CHAPTER –IV

MATERIALS AND METHODS

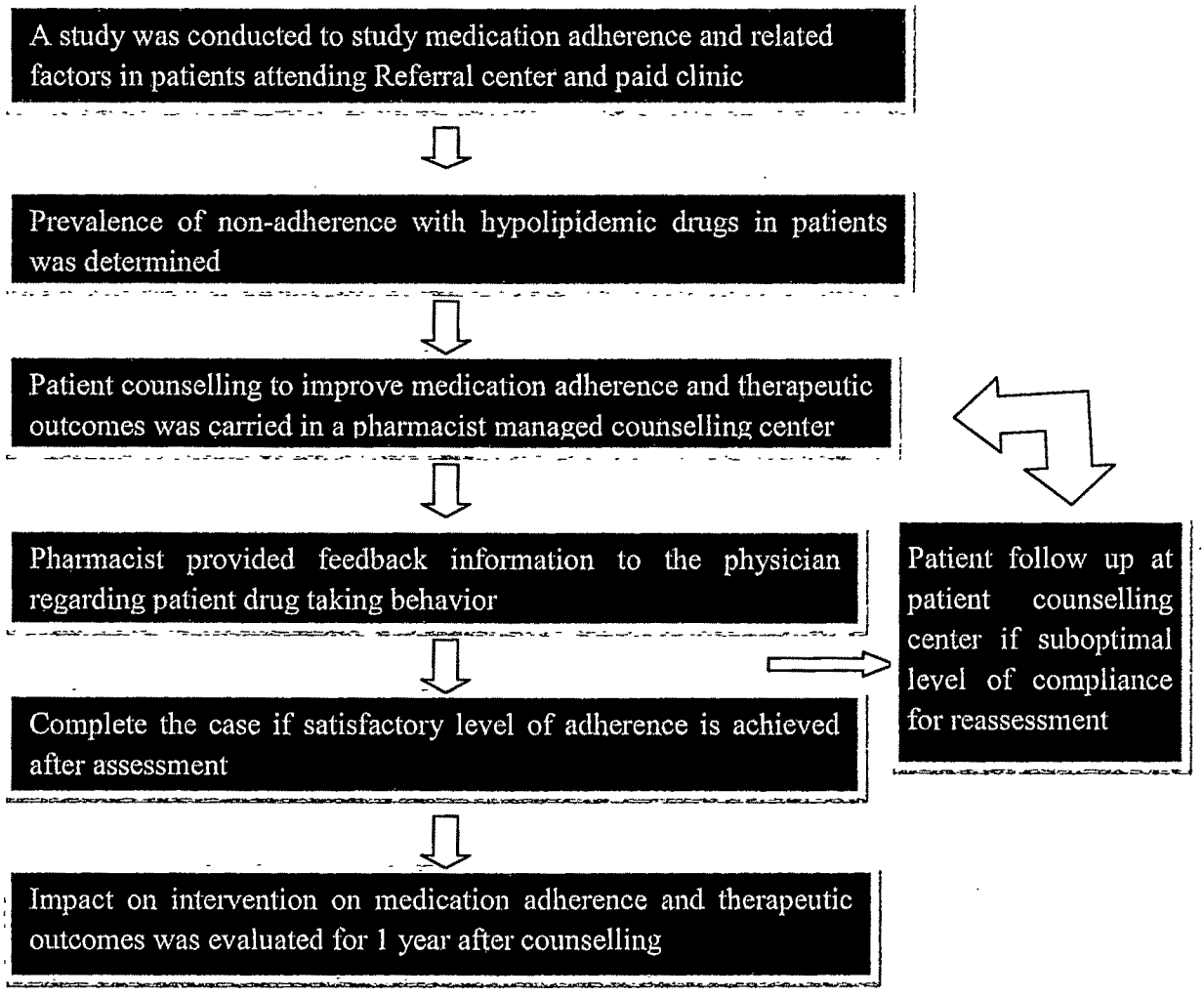
Study site.

The study was conducted at Rajiv Gandhi Institute of Medical Sciences a 1000 bed teaching hospital situated in Kadapa. This hospital provides primary and specialized health care facilities to people in Dr.Y.S.R Kadapa district of Andhra Pradesh of India serving over two million populations from all social classes.

Study design

This study comprises of two parts. The first part was a patient survey to examine the prevalence of non-adherence problem and associated factors in hyperlipidemia patients. Figure 5 shows an overview of the study design.

Figure. 5. Study design outline



The study group consists of 853 patients with hyperlipidemia according to NCEP and accomplice inclusion and exclusion criteria (NCEP, 2002).

Inclusion criteria

Hyperlipidemic patients with LDL-C more than 100mg/dl and HDL-C less than 40mg/dl, attending to Rajiv Gandhi Institute of Medical Sciences (RIMS), Kadapa from November 2008 to December 2010 and have been receiving hypolipidemic drug treatment for at least one year and having same treatment regimen for the past three months.

Exclusion criteria

1. Medications were not administered by the patient himself/herself e.g. administered by attendent.
2. Patients had marked dementia or other unstable psychiatric illnesses.

Study design: A prospective comparative study in a tertiary care teaching hospital.

Place of study: Rajiv Gandhi Institute of Medical Sciences (RIMS), Putlampalli, Kadapa -516004, Andhra Pradesh.

4.1. Patient medication adherence survey

In the first part of the study, a patient survey was conducted to study the prevalence of medication non–adherence and the associated factors in a cohort of patients treated with hypolipidemic agents.

Healthcare system has potential to influence patient medication adherence. It affects the quality, availability, accessibility of services, and also the communication and relationship between physicians and patients. Therefore, we recruited patients attending to referral center and paid clinic to examine the impact of the healthcare system on medication adherence as well as other patient knowledge and health beliefs.

All the patients were out-patients. Ethical clearance from the Institutional Human Ethics committee of Rajiv Gandhi Institute of Medial sciences, Kadapa was taken for the study. The purpose of the survey was explained to patients and informed consents (Annexure 1) were obtained. Each participant's hospital identification number was recorded at the time of presentation, and patient's data were subsequently taken from

their hospital charts and entered into standard data collection form (Proforma) designed for the study (Annexure 2).

The questionnaire consisted of eight sections to collect different types of information including patient demographic data, disease history, treatment regimen, medication adherence assessment, experience of adverse drug effect, patient's health knowledge and beliefs, functional status, patient's comments on health advice provision, and satisfaction with the medical service. COOP/WONCA functional health assessment charts were adopted to assess patient's functional status. The charts covered a core set of functional aspects including physical fitness, feelings, daily and social activities, change in health, and overall health. It is easy to use and is suitable for use in patients with chronic diseases (Andres *et al.*, 1995; Sneeuw *et al.*, 1999; Joao Mazzoncini de Azevedo-Marques *et al.*, 2011; Marta Zwart *et al.*, 2011). The Telugu version of the charts used in our study was developed and validated. For assessment of patient's knowledge and health beliefs, we referred to the Health Belief Model framework (Becker and Maiman, 1975 Becker, 1976). Questions are developed according to the some notable proportions including perceived susceptibility (refer to question 24 in questionnaire), perceived risk (refer to questions 27 and 28 in questionnaire). Perceived benefits (refer to question 29 in questionnaire), and perceived barrier and cost (refer to question 30 in questionnaire). Table 9 lists the parameters collected in the patient's survey.

Table.9. Parameters collected in patient medication adherence survey

Patient demographics:	Age Sex Smoking status Alcohol drinking Educational level Occupational status Duration of hyperlipidemia
Details of lipid lowering regimen:	Duration of hypolipidemic drug treatment Number of hypolipidemic drugs prescribed Number of other chronic drug use prescribed
Assessment of medication adherence:	Knowledge of drug dosage Knowledge of dosing frequency Amount of drug not taken from last 2 weeks Attempt to self-modify the treatment Forgotten to taken the medication any reasons
Patients health knowledge and beliefs:	Whether patient thinks that he has hyperlipidemia, Whether patient know the reasons for taking hypolipidemic treatment Whether patient knows about long-term of treatment Whether patient know increase risk of developing myocardial infarction and heart failure if hyperlipidemia is not treated Whether patient knows the effectiveness of hypolipidemic therapy in lowering hyperlipidemia, Whether patient knows the effectiveness of hypolipidemic therapy in lowering cardiovascular risk, Whether patient thinks drug taking as a waste of money as the patient feels he doesn't have hyperlipidemia.
Patient functional status (COOP/WONCA charts):	Physical fitness Daily activities Social activities Change in health Overall health
Patient satisfaction with medical services provided and relationship with the physician:	Provision of different types of health advice. Physicians are friendly to patients Physicians are listening to words of patients Patients are feeling ease to tell the physician about their problems Patients overall satisfaction with the medical service.

To assess patient's knowledge and health beliefs, functional status and their satisfaction with medical service, a five-point scale was used for data collection (Marjorie *et al.*, 2000; Jeffrey Jackson *et al.*, 2001). Responses were categorized ranging from 1 (strongly agree) to 5 (strongly disagree) for patient's health beliefs assessment. Patient were asked to rate their functional status on the scale form 1 (excellent) to 5 (poor). For patient satisfaction, patients recorded the extent to which they were satisfied ranging from 1 (very satisfied) to 5 (very dissatisfied).

Patients were randomly allotted to two groups, based on health care setting visiting. 516 patients in referral center group and 337 patients in paid clinic group.

Study groups

Group	**Hyperlipidemia patients (number)**
Referral center	516
Paid clinic	337

4.2. Pharmaceutical care Intervention:

The second part was a pharmaceutical care intervention to study the effectiveness of patient counselling in improving patient's medication adherence and control of surrogate endpoints, notably cholesterol levels and metabolic indices. Based on the findings from the first part of study regarding the problems and factors associated with patient medication non–adherence, appropriate counselling and patient follow–up to target these identified problems were delivered through the set-up of a patient counselling center.

Physicians were consulted and discussed about the implementation of patient counselling center to promote the concept of quality improvement. Patients attending as out-patient to referral center with problems in taking their drugs were referred to the Patient counselling center by their attending physicians. These patients have different chronic medical diseases such as diabetes, hyperlipidemia, hypertension, congestive heart failure, and ischemic heart disease. For the purpose of the present study patients with hyperlipidemia were focused. A consecutive cohort of 200 patients with hyperlipidemia

among all patients referred to the counselling center were identified and studied. Patient eligibility was confirmed by review of prescription.

Inclusion criteria

All hyperlipidemia patients with LDL-C more than 100mg/dl and HDL-C less than 40mg/dl, attending to Rajiv Gandhi Institute of Medical Sciences (RIMS), Kadapa from January 2011 to December 2011, identified by health care professionals to have adherence problem with antilipidemic drug treatment.

Exclusion criteria

1. Medications were not administered by the patient himself/herself e.g. administered by attendant.
2. Patients had marked dementia or other unstable psychiatric illnesses.
3. Patients were unable to receive pharmacist education at the counselling center due to functional impairment or other reasons.

4.2.1. Patient counselling center description:

The Patient counselling center was set up adjacent to the out-patient pharmacy of the Rajiv Gandhi Institute of Medical Sciences, Kadapa. The center is a private area of approximately 150 square feet for individualized counselling and education.

Baseline information from study subjects who were referred for pharmacist intervention was collected and individualized counselling and education was conducted. Patient medication adherence and other drug taking behavior were documented using structured monitoring forms (Appendix 3). Data collected included patient demographic data, details of prescribed antilipidemic regimen, assessment of medication adherence, knowledge on indications, directions and precautions of drug use, special drug administration skills, use of over-the-counter and other systems of medicine medications, experience of adverse effects from antilipidemic drugs, health beliefs, other chronic disease control, and dietary and physical exercise interventions. Table 10 lists the parameters collected by pharmacist at the counselling center. On reassessment, pharmacist documented changes in patient medication adherence and other drug-related problems identified, if any.

Table.10. Parameters collected by pharmacist at patient counselling center

Patient demographics:	Age Sex Smoking status Alcohol drinking Educational level Occupational status Presence of functional impairment Duration of hyperlipidemia
Details of therapeutic regimen and drug allergies	
Health beliefs problems:	Whether patient thinks he have hyperlipidemia Whether patient know the reasons for taking hyperlipidemia treatment Whether patient knows about long-term of treatment Whether patient know increase risk of developing myocardial infarction and heart failure if hyperlipidemia is not treated Whether patient knows the effectiveness of hyperlipidemia therapy in lowering hyperlipidemia Whether patient knows the effectiveness of hyperlipidemia therapy in lowering cardiovascular risk Whether patient thinks drug taking as a waste of money as the patient feels he doesn't have hyperlipidemia
Assessment of medication adherence:	Knowledge of drug dosage Knowledge of dosing frequency Amount of drug not taken from last 2 weeks Attempt to self-modify the treatment
Provision of other health advice:	Dietary intervention Recommended physical exercise
Management plan:	Details of adherence problems Details of drug related problems Management plan for the identified compliance and drug related problems

Patient teaching tools such as patient information leaflets, and medication explanation charts (Appendix 5&6&7) were used and provided if necessary. Monitoring form was developed to document the relevant information for appropriate follow–up and data analysis. Important information regarding patient's drug taking behavior was reported to their attending physicians to assist in formulation of disease management plan. The impact of the pharmacist education on patient's medication adherence, control of surrogate endpoints including reduction of serum cholesterol levels and utilization of healthcare resources including rate of hospitalization, duration of hospitalization and usage of causality was examined for a period of one year and compared to the respective data collected one year prior to referral to the Patient counselling center. Improvement in glycemic control and blood pressure control was examined in patients with concurrent diabetes and/or hypertension.

Besides educating the treatment regimen, patient's blood pressures were measured and BMI of the patient was calculated in the counselling center whenever appropriate. A thorough discussion with patients about their current control of hyperlipidemia as well as other chronic diseases such as diabetes and blood pressure and their treatment goals. Finally, lifestyle modifications including dietary interventions such as restriction of calorie and salt intake, regular physical exercise, weight reduction, cessation of tobacco use and alcohol use were reinforced. On average, each visit lasted 20-30 minutes.

If a patient failed to visit counselling center, he or she is contacted by telephone or meet personally. Following the counselling center visit, a telephone follow-up was arranged after 4 weeks. Reassessment of patient medication adherence and drug knowledge was performed in the subsequent visit usually 12-16 weeks later. Reinforcement by further counselling at counselling center would be arranged for those patients with suboptimal adherence in reassessment. A feedback about all the relevant information collected during these visits was given to the attending physician.

4.2.2. Outcome parameters after initiation of counselling and efficacy of patient education:

Hypolipidemic treatment in hyperlipidemia patients aids to reduce blood lipid concentrations, improve glycemic control, and reduce the occurrence and progression of cardiovascular diseases. Biochemical parameters of Hyperlipidemia patients attending patient counselling center were assessed during each visit. All laboratory tests including TC, HDL–C, TG, LDL– C, and FBS were performed for the patients. Blood lipid parameters were measured after an overnight fast of at least eight hours. Patients with hypertension had their blood pressure measured by sphygmomanometer, in the sitting position after resting for five to ten minutes by pharmacist.

Collection of samples

Ten milliliters of venous blood was drawn from each fasting patient (8-12 hours fasting). A tourniquet is placed 15cm above the cubital fossa and venous blood is aspirated slowly via the needle of syringe to prevent hemolysis.

Diagnostic kits used in the study:

- Serum total cholesterol diagnostic kit — Span diagnostics Ltd, Surat, India.
- Serum triglyceride diagnostic kit — Span diagnostics Ltd, Surat, India.
- Serum HDL cholesterol diagnostic kit — Span diagnostics Ltd, Surat, India.
- Serum glucose diagnostic kit — Span diagnostics Ltd, Surat, India.

Instruments used in the study:

- Centrifuge — Remi-India private limited, Mumbai.
- UV visible spectrophotometer — Shimadzu spectrophotometer (Model 1601)

4.2.2.1. Estimation of serum total cholesterol (Allain *et al.*, 1974)

Span diagnostic kit was used for the estimation of total cholesterol, which followed cholesterol oxidase/peroxidase (CHOD-POD) method.

Principle: The enzyme, cholesterol esterase catalyzed hydrolysis of cholesterol esters to free cholesterol and fatty acid molecules. Then free cholesterol gets oxidized in the presence of cholesterol to form cholesten-3-one and H_2O_2. Liberated H_2O_2 reacts with phenol and 4 AAP in presence of peroxidase to form red colored quinoneinine complex the intensity of which was measured at 505 nm.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{FA}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholesterol-3-one} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonimine}$$

Reagents used:

Sl. No.	Reagent composition	Conc. in the final test mixed
1.	Good's butter (pH 6.7)	50 mmol/l
2.	Phenol	5 mmol/l
3.	4 - Aminoantipyrine	0.3 mmol/l
4.	Cholesterol esterase	> 200 U/l
5.	Cholesterol oxidase	> 100 U/l
6.	Peroxidase	> 3 KU/l

Standard: The concentration of standard glucose used was 200mg/dl

Assay &Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Reaction parameters:

1	Reaction type	End point
2	Wavelength	500 nm
3	Optical path	1 cm
4	Temperature	37^0C
5	Measurement	Against reagent blank

Summary of assay details:

Pipetted in to test tube	**Blank**	**Standard**	**Test**
Reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl

The reaction mixtures were mixed well and incubated for 10 min at 37^0C. The absorbance of test and standard are noted against reagent blank at 505nm. The absorbance was measured by using a Shimadzu spectrophotometer (model 1601).

Calculation

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times (\text{Standard conc.}) = \text{----------mg/dL}$$

4.2.2.2. Estimation of serum triglycerides by glycerol-phosphate oxidase method

(Bucolo and David, 1973)

Span diagnostic kit was used for estimation of triglycerides, which followed end point colorimetric enzymatic test using glycerol-3-phosphate oxidase.

Principle: The enzyme, lipoprotein lipase catalyzes hydrolysis of Triglycerides to glycerol and fatty acids. Glycerol is then phosphorylated in an ATP - requiring reaction catalyzed by glycerophosphate. The formed glycerophosphate is oxidized to dihydroxyacetone and H_2O_2 in a glycerophosphate oxidase catalyzed reaction. H_2O_2 then reacts with 4 - Aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase to form colored quinoneimine complex, the intensity of which was measured at 505nm.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + 3\text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol-Phosphate Oxidase}} \text{DHAP} + H_2O_2$$

$$H_2O_2 + \text{4- Aminoantipyrine} + \text{Chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine}$$

The intensity of the color formed is proportional to the triglycerides concentration in the sample.

Reagents used:

Sl.No.	Reagent composition	Conc. in the final test mixed
1.	Pipes butter	50 mmol/l
2.	4-Chlorophenol	5 mmol/l
3.	Mg 2+	5 mmol/l
4.	ATP	1 mmol/l
5.	Lipase	> 5000 U/l
6.	Peroxidase	>1000 U/l
7.	Glycerol Kinase	>400 U/l
8.	Glycerol - 3- phosphate oxidase	>4000 U/l
9.	4- Aminoantipyrine (4-AAP)	0.4 mmol/l

Standard: The concentration of standard triglyceride used was 200mg/dl

Assay & Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Reaction parameters:

1	Reaction type	End point
2	Wave Length	505 nm
3	Optical length	1 Cm
4	Temperature	37^0C
5	Measurement	Against reagent blank

Summary of assay details:

Pipetted in to test tube	Blank	Standard	Test
Reagent	1000 µl	1000 µL	1000 µl
Standard	-	10 µl	250 µl
Sample	-	-	10 µl

The reaction mixtures were mixed well and incubated for 10 min at 37^0C. The absorbance of test and standard are noted against reagent blank at 505nm. The absorbance was measured by using a Shimadzu spectrophotometer (model 1601).

Calculation

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Standard conc} = \text{----------mg/dL}$$

4.2.2.3. Estimation of High Density Lipoproteins in Serum by Precipitation method

(Assmann *et al.*, 1983; Allain *et al.*, 1974)

Span diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase (CHOD-POD) method.

Principle: HDL-C is measured in the supernatant after the precipitation of the lipoproteins including chylomicrons, very low-density lipoproteins, low-density lipoproteins, intermediate-density lipoproteins directly from serum polyanions like phosphotungstic acid and along with $MgCl_2$ are added to an aliquot of serum an immediate heavy precipitation is formed. The precipitate then is sedimented by centrifugation and HDL cholesterol is measured in the clear supernatant, which is estimated by enzymatic method as described earlier in estimation serum of TC.

$$\text{Supernatant HDL-Cholesterol} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{FA}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholesterol-3-one} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonimine}$$

Assay & Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Standard: The concentration of standard HDL cholesterol used was 50mg/dl

Reaction parameters:

1	Reaction type	End point
2	Wave Length	500 nm
3	Optical length	1 Cm
4	Temperature	37^0C
5	Measurement	Against reagent blank

Standard of assay details:

1. 0.5ml of serum was taken into test tube and 0.5ml of precipitating reagent was added, mixed well and kept at room temperature for 1 5min.
2. Centrifuged for 15 min at 4000 rpm.
3. The clear supernatant was separated and immediately used to determine the cholesterol content as follows:

Pipetted in to test tube	**Blank**	**Standard**	**Test**
Precipitating reagent	1000 µl	1000 µl	1000 µl
Standard	-	100 µl	-
Supernatant from step3	-	-	100 µl

The reaction mixtures were mixed well and incubated for 10 min at 37^0C. The absorbance of test and standards was measured against the reagent blank at 505nm. The absorbance was measured by using a Shimadzu spectrophotometer (model 1601).

Calculation

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Standard conc} = \text{----------mg/dL}$$

4.2.2.4. Determination of VLDL- cholesterol

It was estimated from dividing the values of triglyceride concentration by 5.

4.2.2.5. Determination of LDL - cholesterol

The lipid profile does not measure LDL level directly but instead estimates it via the Friedewald equation.

Calculation

In mg/dl: LDL cholesterol = total cholesterol – (HDL cholesterol +VLDL).

4.2.2.6. Estimation of Serum Glucose: Span diagnostic kit was used for the estimation of glucose, which follows enzymatic colorimetric test using glucose oxidase (Trinder, 1969).

Principle: The enzyme, glucose oxidase catalyzes oxidation of glucose to gluconic acid with the simultaneous production of hydrogen peroxide (H_2O_2). H_2O_2 reacts with 4 aminoantipyrine and phenol in the presence of peroxidase to yield a re-coloured quinoneimine compound, the intensity of which was measured at 505nm and directly proportional to glucose concentration.

$$\alpha\text{-D-glucose} \xrightarrow{\text{Mutarotase}} \beta\text{-D-glucose}$$

$$\beta\text{-D-glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{D-gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonemine} + 4\,H_2O$$

Reagents used:

Sl. No.	Reagent composition	Conc. in the final test mixed
1.	Phosphate buffer(pH 7.4)	200 mmol/l
2.	Phenol	5 mmol/l
3.	4 - Aminoantipyrine	0.3 mmol/l
4.	Glucose oxidase	> 15 KU/l
5.	Peroxidase	> 3 KU/l

Standard: The concentration of standard glucose used was 100mg/dl

Assay &Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Reaction parameters:

1	Reaction type	End point
2	Wave Length	505 nm
3	Optical length	1 cm
4	Temperature	37^0C
5	Measurement	Against reagent blank

Assay details:

Pipetted in to test tube	**Blank**	**Standard**	**Test**
Reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl

The reaction mixtures were mixed well and incubated for 30 min at 37^0C. The absorbance of test and standard are noted against reagent blank at 510 nm within 60 min. The absorbance was measured by using a Shimadzu spectro photometer (model 1601).

Calculation:

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100 \text{ (Standard conc.)} = \ldots\ldots\ldots \text{ mg/dL glucose in the sample}$$

4.2.2.7. Other outcome parameters

Other outcomes measures were the healthcare resources utilization including frequency of hospital admissions, length of hospital stay, and frequency of causality visits. Details of hospital admissions (dates of admission and discharge, primary and secondary diagnoses/procedures) were obtained from the hospital medical records database management system and by review of patient medical records. The mean data collected during 1 year before referral to the counselling center was used as control for the patient and compared to data collected during 1 year after discharge from counselling center.

4.3. Statistical analysis:

General comparisons between groups were performed using Student's t-test for normally distributed continuous data. Non–parametric continuous data that could not be successfully transformed into normally distributed data were analyzed by Mann–Whitney test, McNemar's test whereas categorical data, such as gender were analyzed by Chi – Square (x^2) test.