THE CHENNAI URBAN POPULATION STUDY (CUPS) - METHODOLOGICAL DETAILS – (CUPS PAPER NO. 1)

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Field Surveys of diabetes and other non communicable disease (NCD) (hypertension, coronary heart disease) are increasingly being performed in both developed and developing countries [1]. Recent studies have shown that the prevalence of diabetes is very high among migrant Asian Indians [2] and is also rising very rapidly within the Indian sub continent [3]. Indeed, recent World Health Organisation reports show that India already has the largest number of diabetic patients in the world [4]. Indians also have very high rates of premature coronary artery disease [5,6]. Earlier studies have reported on urban-rural differences in the prevalence of diabetes among South Indians [7]. However it is has been assumed by the earlier studies that the "urban" population is homogeneous in its increased susceptibility to diabetes and other components of metabolic syndrome. However, even within the urban environment, there are wide socioeconomic differences which could considerably influence the prevalence of diabetes and its risk factors.

The Chennai Urban Population Study (CUPS) was started in July 1996. The aim of CUPS was to study the prevalence of diabetes, impaired glucose tolerance (IGT) and other features of the metabolic syndrome like insulin resistance, hyperlipidemia, hypertension and upper body obesity in two socioeconomically different sections of society within an urban environment and also to look for differences in risk factor profiles within these two strata of society.

Chennai (formerly Madras), in southern India is the capital of the Tamil Nadu state which has a population of 50 million people. The population of Chennai itself is about 6 million according to the latest 1991 census. Being a city, there are vast differences in the socio-economic strata of the people living within Chennai. This article will discuss the methodological details of the CUPS study.

Description of Population:

Two residential areas were selected, in different areas of urban Chennai, representing middle income and low income group. Asiad colony in Tirumangalam represents the middle income group, whereas Bharathi Nagar slum in T. Nagar represents the low income group. These colonies were chosen (purposive sampling) because of their social differences, geographic convenience and local support available, which would facilitate future incidence studies.

Study Design:

According to World Health Organisation (WHO), in epidemiological research to evaluate the prevalence rates of hypertension, diabetes mellitus, obesity and hyperlipidemia, which are now known to be inter-related disorders and share common risk factors, the most common study design is the cosssectional field survey, hence this was used in the CUPS population.

Training the team:

The epidemiology team consisted of a team leader (epidemiologist) and doctors, laboratory technicians, receptionists, dietitians and social workers from the M. V. Diabetes Specialities Centre, Chennai. All of the team members underwent an intensive training programme at the centre before the survey commenced so that it was ensured that each team member was instructed about the procedures that he/she performed in order to avoid the biases to the procedures employed. We conducted a pilot study which is not a part of the selected sample. The pilot study helped us to overcome the practical difficulties and to perform the actual survey smoothly.

Obtaining approval and pre-survey census [Phase 1 of CUPS]

The first phase of the study was a door to door survey of the families in the colony. A letter requesting participation was written to all families. It was decided to undertake a census of the selected households, immediately prior to the survey, as it provides an opportunity to inform the aim and objectives of the study and about the

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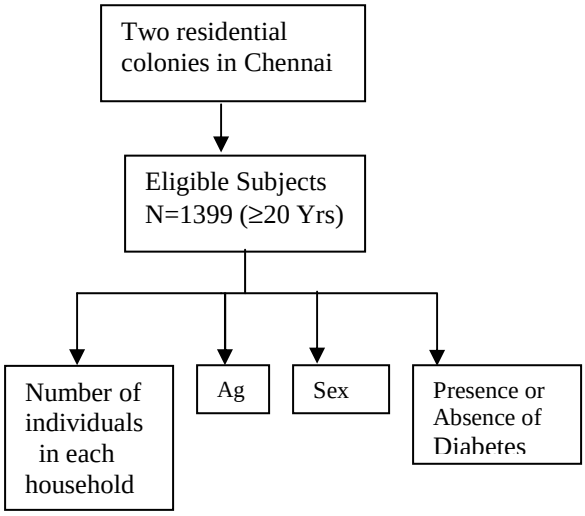
survey procedures, so that it would enhance co-operation. The basic census details including number of individuals in the household, age, sex and presence or absence of diabetes were obtained by using a structured interview [8] (Figure 1).

Figure 1: **Phase I of CUPS**



Conducting the survey [Phase 2 of CUPS]:

In the second phase of the study, all individuals above the age of 20 years living in these colonies were invited to participate in the screening programme. In order to standardise the blood glucose and lipid measurements, subjects were requested to attend the survey early in the morning, after an overnight fast of 8-12 hours. Each participant stayed for at least two hours. The study commenced at 7 am and the last subject entered the system at 10 am, so that all examinations could be completed by 12 noon.

The procedures are shown in the order in which they were performed in Figure 2 and are described in detail below.

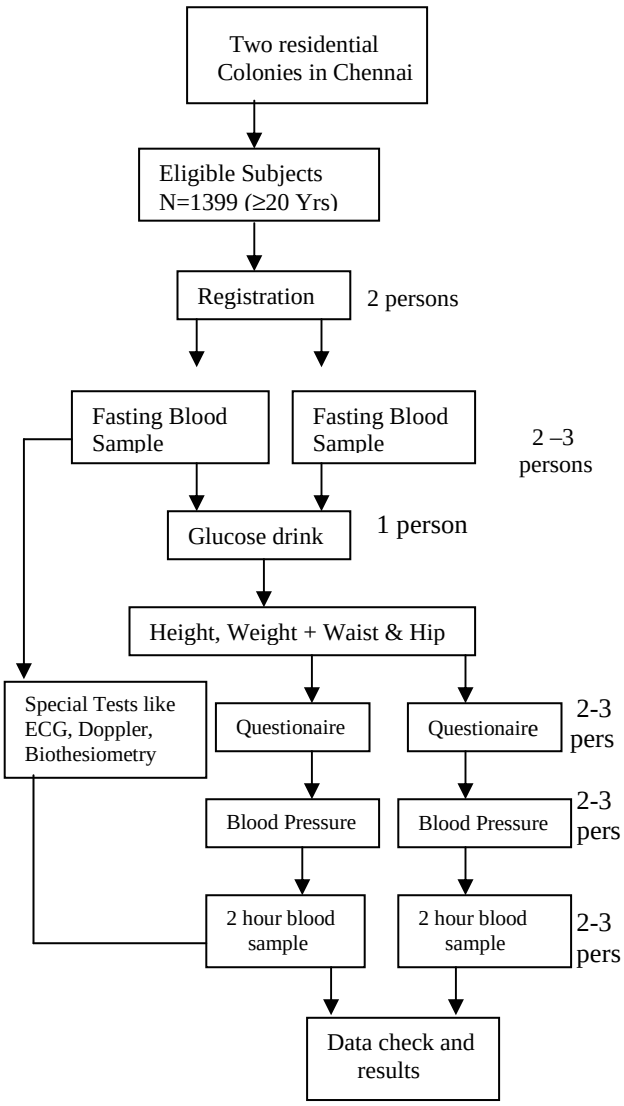
Each subject was located from the precensus list. A sequential survey number was given and this was written besides the name in the master list and then at the top of the individual proforma. At registration, basic personal information, including name, address, sex and age, were recorded on the form. The subject was then given the survey form and instructed to proceed from one department to another for the various examinations.

A fasting blood sample was drawn for blood glucose and lipid estimations. All the adults were given 75 gm of glucose dissolved in 250-300 ml water which was drunk over a period of upto 5 minutes.

Subjects who stated that they had diabetes, were given a standard breakfast and a post prandial blood sample was collected one and half hours after breakfast [9]. All subjects in whom the diagnosis was in doubt were advised to undergo the glucose tolerance test (GTT).

A two hour blood sample was drawn in all those who underwent the GTT and all the blood samples were transported to the M.V.Diabetes Specialties center, within a short period of time in ice packs, for the analysis, which was carried out immediately. Diagnosis of abnormal glucose tolerance was made according to the latest recommendations of WHO consulting group (Appendix 1).

Figure 2: **Phase II of CUPS**



All biochemical assays were carried out using a Corning Express Plus Auto Analyser (Corning, Medfield, MA, USA), using kits supplied by Boehringer Mannheim (Mannheim, Germany).

Fasting and post plasma glucose (GOD-POD method), serum cholesterol (CHOD-PAP method) and serum triglycerides (GPO-PAP method) were measured. HDL cholesterol was estimated by CHOD-PAP method, after precipitating low density lipoprotein and chylomicron fractions by the addition of phoshotungstic acid, in the presence of magnesium ions and very low density lipoprotein (VLDL). Low density lipoprotein (LDL) cholesterol was calculated using friedewald formula [10]. Plasma insulin levels were estimated by enzyme linked immunosorbent assay using the Dako Kit (Dako Diagnostics Ltd., Denmark). Glycosylated haemoglobin (HbA1c) was estimated by high pressure liquid chromatography using the Variant Machine (Bio Rad, Hercules, CA, U.S.A).

ANTHROPOMETRIC MEASUREMENTS:

Height

Height was measured by fixing a tape measure to a wall and measuring the height with a movable headboard, with measures to the nearest centimeter. Subjects were asked to stand upright without shoes, with their back against the wall, heels together and eyes directed forward.

Weight:

Weight was measured with a traditional spring balance which was kept on a firm horizontal surface. The ‘’Zero’’ was checked each day and calibration was done with reference to an individual of ‘’known’’ weight. The subjects were asked to wear light clothing and weight was recorded to the nearest 0.5 kg.

Waist:

Waist circumference was measured using a non stretchable fibre measure tape. The subjects were asked to stand erect in a relaxed position with both feet together. One layer of light clothing was accepted. Waist girth was measured at the mid point between the iliac crest and the lower margin of the ribs. Waist circumference was measured to the nearest centimeter.

Hip

Hip girth was recorded at the greatest trochanter (the widest portion of the hip) on both sides. Measures were made to the nearest centimeter.

SKIN FOLD THICKNESS:

Triceps

The subject stands with his/her back to the measure, with his/her left arm bared of all clothing. The tip of the shoulder is palpated and marked with a pen. With the elbow in the flexed position, a mid point between these two points (measured by the tape) is marked. With the tape in position, a horizontal line is marked on the skin both posteriorly (for triceps) and anteriorly (for biceps) at the level of the mid-arm point. A vertical line is then marked and the point of which its intersects the horizontal line already marked becomes the point of the cross at which the skinfold is to be measured. The subject is asked to flex and extend his/her elbow before applying the caliper. Thus, the muscle that has been picked up is pulled away from the skinfold by the contracting action of the triceps and this reading is noted.

Biceps:

The subject stands erect and faces the observer with the arm relaxed by her side. The horizontal line already marked anteriorly at the mid-arm point, and the point at which this line intersects the vertical line is the point of measurement of the biceps skinfold thickness. It has to be ensured that the palm is relaxed and the palm facing forward when the vertical line is marked. The subject is asked to flex and extend his/her elbow before applying the caliper. Thus the muscle that has been picked up is pulled away from the skinfold by the contracting action of the biceps and this reading is noted.

Subscapular skinfold thickness:

The subjects stands erect with her back to the measurer as in triceps skinfold measurement, with the shoulders and arms relaxed. The medial border of the left scapula is palpated downward until the inferior angle of the scapula is identified. The skin is marked immediately below the inferior angle of the scapula. The skinfold is picked up, though slightly inclined downward and laterally, along with natural tension lines of the skin. The skin fold caliper is then applied and the reading is noted as described.

Study Instrument:

A structured questionnaire was formulated and used in the selected population. Questions were asked by an interview method, in a standard manner, using local language and numbers recorded legibly, in appropriate boxes. The

subjects were asked about personal and family history of hypertension and diabetes. Questions were also administered concerning use of smoking and alcohol. The occupation was recorded according to a score at four levels – sedentary, light, moderate and heavy. Additional questions conserning income, education, physical activity, family history and related risk factorsof diabetes, hypertension, ischaemic heart disease, amputation and strokes, were also obtained from the individual.

Blood Pressure:

Blood pressure was measured after the administration of the questionnaire. The blood pressure (BP) was recorded in the sitting position in the right arm with a mercury sphygmomanometer. Two readings were taken 5 minutes apart and the mean of the two was recorded as the blood pressure. Patients were categorized hypertensive if they were on anti-hypertensive treatment or if they had a systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg. Systolic pressure was recorded at the first appearance of sounds, and diastolic pressure was measured at the disappearance of sounds. After measurement, the cuff was deflated and the measurement was then repeated. Diagnosis of hypertension was made according to the JNCV criteria [11] (Appendix 1).

ECG:

Resting 12 lead ECG's were performed on all subjects. Recording of electrocardiograms and classification according to the Minnesota code assessed the frequency of ischaemic heart disease in the population (12) (Appendix 1).

Doppler:

Doppler studies were done using the KODY Vaslab Machine (Kody Labs, Chennai, India) by a single observer. Blood pressure recordings were made of the brachial pulses in the upper limb. Similar recordings were made of the dorslis pedis and posterior tibial pulses in the lower limb by inflating the cuff proximal to the ankle and the mean of these two readings was taken as the ankle pressure. The ankle/brachial pressure (ABI) index ratio was calculated in every subject. Criteria for diagnosis of pheripheral vascular disease (PVD) was an ABI < 0.9.

Biothesiometry:

Neuropathy was assessed using a biothesiometery (Biomedical Instrument Co., Newbury, Ohio, U.S.A) by a single vibratory perception threshold (VPT) in a standardized fashion. Subjects removed their shoes and socks and lay supine on a couch. The biothesiometer tactor, which vibrates at 100 HZ, with an amplitude proportional to the square of the applied perpendicular to the test site, with a constant firm pressure. Subjects were initially familiarized with the sensation by holding the tractor against the distal plantar surface. VPT was then measured at the distal plantar surface of the right great toe. The voltage was slowly increased at the rate of 1V/S and the VPT was defined as the moment when the subject first felt the vibration. The voltage at which this occurred was recorded. Three further cycles of readings at each site were performed and recorded. Neuropathy was diagnosed if the VPT of the great toe was ≥ 25V.

For logistic reasons, Doppler and biothesiometry studies were restricted to 50% of the individuals i.e. every alternate individual. Thus 631 of the total 1262 individuals in CUPS participated in these studies (Figure 2).

Final assessment:

Each subject surrendered the form to the team members after completing all the procedures. The team members then rechecked all record forms for completeness before the subject is sent off.

Non response rate:

Non response rate is one of the most important potential bias, usually inflating prevalence estimates and its effect should never be ignored. Initially, we could only get a 60-70% response rate. Our survey team members then approached each non-respondent for the reason for their absence and they were then given a second and third opportunity to participate in the survey according to dates of their convenience. In T.Nagar population (low income group) initially we found that female population was higher than the male population. This is because the male population is often absent from the community, seeking emploment (majority of these were coolies, construction workers, manual labourers etc.). We increased the male population in T.Nagar group by continuously approaching either their family members or the subjects at their workspots. Finally, a total of 479, out of 524 individuals in the Tirumangalam colony (91.4% response rate) and 783 of the 875 individuals in the T.Nagar colony

(89.4% response rate) participated in the study. Thus the overall response rate was 90.2%.

Data entry and validation:

At the completion of survey, the team coordination and members rechecked all record forms for their completeness and then entered it into the computer using EPI INFO and Fox pro data bases. Range and consistency checks were undertaken on all data, which was double entered before the data was analysed.

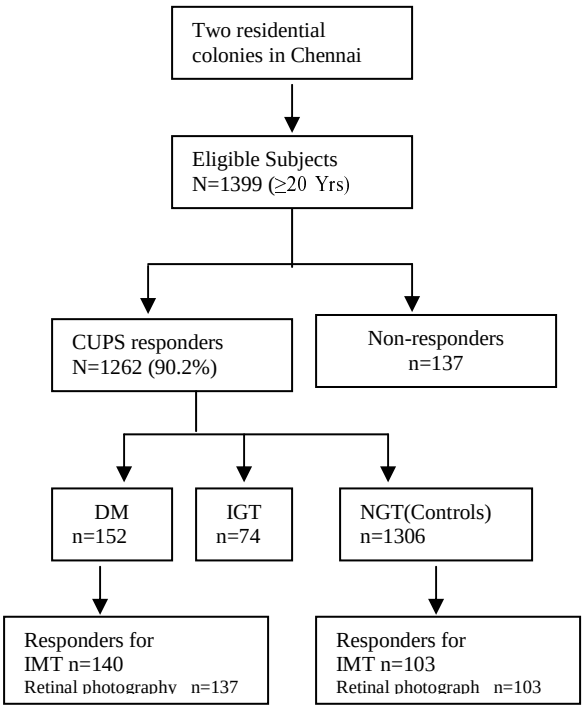
Statistical Analysis:

The prevalence of diabetes and its risk factors eg. NIDDM (known diabetes and/or newly diagnosed diabetes), IGT, cigarette smoking, obesity, hypertention andECG for ischaemia, by Age standardization was performed whenever necessary using the 1991 population census of Chennai. Mean or median values and distributions of continuous variables were described.

Chi square and "t" tests were used to identify the differences between groups. Relative risk estimates for risk factors were done using Mantel-Haenszel techniques. Multivariate statistics,including multiple liner and logistics regression were carried out quantify the impact of variables like social class,age,sex,BMI and WHR on the components of metabolic syndrome.

STUDEIS OF DIABETES COMPLICATIONS (PHASE III OF CUPS)

Figure 3: **Phase III of CUPs**



In third phase of the study, all the known diabetics and newly diagnosed diabetes along with age and sex matched controls were brought into the M.V.Diabetes specialities Centre, in a van, for special studies to look for complications like retinopathy, microalbuminuria, echocardiogram, intimo-medial thickness as described below (Figure 3).

RETINOPATHY STUDIES

Retinal Photography:

Retinopathy was assessed after adequate mydriasis by colour photography. Four field colour photography of the retina was taken of both eyes using a 50VT Topcon retinal camera using 35 mm colour transparencies. The four fields taken were stereoscopic pictures of the macula, superior temporal, nasal and inferior temporal quadrants [13]. Four fields were chosen to reduce the costs and improve compliance. The nasal field was centered on the nasal margin of the disc to assess the disc, superior and inferior nasal fields in one photograph. Photographs were coded and assessed in a masked manner for the presence and severity of retinopathy and quality of photographs. The photographs were graded twice by the same observer 4 weeks apart and the mean of the two readings was taken. A modified version of the Early Treatment Diabetic Retinopathy Study (ETDRS) grading system was used to grade the photographs (14-16).

Carotid Intimal Medial Thickness Study:

Measurement of carotid intimal medial thickness (IMT) was used as a non-invasive marker of atherosclerosis [17, 18]. It has also been shown that the IMT measurements correlates strongly with future development of myocardial infarction and stroke [19, 20].

The intimal plus medial thickness of the carotid arteries was determined by using a high resolution B mode ultrasonography system (Logic 400 GE, Milwauker, Wis., USA) having an electrical linear transducer mid frequency of 7.5 MHz. The axial resolution of the system was 0.5 mm. The images were recorded as well as photographed. The mean intimal medial thickness values of the diabetic subjects (0.95 ± 0.32 mm) were significantly higher than the non-diabetic subjects (0.74 ± 0.14 mm).

NEPHROPATHY

Microalbuminuria and Proteinuria:

Microalbuminuria was estimated only in patients who had proteinuria of less than 500 mg/ day and was measured using the immunoturbidometric assay (Randox, Crumlin, UK). The criteria for microalbuminuria was an albumin excretion>30 µg/mg creatinine, without any evidence of urinary track infection clinically and a sterile urine culture. Protein/creatinine ratio was calculated [21]. Patients with proteinuria i.e. ≥ 500 mg/ day of proteinuria were called as "macroproteinuric". Patients were classified as "microproteinuric" in the range of 150-499 mg/day. Renal insufficiency was defined as serum creatinine > 133 µmol/L.

Echocardiography:

This was done to evaluate the cardiac status including left ventricular function parameters. The male patients were instructed to remove the shirt and banian and the female patients were requested to unbutton their blouse and push it side ways. The ECG electrodes were placed on the right arm, left arm and in the right subcostal regions. They were connected to the unit through the ECG cable of the ultrasound unit. Patients were positioned in the left lateral position.

The transducer was kept over the pre-cardial region of the chest after applying sufficient amount of ultra sound gel over the transducer. Through appropriate maneuvering of the transducer head the heart was evaluated in two dimensional echo mode in the apical long axis, parasternal long and parasternal short axis views. The M-mode evaluation of the mitral and aortic valve regions were captured. The Doppler evaluation at the mitral and pulmonary valve regions were also captured. The left ventricular functional parameters were evaluated using the M-mode tracings of the parasternal long axis view at the level of the tip of the mitral valve. The regional wall motion analysis of the heart was then evaluated.

CONCLUSION:

CUPS is one of the first study from any developing country to study so exhaustively the prevalence of diabetes and virtually all its complications. The data will be published in a series of publications. This articles provides all the methodological details, which it is hoped, will benefit other researchers in India to undertake similar studies.

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APPENDIX – 1

Definitions and Diagnostic Criteria

Diabetes :

Diabetes was diagnosed based on drug treatment for diabetes (insulin or oral hypoglycemic agents) and/or criteria laid by the WHO consultation group report i.e. fasting plasma glucose (FPG) ≥7.0 mmol/L or 2hr post glucose value ≥ 11.0 mmlo/L.

Hypertension

Hypertension was based on drug treatment or if the blood pressure is greater than 140/90 mmHg.

Hyperlidemia

Hypercholesterolemia was diagnosed if serum cholesterol levels were greater than 5.2 mmol/L and hypertriglyceridemia if serum triglyceride levels were greater than 2.26 mmol/L.

Coronary Artery Disease:

Coronary artery disease (CAD) was diagnosed based on a documented past history of myocardial infarction or drug treatment for coronary artery disease and/or Minnesota codes 1-1-1 to 1-1-7, (Q wave changes) 4-1 to 4-2 (ST segment depression) or 5-1 to 5-3 (T wave abnormalities).

Anthropometry:

The most common anthropometric measures are body mass index (BMI) which is defined as weight in kg/height in meters squared. According to WHO Expert Committee, obesity was defined as BMI > 25 kg/m^2 for both males and females.

Waist-hip-ratio (WHR) was defined as waist circumference (cm) divided by hip circumference (cm). Central obesity was defined as a WHR of 0.85 or greater for women and 0.95 or greater for men.

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