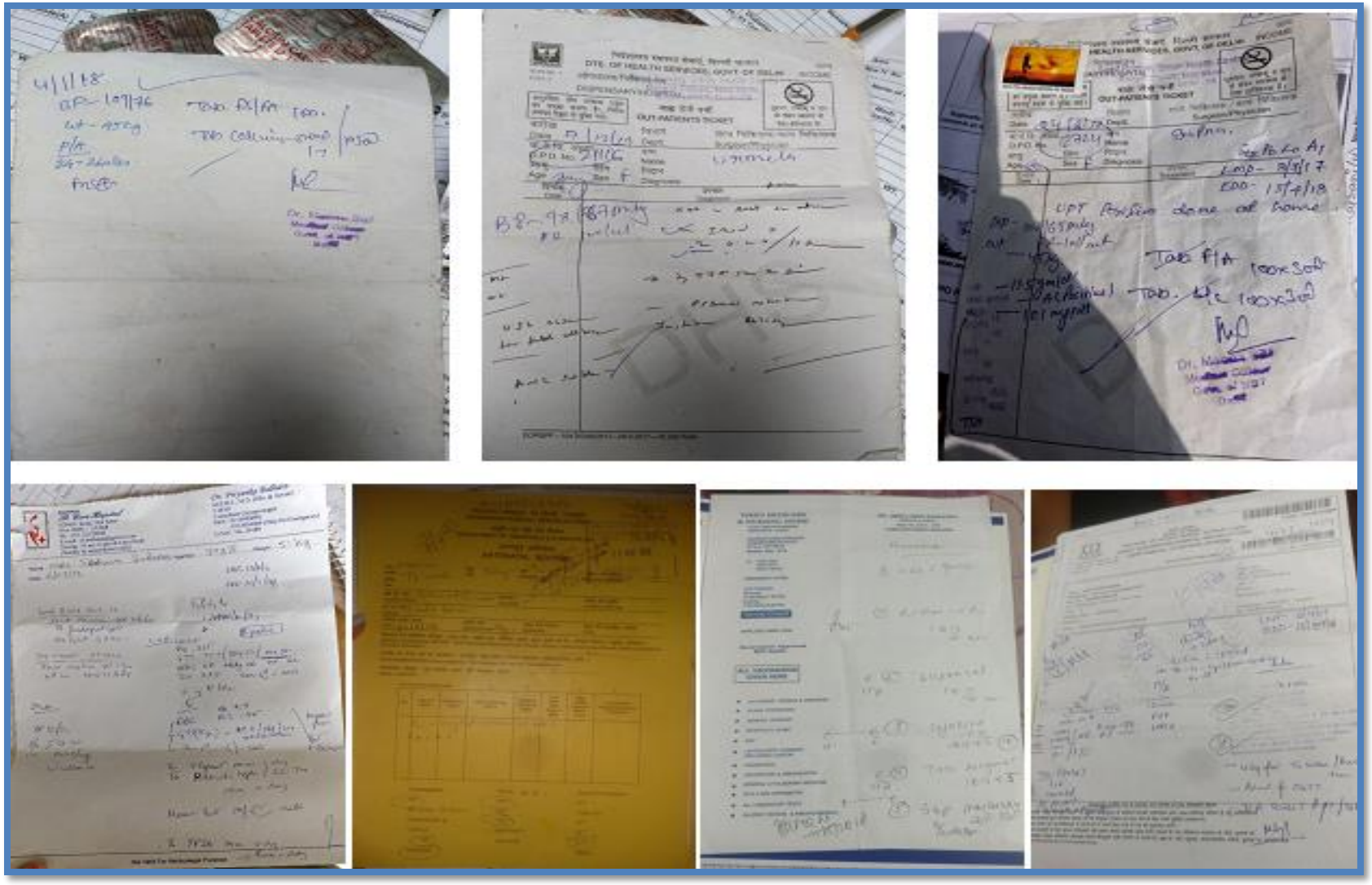

Plate 4.10: Some of the ANC cards/ prescriptions from where the OP findings and recommended supplements were recorded

4.10 Proformas used for data collection

The proformas used to collect the data of the present study are attached in the annexures. Brief of the proformas used are as follows:

- Proforma 1- Socio-demographic profile (SDP)
- Proforma 2- Obstetric history and antenatal care findings
- Proforma 3- Course and outcome of pregnancy: used while recording information regarding anthropometry, blood pressure, haemoglobin and antenatal care in follow-up visits.
- Proforma 4 - Compliance with Calcium and Vitamin D supplements: Information on the number of Calcium and Vitamin D supplements accessed (from where) number consumed and compliance rates, delivery details
- Proforma 5- Household food security and food frequency questionnaire
- Proforma 6-Proforma for Diet survey using 24-hour diet recall method

Nutrition Foundation of India has already been working both in the primary health care institution and community setting for over a decade. They were pretested and used proforma for collection of information on socio-demographic profile, obstetric history and antenatal care findings, and course and outcome of pregnancy. These proforma were used to ensure uniformity in data collection and comparison between studies. Proforma 4, 5 and 6 were designed and pretested and then used for data collection in the present study.

4.10.1 Proforma 1 *Socio demographic proforma (SDP)*

At the time of enrolment of each of the subjects, sociodemographic profile data was collected and recorded using proforma 1. SDP had information on various socioeconomic and demographic parameters of the subjects.

The sociodemographic profile consisted of a Unique Identification number UID number, name of the subject as well as husband, address, age of the subject, place of origin, family type (join/nuclear), family size, age at marriage, literacy and work status of both the subject and the husband, dietary habits, monthly family income, type of locality they

reside, type and ownership of house, number of rooms in the house, toilet facility, means of transport, cooking fuel, drinking water facility, means of entertainment and the type of kitchenware used in the household.

4.10.2 Proforma 2 *Obstetric History and antenatal care findings*

Obstetric history was collected and recorded using proforma 2, at the time of enrolment of each of the subjects.

LMP and EDD of the current pregnancy were recorded along with the information on the history of gravida, para, abortion, live birth, stillbirth, and the date of last termination of pregnancy. The inter-pregnancy interval was calculated using the LMP and the date of the last termination of pregnancy.

4.10.3 Proforma 3 *Course and outcome of pregnancy*

Anthropometry:

Anthropometric measurements include the weight and height of all the subjects; these were collected using the standard techniques (WHO, 1995) and were recorded in the proforma 3.

The height of all the subjects was measured by a standardized stature meter at the time of enrolment. As the subjects of the study were adults, height was measured only for a single time point.

The weight was collected at each visit for all of the subjects with an accurate digital balance (accuracy of 100 g). Every day, the accuracy of the balance was checked. All the information was recorded in the proforma 3. In Group 1, the weight of the pregnant women was measured by the staff-ANM of the PHCI using the hospital's digital weighing balance. The research team recorded the weight from the antenatal card. In Group 2 and 3, the weight of the pregnant women was measured by the research team using a NFI's digital weighing balance.

To minimize the measurement errors all instruments were calibrated and accuracy was checked every day.

Blood Pressure (BP):

Proforma 3 was used to record the blood pressure (BP) of all the subjects during each visit**.**

In Group 1, the BP of the pregnant women were measured by the staff-ANM of the PHCI using the hospital's manual sphygmomanometer.

In Group 2 and 3, the BP of the pregnant women was measured by the research team using a digital blood pressure monitor.

Haemoglobin estimation

Haemoglobin estimation was done using the indirect cyanmethaemoglobin method.

At the time of the initial visit to the antenatal OPD, all pregnant women have their blood drawn for the blood grouping and VDRL test. In those who agreed to participate in the study 20 µl blood was pipetted from the collected blood and put on the filter paper and dried. The dried blood spot was eluted in 5 ml Drabkin's solution and Hb estimation was done by using a colorimeter at NFI. Hb estimation was repeated after three months.

Information on outcome of pregnancy (delivery details):

Efforts were made to follow up with all the enrolled women till delivery; information on the outcome of pregnancy i.e. delivery details were collected and recorded using proforma 3. The information on the date of birth of the newborn, gender of the newborn, place of delivery, type of delivery, and birth weight of the newborn was collected under this segment.

4.10.4 Proforma 4 *Compliance with Calcium and Vitamin D supplements*

Proforma 4 was designed for the current study at NFI and was pre-tested before using it for the data collection for the study.

Information on number of tablets given/purchased (including composition) and compliance with calcium and vitamin D supplements were collected and recorded using proforma 4 for each of the subjects at each visit. Information was collected at each visit (date) on the number of tablets given (obtained), the number taken, number of tablets remaining, number of tablets skipped, number of tablets given in the current visit and the total number of tablets available. The subjects were requested to keep the empty strips of the supplements and to show them to the data collecting team so that these could be used to verify the compliance with the supplements. For Group 2 and 3 the source of the calcium and vitamin D supplements was also recorded along with the composition.

4.10.5 Proforma 5 *Household food security and food frequency questionnaire*

A Household Food Security proforma was designed and pretested at NFI. This proforma was used to collect information from a small subsample of women who purchased food for the family and cooked for the family and was willing to answer questions about the food purchased and food cooked and consumed by the family and individuals in the last 24-hours. The respondents were asked about the purchase of foodstuffs (previous day, week or month) (NSSO Method) and the amount of food cooked in the previous 24-hour period (NNMB Method).

NSSO Method: The actual amount of the foodstuffs purchased and the period over which it was consumed were ascertained. Based on the information collected, carbohydrate, protein, fat consumption and energy intake of the family per day were computed. Total energy intake per consumption unit per day was calculated and compared with the Nutrient Requirement of Indians (2020) to assess the food security status of the family.

NNMB Method: In the same households information on food cooked for the family on the previous day was collected using the 24-hour dietary recall method. Based on this information carbohydrate, protein, fat consumption and energy intake of the family on the previous day were computed. Energy intake per consumption unit was calculated to assess the food security status of the family. The food security as assessed by these two methods were compared and found to be concordant in the majority of families.

Note: Though tomatoes are considered fruits, but in majority of the households it is used as vegetables and thus information regarding tomatoes was collected in the other vegetable segments in the proforma used by the NFI research team.

4.10.6 Proforma 6 *Proforma for Diet survey using 24-hour diet recall method*

The dietary intake of an individual depends upon a number of factors like dietary habits and customs of their community, socio-economic status, personal food choices, seasonal availability of foods etc. The food frequency questionnaire (FFQ) is a relatively simple and easy method which involves less time to collect data on the food consumption pattern of an individual or a family. Data on specific food choices and avoidance may be obtained by this method. FFQ provides data on the consumption pattern but not on the quantity of food consumed.

Information on the dietary intake of an individual as well as the family cooking pattern and intra-family distribution of food is obtained by diet survey using 24-hour diet recall, using proforma 6. A diet survey using 24-hour recall can be done only by a well-trained person and the procedure is time-consuming.

The proforma for collecting data on diet survey using 24-hour recall was developed and pre-tested by NFI. The proforma is of two pages. The first page of the proforma consisted of columns to collect data on the timing of food consumption, the type of food prepared, the raw ingredients used with their quantity and the total volume of the cooked item. On the second page of the proforma columns were made to collect data on intra-family distribution of food items along with the amount of food left over.

4.11 Instruments/tools used, procedure of operation and procedure of assessing the accuracy

Ensuring accuracy in measurements is important in all research studies; and is vital for longitudinal studies aimed at assessing changes over time. A detailed knowledge of the instruments used in data collection is required to ensure the accuracy of measurements. In the present study, the investigator and team were trained in taking anthropometric measurements (height, weight), blood pressure (BP) measurements, pipetting of blood

and haemoglobin estimation. Only those who were accurate and consistent were given the responsibility of taking these measurements.

4.11.1 Height

The small readily portable wall-mounted stature meter is used for measurement of height.

Plate 4.11: Wall-mounted stature-meter

4.11.1.1 Testing the accuracy of the Stature meter

The accuracy of the tape in the stature meter is to be assessed by comparing it with the standard steel tapes certified by the Department of Weights and Measures. To check the accuracy of the tape, firstly, the tape was fully unwound and then the zero mark of the stature meter should be against the red line in the reading window. If the zero mark on the tape crosses or does not coincide with the red line in the reading window the tape will be

rejected. Next, the tape was fully wound, in this state, the red mark in the reading window should coincide with the 200 mark in the tape, otherwise, the tape is rejected.

Comparison of the height, measured by the standard stature meter and the test stature meter, of the same individuals also helps to check the accuracy of the test stature meter.

Plate 4.12: Comparison of the standard stature meter and the test stature meter

4.11.1.2 Fixing of stature meter to the wall

Depending on the structure of the wall where the height was being measured, the stature meter was fixed in one of the two ways described below-

Wall without skirting: The horizontal limb of the stature meter was placed on the floor and the vertical limb was pulled fully so that the read mark in the reading window coincides with the "0" cm mark. The vertical limb was then fixed to the wall using brown adhesive tape. Stature meter could be fixed with three screws inserted in the three screw holes in the vertical arm. Then the stature meter was allowed to unwind.

Wall with skirting: Skirting in the wall is almost present in all Indian households and constructions. As the skirting creates a gap in the wall and the tape and thus the readings get different, it was important to measure the skirting first. For example, if the skirting was 10 cm, the tape was pulled out and fixed with tape at 10cms reading in the window. Then the horizontal limb of the stature meter was placed on the skirting and the vertical limb was pulled fully and fixed on the wall with brown adhesive tape. Stature meter could be fixed with three screws inserted in the three screw holes in the vertical arm.

Then the tape used to fix the tape at 10cms was removed and the stature meter was allowed to wind.

Some stature meter tapes did not unwind fully or smoothly. Such stature meters did not function under field conditions and so were rejected and replaced (https://www.youtube.com/watch?v=VbJEyyCX3sY).

4.11.1.3 Measurement of height using Stature meter

The subject was made to stand barefoot, feet together against the wall. The hair was made flat, i.e. without any puff, bun, ponytail or any other hairdo must be removed before measuring the height. To ensure that the subject was standing straight, the following points were checked-

- If the subject was thin, heels buttocks and shoulders all three should be touching the wall;
- Whereas if the subject was overweight, only the buttock should be touching the wall.

The position of the head was checked to see that the tragus of the ear and the lower orbital margin i.e. Frankfurt Plane was in the horizontal plane.

After checking the subject was in the right position, the horizontal limb of the stature meter was brought down to rest firmly on the top of the head. Care was taken in placing the horizontal limb firm but not pressed tightly. The measurements were taken nearest to ±0.1 cm. The reading was recorded keeping the reading window at eye level. For this, if the subject was shorter than the person measuring the height, the person had to bend the knee to keep the reading window at eye level and if the subject was taller, the person measuring had to stand on a stool to keep the reading window at the eye level (https://www.youtube.com/watch?v=VbJEyyCX3sY).

Plate 4.13: Measuring height with wall-mounted stature-meter

4.11.1.4 Quality control of investigator and the team

The investigator and the team were trained by the expert trainer at NFI before going to collect the data to minimise intra- and inter-individual variations in measurements. All the team was taught how to measure height using a stature meter. The team took the height of each other in five rounds. The mean of the five rounds and the standard deviation of the individuals were evaluated. Only those who were accurate at the end of training measured height in pregnant women.

4.11.2 Weight

In the present study, weight was measured using the Lithium battery operated digital weighing balance which is lightweight, portable, accurate and sensitive. The range of the weighing balance was 5 kg to 150 kg with an accuracy of ± 100g. Subjects were made to stand erect on the digital weighing balance, barefoot, with minimal clothing, without taking any support (https://www.youtube.com/watch?v=Dla4oYnV0Es).

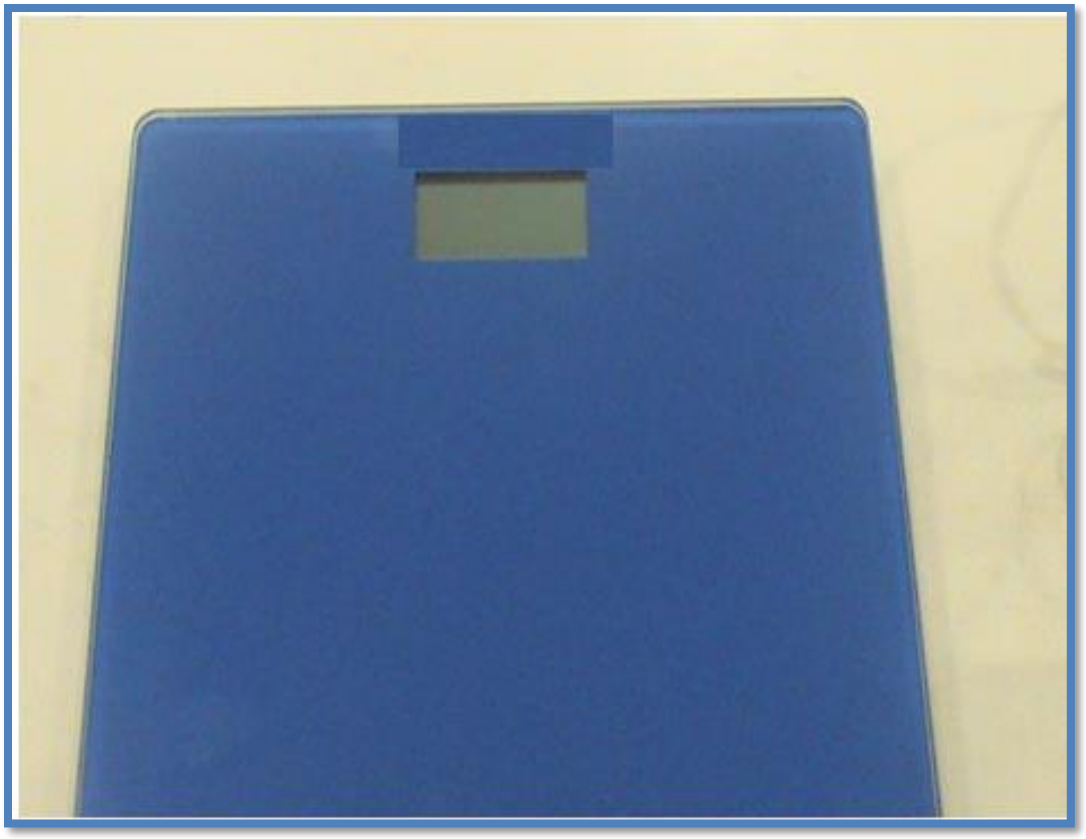

Plate 4.14: Digital weighing balance

4.11.2.1 Testing accuracy of Electronic Balances

Weighing is the most commonly used measurement for assessment of nutritional status and weight gain during pregnancy. Digital weighing machines minimize errors in weighing, but they need to be checked for accuracy every time before use.

The accuracy of the digital weighing balance was tested by two methods:

- Using, 5kg, 2kg, 1kg, 0.5kg, 0.2kg and 0.1kg standard weight certified by the Department of Weights and Measures. Initially, the 5kg standard weight was placed on the weighing balance and subsequently, the other standard weights were placed sequentially. Thus, ideally, the weighing balance should show 5 kg, 7kg, 8kg, 8.5kg, 8.7kg and 8.8 kg. The acceptable variation in this was ± 0.1 kg.

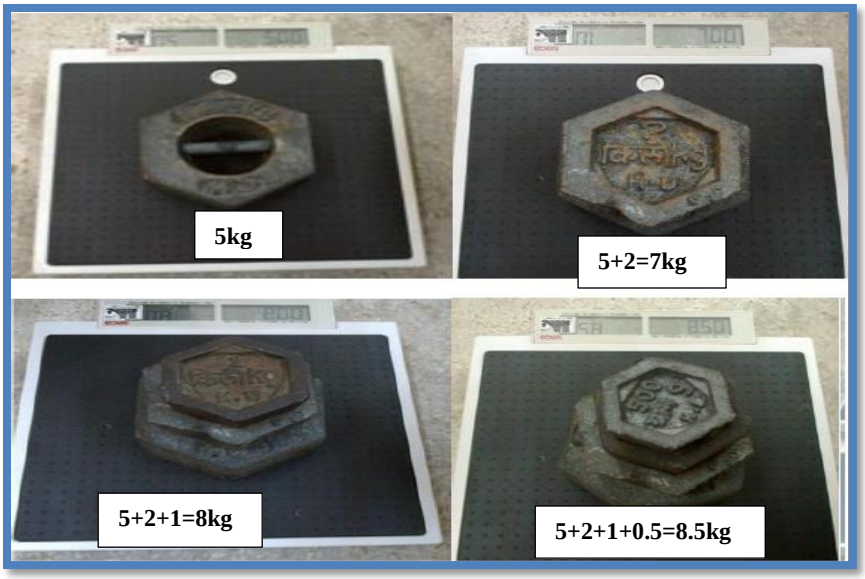

Plate 4.15: Testing accuracy of weighing balance using standard weights

- By weighing two persons of varying weights five times in the test balances and comparing it with the weight of the same person weighed using the standard balance. The acceptable variation between the weight recorded by the standard weighing balance and the test weighing balance was ± 0.1 kg. The five measurements recorded for each individual, in the same weighing balance should be within ± 0.1 kg.

  (https://www.youtube.com/watch?v=Dla4oYnV0Es)

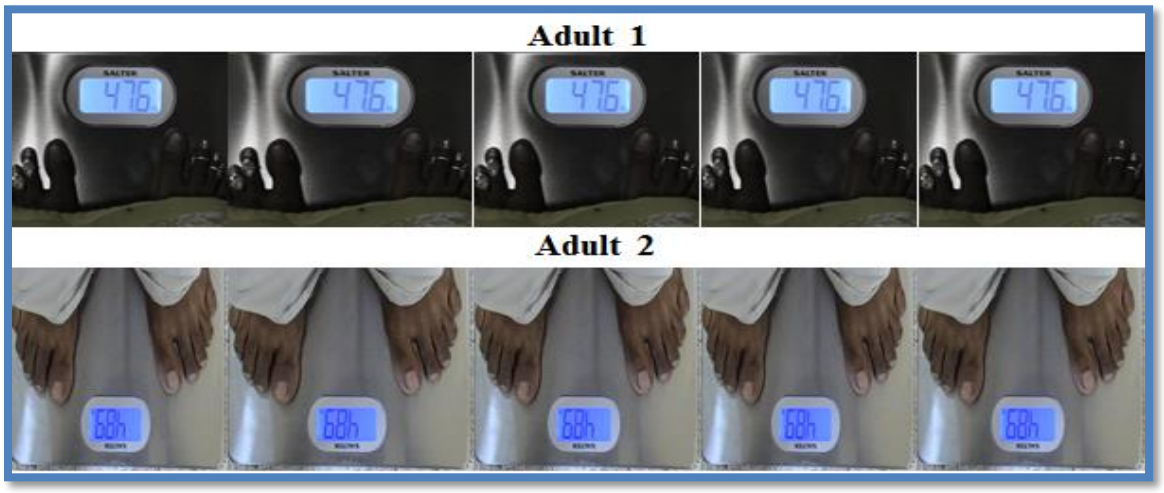

Plate 4.16: Testing accuracy of weighing balance by weighing two adults

4.11.2.2 Checking the sensitivity of the weighing balances

To check the sensitivity of the digital weighing balance initially a volunteer was asked to stand on the weighing balance and the weight was recorded. Then the volunteer was requested to carry the standard weight of 5kg, and then standard weights of 2kg, 1kg, 0.5kg, 0.2kg and 0.1kg were added one by one. At each step, the weighing balance should show the weight of the volunteer plus the standard weights. The acceptable variation of the combined weight of the volunteer and the standard weights was ± 100g. (https://www.youtube.com/watch?v=Dla4oYnV0Es)

Plate 4.17: Testing sensitivity of balances (adult + standard weight)

4.11.3 Blood Pressure

Automated digital blood pressure monitor is fully automated and provides systolic, diastolic blood pressure and pulse rate readings.

Plate 4.18: Automated digital BP monitor

4.11.3.1 Measurement of blood pressure using automated digital blood pressure monitor

The air plug was inserted into the air jack at first. The subject should sit comfortably on a chair. The instrument was put on a level with the heart of the person whose blood pressure was to be measured. The subject's arm was put through the cuff loops. The bottom edge of the cuff was 1 or 2 cm above the elbow. The marker (arrow under tube) was centered on the middle of the inner arm. The Velcro fastener was closed after the cuff snugly encircled the upper arm. Then the cuff inflated automatically after pressing the START / STOP button. After reaching the required level of inflation, the cuff would start to deflate automatically. After the full deflation the reading of systolic, diastolic pressure and pulse rate, shown on the monitor was recorded. The entire procedure was repeated after five minutes.

In a home situation at community setting, if a chair and table were unavailable then the subject was made to sit on the floor. BP apparatus and arm were kept on a cot, sofa or stool so that the heart, arm and BP apparatus were in the same horizontal plane.

4.11.4 Haemoglobin estimation

Anaemia in pregnancy has been recognized as a major public health problem with adverse impacts on the mother-child dyad since the 1960s.

For this study, the haemoglobin was estimated using cyanmethaemoglobin method, the gold standard method for estimating Hb.

4.11.4.1 Estimation of haemoglobin by cyanmethaemoglobin method

The cyanmethaemoglobin method can be of two types- direct method and indirect method (https://www.youtube.com/watch?v=-5EpYmU0erc). Indirect method was used for this study.

Indirect Method:

Estimating haemoglobin by cyanmethaemoglobin method required the following steps:

Sample collection and storage: 20µl blood sample was drawn using a Hb pipette, either from the finger prick blood sample of a woman or from an intravenous blood sample collected in tubes containing anticoagulants.

For collection of the blood sample from finger prick, the following items were required:

- Ether and cotton - to wipe the finger;
- Lancet for pricking finger;
- 20 µl pipettes for blood collection,
- Filter paper for collection of blood spots and pencil to number the filter paper for identification; and
- Drabkin's solution and distilled water to rinse the pipette, and ether to dry the pipette after collection of the sample.

Plate 4.19: 20 µl pipette and lancet

Plate 4.20: Drabkin's solution with 5ml dispenser

In the indirect method, the 20 µl pipetted out blood was then deposited on the filter paper on which the identification of the subject was labelled in pencil and the stained filter paper was then allowed to dry. After getting fully dried the filter paper was stored in a zip-lock bag.

Plate 4.21: Pipette containing 20 µl of blood

Plate 4.22: Filter paper with blood

As soon as the blood was deposited on the filter paper, the pipette was rinsed twice with Drabkin's solution and then the remaining Drabkin's solution was blown out into tissue paper/cotton. Then the pipette was rinsed twice in distilled water and the remaining distilled water was blown out into tissue paper/cotton. Next, the pipette was dried by pipetting ether twice and blowing that out. Once the pipette was dry, that was ready for use.

Estimating haemoglobin of the collected blood sample with colorimeter:

As the first step, the standards for Hb were run in the colorimeter and the standard graph depicting optical density deviation on the x-axis and Hb on Y-axis was prepared.

For Hb estimation, the test tubes were labelled, for identification, with a marker; 5 ml Drabkin solution was dispensed with the help of the dispenser. The filter paper containing dried blood was put in the test tube containing 5ml Drabkin solution; and waited for 30 minutes; then it was checked whether the blood in the filter paper had fully eluted.

The colourimeter was switched on at least 15 minutes before use. A cuvette containing 2 ml Drabkin solution (blank) was placed in the cuvette holder of the colourimeter and the reading of the colourimeter was set to zero. The reading of the colourimeter should range within ±0.02 after removing the blank. The Drabkin's solution in which blood had fully eluted was transferred into a fresh cuvette, and was placed in the cuvette holder of the colourimeter to record the reading. The optical density is directly proportional to the haemoglobin content of the sample. Using the standard graph, the haemoglobin value at the optical density deviation was read.

Plate 4.23: Colourimeter with voltage stabilizer

Preparation of standard graph: A standard graph was prepared at regular intervals. For this, a set of five solutions with different fixed concentrations were made by mixing a standard haemoglobin solution and Drabkin's solution. Each solution was of 2ml. The standard haemoglobin solution was available in the market and the concentration was 15g/dL. The set of five solutions was of the following concentrations- 15g/dL, 12.5g/dL, 10g/dL 7.5g/dL, 5g/dL and 2.5g/dL. Then the optical density of these solutions was measured in a colourimeter in which the reading of the samples was to be taken to estimate the haemoglobin levels and the obtained optical densities were plotted against the concentrations of the solutions respectively. After plotting the points were joined and the line should be straight.

This standard graph helped to estimate the haemoglobin concentration of an unknown sample. The standard graph also helped to calibrate the colourimeter.

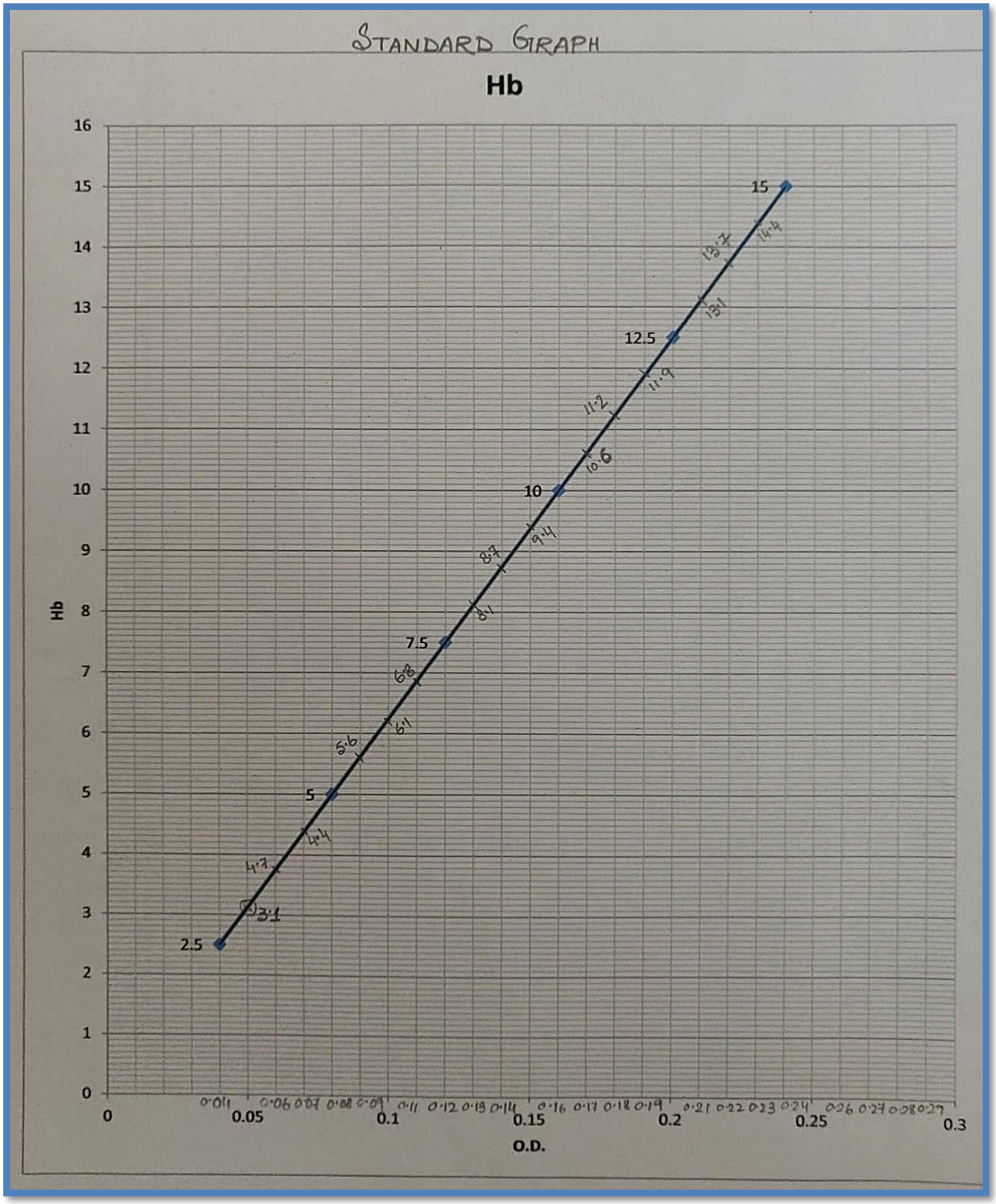

Figure 4.9: Standard graph for haemoglobin estimation

Accuracy checking: 1. The accuracy of the pipette was tested against a standard pipette. The expected accuracy of the colorimetric reading was ± 0.01 OD.

2. The accuracy of the 5ml dispenser was tested against a standard dispenser. The expected accuracy was the volume of ± 0.1 ml or the colourimetric reading ± 0.01 OD.

3. The accuracy of colourimeter was tested against a standard colorimeter. The expected accuracy of the colourimetric reading was within ± 0.01 OD.

Precautions: 1. At the time of blood sample collection, the finger to be pricked was wiped only with ether and not with alcohol/ spirit because alcohol denatures proteins. If alcohol was used for wiping the finger and then blood was collected on filter paper, the blood spot would not elute fully.

2. During pipetting, excess blood was removed using a tissue paper and was never blown out.

3. Blood in the pipette was fully blown out on to a filter paper.

4. During pipetting, air bubbles in the blood column in the pipette were carefully eliminated.

Direct Method:

The direct method of estimating haemoglobin by cyanmethaemoglobin method is the same as the indirect method, except after the collection of the 20 µl blood sample, is directly transferred into a test tube contacting 5 ml drabkin solution and not transferred onto a filter paper. The test tube is labelled with the identification of the sample before the transfer of the sample into it.

4.11.5 Diet survey using 24-hour recall

Collecting detailed information about all food items cooked in the family and consumed by various members of the family in the previous 24-hour using a structured interview is known as 24-hour dietary recall.

4.11.5.1 Collecting data on dietary intake by 24-hr recall method

A diet survey using 24-hour recall (Thimayamma and Rao, 2016) was done in a sub-sample in the primary health care institution (PHCI) institutions as well as in the community setting.

A diet survey should not be done immediately after any festival or a day when a fast was observed by any member of the family or holiday. On the day of feasting people consume special food and even sometimes consume more food than regular days; the days of fasting are when people either do not consume any food or consume less quantity of fruits, milk and foods specific for "Vrat" (fast). Thus, the day on which we collected diet survey data by 24-hour recall method, was specifically chosen and carefully avoided any day preceded by feasting or fasting. There are some festivals in different religion, which continues for several days, on which fasting or feasting may be observed. Data were not collected during those festival times if any family was celebrating those occasions. If any special social phenomena like a wedding ceremony, pre-decided occasions like a picnic, get-together or death in the family were noted, the investigator avoided conducting a diet survey.

After ensuring the comfort of the subject, she was asked about the food prepared and consumed on the previous day from early morning till bedtime. Data was collected on the type of food prepared, the raw ingredients used with its quantity and the total volume of the cooked item. If any food item was bought from outside the quantity purchased and the quantity used on the particular day was recorded. The total quantity of the cooked food item was recorded along with the left-overs. Then the information on the amount of each item of food consumed by individual members at different times was collected. This provides the intra-family distribution of the volume of the cooked food. Utilization of the left-over food also was recorded; if it was consumed later in the same day, given to someone else or wasted. Food bought from outside was recorded. Investigators in the current era have to enquire about the food intake from outside sources in addition to home-cooked food shared by the members of the family. This is especially important in

pregnant women who may be provided either with cooked food or ready-to-eat food under different food supplementation programmes.

It is also essential to probe and get information from the individual about the frequency and quantity of snacks, beverages and sweets purchased outside and consumed on the previous day and over the last week. This probing will not be able to provide an accurate estimation of all the nutrients consumed but can provide some idea about energy consumption and frequency of consumption of high-fat and high-sugar snacks and beverages in pregnant women. This may come in very useful in providing appropriate nutrition counselling according to the nutritional status of the women.

After collecting the data, it was again cross-checked with the subject so that no data was missed out. After collecting the data, the food consumed by the individual members of the family was converted into nutrients to assess the nutrient consumption.

Single day 24-hour dietary recall may not give very accurate information about the dietary intake of the individual but the alternative of the use of three-day recall (one on holiday and two on weekdays) was not feasible either in the community or in hospital settings. Collecting data using 24-hour dietary recall method takes on average 30 to 45 minutes to be completed properly and the subjects were not ready to spare such a long time and spend it with the research team.

Plate 4.24: Recording information on dietary intake by 24-hour recall method

4.11.5.2 Standardisation of the utensils used to collect the data

NFI has a set of utensils which were pre-standardised and labelled accordingly. This set of utensils was used for the current study. For standardising the utensils, measuring cylinders and water were used to measure the volume of the utensils and according to the volume the utensils were labelled. The water was first poured into the utensils and then the water was very carefully transferred to the measuring cylinder, and the measured volume was then recorded and labelled on the utensils measured. The measuring procedure was repeated to crosscheck the volume and avoid any measurement error.

Plate 4.25: Standardized and labelled utensils to collect information on dietary intake by 24-hour recall method

4.12 Data entry and data cleaning

The data was entered using MS excel. Efforts were made to enter the data on a day-to-day basis i.e. entering the data on the same day on which it was collected.

To ensure uniformity of data, cleaning of the same was done carefully.

4.12.1 Allotting unique identification number

All the subjects were allotted a unique identification (UID) number. The subjects enrolled in the primary health care institution setting were given serial numbers as they were enrolled. Subjects enrolled in the community setting were given an eight-digit number as

an UID number. The first digit indicated the area code, the second and third digits together indicated the Anganwadi number, the fourth, fifth and sixth digits together indicated the household number, the seventh digit indicated the sequence of the subject in the household and the eighth digit indicated if the subject was women, child, men. For this study, the subjects were pregnant women so the last digit was always "2".

4.12.2 Calculating Gestational age

In the study group almost, all women were able to give their LMP accurately at enrolment. Majority of them had an ultrasound confirmation of the gestational age in the second trimester. It was therefore possible to compute gestational age with reasonable accuracy.

4.12.3 Comparing nutrient intake with ICMR-NIN 2020 recommendations

Due to increasing Basal Metabolic Rate (BMR), pregnancy weight gain and maternal placental and foetal tissue formation, pregnant women require additional calories and nutrients. Expert Group on Nutrient Requirements recommended 350 Kcal of additional energy for pregnant women using the factorial approach. The report by the Expert Group on Nutrient Requirements has recommended the EAR of nutrients for women with a pregnancy weight of 55 kgs and pregnancy weight gain of 10 to 12 kgs. The actual mean height of the population from which the subjects for the current study was drawn is 151.7 cm, thus the calculated optimal weight is 48 kg. The calculated EAR for the NPNL women is 1450 Kcal. The mean weight gain during pregnancy in the current study population is around 8 kg. Taking this into account the additional requirement during pregnancy is only 250 Kcal and the total energy requirement is 1700 Kcal. Similarly, EAR was calculated for the other selected nutrients.

4.13 Data analysis

The data analysis was done using MS Excel.

Frequency distribution and percentage calculations were done for the variables of SDP, obstetric profile, nutritional profile and haemoglobin status.

Mean, standard deviation and percentages were calculated for the continuous variables (e.g. compliance, weight gain in pregnancy, maternal anthropometric indicators & birth weights, post-pregnancy weight gain, pregnancy outcome and dietary data collected by FFQ and 24-hour dietary recall methods); centiles and tertiles were calculated for weight gain during pregnancy.

The T-test was used to assess the statistical significance of the difference in means between groups and the chi-square test was used to assess the statistical significance of comparison in categorical variables between groups.