2.1 Place of study: This study was conducted at Itaunja. Itaunja is a town and a nagar panchayat in Lucknow district of the north Indian state of Utter Pradesh (fig 2.1). The distance of Itaunja and Lucknow is approximately 25 Km. Geographically, the town is located between 27.08° North latitude and 80.92° East longitude at a height of 124 meters above mean sea level. Itaunja is characterized by an unplanned construction of separate houses, mostly made of mud (kachcha houses) or cement blocks (pucca houses) or mix but some were made from reeds and flattened oil drums and discarded metallic advertisement boards. In some of the places there were double-storey houses. As per the 2001 India census report, Itaunja had a small population of 6249. Males and females account for 54% and 46% of the population. In Itaunja, 15% of the population is less than six years of age. Itaunja has a literacy rate of 61%. Out of it the female literacy rate is 54%.

2.2 Study design: This cross-sectional study was planned and conducted in various villages of Itaunja, involving 147 randomly selected females of the age group 15-65 years using biomass fuel for domestic cooking and exposed to its emissions. This village had no industrial activities. One hundred and two control subjects were selected from such areas of Lucknow which had nearly the same environmental conditions, approximately the same socio-economic status and matching age, height and weight but not using the biomass fuel for domestic cooking and thus not exposed to emissions of bio mass fuel. These
subjects were using clean fuels e.g. LPG or electric stove for domestic cooking. The project was cleared by Institutional Human Ethics Committee. Verbal consent was taken from each subject.

Fig. 2.1- The map of Lucknow district showing the location of ITAUNJA.
2.3 **Sample size**: Sample size was calculated assuming prevalence of overall morbidity from the previous studies. Level of significance \( \alpha = 0.05 \) and the precision of the estimate \( d = 0.05 \) were considered while calculating the sample size.

2.4 **Selection of exposed and non exposed subjects**: A representative sample comprising females of 15-65 yrs of ages was selected through random sampling technique. The subjects using *pan masala*, tobacco and receiving treatment for more than one month were not included in the present study.

**Criteria of inclusion for the exposed:**
- Cooking on bio mass fuel, Age >15 years
- Residence of local area, willing to participate

**Criteria of exclusion for the exposed:**
- Cooking on clean fuel, Occasional cooking
- Migratory subjects, Age < 15 years
- Subjects not willing to participate

**Criteria of inclusion for the control:**
- Cooking on clean fuel, Age >15 years
- Residence of local area, willing to participate

**Criteria of exclusion for the control:**
- Cooking on bio mass fuel, Age <15 years
- Occasional cooking, Migrant subjects
- Subjects not willing to participate
2.5 **Statistical analysis: Data Analysis:** The data of completed questionnaire were entered onto the computer daily in MS excel sheet which made it possible to check inconsistencies and update information from the respondent in the field. Data-analysis was carried out, using the SPSS statistical package (SPSS Inc., Chicago, IL). \( t \)-tests were used for comparisons between means in exposed and controls groups. Analysis of variance was also carried out to compare the means where numbers of groups are more than two. Statistical significance of prevalence of sign and symptoms pertaining to various symptoms in exposed and control groups was done using Chi square test after ascertaining the fact that expected cell frequencies are more than five. Fisher exact test was used where expected cell frequencies were less than five.

2.6 **Measurement of height and weight:** Height was measured in centimeter by portable stadiometers against a vertical surface. Body weight was measured to 0.1 kg in light clothing without shoes using body fat analyzer.

2.7 **Measurements of body mass index (BMI):** The BMI (kg/m\(^2\)) was calculated by body fat analyzer (HBF 357/352, OMRON Ltd, Japan), based on bioelectric impedance analysis, among subjects >18 years of age (fig. 2.2). BMI of <18 years was calculated manually from height and weight measurements. The height, age and sex were given as inputs in the body fat analyser. The subject was asked to stand upright (after cleaning the sole) on foot electrodes of the instrument and hold the arm electrodes (posture looks like holding the handle of scooter while driving). By pressing the respective keys of body fat analyser, the reading displayed on the screen can be viewed and entered in the register. The parameters measured were body fat (BF %), visceral fat level and BMI. The following precautions were taken during measurement:
1. Subjects should not carry metallic object like coins, watch etc.
2. Subjects should not carry mobile phones or any other electronic devices.
3. Pregnant women and patient using pacemaker were restricted to use the instrument.

2.8 **Calculation of Socio-economic status:** The Socio-Economic Status (SES) of respondent was calculated by modified Pareek’s socio-economic scale for rural area. It has a twenty-point scale questionnaire and classified the class of subjects. *(Appendix – II)*

2.9 **Calculation of life time exposure by cumulative exposure index (CEI)** *(Regalado et al (2006):* An estimate of the lifetime exposure from cooking fuels was made. It was made by details of cooking history like no. of cooking of meals, duration of cooking in years and no. of hours expert per meal per day consumed for cooking, and type of fuel used. An estimate of the lifetime cumulative exposure from cooking fuels was assessed by calculating cumulative exposure index (EI) in hour-years. It was computed as the product of the exposure with the fuel, the duration of daily cooking, and the number of years it had been used. Location and ventilation of kitchen were also recorded.

2.10 **Team for interview of subjects:** The survey was undertaken by a team comprising scientists having medical degree with post graduate diploma having twenty year experience in conducting environmental health survey, one scientist having the doctorate degree and female post graduate students. The questionnaire was developed in English, based on a questionnaire used in previous environmental studies. Subjects were free to withdraw their participation from study at any time. In no case was there any indication of false reporting. Presence of a chaperone/ female volunteers was arranged to avoid medico legal problems.
Fig. 2.2- Body Fat Analyser (HBF 357, Omron) for measuring Body Mass Index, Body Fat%, Visceral Fat Level
2.11 HISTORY TAKING: Arranged the seating in a non-confrontational way to see my subject easily and gain eye contact. The first non-verbal communication was assessed. After that verbal communication occurred.

2.11.1 The physical state: A quiet, well ventilated and well-lit room was arranged for physical examination for subjects. Source of light was sunlight in day time during sunny days. Attempts were made to reassure and relax the subject. Picked-up non-verbal cues from the subject e.g. distress, mood, behavior and change in body language during interview. The mental and emotional status (restless and sweating palms) of subjects was assessed.

2.11.2 The physique: Is she tall, fat, thin or muscular according to her age? The obvious deformity was noted. Visible obesity and malnutrition were recorded.

2.11.3 The skin: Inspected the skin for pallor (anemia), yellowness, dark yellow, cyanosis, cutaneous eruptions etc. Noted the various types of body smells e.g. the sweetness of breath, the smell of wound or ulcers.

2.11.4 Facial expression, complexion and general behavior: Facial expression and eye-to-eye contact was made to find out the physical and psychological well-being. The unusual skin colour was noted.

2.11.5 Confidentiality: The subject’s information was kept confidential and secured. The details are not being shared with anyone.

2.11.6 Abnormality in speech/ sound: Found out and noted any abnormality in speech.

2.12 History: Face to face interview (in Hindi) was taken of each subject. Here I asked full identification, food habit, educational status, marital status,
occupation including field work and part time work. A complete history of present illness including main complaints with duration was noted. Detailed past history with duration was also recorded.

2.12.1 **Family history** included type of family, total number of family members including adult and children. The total family income including person’s income in the form of rupees and terms was also noted. General health of family was also recorded to find out the chronic diseases running in the family. Any mortality in the family for the last 5 years was noted down to know the diseases running in the family or factors which caused death in the family.

2.12.2 **Menstrual history**: A detailed menstrual history was recorded. Menstrual history included age of menarche (age of menstruation began), if menstruation has ceased- asked how long it was absent, age of menopause (cessation of menstrual cycle for at least one year) in older subjects, length of cycle (interval of two cycles in days), duration of cycle or bleeding duration (number of days cycle run), amount of flow (number of pads used per day), regularity of cycle, complaints during period, history of miscarriage/ still birth/premature delivery was noted, abnormalities in the children born; date of last delivery. Noted gynecological problems if any e.g. type of discharge, blood stained secretion or any growth etc.

2.12.3 **Collection of environmental information**: General information: It included type of houses, number of rooms/ partition in the house, visible pollution, ventilation, sources of drinking water etc. Distance of water ponds/ animal shed if the source of water is hand pump (IM2/shallow).

2.12.4 **Location of kitchen and type of stove**: Location (separate kitchen or kitchen located within the room), type of kitchen, ventilation and area of kitchen
was recorded. Type of stove used for cooking was also noted down e.g. use of improved chulha, indigenous chulha (made of mud- Fig. 2.3) / LPC Stove etc.

2.12.5 Information of micro pollution including social history: History of smoking, alcohol consumption, and tobacco use was taken. These subjects were not included in the study.

2.12.6 Information of macro pollution including the history of fuel consumption: History of fuel consumption e.g. type of fuel used for domestic cooking (LPG, wood, cow dung cake etc.), cooking periods, and number of cooking time was taken.

2.12.7 Occupational history: A thorough occupational history including all occupations was noted to find value-added economic activities within the community. Many women were involved in various types of jobs in unorganized sectors. A few females and adolescent girls were involved in cottage industry work e.g. basket-making, stitching, decorative items etc. The women and adolescent girls were also engaged in collecting traditional fuels (e.g. wood, biomass etc.), etc. Some women were also engaged in animal tending. They also collected manure of their animals which were brought home for making dung. History of use of protective devices was also asked. All the information were noted in the questionnaire.

2.12.8 Present status of health: By asking many questions about all system organs, I noted all complaints mentioned in case sheet with duration, how it started, and when subjects was asymptomatic. Asking such questions I helped me to find out the symptoms not described by the subjects previously. A detailed respiratory history was obtained in subjects complains of respiratory problems. It included history of dry/ productive cough with its duration, frequency, time, relation to season, cough associated with fever, relieving factor etc, history of
sputum production including its color/ blood stained, quantity etc, breathlessness and its time, tightness in chest, history of allergy etc.

Detailed history of skin disease was recorded in subjects having skin problems. It included types of problem, areas affected, predominial symptoms, history of discharge, aggravating/ relieving factors etc.

The history of eyes problems was also recorded. This includes irritation in eyes, redness, itching, pain in eyes, lacrimation (watery discharge), sticky eyes etc. Vision impairment if any was also recorded.

**Fig. 2.3- Chulha (u shaped construction made of mud)**
2.13 **General Examination:** Physical examination was performed by following methods with the help of stethoscope, torch, measuring tape, ophthalmoscope, sphygmomanometer, tendon hammer, tuning fork, cotton wool and magnifying glass, diagnostic set etc.

2.13.1 **Measurement of arterial pulse:** Radial pulse was measured in beats per minute (bpm) by placing three middle fingers over the radial pulse. I used the pad of our fingers to assess the beats, rhythm and volume. It is measured by counting over a time-period of 15 seconds and is multiplied by four. While counting radial pulse, one should also be careful in following pulse variables: pulse pressure, whether it was regular or irregular, pulse deficit present or not, the type of pulse (Mehta and Joshi, 2008).

2.13.2 **Measurement of blood pressure** (Hutchison, Clinical Methods, 2007): Blood pressure is measured indirectly by mercury sphygmomanometry. Rest the subjects for five minutes. Support the subjects’ arm at about heart level. Apply the cuff to the upper arm with the centre of the bladder over the brachial artery. Palpate the brachial pulse. Inflate the cuff until the pulse is impalpable. Inflate the cuff another 10mm Hg. Now deflate the cuff slowly until regular sounds are heard. Note the reading to the nearest 2mm Hg. This is systolic pressure. Continue to deflate the cuff slowly until sounds disappear. This is diastolic blood pressure. Before measurement the following precautions should be kept in our mind. Mercury of sphygmomanometer should be at zero level. The mercury should not strike in inner wall of the mercury tube. A bladder length should be 30-35 cm and width should be 12 cm. The meter of mercury should be perpendicular to the earth level.
2.13.3 Examination of heart

**Auscultation of heart sounds:** Auscultate heart for 1st and 2nd (S₁ & S₂) heart sound, extra heart sounds (3rd & 4th – S₃ & S₄ heard in diastole, pericardial rub, murmurs in systole and/or diastole).

**Method of auscultator of heart sounds:** Allow the patient to sit comfortably at approximately 45° angle to horizontal, listen over the precardium at the base of the heart, apex and upper left and right sternal edges. Identified the 1st and 2nd heart sounds and assessed their character and interisity. Noted splitting of second heart sound. Listen for added sounds and then for murmurs.

2.14 EXAMINATION OF RESPIRATORY SYSTEM

Respiratory system starts from anterior nares to the terminal alveoli.

2.14.1 Examination of nose: Examining the external surface and appearance of the nose. Noted any skin disease. Examine the anterior nares including nasal vestibule, nasal septum for any bleeding point, crusting and perforation etc. The lateral wall and its mucosa examination were done for its hypertrophy, swelling and redness of mucosa. Asked the number of sneezing per day. Relationship of sneezing with cooking fuel was also recorded. I asked the disturbance in smell. Is she not feeling smell, reduced sense of smell or unpleasant smell? Examine for any facial swelling. Tenderness was also noted.

2.14.2 Examination of throat

**Inspection:** Asked subject to open the mouth for examination of oral cavity and oropharynx, using torch and tongue depressor looking for surface appearance and asymmetry in the tonsils or any redness around it. Difficulty in
opening of mouth was noted if present. Change in the quality of voice was noted if any. It might be due to inhalation of kitchen smoke.

I examined subjects for 5 principal symptoms of respiratory diseases: (1) cough, (2) sputum production including blood stained sputum (3) chest pain (4) breathlessness i.e. dyspnoea and (5) wheezing. Apart from these, I also examined them for respiratory rate, respiratory rhythm, and movement of chest and use of accessory muscles etc.

(1) **Cough:** Type of cough (bovine, cough with hoarseness) and cough syncope were noted. Asked about cough associated with hoarseness and strider or any chronic dry cough. A history of nocturnal cough causing sleep-disturbance was also enquired into.

(2) **Sputum production:** History of expectorated respiratory secretions (sputum or phlegm) with following specifications e.g. amount of phlegm in teaspoons produced per day, time of sputum production and colour was recorded. A separate history of blood stained sputum, its amount, duration and frequency was also obtained.

(3) **Chest Pain (Chest wall pain):** The complaint of chest tightness was noted to rule out COPD or asthma.

(4) **Breathlessness:** A complete history of breathlessness inappropriate to the level of physical including its mode of onset, duration, progression was recorded. Whether it was present in lying condition or breathlessness that waked the subjects from sleep. The time of breathlessness and its severity was noted i.e. breathlessness was more during cooking time or breathlessness was improved at non cooking time.
(5) **Wheeze:** History of wheeze was obtained. Wheeze is a high pitched whistling sound produced by the air passing through narrowed small airways. Whether wheeze occurs on resting or during cooking/after cooking or wheeze causing night wakening.

**Respiratory rate:** Observed the movement of chest carefully while feeling the pulse and counted number of respiratory rate per minutes.

**Respiratory rhythm:** The respiratory rhythm was noticed whether it is cheyne-stroke respiration, kussmaul's respiration, Bilot's respiration or wheezing.

**Movement of chest and use of accessory muscles:** To find out the abnormal movement of the chest wall the movement of chest was inspected (unilateral or bilateral). The prominence of accessory muscles of respiration, alae nasi and sternocleidomastoids, platysma and pectoral muscles was noticed. Looked for cyanosis on lips and tongue if any.

2.14.3 **Auscultation of chest:** Auscultation is done to note the type of breathing plural or pericardial rubs murmurs etc. A stethoscope was placed firmly over the chest. Auscultate the chest on both sides. Anteriorly it was done from above the clavicle down to the 6th rib, laterally from axilla to 8th rib and posteriorly down to the level of 11th rib. In each area quality and amplitude of the breath were noted. Assessed the quality and amplitude of vocal resonance by asking the patient to say 'one, one, one' or 'ek do tin' (1, 2, 3). Noted added sounds if present. The pleural friction rub was noticed with the help of stethoscope and only on deep breathing at the end of inspiration and beginning of expiration by hearing creakling sound.
2.15 Examination of hands

**Examination of nail:** The nails including the nail bed of the subjects were examined. Pachyonychia (thick nail due to disturbance of the nail bed), oncholysis (separation of nail from nail bed), Onychomycosis (Fungal infestation of nail), Clubbing and koilonychias were recorded.

**Examination of fingers**

*Finger Clubbing:* Clubbing of fingers is observed for thoracic diseases. Clubbing can be confirmed by the following four criteria:

- Loss of normal angle between the nail bed;
- Increased nail bed fluctuation;
- Increased nail curvature;
- Increased bulk of the soft tissue over the terminal phalanges.

To examine finger clubbing I first looked across the nail and nail bed at the 'nail bed angle'. Nail bed fluctuation was done by placing both thumbs under the pulp of terminal phalanx and attempt to move the nail within the nail bed using our index fingers. A 'spongy feel' confirms nail bed fluctuation.

**Tremors:** Regular or irregular distal movements having an oscillatory character are classified as tremors. Asked subject to hold out the arms with the hands extended at the wrist. This posture is periodically dropped, usually every 2-3 seconds and then resumed resulting in a jerky, flapping tremors.

2.16 Examination of cervical lymph glands: From behind, examined the submental, sub mandibular, preauricular, tonsillar, supraclavicular and deep cervical glands in the anterior triangle of the neck. From the front of the subject, examined the posterior triangle up to back of neck and occipital nodes. While examining lymph nodes following points were noted.
Number of lymph nodes, position, size, shape, surface, skin over lymph node, local temperature, tenderness, surface margin (discrete or indistinct), mobility, consistency, matted together or separate, number, fixity, location and extent (Das, 1976). Also examined the cervical supraclavicular lymph nodes and scalene lymph nodes from behind with the subject sitting. The head of subject was titled to examine side and palpated scalenus lymph nodes.

2.17 Examination of thyroid gland: It was done by inspection and palpation.

**Inspection:** Noted down the position, extent, size, shape, surface, condition of overlying skin. Asked the subjects to swallow and watch whether swelling moves on swallowing.

**Palpation:** Thyroid gland felt from the front as well as from behind with the subject’s neck slightly flexed. During palpation the following points were recorded: position, extent, size, shape, consistency, pulsation, mobility, relation with the neighboring structure, position of the trachea, the larynx, the infrahoid and sternomastoids muscle, subcutaneous tissue and skin.

2.19 Examination of eyes: The eyes were examined for redness, more diffuse redness in the eyes, yellow discharge, watery discharge, itching, cataract, any vision impairment etc. The inner eyelid was also examined for inflammation and redness and any discharge.

**Examination of sclera for jaundice and pallor:** The sclera, mucous membranes and skin were examined for jaundice. Asked the subjects to look downward. Elevated the upper eye lid. Yellow coloration of sclera showed presence of jaundice. Again asked the subjects to look upward. Pressed the lower eye lid. Reduced redness of sclera showed the presence of clinical anemia.
Examination of lens: The presence of lens opacities is suggested by the loss of the normally bright red reflex. The opacity of lens may be observed among subjects chronically exposed to biomass fuel smoke. The subjects were asked to look straight. A torch light is put on eye from the lateral side. A shining silver colored lens shows opacity of lens (cataract) associated with subjects’ complaint of vision impairment.

2.20 EXAMINATION OF CENTRAL NERVOUS SYSTEM

Examination of following cranial nerves

1. Olfactory nerve: It conveys the sense of smell. Asked the subject to close his eyes and shut one nostril with a finger. Presented chocolate and asked the patient to sniff. This showed the presence or absence of sense of smell.

2. Optic nerve: This tests the degree of loss of vision. It can be tested by the testing of visual field in each eye. Testing was done by asking the patient to read different letter-types or to count fingers. The field of vision can be roughly estimated by asking the patient to gaze straight ahead at a fixed object and then moving a small object from the periphery to the centre of the visual field, first horizontally and then vertically

3. Oculomotor nerve: On emerging from the brain, it divides into two branches, which enter the orbit through the superior orbital fissure. The superior ramus (smaller) and supplies the Rectus superior and Levator palpebræ superioris. The inferior ramus (larger) divides into three branches. One passes beneath the optic nerve to the Rectus medialis; another, to the Rectus inferior; the third and longest, to the Obliquus inferior. Any problem in oculomotor nerve
leads to disturbances in function of previously mentioned muscles. The impairment of the function can be assessed by the following method:

Examined the size and shape of the pupils for any abnormal dilatation. Reaction to light and accommodation was tested by throwing light on to the pupil. Asked the subject to look first at some distant object and then at the finger held in front of the eyes. Normally, the pupils contract in each case. The ocular movements was tested by fixing the head and asked to move the eyes ‘to the right’, 'to the left, upwards, and downwards as far as possible in each direction. Any limitation of movement was noted if any. Asked the subject about the number of objects seen double. Squint, ptosis or nystagmus was assessed.

4. **Trochlear nerve**: In the orbit the nerve innervates the superior oblique muscle. Any defect in this nerve impairs the function of superior oblique muscle i.e. impaired downward movement and outward movement of the eyeball. Diplopia (double objects) occurs when such movement is attempted.

5. **Trigeminal nerve**: The functions of trigeminal nerve include the sensation of the face, mouth and part of the dura and motor supply to the muscles of the jaw involved in chewing. This can be assessed in following ways:

*Assessment of motor function*: Asked the subjects to clinch her mouth and felt the masseter and the temporalis muscles. The paralysis of the pterygoids was assessed by asking subjects to open their mouth, the jaw deviate towards the affected side (paralysis of the pterygoids).

*Assessment of sensory function*: Sensations over the face supplied by the three divisions of the trigeminal nerve is lost. The sensation of the conjunctiva, nasal mucosa and anterior 2/3rd of the tongue also impaired.
It was done with a piece of cotton, a safety pin as described by H. K. Walker (1990).

6. **Abducens nerve:** The nerve supplies lateral rectus muscle. Any defect in this nerve leads to inability to move the eye outwards (laterally). The subject was advised to move eye outwards. When such movement was attempted, diplopia occurs.

7. **Facial nerve:** It supplies all the muscles of face except the levator palpebrae superiors. It also recognizes the taste of anterior 2/3rd of tongue. Any defect in this nerve may lead to change in taste of anterior 2/3rd of tongue or change in facial expression. The facial nerve impairment can be assessed by following manner.

   Asked the subject to show her teeth. On showing teeth, the angle of the mouth is drawn to the healthy side. (2) Asked the subject to close her eyes. Subject was unable to close the eye on the affected side and on attempting to do so the eyeball rolls upwards. (3) Asked the subject to move his eyebrows upwards; the paralysis side remains immobile and (4) Asked the subjects to smile. The mouth is withdrawn to the healthy side during smiling.

8. **Vestibulocochlear nerve:** This special sensory nerve consists of two components. One innervates the cochlea and subserves hearing; the other supplies the labyrinth and semicircular canals and subserves equilibration balance and sensation of bodily displacement. Any defect in this nerve may lead to complaints of 'ringing in the ear', vertigo (giddiness, dizziness or unsteadiness) or positional vertigo. To test the nerve defect a watch is gradually brought to the ear of the subject with the eyes closed; noted the distance at which she could hear.
9. **Glassopharyngeal nerve:** This nerve carries sensation from the pharynx and tonsils and sensation from the posterior third of the tongue. Asked subjects to swallow her saliva. Defect in this nerve may lead to difficulty in swallowing.

10. **Vagus nerve:** It innervates the muscles of palate, pharynx and larynx. Defect in this nerve may lead to regurgitation of fluid through the nose. Subject asked to cough. Subject cannot cough clearly (boring cough).

11. **Accessory nerve:** Defect in this muscle may lead to weakness of rotation of the chin towards the opposite side shows. The sternomastoid muscle was tested for paralysis by asking the subject to turn her face to the other side, while resistance is offered to the act by the hand over the chin. This shows the paralysis of sternomastoid muscles.

12. **Hypoglossal nerve:** The nerve supplies the tongue muscle. Asked the subject to put out her tongue and move side to side. Defect in this muscle leads to deviation of tongue, unable to move the tongue side to side properly.

2.21 **ASSESSMENT OF MOTOR SYSTEM:** The assessment of the motor system was considered under the following headings:

(1) Inspection and palpation of muscles

(2) Assessment of tone

(3) Examination of reflexes

(4) Testing movement and power, and

(5) Testing coordination.

(1) **Inspection and palpation of muscles:** Here I saw the wasting of muscles, fasciculation (irregular ripple under the skin), sudden shock like contractions (myoclonic jerks), oscillary movement of joints (tremors).
(2) **Assessment of tone:** To find out hypotonia or hypertonia (spasticity and rigidity) the assessment of tone is required.

(2) **Examination of reflexes:** The tendon reflexes may be

- hyperactive (+++),
- normal (++),
- diminished (+),
- absent (-).

Generally done at upper and lower limbs to find out the lesion at C₅ (cervical 5), C₇ (cervical 7), L₃ (lumber 3), L₄ (lumber 4), and S₁ (spinal 1) nerve.

(3) **Testing movement and power:** Conducted according to the guidelines of Medical Research Council Scale:

- 0- No muscle contraction visible
- 1- Flicker of contraction but no movement
- 3- Joint movement when effect of gravity eliminated
- 4- Movement against resistance but weaker than normal.
- 5- Normal powder

(4) **Testing Coordination:** It was done by finger nose test and heals.
2.22 LUNG FUNCTION STUDIES (SPIROMETRY)

Spirometry is a physiological test that measures how much volume an individual inhales or exhales at a defined time. The primary signal measured in spirometry may be volume or flow. Spirometry is invaluable as a screening test of general respiratory health. Spirometry can be undertaken with many different types of equipment, and requires cooperation between the subject and the examiner, and the results obtained will depend on technical as well as personal factors. If the variability of the results can be diminished and the measurement accuracy can be improved, the range of normal values for populations can be narrowed and abnormalities detected more easily. The Snowbird workshop held in 1979 resulted in the first American Thoracic Society (ATS) statement on the standardisation of spirometry (Renzetti, 1979). This was updated in 1987 and again in 1994 (ATS, 1987; 1995) and protocols mentioned in ATS statement were followed in the present study.

Lung function parameters like Peak Expiratory Flow rate (PEER) and Forced Expiratory Volume in 1 sec (FEV₁) were recorded, using calibrated Spirometer (PIKO-1, Ferraris Respiratory Europe Ltd, UK) (fig. 2.4) in standing position and manoeuvre recommended by American Thoracic Society recommendations (Pellegrino et al., 2005).

**Forced expiratory volume in 1 sec (FEV₁):** FEV₁ is the maximal volume of air exhaled in the first second of a forced expiration from a position of full inspiration, expressed in litres at basal temp pressure saturated with water vapours (BTPS).

**Peak expiratory flow (PEF):** It is the maximum expiratory flow achieved from a maximum forced expiration, starting without hesitation from the point
of maximal lung inflation, expressed in L/Sec [Quanjer et al., 1997]. When PEF is obtained with portable spirometer, it is expressed in L/min.

**Standard operating Procedures (major points) for recording lung function test**

- Check the spirometer calibration
- Explain the test and prepare the subject
- Ask about smoking, recent illness, medication use, etc.
- Measure weight and height without shoes
- Wash hands
- Subjects should have correct posture with head slightly elevated
- Exhale with maximal force
- Place mouthpiece in mouth and close lips around the mouthpiece
- Exhale maximally until no more air can be expelled while maintaining an upright posture
- Repeat instructions as necessary, if coaching vigorously
- Repeat for a minimum of three manoeuvres; no more than eight ones are usually required.
- Check test repeatability and perform more manoeuvres as necessary.

Throughout the manoeuvre, enthusiastic coaching of the subject, using appropriate body language and phrases, such as “keep going”, is required. It is particularly helpful to observe the subject with occasional glances to check for distress, and to observe the tracing or computer display during the test to help ensure maximal effort. If the patient feels “dizzy”, the manoeuvre should be stopped, since syncope could follow due to prolonged interruption of venous return to the thorax. This is more likely to occur in older subjects and those with airflow limitation. (Stoller et al., 1993). Well-
fitting false teeth should not be routinely removed, since they preserve oropharyngeal geometry and spirometry results are generally better with them in place (Bucca et al., 2001).

**Summary of acceptable blow criteria:** The researcher should observe that the subject understood the instructions and performed the manoeuvre with a maximum inspiration, a good start, a smooth continuous exhalation and maximal effort (ATS, 1995).

**Fig. 2.4-** PiKO-1
2.23 HEMATOLOGICAL AND BIOCHEMICAL STUDIES

Collection of samples: A few days before the visit, people were informed about the visit. They were explained the whole procedure of the blood and urine collection, objectives, merits & demerits of the collection. Individuals were taken into confidence before conducting any procedure, so that they may not be afraid of the procedure and be cooperative with us. The first-aid box was ready for providing the first aid. The Medical officer of Community Health Centre, Itaunja, was informed about our aim and visit, and requested to extend hospital facilities if required. The place chosen was a clean room. The subjects were informed about the confidentiality of their reports.

Blood collection

Preparation: Materials required for the blood collection:
- Sterilized, disposable, hypodermic syringes -5ml
- Sterilized cottons
- Tourniquet
- Coded heparinized and non-heparinized vials
- Ethyl alcohol
- Ice box with ice cubes.

Method: The blood was collected with the procedure called phlebotomy by a trained lab technician using all the precautions. Hand gloves were used.
- Ethyl alcohol was applied on the left arm of the subject over the area of medial cubital vein.
• Tourniquet was applied on the left upper arm of the subject slightly above the elbow joint.
• A dry, sterilized, disposable hypodermic syringe of 5 ml was used to withdraw the blood. Needle of the syringe was smacked in the skin over the site of the vein. Three (3) ml of blood was withdrawn and transferred in to coded non-heparinized vial. Tourniquet was removed and pressure is applied for few minutes.
• Blood slides were prepared for differential count, and were stained with Leishman stain. The hematological parameters were done as per Dacie and Lewis (1975).

• **Separation of serum:** Processing was done on the same day. The vials containing blood were left for one hour at 37°C. After that samples were kept at 4°C overnight (for clot to contract). Loosen the clot from the sides of the tubes with the help of glass pasteur. Centrifuged the serum at 4000 rpm for 20 minutes. Removed the serum from the clot by pipetting off into the clean tubes with the help of a glass pasteur. Coded the specimens, its volume and date of collection and stored at -20°C. (http://www.protocol-online.org/cgi-bin/prot/view_cache.cgi?ID=3096).

Serum was transferred into eppendorf tubes for further analysis of the following four parameters with the fully automated biochemical analyzer (Chemwell 1520, USA) (Fig 2.5).
(a) Serum glutamic oxaloacetic transaminase (SGOT), or Aspartate aminotransferase (AST)
(b) Serum glutamic pyruvic transaminase (SGPT), or Alanine aminotransferase (ALT)
(c) Creatinine, and (d) Glucose.
(a) Serum glutamic oxaloacetic transaminase (SGOT), or aspartate aminotransferase (AST):

**Principle:** AST or GOT catalyses the reversible transfer of an amino group from aspartate to \( \alpha \)-ketoglutarate forming glutamate and oxaloacetate. The xaloacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

\[
\text{Aspartate} + \alpha \text{-ketoglutarate} \rightarrow \text{Glutamate} + \text{Oxaloacetate} \\
\text{MDH} \\
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{Malate} + \text{NAD}^+
\]

The rate of decrease in the concentration of NADH measured photometrically is proportional to the catalytic concentration of AST present in the sample.

**Procedure:** Kit was provided with \( R_1 \) buffer (containing TRIS pH 7.8, 80mmol/L; L-aspartate, 200mmol/L) and \( R_2 \) substrate (containing NADH, 0.18 mmol/L; lactate dehydrogenase [LDH], 800 U/L; malate dehydrogenase (MDH), 600U/L; \( \alpha \)-ketoglutarate, 12 mmol/L). Working reagent was made by dissolving
one tablet of R₂ substrate in one vial of R₁ buffer followed by mixing. One (1.0) ml of working reagent was taken in reaction vial with 100 µl of sample and incubated for one minute at 37°C temperature. Read initial absorbance (A) of the sample at 340nm, start the stopwatch and read absorbance at 60 second intervals thereafter for three minutes. The difference between absorbance and the average absorbance (ΔA) differences per 60 seconds (ΔA/60s) was calculated and multiplied by factor 1750. Value is expressed as activity in U/L of AST (Ref value- up to 31 U/L) (Murray, 1984a).

(b) Serum glutamic pyruvic transaminase (SGPT) or Alanine aminotransferase (ALT):

Principle: GPT or ALT catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutarate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH:

\[
\text{ALT} \\
\text{Alanine} + \alpha\text{-ketoglutarate} \rightarrow \text{Glutamate} + \text{Pyruvate} \\
\text{LDH} \\
\text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{Lactate} + \text{NAD}^+
\]

The rate of decrease in the concentration of NADH measured photometrically is proportional to the catalytic concentration of ALT present in the sample.

Procedure: Kit was provided with R₁ buffer (containing TRIS pH 7.8, 100mmol/L; L-alanine, 500mmol/L) and R₂ substrate (containing NADH, 0.18 mmol/L; lactate dehydrogenase (LDH), 1200 U/L; α-ketoglutarate, 15 mmol/L). Working reagent was made by dissolving one tablet of R₂ substrate in one vial of R₁ buffer followed by mixing. One (1.0) ml of working reagent was taken in reaction vial with 100 µl of sample and incubated for one minute at 37°C temperature. Read initial absorbance (A) of the sample at 340nm, start the stopwatch and read absorbance at sixty second intervals thereafter for three
minutes. Calculate the difference between absorbance and the average absorbance \((A)\) differences per 60 seconds \((\text{delta}A/60s)\) was calculated and multiplied by factor 1750. Value is expressed as activity in U/L of ALT [Ref value- up to 32 U/L] (Murray, 1984b; Burtis et al., 1999; Tietz et al., 1995).

(c) Serum creatinine (Jaffe’s method):

**Principle** The assay is based on the reaction of creatinine with sodium picrate. Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents. The intensity of the colour formed is proportional to the creatinine concentration in the sample.

**Reagents**

\[\text{R}_1\] - Picric reagent-picric acid (17.5mmol/l)

\[\text{R}_2\] - Alkaline reagent-sodium hydroxide(0.29mol/l)

Creatinine cal-creatinine aqueous primary standard (2mg/dl)

**Working reagents:** Mix equal volumes of \(\text{R}_1\) picric reagent and \(\text{R}_2\) alkaline reagent. The working reagent is stable for ten days at 15-25°C.

**Procedure:**

1. Assay conditions
   - Wavelength – 492 nm
   - Cuvette – 1cm light path
   - Temperature - 37°C/15-25°C
2. Adjust the instrument to 0 with distilled water.
3. Pipette into a cuvette.

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
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<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard(µL)</td>
<td>--</td>
<td>100</td>
<td>--</td>
</tr>
<tr>
<td>Sample (µL)</td>
<td>--</td>
<td>--</td>
<td>100</td>
</tr>
</tbody>
</table>

4. Mix and start stop watch.
5. Read the absorbance \(A_1\) after 30secs and after 90secs \(A_2\) of the sample.
6. \(\Delta A = A_2 - A_1\)
Cal = ΔA sample/ΔA standard x 2(standard conc.) = mg/dl of creatinine in the sample.
Conversion factor- mg/dl x 88.4 = µmol/l

[Ref value- 0.6-1.1 mg/dl or 53.0-97.2 µmol/L]

(d) Serum glucose (Trinder’s GOD-POD method):

Principal: Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H₂O₂), is detected by a chromogenic oxygen acceptor, phenol, 4-amino phenazone (4-AP) in the presence of peroxidase (POD)

\[
\begin{align*}
\alpha-D-glucose & \xrightarrow{\text{Mutarotase}} \beta-D-glucose \\
\beta-D glucose + O_2 + H_2O & \xrightarrow{\text{GOD}} D-Gluconic acid + H_2O_2. \\
H_2O_2 + phenol + 4-AP & \xrightarrow{\text{POD}} \text{Quinone (quinonemine)} + 4H_2O.
\end{align*}
\]

The intensity of the colour formed is proportional to the glucose conc. in the sample.

Reagents:  R-Tris pH 7.4 (92mmol/l)
Phenol (0.3mmol/l)
Glucose oxidase (GOD) (15000u/l)
Peroxidase (POD) (1000u/l)
4-Aminophenazone (4-AP) (2.6mmol/l)
Glucose cal- glucose aqueous primary standard (100mg/dl).

Procedure
1. Assay conditions
   Wavelength – 505nm
   Cuvette – 1cm light path
   Temperature – 37°C/15-25°C
2. Adjust the instrument to 0 with distilled water.
3. Pipette into a cuvette.
4. Mix and incubate for 10min at 37°C. Read the absorbance of the samples and standard against the blank. The colour is stable for at least 30min.

**Calculation (as per kit):**

\[
\text{(A) sample} \quad \frac{X}{\text{(standard concentration)}} = \text{mg/dl glucose in the sample.}
\]

\[
\text{(A) standard}
\]

Conversion factor: \(\text{mg/dl} \times 0.0555 = \text{mmo/L}\)

[Ref value- Fasting: 70-110 mg/dl;  
Post-parandial: 80-140 mg/dl  
Random: 60-140 mg/dl]

(Kaplan, 1984; Trinder, 1969; Barham and Trinder 1972).
2.24 BIOCHEMICAL STUDIES OF URINE

Urine samples of female subjects were collected and analyzed for the qualitative presence of urobilinogen, glucose, pH, protein, bilirubin by SD Urocolor strips (Fig 2.6). SD Urocolor are plastic strips to which are attached several separate reagent areas.

Fig. 2.6- SD uro color strips

Fig. 2.7- Uro meter-600
Preparation for collection of urine:

Clean and covered place for sample collection.

1. Disposable sterile coded specimen container (70 ml capacity).
2. Zip envelops for keeping specimen.
3. Ice box containing ice cubes.

Method: (http://www.webmd.com/a-to-z-guides/urine-culture)

- The subjects were instructed to wash the part properly with water and then collect the urine in the vials after discharging small amount of urine i.e. mid stream sample (MSS).
- Coded specimen container transferred into another ice box containing ice cubes.

Precautions:

1. The specimen container should be held from bottom side while collecting specimen. The finger should not touch the specimen.

2. The menstruating subjects were exempted from collection of urine.

Procedure: One strip taken from bottle and completely emerge the test area of the strip in the urine and remove immediately (to avoid the dissolving out reagent). While removing, run the edge of entire strip against the rim of urine container and put the strip on its slide. Remove excess urine with the help of tissue paper and gauge. Hold the strip in horizontal position to prevent the mixing of chemical from adjacent test area or contamination the hand with urine.

Insert the strip in to the urine analyzer (Uro meter-600) (Fig. 2.7) carefully for obtaining the result. After test strip is accepted by urine analyzer, it is measured by means of reflectance photometry. The results are automatically calculated in terms of normal, negative, positive or concentration values.
2.25 AIR MONITORING:

The indoor air quality was conducted in the houses near chulha (earthen stove) at breathing zone with the help of battery operated analyzer, Haz-dust Environmental Particulate Air Monitor model EPAm- 5000, from Environmental Device Corporation USA for measuring PM$_1$ and PM$_{2.5}$ and electrically operated Aeroqual Air Quality Monitor (IQM 60) model number 2602009-024, from Auckland Newzeland for measuring CO, CO$_2$, NH$_3$, relative humidity and temperature. The average values were taken as the final result. Instruments were placed 0.5 m above the ground and at least 0.5 m away from walls and 1 meter away from kitchen (sources of pollutants).

Fig 2.8- Air monitoring near chulha (earthen stove)