

7) Informed consent

Participation in the examinations totally depended on free will, without any enforcement. All participants were fully informed of the following items. Only subjects who understood and accepted examination procedures, and signed their names on a written form to participate in the study (informed consent) were included. This informed consent included; (1) purpose of the study; (2) detailed procedures for each examination; (3) gene analysis; (4) preservation of blood, urine and DNA samples for future examinations; (5) to send examination report to the participants; (6) to keep personal data secret. The Ethical Committee of the National Center for Geriatrics and Gerontology had already approved all procedures of the NILS-LSA.

8) Examinations and tests

The normal aging process was assessed by detailed examinations including clinical evaluation, sensory functions, body composition and anthropometry, physical functions, nutritional analysis, and psychological tests (Table 4).

The NILS-LSA is a longitudinal study to observe age related changes of various examination and tests. Thus examinations and tests of the third wave were basically same with the first and second wave except oral examinations such as assessment of dental status, periodontal status, denture wearing habit, and tongue coating status.

Table 4. The third wave examinations and tests in the NILS-LSA

Health related questionnaire

Self-rated Health (SRH), Medical history, Clinical symptoms, Life-style, Personal history (job, marriage, education, etc.), Family history, Environment, Smoking, Social and economical back ground

Routine clinical evaluations

Physical examination

Blood pressure

Blood chemistry (fasting)

GOT, GPT, gamma-GTP, Total protein, Albumin, LDH, Alkaline phosphates, Choline esterase, Uric acid, Creatinine, Calcium, Total cholesterol, Triglyceride, HDL-cholesterol, Lipid peroxide, Fasting glucose, HbA1c, Insulin, Vitamin A, Serum sialic acid, Fe, Cu, Mg, free T3, free T4, TSH, DHEA-S, DHA, EPA, arachidonic acid, Dihomo-gamma-linolenic acid

CBC: Red cell count, White cell count, Hb, Hematocrit, Platelet count

Urine analysis: Protein, Sugar, Urobilinogen, Ketone, pH, Occult blood, Hemoglobin, Nitrite

Sensory examinations

Visual system

Visual acuity: Presenting Visual Acuity, Best-corrected Visual Acuity (5 m), Refraction, Retinal fundus camera, Intraocular pressure, Stereoscopic vision, Contrast sensitivity, Corneal thickness, Retina tomography

Auditory system

Audiometry (air and bone), Middle ear functions (Single frequency and Multifrequency tympanometry), Video recording of tympanic membrane

Medical examinations

ECG (Automatic ECG analyzer)

Cardiac ultrasonic tomography

Intima-media thickness of carotid artery

Head MRI (Magnetic resonance imaging system)

Thoracic and lumbar radiography

Dual energy X-ray Absorptiometry (DXA)

Lumbar spine, Right and left femur neck, Total bone density, Body fat (total and segmental fat)

High Quality Peripheral Quantitative CT (pQCT)

Radial bone mineral density

Resting metabolic profiles (Computerized indirect calorimetry system)

Resting energy expenditure and Respiratory exchange ratio

Oral examinations

Dental caries

Periodontal index

Denture use

Tongue coat

Aging and geriatric disease related genotypes

Alzheimer disease and dementia related genotypes

Osteoporosis related genotypes

Obesity and diabetes related genotypes

Parkinson disease related genotypes

Cardiovascular disease related genotypes

Anthropometry and body composition

Anthropometric measurements

Body fat measurement

Dual energy x-ray absorptiometry (DXA)

Body fluid measurement (Bioimpedance spectroscopy)

Intra- and Extra-cellular fluid

Thickness of fat and muscle tissue (Ultrasonic tomography)

Intra-abdominal fat, Muscle thickness, Subcutaneous fat thickness

Abdominal fat distribution (Computed tomography)

Intra-abdominal and Subcutaneous fat area

Physical function

Exercise test system

Grip strength, Sit-up, Trunk flexion, Static balance, Leg extension power,
Isometric knee extension strength, Reaction time, Maximum step length.

10m walking test (pitch, step length, velocity),

3-D motion analysis system (four cameras and two force plates)

Stabilometer (with or without eye closed conditions)

Physical activity questionnaire

Electric pedmeter (7 days average)

Psychological tests

Interview

Cognition (MMSE, WAIS-R), Life events, Stress, Social relations, Basic
ADL (Katz Index)

Questionnaire

Depression (CES-D, GDS), Personality (Rosenberg Self-Esteem Scale,
Locus of control, Scale of Attitude toward Death), Social relations,
Subjective well-being (LSI-K), Stress checklist, Stress coping scale,
Instrumental ADL

Nutrition analysis

Food and nutrition intake

Three-day dietary record using scale and disposable camera

Dietary supplement frequency interview

Beverage frequency questionnaire

1. Routine clinical evaluations

First of all, physical examinations including history taking, auscultation and blood pressure were taken by a physician, and during the medical examination the physician reconfirms every participant willingness to participate in examinations. Venous blood and urine samples were collected early in the morning after at least 12 hours' fasting.

Life-style, personal history (job, marriage, education, etc.), family history, environment, smoking, social and economical back ground, health status, clinical symptoms, medical history and medication were examined by questionnaires. These questionnaires are checked by a physician at the medical examination. All drugs were to be documented by participants; the physician confirms them by interview and codes drugs used during the last two weeks.

Blood and urine analysis including renal and liver functions, serum protein and lipids, minerals, glucose, HbA1c, Insulin, Vitamin A, sialic acid, lipid peroxide, fatty acid fractions, thyroid hormones, DHEA-S, and complete blood count were also examined. DNA of the first visit participants was stored in deep freezers for future examinations. As for DNA analysis, genotypes related geriatric diseases such as Alzheimer's disease, arteriosclerosis, osteoporosis, benign prostate hypertrophy and diabetes mellitus were examined with the agreement of the participants.

2. Physiological examinations

For physiological examinations, a head MRI was taken for the each participant and stored in an image database. Intracranial tumors and vascular lesions are checked and brain atrophy was assessed via a computerized trace of the MRI. Electrocardiograms are assessed by computerized automatic diagnosis and Minnesota codes of the diagnosis were stored in a database. Cardiac functions and intima-media thickness of the carotid artery were assessed by ultrasonic tomography. Blood pressure was measured by a physician as well as with an automatic blood pressure manometer.

Osteoporosis is one of the major geriatric diseases. Osteoporosis causes chronic lumbago and bone fracture that disturbs activity in daily life in the elderly. Bone mineral density was measured by dual x-ray absorptiometry (DXA, Hologic QDR-4500). Four scans, including whole body, lumbar spine L2 to L4, right and left femoral bone neck, were taken. Moreover, bone density was also measured by high quality peripheral quantitative computed tomography (pQCT, Dinsiscan 1000).

Resting metabolic profiles were presented as resting energy expenditure,

oxygen intake and carbon dioxide excretion, and respiratory exchange ratio on resting using a computerized, open-circuit, indirect calorimetry system.

3. Sensory examinations

Sensory functions are profoundly associated with QOL in the elderly. Visual and auditory disturbance causes various difficulties in the daily lives of the elderly. Sensory functions, including visual and auditory functions were examined in detail. Distant visual acuity was measured for each eye with a Landolt C letter at 5m. Contrast sensitivity and intraocular pressure were also examined. Fundus photographs were taken with a Topcon fundus camera (TRC-NW5S). Autorefraction was done with the NIDEK-ARK700A. Refractive errors, in the spherical equivalent, were assessed. Corneal thickness and endothelial cell density were obtained with the Topcon SP-2000 specular microscope.

Auditory function assessed by pure-tone audiometry (Audiometer RION AA-73A), and impedance audiometry (Middle Ear Analyzer, Grason-Stadler model 33, version 2). Air conduction thresholds at 125Hz to 8000Hz were examined in all participants. Bone conduction thresholds at 250Hz to 4000Hz were examined in participants with elevation of air conduction thresholds. Middle ear function was evaluated by impedance audiometry.

4. Oral examinations

Caries, periodontal index, and tongue coat were evaluated by a well trained dentist with a dental mirror and WHO CPI probe. All teeth were classified into intact, decayed, filled, missing, or caries observation. Periodontal index (CPI score) on 10 index teeth (17/16, 11, 26/27, 36/37, 31, 46/47) were derived according to World Health Organization standard. Score of tongue coat was given by its area on the tongue dorsum. Denture use was asked by a dentist on verbal.

5. Anthropometry and body composition

For anthropometry measurements, height, weight, abdominal sagittal diameter, circumferences of waist, hip, thigh and upper arm and other parameters were taken. Using ultrasonic tomography, intrabdominal and subcutaneous fat thickness and muscle thickness were evaluated. Intra- and extra-cellular fluid was measured via bioimpedance spectroscopy. Body fat was assessed by DXA. Abdominal fat distribution was evaluated as intra-abdominal and subcutaneous fat areas at the level of umbilicus using a computed tomography

6. Exercise examinations

Grip strength, leg extension power, sit-up and static balance, reaction time, and trunk flexion were measured with a computerized automatic diagnosis system. Step length, pitch, and velocity of walking were assessed by the 10m walking test using four video cameras and two force plates. Physical activities were checked by detailed interview using job-specific questionnaire sheets. Seven-day averages of physical activity were also measured with an electric pedmeter.

7. Nutritional survey

Nutritional intakes were assessed by three-day dietary record using a scale. The scale was handed out to each participant to record the weight of each food taken over the recording period. If it was impossible to weigh each food, approximate size and amounts of food were noted. Dieticians explained to each participant how to weigh foods and how to determine the size and approximate amount. For more accurate assessment, disposable cameras were also handed out to all participants. Before and after each meal, participants were asked to take pictures of all dishes to record what kind of foods and how much food were eaten, and how much food was not eaten. Using these dietary records and photographs, dieticians estimate actual food intake.

8. Psychological test

All participants were interviewed by psychology specialists. Cognition and intelligence were assessed using the Wechsler Adult Intelligence Scale-Revised Short Form (WAIS-R-SF) in all participants and the Mini-Mental State Examination (MMSE) in participants aged 60 years and over. Life events, hassles, and stress coping were also assessed by interview. Basic ADL was checked via the Katz index.

Depressive symptoms, personality, subjective well-being, social relations, and ADL were assessed using a questionnaire.

Over 1,000 variables, including various areas of gerontology and geriatrics will be checked repeatedly every two years in almost 2,400 participants. The staff of the NILS-LSA were consisted of full time researchers, researchers from hospitals and universities, research assistants such as administrators, clinical technicians, dieticians, psychologists, and radiologists. The total number of staff was about 90.

9) Future of the NILS-LSA

We will continue the NILS-LSA to investigate the natural course of aging and the changes that lead to disease. The third wave examination was completed in May 2004. The participants will be examined every 2 years. The cohort of the NILS-LSA is a dynamic cohort, that is, new subjects participate in the study instead of those who do not attend their next examination. Participants who move out of the area are to be followed up by telephone interview or postal questionnaire. Medical records of the participants who die during follow-up will be checked to find out the cause of death.

Extensive tests and examinations should be repeated in longitudinal studies on aging. However, it is actually impossible to repeat many tests and examinations in multiple research facilities with the same protocols and methods. Thus, there are almost no comprehensive longitudinal studies on aging that have been followed up for a long period by multi-center collaboration in the U.S. or other countries.

However, cohort studies with common end points such as dementia and disturbance of ADL are also important for aging studies. For these studies, a relatively large number of subjects and cases during follow-up will be required to get significant analysis results. We are collaborating with other research facilities in Japan and other countries as shown in Fig. 6.

Comparative studies of the aging process accounting for regional and cultural differences between northern and southern areas, or between urban and rural areas, are also important. In these comparative studies, the number of common examinations and tests should be limited. The study design should be a cross-sectional or short-term longitudinal study, considering the difficulties involved continuing and repeating the examinations in all facilities with same protocols.

We are going to make the data of this study public through the Internet. We hope that the results from this large longitudinal study of aging can serve the development of health science on aging.

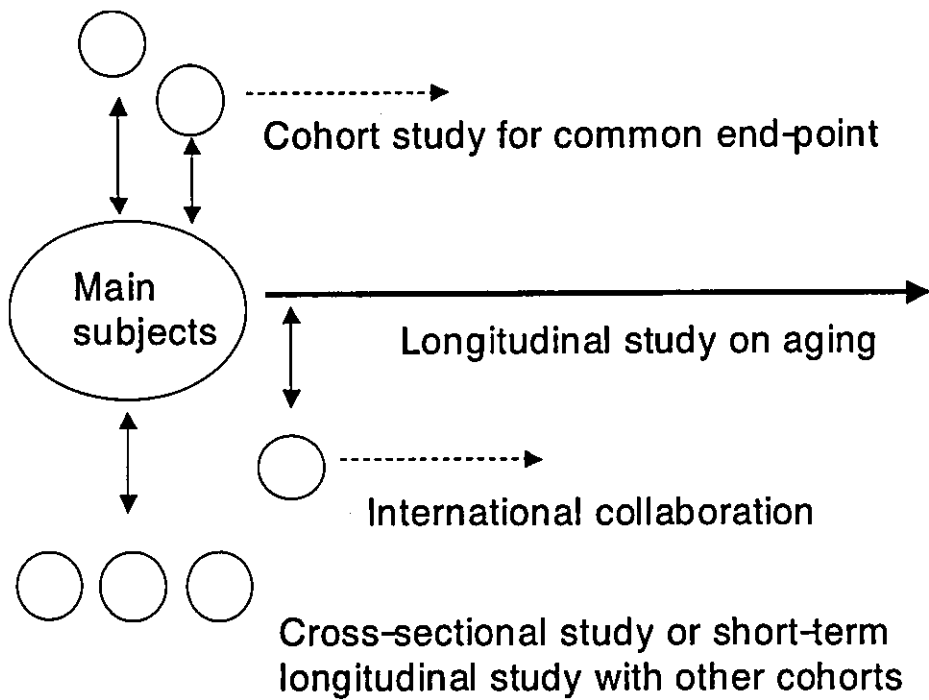


Fig. 6 Design of the longitudinal study by multi-center collaboration

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II. Background Examinations

II . Background Examinations

- 1) State of sleep
- 2) Smoking
- 3) Health status
- 4) Past history and present illness
- 5) Family history
- 6) Motor function
- 7) Dental status
- 8) Menstruation (for only women)
- 9) Hearing
- 10) Eyesight
- 11) Urinary disturbance
- 12) Job
- 13) Educational background
- 14) Ikigai or hobby
- 15) About family

1) State of sleep

Sleeping hours (hour)

	40-49yr			50-59yr			60-69yr			70-79yr			80yr-			Total		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Male	6.6	0.8	261	6.8	1.0	327	7.1	1.0	297	7.5	1.4	266	7.8	1.3	42	7.0	1.1	1193
Female	6.5	0.9	292	6.6	0.9	284	7.0	1.1	285	7.0	1.3	273	7.0	1.3	33	6.8	1.1	1167
Total	6.6	0.9	553	6.7	0.9	611	7.1	1.1	582	7.3	1.3	539	7.4	1.4	75	6.9	1.1	2360

Sleeping state

		40-49yr		50-59yr		60-69yr		70-79yr		80yr-		Total	
		N	%	N	%	N	%	N	%	N	%	N	%
Hard to sleep in	Male	36	13.8	43	13.1	62	20.9	75	28.2	8	19.0	224	18.8
	Female	45	15.4	60	21.1	128	44.9	105	38.5	13	38.2	351	30.1
	Total	81	14.6	103	16.8	190	32.6	180	33.4	21	27.6	575	24.3
Awake in midnight	Male	33	12.6	58	17.7	107	36.0	88	33.1	17	40.5	303	25.4
	Female	27	9.2	59	20.8	115	40.4	120	44.0	13	38.2	334	28.6
	Total	60	10.8	117	19.1	222	38.1	208	38.6	30	39.5	637	27.0
Big snoring	Male	99	37.9	131	39.9	101	34.0	62	23.3	10	23.8	403	33.8
	Female	32	11.0	62	21.8	45	15.8	39	14.3	5	14.7	183	15.7
	Total	131	23.7	193	31.5	146	25.1	101	18.7	15	19.7	586	24.8
Often see dream	Male	43	16.5	49	14.9	66	22.2	71	26.7	16	38.1	245	20.5
	Female	39	13.4	43	15.1	52	18.2	47	17.2	7	20.6	188	16.1
	Total	82	14.8	92	15.0	118	20.3	118	21.9	23	30.3	433	18.3
Deep sleep	Male	94	36.0	145	44.2	127	42.8	121	45.5	17	40.5	504	42.2
	Female	95	32.5	119	41.9	114	40.0	130	47.6	15	44.1	473	40.5
	Total	189	34.2	264	43.1	241	41.4	251	46.6	32	42.1	977	41.4
Drowsy in daytime	Male	61	23.4	75	22.9	96	32.3	100	37.6	20	47.6	352	29.5
	Female	108	37.0	107	37.7	71	24.9	112	41.0	16	47.1	414	35.4
	Total	169	30.6	182	29.7	167	28.7	212	39.3	36	47.4	766	32.4
No adequate situation	Male	43	16.5	42	12.8	22	7.4	14	5.3	2	4.8	123	10.3
	Female	58	19.9	35	12.3	25	8.8	13	4.8	1	2.9	132	11.3
	Total	101	18.3	77	12.6	47	8.1	27	5.0	3	3.9	255	10.8
Total	Male	261	100.0	328	100.0	297	100.0	266	100.0	42	100.0	1194	100.0
	Female	292	100.0	284	100.0	285	100.0	273	100.0	34	100.0	1168	100.0
	Total	553	100.0	612	100.0	582	100.0	539	100.0	76	100.0	2362	100.0

Nap

		40-49yr		50-59yr		60-69yr		70-79yr		80yr-		Total	
		N	%	N	%	N	%	N	%	N	%	N	%
Not recorded	Male	0	0.0	1	0.3	0	0.0	0	0.0	1	2.4	2	0.2
	Female	0	0.0	0	0.0	1	0.4	0	0.0	0	0.0	1	0.1
	Total	0	0.0	1	0.2	1	0.2	0	0.0	1	1.3	3	0.1
More than 6 times a week	Male	2	0.8	10	3.0	16	5.4	19	7.1	5	11.9	52	4.4
	Female	6	2.1	6	2.1	10	3.5	23	8.4	6	17.6	51	4.4
	Total	8	1.4	16	2.6	26	4.5	42	7.8	11	14.5	103	4.4
3 to 5 times a week	Male	30	11.5	41	12.5	46	15.5	67	25.2	18	42.9	202	16.9
	Female	44	15.1	53	18.7	48	16.8	69	25.3	7	20.6	221	18.9
	Total	74	13.4	94	15.4	94	16.2	136	25.2	25	32.9	423	17.9
1 or 2 times a week	Male	78	29.9	115	35.1	110	37.0	87	32.7	9	21.4	399	33.4
	Female	121	41.4	123	43.3	109	38.2	92	33.7	13	38.2	458	39.2
	Total	199	36.0	238	38.9	219	37.6	179	33.2	22	28.9	857	36.3
None	Male	151	57.9	161	49.1	125	42.1	93	35.0	9	21.4	539	45.1
	Female	121	41.4	102	35.9	117	41.1	89	32.6	8	23.5	437	37.4
	Total	272	49.2	263	43.0	242	41.6	182	33.8	17	22.4	976	41.3

2) Smoking

Smoking habit

		40-49yr		50-59yr		60-69yr		70-79yr		80yr-		Total	
		N	%	N	%	N	%	N	%	N	%	N	%
Not recorded	Male	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Female	0	0.0	0	0.0	1	0.4	0	0.0	0	0.0	1	0.1
	Total	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0	1	0.0
Non-smoker	Male	69	26.4	85	25.9	82	27.6	50	18.8	8	19.0	294	24.6
	Female	243	83.2	247	87.0	263	92.3	258	94.5	34	100.0	1045	89.5
	Total	312	56.4	332	54.2	345	59.3	308	57.1	42	55.3	1339	56.7
Ex-smoker	Male	91	34.9	117	35.7	136	45.8	162	60.9	24	57.1	530	44.4
	Female	25	8.6	11	3.9	7	2.5	7	2.6	0	0.0	50	4.3
	Total	116	21.0	128	20.9	143	24.6	169	31.4	24	31.6	580	24.6
Current-smoker	Male	101	38.7	126	38.4	79	26.6	54	20.3	10	23.8	370	31.0
	Female	24	8.2	26	9.2	14	4.9	8	2.9	0	0.0	72	6.2
	Total	125	22.6	152	24.8	93	16.0	62	11.5	10	13.2	442	18.7

Age at starting to smoke in ex-smoker (years old)

	40-49yr			50-59yr			60-69yr			70-79yr			80yr-			Total		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Male	19.8	2.8	90	21.1	6.0	116	23.2	10.1	133	23.9	12.2	158	24.7	14.4	24	22.4	9.6	521
Female	21.6	4.8	25	24.7	9.1	11	31.2	9.3	6	24.5	2.7	6	.	.	.	23.9	7.0	48
Total	20.2	3.4	115	21.4	6.4	127	23.5	10.1	139	23.9	12.0	164	24.7	14.4	24	22.5	9.4	569

Age at stopping to smoke in ex-smoker (years old)

	40-49yr			50-59yr			60-69yr			70-79yr			80yr-			Total		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Male	31.4	6.6	90	39.4	9.8	115	48.0	11.8	132	55.8	13.3	153	61.4	14.7	24	46.1	14.6	514
Female	30.1	9.0	25	34.2	13.0	11	53.5	6.1	6	53.5	11.4	6	.	.	.	36.9	13.8	48
Total	31.1	7.1	115	38.9	10.2	126	48.2	11.6	138	55.7	13.2	159	61.4	14.7	24	45.3	14.8	562

Previous amount of consumption in ex-smoker (pieces/day)

	40-49yr			50-59yr			60-69yr			70-79yr			80yr-			Total		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Male	18.3	8.4	90	22.0	10.6	115	23.5	11.0	134	19.8	10.5	160	16.3	6.3	24	20.8	10.4	523
Female	11.0	8.6	24	12.4	10.9	11	16.0	13.8	6	11.3	7.3	6	.	.	.	12.0	9.6	47
Total	16.7	8.9	114	21.1	11.0	126	23.2	11.2	140	19.5	10.5	166	16.3	6.3	24	20.1	10.6	570

Age at starting to smoke in current-smoker (years old)

	40-49yr			50-59yr			60-69yr			70-79yr			80yr-			Total		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Male	19.7	3.2	100	21.0	6.3	125	21.6	6.6	77	22.8	10.0	53	21.3	3.3	10	21.1	6.4	365
Female	24.4	8.2	24	24.1	6.1	26	40.6	12.8	14	34.9	14.0	8	.	.	.	28.6	11.4	72
Total	20.6	4.9	124	21.6	6.3	151	24.5	10.4	91	24.4	11.2	61	21.3	3.3	10	22.3	8.0	437

Amount of consumption in current-smoker (pieces/day)

	40-49yr			50-59yr			60-69yr			70-79yr			80yr-			Total		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Male	22.2	9.9	101	23.3	9.4	126	20.0	9.5	79	16.4	8.9	53	13.9	6.0	10	21.1	9.8	369
Female	11.0	5.7	24	15.2	9.1	26	12.1	4.9	14	13.0	6.7	8	.	.	.	12.9	7.2	72
Total	20.1	10.3	125	21.9	9.8	152	18.8	9.4	93	16.0	8.7	61	13.9	6.0	10	19.7	9.8	441

Reason for quitting to smoke

		40-49yr		50-59yr		60-69yr		70-79yr		80yr-		Total	
		N	%	N	%	N	%	N	%	N	%	N	%
Not want to smoke as previously	Male	16	17.6	12	10.3	11	8.1	17	10.5	5	20.8	61	11.5
	Female	7	28.0	2	18.2	1	14.3	1	14.3	0	0	11	22.0
	Total	23	19.8	14	10.9	12	8.4	18	10.7	5	20.8	72	12.4
Disease	Male	21	23.1	24	20.5	39	28.7	66	40.7	6	25.0	156	29.4
	Female	0	0.0	1	9.1	1	14.3	4	57.1	0	0	6	12.0
	Total	21	18.1	25	19.5	40	28.0	70	41.4	6	25.0	162	27.9
For health	Male	49	53.8	72	61.5	83	61.0	96	59.3	10	41.7	310	58.5
	Female	11	44.0	7	63.6	5	71.4	2	28.6	0	0	25	50.0
	Total	60	51.7	79	61.7	88	61.5	98	58.0	10	41.7	335	57.8
For family	Male	21	23.1	48	41.0	60	44.1	33	20.4	5	20.8	167	31.5
	Female	1	4.0	1	9.1	0	0.0	0	0.0	0	0	2	4.0
	Total	22	19.0	49	38.3	60	42.0	33	19.5	5	20.8	169	29.1
Economical problem	Male	15	16.5	4	3.4	5	3.7	3	1.9	0	0.0	27	5.1
	Female	1	4.0	0	0.0	0	0.0	0	0.0	0	0	1	2.0
	Total	16	13.8	4	3.1	5	3.5	3	1.8	0	0.0	28	4.8
Others	Male	15	16.5	14	12.0	22	16.2	28	17.3	3	12.5	82	15.5
	Female	12	48.0	4	36.4	0	0.0	1	14.3	0	0	17	34.0
	Total	27	23.3	18	14.1	22	15.4	29	17.2	3	12.5	99	17.1
Total	Male	91	100.0	117	100.0	136	100.0	162	100.0	24	100.0	530	100.0
	Female	25	100.0	11	100.0	7	100.0	7	100.0	0	0	50	100.0
	Total	116	100.0	128	100.0	143	100.0	169	100.0	24	100.0	580	100.0