ABSTRACT  

**Purpose:** To describe the methodology of the Sankara Nethralaya–Diabetic Retinopathy Epidemiology and Molecular Genetic Study (SN–DREAMS 1), an ongoing population-based study to estimate the prevalence of diabetes and diabetic retinopathy in urban Chennai, Tamil Nadu, South India, and also to elucidate the clinical, anthropometric, biochemical and genetic risk factors associated with diabetic retinopathy.  

**Methods:** In this ongoing study, we anticipate recruiting a total of 5830 participants. Eligible patients, over the age of 40 years, are enumerated using the multistage random sampling method. Demographic data, socioeconomic status, physical activity, risk of sleep apnea, dietary habits, and anthropometric measurements are collected. A detailed medical and ocular history and a comprehensive eye examination, including stereo fundus photographs, are taken at the base hospital. Biochemical investigations (total serum cholesterol, high-density lipoproteins, serum triglycerides, hemoglobin, glycosylated hemoglobin HbA1c) and genetic studies of eligible subjects are conducted. A computerized database is created for the records.  

**Conclusion:** The study is expected to result in an estimate of the prevalence of diabetes and diabetic retinopathy and a better understanding of biochemical and genetic risk factors associated with diabetic retinopathy in an urban South Indian population. Worldwide, the prevalence of diabetes mellitus, in particular type II diabetes, is rising at an alarming rate. The World Health Organization (WHO) and International Diabetes Federation (IDF) have predicted that the number of cases of adult-onset diabetes would more than double by 2030 from the present level of 171 million to 366 million—an increase of 214%. In developed countries, this increase in diabetic population would be around 42% and in developing countries, particularly in India, it is even higher; i.e. 150%. In India, the prevalence of diabetes mellitus in the urban population is around 12.1%, as reported by the national urban diabetes study conducted in six major cities. Studies have shown the prevalence of diabetes to be higher among the high-income groups (25.5%) as compared to low-income groups.
The assessment of socioeconomic status was based on income, education, occupation or caste—which are not representative of the actual socioeconomic status. In the present study, however, the sample was stratified on socioeconomic scoring. This scoring was calculated on the basis of several parameters such as the residence being rented or owned, the number of rooms in the house, the highest educational status, the highest salary, the highest occupation, material possessions (cycle, TV, audio, car, etc.) and house/land value. To the best of our knowledge, this kind of comprehensive socioeconomic scoring has not been done before for prevalence studies on diabetic retinopathy in the general population.

KEYWORDS  Diabetic retinopathy; diabetes mellitus; epidemiology; genetics; prevalence; risk factors; India

INTRODUCTION

The prevalence of diabetic retinopathy in a diabetic population ranges from 10.2 to 48%. In India, the prevalence of diabetic retinopathy among diabetics ranges from 22.4 to 34.1%; since this prevalence has been estimated primarily in self-reported diabetics or in specialist diabetic centers, it does not truly reflect the prevalence of diabetic retinopathy in previously diagnosed and newly diagnosed diabetics in the population. The present study addresses this issue by including both newly diagnosed and previously diagnosed diabetics.

Genetic factors play an important role in the pathogenesis of diabetic retinopathy. In India, until now, no population-based studies have evaluated the genetic factors influencing diabetic retinopathy. The important candidates are the vascular endothelial growth factor (VEGF) gene, the receptor for advanced glycosylation end products (RAGE) gene, the protein kinase C (PKC–β) gene, and the apolipoprotein E (ApoE) gene. In our previous work in the Asian Indian population, we found that the Z-2 allele of the aldose reductase gene is a risk factor for developing diabetic retinopathy, and also observed that Gly82Ser polymorphism in the RAGE gene was a protective allele for retinopathy whereas the tumor necrosis factor 11 bp allele of dinucleotide microsatellite repeat was associated with proliferative diabetic retinopathy. Our study on the inducible nitric oxide synthase gene showed that a 210 bp pentanucleotide repeat 2.5 kb upstream from the transcription site was a low risk allele for developing retinopathy.

The present population-based study has been designed using a multistage random sampling technique to answer the following questions:

What is the prevalence of diabetes mellitus in an urban population of Chennai, Tamil Nadu, India?
What is the prevalence of diabetic retinopathy in this well-defined population of diabetics, both newly diagnosed and previously diagnosed?
What are the risk factors—clinical, sociodemographic, anthropomorphic, and biochemical—associated with diabetic retinopathy?
What sequence variants or polymorphisms in the RAGE, VEGF, PKC–β, and ApoE genes are associated with diabetic retinopathy?

This paper will elucidate the research methodology designed for SN-DREAMS.

MATERIALS AND METHODS

The study procedure is divided into five phases (see Fig. 1):

Phase 1: Epidemiological field work
Phase 2: Training program, quality control, pilot study, definitions
Phase 3: Medical history questionnaire
Phase 4: Ophthalmic examination, fundus photography, diabetic retinopathy classification
Phase 5: Biochemical investigations and genetic study

Phase 1: Epidemiological Fieldwork

The epidemiological fieldwork, commenced in October 2003, is expected to be completed by December 2005. The clinical examination, biochemical investigations and genetic study, commenced in January 2004, will be completed by April 2006. The Institutional Review Board at Sankara Nethralaya has approved this project.

Study Design

SN-DREAMS is a population-based cross-sectional survey, which measures the prevalence of diabetes and diabetic retinopathy and estimates the risk or protective factors associated with diabetic retinopathy.
FIGURE 1  Study Design. RAGE = Receptor for Age-related End Products; VEGF = Vascular Endothelial Growth Factor; PKC-β = Protein kinase C-β.

Sample Size Estimation

In India, Dandona et al. estimated the prevalence of diabetic retinopathy to be 1.78% in a population-based sample of adults aged 30 years or older, and Narendra et al. reported a prevalence of 1.4% in self-reported diabetics aged 50 years or above. Based on these published studies, it is appropriate to assume that the prevalence of diabetic retinopathy in the general population may range from 1 to 2%.

In our study, the computed sample size is 5830. This estimation is based on the following assumptions: the prevalence of diabetic retinopathy in the general population is assumed to be 1.3%, with a relative precision of 25%, a drop-out rate of 20%, and a design effect of 2. The sample size for the prevalence survey was calculated by using the formula \( n = \frac{P(1-P)}{d^2} \), where \( n \) is the sample size, \( P \) is the expected prevalence, \( Q = 1-P \), and \( d \) is the precision.\(^27\)\(^-\)\(^29\)

Study Area

Chennai (formerly Madras), the fourth largest city in India, is located on the Coromandel Coast of the
Bay of Bengal. Chennai city has an area of 174 sq. km, accounting for 0.13% of the State’s total area of 1,300,058 sq. km; the entire area of the district has been classified as urban. It has a population of around 4.3 million with 2.2 million males and 2.1 million females according to the census of 2001; the literacy rate is 76.8% (Table 1).

### TABLE 1 Demographic Data on the Study Area

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chennai District</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>174 sq. km</td>
</tr>
<tr>
<td>Population</td>
<td>4.3 million</td>
</tr>
<tr>
<td>Male</td>
<td>2.2 million</td>
</tr>
<tr>
<td>Female</td>
<td>2.1 million</td>
</tr>
<tr>
<td>Literacy rate</td>
<td>76.8%</td>
</tr>
<tr>
<td>% Population &gt;40 yrs</td>
<td>28%</td>
</tr>
<tr>
<td>Major religions:</td>
<td></td>
</tr>
<tr>
<td>Hindus:</td>
<td>3.6 million (82.3%)</td>
</tr>
<tr>
<td>Muslims:</td>
<td>379,206 (8.7%)</td>
</tr>
<tr>
<td>Christians:</td>
<td>331,261 (7.6%)</td>
</tr>
<tr>
<td>Others:</td>
<td>51,791 (1.2%)</td>
</tr>
<tr>
<td>Religion not stated:</td>
<td>8,031 (0.2%)</td>
</tr>
</tbody>
</table>

Bay of Bengal. Chennai city has an area of 174 sq. km, accounting for 0.13% of the State’s total area of 1,300,058 sq. km; the entire area of the district has been classified as urban. It has a population of around 4.3 million with 2.2 million males and 2.1 million females according to the census of 2001; the literacy rate is 76.8% (Table 1).

### Sampling Method

Chennai city is divided into 10 corporation zones of 155 divisions (Fig. 2). The sampling is based on multi-stage systematic random sampling. Sampling is done in two stages: selection of divisions and selection of study subjects. Selection of divisions is done using computer-generated random numbers; of 155 divisions, 10 are selected ensuring that one division per one corporate zone is represented in the sample. Eligible study subjects are randomly selected from each division. To meet the target, 600 individuals are enumerated for each division (a total of 6000 in 10 zones). This sample is thus truly representative of urban Chennai, Tamil Nadu, India.

The sampling is done to ensure that data is collected from all socioeconomic groups. Socioeconomic data are collected from every 5th household on both sides of the street. Using a scoring system, each street is categorized as being a low (score 0–14), middle (score 15–28) or high (score 29–42) socioeconomic status street. To obtain a sample of 600 from each division and distribute it evenly based on socioeconomic criteria, the population proportion to size sample (PPS) from each socioeconomic stratum is calculated.

### Household Enumeration

Family members living on the same premises and sharing a common kitchen are defined as being members of one household. A door-to-door survey of all the households on the right side of the street is conducted in the selected division until a number of 600 subjects is reached. The household data sheet contains details of demography, educational qualification, occupation, residential status, and diabetic status. Each subject is assigned a unique eleven-digit identification number. For the population, the first two digits indicate the division, the third and the fourth represent the street number, the next three represent the door number, the following two represent the household number, and the last two denote the individual serial number.

### Eligibility Criteria

Individuals aged 40 years or above or turning 40 in the current year and residing for a minimum of six months at the same residence.

### Exclusion Criteria

Individuals residing for a period of less than six months at the same residence, temporary residents
(whose permanent residence is elsewhere), a resident who dies after the enumeration but prior to examination, and those who cannot be contacted after five attempts by the social worker at their residence. Very old or invalid persons (blind subjects are not considered as invalid) who cannot be transported to the examination center are also excluded.

**Refusals**

When the occupants of the household refuse to provide any information or to comply with the examination, it is taken as a refusal. A maximum of five attempts are made to convince them before considering them as dropouts. The strategy to minimize refusals includes informing all the study participants about the study benefits plus sending reminders via the telephone or making a personal visit by the social worker.

**Estimation of Fasting Blood Glucose**

The team visits the selected households a day in advance to request eligible individuals to observe a minimum of 8 hours of overnight fasting prior to estimation of the fasting capillary blood glucose levels the next morning. Using Accutrend Alpha, blood glucose estimation is done on a sample of capillary blood obtained by finger-prick (glucose oxidase method). In accordance with the ADA criteria, known diabetics and new asymptomatic subjects with a fasting blood glucose $\geq 110$ mg/dl (provisional diabetics) are selected. For patients who forget the fasting instructions and for dropouts, the fasting blood glucose is estimated on Sundays or at their convenience.

All known diabetics and provisional diabetics from the field area are given appointments for further evaluation at the base hospital. On the day of the examination, the subjects are instructed to assemble at a specific place with their prescriptions or medicine that they are taking for ophthalmic or systemic conditions; the social worker accompanies the subjects in the project vehicle to the hospital. Once the comprehensive evaluation is over, the subjects are transported back to their residences.

**Phase 2: Training Program, Quality Control, Pilot Study, Definitions**

**Training Program**

The epidemiology team of Sankara Nethralaya provided intensive training on a one-to-one basis to all the team members. This lasted for 7 days with 8 hours a day of training sessions. The aim was to ensure that each member of the team was well trained in doing a household survey, enumeration, and filling out the study data sheet. The trainees were instructed to use the BP apparatus and glucometer for estimating fasting capillary blood glucose. The main objective was to avoid bias or errors in any of the procedures employed. Each trainee was evaluated individually and allowed to participate in the study only after he or she displayed minimum error rates for the tasks involved in the study.

**Quality Control**

In order to ensure accurate and reliable data, a comprehensive instruction manual has been prepared. A start-up training session helped us to standardize all the examination and diagnostic procedures. The glucometer is calibrated every day and its reproducibility is assessed by measuring the blood glucose for the same patient six times and also with two machines. A similar procedure is undertaken for the sphygmomanometer. The scale for measuring the weight is calibrated with a known weight once a week. The collected data is scrutinized manually before its entry into the computer.

**Pilot Study**

A pilot study was conducted taking 139 patients from the first division (Chintradipet); this was done to identify the initial practical difficulties in carrying out the study. The first step was the selection of the participants. Next, informed consent was obtained after the study had been explained to each participant and the interviewer was certain that the participant understood and accepted the contents. The subjects underwent the entire sequence of examination and laboratory procedures as planned for the actual study.

During the pilot study, inter- and intraobserver variations for the photographic classification of diabetic retinopathy were compared (in order to minimize bias); kappa was 0.83 and 1.0, respectively. A Kappa value of $>0.8$ suggests good agreement between the observers.

A time-motion study was done to determine the time needed for a complete examination. The study revealed that it took 2 hours and 45 minutes for the collection of all the data, which included medical history, questionnaires and anthropometric measurements (45 minutes); ophthalmic work-up with corneal examination (1 hour); dilatation, fundus examination, and fundus
photography (30 minutes); vibration perception threshold (VPT) testing and genetic work-up (30 minutes). The pilot study concluded that there was no questionnaire fatigue and a good response rate, creating the necessary confidence to proceed with the study.

Overview

All known diabetics and provisional diabetics (first FBS $\geq 110$ mg/dl) are invited to the diabetic retinopathy project site (base hospital) of Sankara Nethralaya for further evaluation; provisional diabetics are instructed to come fasting on the day of examination. On arrival at the reception, the subjects are registered and issued the previously designed eleven-digit ID number. A new four-digit hospital diabetic retinopathy (DR) number is given. The subjects then undergo blood collection by the laboratory technician. Various definitions used in the study are summarized in Table 2.

Phase 3: Medical History, General Physical Examination and Questionnaires

Medical and Ocular History

In this phase, a detailed questionnaire is administered regarding the medical history and a general physical examination is given. The data in the medical history include duration and treatment of diabetes or hypertension, a family history of diabetes, coronary artery disease, symptoms related to diabetic nephropathy or neuropathy (tingling, numbness, foot ulcers and amputated toes or foot), the presence of an ear lobe crease, smoking or alcohol consumption, pregnancy, and duration of total sleep. The ocular history includes details of the first and last eye examination, present or past ocular complaints, and laser treatment or ocular surgery.

Table 2: Definitions

<table>
<thead>
<tr>
<th>Definition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provisional diabetics</td>
<td>New asymptomatic individual with a first fasting blood glucose level $\geq 110$ mg/dl (Accutrend alpha).</td>
</tr>
<tr>
<td>Known diabetics</td>
<td>Diagnosis of diabetes made by a medical practitioner, or patient using hypoglycemic medication, either oral or insulin or both.</td>
</tr>
<tr>
<td>Newly diagnosed diabetics</td>
<td>Fasting blood glucose level $\geq 110$ mg/dl on two separate days; provisional diabetics were retested at the base hospital by the laboratory method.</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>Time interval between the date of diagnosis of diabetes (as made by a diabetologist or when the antidiabetic treatment started) and the date of eye examination.</td>
</tr>
<tr>
<td>Hypertension</td>
<td>If the systolic BP is $\geq 140$ mm Hg or the diastolic BP is $\geq 90$ mm Hg or the patient is on antihypertensive treatment.</td>
</tr>
</tbody>
</table>

General Physical Examination

This includes measurement of height and weight, after which the body mass index (BMI) is calculated using the formula: weight (kg)/height (m$^2$). Based on the BMI, individuals are classified as lean (male $< 20$, female $< 19$), normal (male 20–25, female 19–24), overweight (male 25–30, female 24–29) or obese (male $> 30$, female $> 29$).

The waist and hip ratio (WHR) is calculated by dividing the waist circumference (cm) by the hip circumference (cm). The neck circumference and thigh length are also measured. The resting heart rate per minute is taken after making the patient sit for at least five minutes. The blood pressure is recorded in the sitting position on the right arm; two readings are taken 5 minutes apart and the mean of the two is taken as the blood pressure.

The Epworth sleepiness score is calculated by eliciting the chance of dozing off (never, slight, moderate or high) during the day while reading, watching TV, sitting inactive in a public place, traveling in a car for half an hour without a break, lying down in the afternoon and so on. Sleep apnea is considered if any of the following two variables are present: obesity (BMI male $> 30$, female $> 29$) or hypertension ($> 140/90$), a history of snoring and an Epworth sleepiness score $\geq 5$.

Questionnaires

Physical activity is assessed by means of a simple questionnaire, which has been validated in the South Indian population. Socioeconomic scores are noted on the basis of the questionnaire used in the first phase.

A knowledge, aptitude, and practice (KAP) questionnaire about diabetic retinopathy is filled in for each individual, as well as a diet questionnaire. For all
patients, peripheral neuropathy foot testing is done by measuring the vibration perception threshold.44

**Phase 4: Ophthalmic Examination, Fundus Photography, Diabetic Retinopathy Classification**

**Visual Acuity and Refraction**

The modified ETDRS chart (Light House Low Vision Products, New York, NY, USA) is used to test the distance visual acuity; and for those who cannot read the English alphabet, Landolt’s ring test is used. If the visual acuity is less than 4/4 (logMAR 0.0), the pinhole visual acuity is assessed.

Objective refraction is performed with a streak retinoscope (Beta 200, Heine, Germany) followed by subjective refraction. If the subject is unable to read the 4/40 (logMAR 1.0) line, vision is checked at one meter. If he or she is still unable to identify any of the largest optotypes, perception of hand movements is observed. If hand movements cannot be identified, the examiner checks for perception of light, which is recorded as present or absent.

**External Examination**

External examination is performed using a handheld flashlight. The face and eyes are examined for the presence of strabismus, extraocular movement abnormalities or any other gross abnormality.

**Corneal Examination**

This consists of corneal pachymetry, Schirmer’s test, specular microscopy, and Rose Bengal and fluorescein staining. It is believed that corneal thickness plus endothelial cell density, pleomorphism and polymegathism are positively correlated with the type, duration and severity of diabetes; as is the case for tear film and ocular surface dysfunction.45−47 However, these relationships have not been substantiated in the population-based studies.

Corneal pachymetry48 (Alcon ultrasound pachymeter) is performed to measure the corneal thickness. Measurements are taken for the central and inferior peripheral cornea. If measurements of the inferior peripheral cornea are not possible, a temporal peripheral reading is taken. An average of three readings are taken for each eye.

Schirmer’s test49,50 is done to assess the aqueous component of the tears. Whatman’s no. 41 filter paper is bent at the notch and inserted in the fornix at the junction of the medial 2/3 and lateral 1/3 of the lower eyelid, into each eye in succession with the shortest possible interval; care is taken not to touch the cornea. The patient is allowed to keep the eye either closed or open. At the end of 5 minutes, the length of the strip that has become wet is measured; more than 10 mm of wetting is considered normal.

Corneal specular microscopy51−53 is performed to assess the corneal endothelial status. With the patient sitting and the head position adjusted to obtain a complete and well-focused corneal reflex on the center of the cornea, the record button is pressed; if the central corneal pictures are not good, the procedure is repeated in any of the peripheral quadrants. The data are analyzed with regard to cell density, percentage of hexagonality, coefficient of variance, and number of cells including the standard deviation.

Rose Bengal and fluorescein staining54−56 is used for the diagnosis of ocular surface diseases, especially for the screening of dry eye. A drop of 1% Rose Bengal is placed on a strip of fluorescein paper which is instilled into the cul-de-sac of the patient’s eye; separate strips are used for each eye. The patient thereafter blinks naturally and the staining pattern is studied after 1–2 minutes (to allow excess stain in the preocular film to disappear) by slit-lamp biomicroscopy with a red-free filter. The cornea, the nasal part of the conjunctiva and the temporal conjunctiva are scored separately. A score of 1 is given if a few separated spots of stain are seen, a score of 2 if multiple but separated spots appear, and a score of 3 if there are confluent spots. Thus, the total score ranges from 0 to 9.

Using a blue filter, fluorescein staining of the cornea is also studied by the slit-lamp. For documentation purposes, the cornea is divided into 3 zones: superior, middle and inferior 1/3. The grading of the staining is the same as that used for Rose Bengal.

**Slit-Lamp Examination**

The Zeiss SL 130 (Carl Zeiss, Jena, Germany) slit-lamp is used. Using a moderately wide beam, the eyelids, margins, lashes, canthi, and puncta are systematically examined, followed by the palpebral and bulbar conjunctiva, sclera and cornea. Then, using a narrow parallelopiped beam, the cornea, anterior chamber and iris are examined for abnormality. Grading of peripheral anterior chamber depth is done according to the Van Herick grading.57 The iris and pupilary margins
are examined under high magnification to look for iris neovascularization.

**Applanation Tonometry**

Using the Goldmann applanation tonometer (Zeiss AT 030 Applanation Tonometer, Carl Zeiss, Jena, Germany), the intraocular pressure (IOP) is measured in both eyes; 0.5% proparacaine eyedrops are used for topical anesthesia, and a 2% fluorescein strip to stain the tear film.

**Gonioscopy**

Gonioscopy is performed in dim ambient illumination with a shortened slit that does not fall on the pupil. A Sussmann-type 4-mirror handheld gonioscope (Volk Optical Inc, Mentor, Ohio, USA) is used. Gonioscopy is performed only on those patients who have an elevated intraocular pressure, iris neovascularization or a shallow anterior chamber (Van Herrick’s grading ≤ 2).

The angle is graded according to the Shaffer grading, the peripheral iris contours, degree of trabecular meshwork pigmentation and angle neovascularization are also recorded. In eyes with an occludable angle, laser iridotomy is performed before dilatation; the rest of examination is done at a later date.

**Grading of Lens Opacities**

The subject’s pupils are dilated with 5% phenylephrine and 1% tropicamide eyedrops; if phenylephrine is contraindicated, 1% cyclopentolate eyedrops are used.

Grading of lens opacities is performed using the Lens Opacities Classification System (LOCS chart III, Leo T. Chylack, Harvard Medical School, Boston, MA). Lenticular opacities are graded by comparison with the standard set of photographs, which are retroilluminated by mounting on a light box.

With the slit beam at a 45° angle, the slit height and width are adjusted to approximate the overall brightness of the corneal image and anterior subcapsular zone to nuclear color/opalescence standard N1; 0° brightness is allowed to visualize all the lens opacities without causing discomfort to the patient. Keeping the slit width at 0.2 mm, nuclear opalescence (NO) and nuclear color (NC) are graded by comparing the slit-lamp examination image with the nuclear standards NO1 to NO6 and NC1 to NC6.

Cortical cataract (C) and posterior subcapsular cataract (P) are graded by examining the opacity in retroillumination images (0° angle) focused either anteriorly (at the iris plane) or posteriorly (at the plane of the posterior capsule) and comparing these examination pictures to the cortical standards C1 to C5 and posterior subcapsular standards P1 to P5.

**Fundus Examination and Clinical Grading of Diabetic Retinopathy**

The binocular indirect ophthalmoscope (Keeler Instruments Inc., Pennsylvania, USA) and +20 D lens (Nikon) are used to examine the fundus. Diabetic retinopathy is clinically graded according to the new disease severity scale given by the American Academy of Ophthalmology; the macular area is specifically examined with a +78 D lens (Nikon) to diagnose clinically significant macular edema as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS).

**Fundus Photography Using Stereo-Pictures**

Irrespective of the presence or absence of diabetic retinopathy, 45° four-field stereoscopic digital photographs (posterior pole, nasal field, superior and inferior) are taken for all subjects with a Carl Zeiss fundus camera (Visucamlite, Jena, Germany). However, for those who are found to have diabetic retinopathy on clinical examination, additional 30° seven-field stereo-digital pairs are taken.

**Evaluation of Photographic Grading and Quality**

The photographic grading and quality are assessed using the Visupac digital image archiving system. The “Screenscope” (Berezin Stereo Photography Products, Mission Viejo, CA, USA), a stereo viewer that can be fixed on a computer monitor, is used to examine the stereo pairs. Photographs of each eye are reviewed and given grades for overall quality. Field definition and image clarity are graded as inadequate for reading or grading, adequate (sufficient visualization of disc, macula, and vessels to grade), and good (small vessels or retinal details visible across 90% of the image).

**Photographic Grading of Diabetic Retinopathy**

The modified classification of diabetic retinopathy based on the degrees of retinopathy used by Klein et al. is used (Table 3); digital photographs are assessed and graded by three independent observers (experienced retinal specialists) in a masked fashion. The
TABLE 3  Diabetic Retinopathy is Graded as Follows

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>No DR</td>
<td>No abnormality</td>
</tr>
<tr>
<td>Mild NPDR</td>
<td>Only microaneurysm</td>
</tr>
<tr>
<td>Moderate NPDR</td>
<td>More than mild, but less than severe</td>
</tr>
<tr>
<td>Severe NPDR</td>
<td>Any of the following: 20 or more intraretinal hemorrhages in 4 quadrants, venous beading in &gt;2 quadrants or intraretinal neovascularization in 1 quadrant</td>
</tr>
<tr>
<td>Proliferative DR</td>
<td>One or more of the following: neovascularization or preretinal or vitreous hemorrhage</td>
</tr>
</tbody>
</table>

photographs are graded against the standard photographs of the ETDRS grading system for severity of retinopathy. In case of any disparity in the findings, the first grader consults the second grader before reaching conclusions. If the two graders do not agree, the third grader’s opinion is sought.

**Phase 5: Biochemical and Genetic Studies**

**Biochemical Investigations**

In case of patients with provisional diabetes, confirmation is done by re-estimation of the fasting blood glucose by enzymatic assay; glucose is oxidized by glucose oxidase to produce gluconate and hydrogen peroxide, which is detected photometrically.

Biochemical analysis is done on the Merck Micro Lab 120 semi-automated analyzer. Total serum cholesterol (CHOD-POD method), high-density lipoproteins (CHOD-POD method after protein precipitation), serum triglycerides (CHOD-POD), hemoglobin (calorimetric hemoglobinometer), packed cell volume (capillary method) and the lycosylated hemoglobin fraction (Bio-Rad DiaSTAT HbA1c Reagent Kit) are estimated. Microalbuminuria estimation is done by a semi-quantitative procedure (Clinetek 50 Bayer Urine Analyzer) with the first morning urine sample.

**Genetic Analysis**

From the blood sample collected, the DNA is extracted from the heparinized blood by the phenol/chloroform method. The extracted DNA is quantified spectrophotometrically, used for the polymerase chain reaction and amplified using specific primers. Further screening of polymorphisms and sequence variants and their associations with the disease (diabetic retinopathy) is the candidate genes is done by observing the restriction fragment length polymorphism and direct sequencing (VEGF, PKC-β, receptors for AGEP and Apo E). The presence of a risk allele in the genes for aldose reductase and inducible nitric oxide synthase and their association with the disease are studied. The investigations on the patients are performed in accordance with the Declaration of Helsinki.

**Data Management**

**Data Collection Forms**

Identification data, history, the results of the medical and general physical examination along with the results of all the biochemical tests are entered onto the study data sheet designed in accordance with the OmniExtract ICR service package (Newgen Software Technologies Ltd). After the examination is over and before the patient leaves the base hospital, the records are checked for completeness.

**Data Entry and Processing**

All of the data are fed into the computer using the Omni Extract ICR service package and scanner. Two data operators validate the data entry.

**Statistical Analysis**

The prevalence of diabetes and diabetic retinopathy will be expressed as percentages with the 95% confidence interval. Logistic regression analysis will be done to elucidate the association between risk factors and diabetic retinopathy. Odds ratios with 95% confidence intervals will be calculated for the studied variables.

**Major Challenges**

As we plan to have an equal share of individuals from all socioeconomic strata, poor compliance with the examination for the study is expected particularly from those in the higher strata. The solution to this challenge is to provide some flexibility in scheduling their eye examination based on their convenience and availability. Other challenges include refusals and dropouts and uniformity in the data collection. The issue of refusals and dropouts has been addressed by showing the benefit of this study to all the participants prior to their enrollment. To ensure uniformity in examination and data collection, the team underwent a comprehensive training program prior to commencement of the study.
Limitations

The present study, being a cross-sectional survey, has its inherent limitations. As the sample size was calculated for the estimation of the prevalence of diabetic retinopathy in the general population, the power to elucidate associated risk factors in the subgroup analysis may be inadequate. Secondly, this study is unable to provide data on the progression of diabetic retinopathy over time, as no follow-up is envisaged.

CONCLUSION

SN-DREAMS is an ongoing population-based study. Data collection and analysis are expected to last for about three years from commencement. The project is designed to provide valuable information on the prevalence of diabetes and diabetic retinopathy in a representative sample of an urban population and the risk factors associated with diabetic retinopathy. Additional insights will be obtained into the influence of genetic aspects on diabetic retinopathy. The study results will help plan service delivery in this rapidly spreading epidemic of diabetes.

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Photographer: Mr. M. S. Krishna
Field Operation Incharge: Mr. T. Arokiasamy
Social workers: Mr. J. Singaraj, Mr. E. Chandrasekaran,
Mr. K. Jayandrakumar
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