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COHORT PROFILE

The profile of Rafsanjan Cohort Study

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Abstract
Owning the largest human-made jungle of pistachio, the second largest copper mine, and being located on the trade route of opium transit, distinguish Rafsanjan from many other cities in Iran. The environmental exposures and lifestyle factors associated with these characteristics of Rafsanjan, have raised concern about possible health outcomes for individuals living in and around this city. Thus, local health authorities initiated the Rafsanjan Cohort Study (RCS), as part of the prospective epidemiological research studies in IrAN (PERSIAN). RCS is a population-based prospective cohort of men and women aged 35–70 years, launched in August 2015. Individuals from diverse socioeconomic levels and lifestyles were recruited from four urban and suburban areas of Rafsanjan (participation rate 67.42%). Questionnaire-based interviews regarding demographics, dietary and environmental exposures, medical and occupational history, as well as anthropometric measurements were completed for all participants. Additionally, bio-specimens (blood, urine, hair, and nail) were collected, and dental and eye examinations were performed. The enrollment phase ended in December 2017, and a 15-year follow-up is planned. A total of 9990 individuals were enrolled in RCS (53.41% females). About 26% of men are pistachio farmers. The baseline prevalence of major non-communicable disease (NCD) risk factors such as cigarette smoking, alcohol consumption and opium use were 25.45%, 10.02%, and 23.81%, respectively. The mean ± SD of other common risk factors are as follows: body mass index (27.83 ± 4.89 mm Hg), systolic blood pressure (107.18 ± 17.56 mm Hg) diastolic blood pressure (71.13 ± 10.83), fasting blood sugar (113.27 ± 39.11 mg/dL) and plasma cholesterol (198.78 ± 41.89 mg/dL). These results indicate a concerning prevalence of NCD risk factors in Rafsanjan city, warranting further detailed investigations, particularly regarding the association of NDC with agricultural/industrial pollutants and drug abuse.

Keywords Cohort · NCDs · PERSIAN · Rafsanjan

Introduction

Non-communicable diseases (NCD) have become the main cause of morbidity and mortality in Iran. Therefore, research—especially population based studies—on NCD risk factors and the subsequent policies to control them have high priorities.

Rafsanjan, a city in southern Iran, has unique characteristics that make it a suitable area for conducting cohort studies. This region, in terms of agriculture, owns the largest man-made pistachio jungle in the world. It also has the second largest copper mine worldwide (Sarcheshmeh Copper Complex). Therefore, farmers and miners are the main residents of Rafsanjan (population 160,000), who are highly exposed to environmental and industrial pollutants, and

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various agricultural toxins. In addition, international opioid transit routes pass around Rafsanjan, causing a relatively high rate of opium addiction among residents.

Over the past three decades, industrial development, including the establishment of new industrial towns and factories, activities of the Sarcheshmeh copper complex, and overuse of pesticides and fertilizers in pistachio orchards and farmlands has increased pollution in and around Rafsanjan [1]. The 80,000 hectares of pistachio orchards in the area, receive about 100,000 L of pesticides—mostly Organophosphorus—each year [2]. Surprisingly, around 70% of farmers use no personal protective equipment when applying pesticides and about 60% of them discard the pesticide containers in the environment with no special care [3], raising health threatening concerns for all Rafsanjan residents. A limited number of studies investigating the health profile of pistachio farmers point to high incidence rates for infertility, tiredness and some cancers [3, 4]. More comprehensive studies in this regard are necessary to assist local health policymakers.

The Sarcheshmeh Copper mine is located only 40 km from Rafsanjan. Previous reports have demonstrated high levels of heavy metals (copper, cadmium, molybdenum, zinc and gold), as well as sulfur dioxide, nitric oxide, ozone and aerosols in the mine and around it [5, 6]. Only a few studies have previously been performed on the health profile of copper miners and the possible air, soil and water polutants arising from mining activities. More complete and inclusive studies are needed in this regard.

In addition to unique environmental exposures, distinct health characteristics reported in previous studies also show that Rafsanjan has one of the highest diabetes incident rates in Iran (19.1%) [7]. About 30% of Rafsanjan's urban population (over the age 22) have at least one lipid abnormality, which is also among the highest rates in Iran [8].□□□□□ □□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□

Considering the unique characteristics of Rafsanjan and also the lack of adequate comprehensive studies on the health profile of its residents, a cohort study can be very beneficial in the identification of the most common NCD and their associated risk factors. Therefore, Rafsanjan was chosen as one of the cohort sites included in the prospective epidemiological research studies in IrAN (PERSIAN Cohort) [9], a nationwide cohort launched in 2014, funded by the Iranian ministry of health and medical education. The data collected in the PERSIAN Cohort Study thoroughly captures many lifestyle and environmental exposures that may affect NCD development and the Rafsanjan Cohort Study (RCS) follows the protocols provided by the PERSIAN Cohort Central Scientific Committee, however, given that there are many reports about the effects of farming [10], industrial activities [11–13] and addiction [14, 15] on dental and eye health, additional data collection on these categories were also performed in RCS. In the current article, the methodology and profile of RCS cohort is presented.

The main objectives of the Rafsanjan Cohort Study

While the overall objectives of RCS are in line with the PERSIAN Cohort, special attention was paid to the unique environmental and lifestyle factors of the participants in determining the prevalence and burden of NCD and their associated risk factors. The main objectives of RCS are as follows:

A. To determine the effects of risk factors including environmental factors (e.g. pesticide, industrial pollution), nutritional habits (e.g. pistachio consumption), occupational status (e.g. pistachio farmer, copper miner), socioeconomic factors, lifestyle habits (opioid addiction) and family history on the incidence and prevalence of NCDs
B. To investigate the prevalence, incidence and progression of dental and ocular diseases
C. To determine the cause-specific mortality rate
D. To analyze the trajectory of risk factors and the natural course of diseases, with an emphasis on occupational and environmental factors
E. To establish a bio-bank for the collected specimen, to be used in future biomedical research, cross sectional and nested case–control studies
F. To establish a shared and collaborative database with other similar studies at national and international levels

The participants' characteristics

As one of the 19 geographically district areas of Iran included in the PERSIAN Cohort [9], RCS was designed to recruit a total of 10,000 participants of both genders aged 35–70 years, living in four pre-determined districts of Rafsanjan (2500 participants from each site) including both urban and suburban areas. This sample size estimated by the PERSIAN Cohort Central Scientific Committee for each PERSIAN cohort site supports adequate statistical power. A population of 10,000 individuals would be large enough to detect for detection of a relative risk of 2 for an exposure, (α level of 0.05, β of 90%, for a given 10% prevalence) with 150 incident cases of the outcome of interest during the follow up phase. A total of 150 incident cases for 15 years of follow up equates 10 case/year, which is very much realistic to achieve in RCS [9].

The study districts were selected based on two main criteria: (1) inclusion of a population with varied socioeconomic levels, as well as environmental and occupational exposures, (2) regions with minimum migration rates that housed relatively non-mobile permanent residents of Rafsanjan city and its suburban areas, providing an advantage for the follow-up

studies. These districts were selected based on the records provided by the vice chancellor for health affairs at Rafsanjan University of Medical Sciences (RUMS) (Fig. 1).

Recruitment methods and measured indices

Active recruitment of the study population was conducted by trained personnel and local health volunteer communicators, using a population census, and following PERSIAN Cohort protocols [9]. The recruitment team employed a door to door, face-to face approach to invite residents of each Rafsanjan district to participate in RCS. The recruiters explained the aims of the study, and collected phone numbers from individuals who agreed to participate. Next, the participants were contacted by phone and were assigned an appointment date to attend the Rafsanjan cohort center. Given the target population of 10,000, the recruitment team invited 14,827 individuals until the target sample size was reached (participation rate: 67.44%).

The work flow at the cohort center consisted of 4 steps: registration, biological sampling, anthropometric measurements, and completion of interviewer-based questionnaires, which will be briefly explained in the following paragraphs. A team consisting of 27 trained personnel including enumerators, medical doctors, laboratory technicians and interviewers performed the data and biospecimen collection and storage in accordance with the PERSIAN cohort protocols [9].

Participants recruited to the cohort center were first registered in the study after providing valid identification, and signing an informed consent to participate. A PERSIAN Cohort Identification (PCID) code was next specified for each individual, which was used to label all the corresponding bio-specimens and documentation.

Next, 25 mL of fasted blood were taken from each individual. Shortly after sampling, blood was centrifuged and the obtained fractions were aliquoted, labeled with the participants' PCID and stored (− 70 °C) for future use. Urine samples were also taken and stored at − 30 C. Blood and urine biochemical, hematological and microscopic tests were performed for all participants (see Table 1 for more details). As an incentive, all participants received the results of their blood and urine tests. Individuals with abnormal laboratory findings were advised to visit their family physician. Nail and hair samples, as biomarkers for metal exposures and remnants of toxins and drug

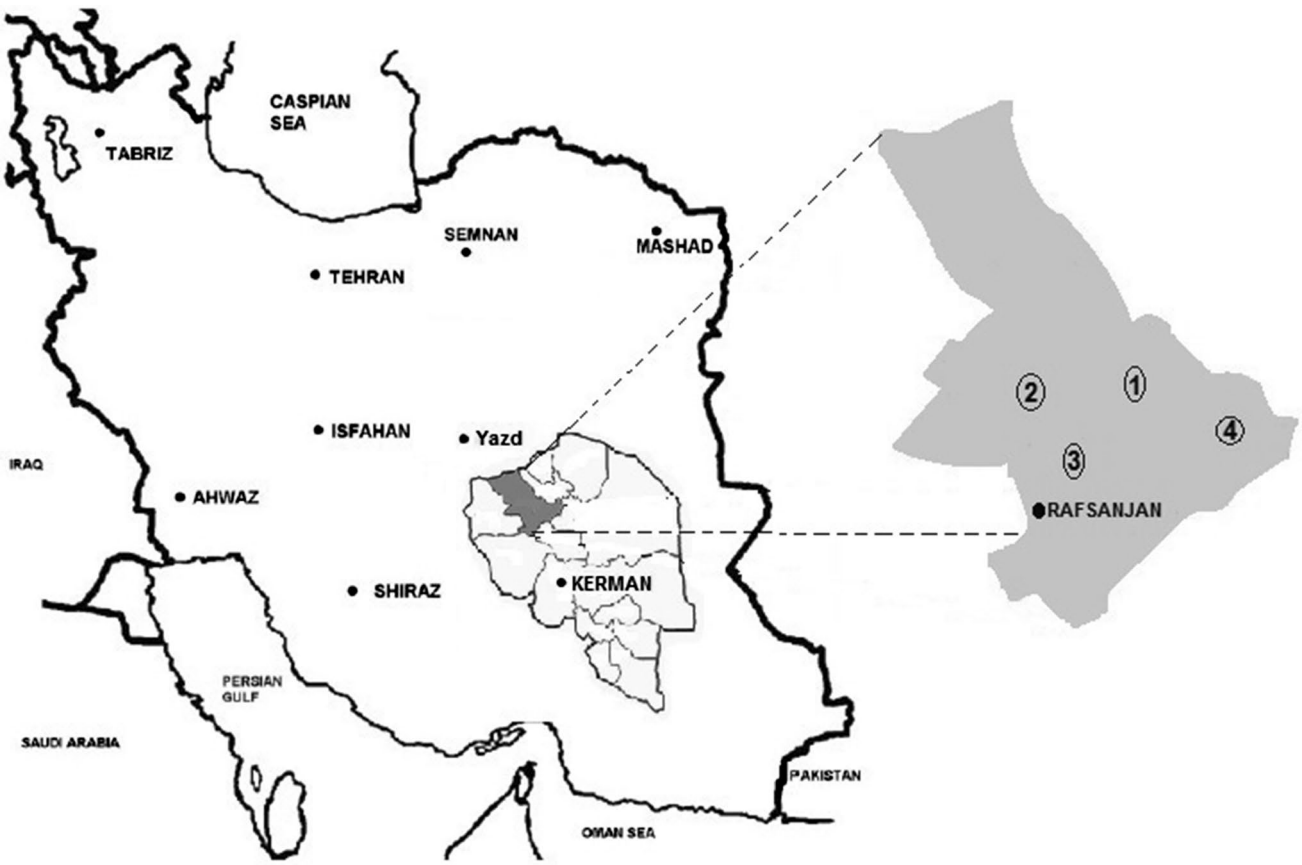

Fig. 1 Geographic location of Rafsanjan city in southeastern Iran. The four districts selected to be part of RCS are shown

Table 1 Data domains and major subcategories addressed in the Rafsanjan Cohort Study (RCS)

General questionnaire (104 items)	Demographic factors, socioeconomic status, marital status, educational level, lifestyle
Occupational status	Full job history of the participants and their partner
Environmental exposures	Frequency and duration of toxin exposure (domestic and agriculture-related pesticide, insecticide, etc.), personal protective equipment
Biological samples and tests	Complete blood count, fasting blood sugar, BUN, creatinine, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, LDL cholesterol/HDL cholesterol, total cholesterol/HDL cholesterol, S.G.O.T. (AST), S.G.P.T. (ALT), alkaline phosphatase, urine analysis, nail and hair samples
Medical questionnaire (182 items)	Personal medical history, family medical history, medication and physical activity
Personal habits	Smoking, alcohol consumption and opium consumption
Nutrition questionnaire (161 items)	Food frequency and dietary habits
Anthropometric features	Weight (kg), height, waist, hip and wrist circumferences (cm)
Ophthalmologic examinations	Refractive errors, intra-ocular pressure, diabetic retinopathy, Marcus Gunn pupil, red reflex, keratoconus, xerophthalmia, lacrimation, blepharoptosis and entropion, ectropion
Oral and dental health	Decayed teeth, missing teeth, filled permanent teeth and oral lesions

metabolites, were also collected and shelved using labeled zip-lock bags in a dark, dry and cold room for further investigations.

After sampling, common anthropometric measurements including; height, waist, hip, and wrist circumferences (in cm) and weight (in kg), were measured based on the US National Institutes of Health protocols [16]. These measurements were taken in the morning while the participants were still fasting since these characteristics have minimum bias at this time. Due to the relatively long duration of the 4-step enrollment process, participants were warmly welcomed with breakfast prior to proceeding to questionnaire completion.

Various questionnaires regarding demographic and socioeconomic information, medical history, oral and dental health, nutrition, personal habits and type and duration of occupational and environmental exposures were completed, details of which are provided in Table 1. All data was entered directly into the PERSIAN Cohort data server.

While the abovementioned data/samples are collected in all PERSIAN Cohort sites, Rafsanjan is also part of the PERSIAN Eye Cohort Study (including 6 cities in Iran) with the aim to determine the prevalence of major eye diseases. Therefore, eye examinations were also performed for all RCS participants. Two trained optometrists performed Fundoscopy and slit lamp examinations for all participants and completed a nationally-standardized ophthalmologic questionnaire to screening for common ophthalmologic conditions. The screening criteria consisted of the following ten major ophthalmologic health determinants: 1. diabetes mellitus 2. family history of glaucoma, 3. relative afferent pupillary defect (RAPD), 4. strabismus, 5. keratoconus suspect (Scissor reflex), 6. belphar abnormality, 7. xerophthalmia, 8. poor red reflex, 9. corrected refractive errors less than 8/10, and 10. Any other suspected ophthalmologic abnormality. Participants who met at least one of the above-mentioned criteria received a complete ophthalmological examination by an ophthalmologist cooperating with the RCS team.

A unique characteristic of RCS among all other PERSIAN cohort sites is the addition of dental examinations, which were performed in a manner similar to the eye exams. A locally designed dental questionnaire covering virtually all aspects of a complete dental examination, as well as a complete dental exam by a trained dentist were completed for each participant. Detailed information on the dental and eye examinations performed are presented in Table 1.

Quality assurance and quality control

All stages performed in RCS including recruitment, interviews, measurements and physical examinations are in accordance with the PERSIAN cohort protocols [9]. In order to reach the minimum rate of error in data collection, a smart data server was used. This server was monitored for daily enrollment, and recurrently checked for incomplete data entry by the central and local quality control (QC) teams. Evaluation reports of this monitoring process were sent to the principal investigators of RCS. Random recordings of the interviewing processes (with the consent of the interviewee) were also made and audited by the central and local training teams as a QC measure. Furthermore, unexpected inspections were made to the cohort center by the central QC team to assess the quality of data collection procedures and to collect random participant opinion surveys. In addition, 100 individuals who had been enrolled, were randomly invited to attend the cohort center for a second time to repeat various sections of data collection, for comparison purposes. The kappa statistics for agreement were above 0.8 for most variables.

All laboratory tests were performed under the supervision of a pathologist. Laboratory measurement quality was ensured by: 1. personnel training and monitoring for

adherence to the protocol; 2. verification and calibration of all equipment according to standardized protocols; and 3. regular measurement of Inter-assay coefficients of variation for blood chemistry parameters according to the standard QC protocols (a sample of these measurements for 1 month are presented in Table 2).

Follow-up phase

Participants of RCS will be followed for at least 15 years, and a reassessment in a subsample will be conducted every 5 years. During this period, participants and/or their close relatives will receive annual phone calls and would be asked about the outcomes of interest in RCS, and any serious medical conditions and/or hospitalization. For the expired participants, a verbal autopsy form is also completed [9].

Ethical considerations

This study was conducted by local investigators at RUMS, under the supervision of the Iranian Ministry of Health and Medical Education and the PERSIAN Cohort Central Scientific Committee. Ethical approval for the study was obtained from the Ethical Committee of RUMS (ID: IR.RUMS.REC.1394.254), and informed consent was obtained from all participants for the interview, physical examinations, bio-specimen collection and future research using the collected data. All necessary measures were taken to ensure confidentiality of personal data.

Table 2 Inter-assay CV for blood chemistry measurements

Analyte	Level (normal/high)[a]	CV[b] (normal/high)
Fasting blood sugar (mg/dL)	94.2	1.22
	252.76	1.34
Urea (mg/dL)	39.28	2.14
	125	1.51
Creatinine (mg/dL)	1.34	1.73
	4.25	1.27
Total cholesterol (mg/dL)	94.56	1.49
	192.36	1.23
Triglycerides (mg/dL)	110.96	1.92
	205.72	1.17
HDL cholesterol (mg/dL)	39.92	2.49
	95.08	2.22
Aspartate aminotransferase (U/L)	46.28	2.53
	143.88	1.53
Alanine aminotransferase (U/L)	54.24	1.93
	154.68	1.23
Alkaline phosphatase (U/L)	174.04	1.74
	365.6	2.28
Gamma-glutamyl transferase (U/L)	43.68	1.58
	205.28	1.42

[a]Normal, control material values that is deemed normal for a physiologic measurement in healthy persons; high, control material values that is deemed high for a physiologic measurement in healthy persons

[b]*CV* coefficient of variation

Results

The enrolment phase started in August 2015. The target population (n = 10,000) was reached in December 2017 after 14,827 individuals were invited to participate (participation rate: 67.44%). After the data was cleaned and individuals missing major sections of data and/or laboratory tests were omitted, the final sample size declined to 9990 (5335 women and 4655 men) (Table 3). The participation rate was lower in young men compared to women and also in the elderly. More details about participant and non-participant samples including the main baseline characteristics of the study population are shown in Table 3.

Major lifestyle risk factors are also presented in Table 3. The rates of cigarette smoking and alcohol consumption among men were 51.73% and 21.36%, respectively. The rates of opium use, cigarette smoking and alcohol consumption were noticeably lower in women: 4.27%, 2.51% and 0.16%, respectively.

Table 4 shows some of the main clinical and laboratory characteristics of the RCS population. While, lifestyle risk factors mentioned above were higher in men, serum levels of cholesterol (194.2 ± 37.6 mg/dL in men vs. 202.7 ± 44.8 in women), FBS (111.4 ± 36.3 in men vs. 114.8 ± 41.3 mg/dL in women), as well as BMI (26.1 ± 4.3 in men vs. 29.3 ± 4.8 in women), are higher in women.

As expected, a considerable proportion (about 26%) of the employed male population in RCS are pistachio farmers. Preliminary results indicate that the rate of cardiac diseases and serum triglyceride levels—among other clinical and laboratory findings—are different in pistachio farmers compared to others (Table 5).

Notably, the rate of opium use is high among RCS participants; about 46.3% of men reported opium use of at least once per week for 6 months (Table 3). Differences in some measured clinical and laboratory indices between opium users and non-users are shown in Table 6. Ischemic heart diseases, myocardial infarction, and serum levels of triglycerides and alkaline phosphatase activity are higher among opium users.

Discussion

RCS, as one of the PERSIAN cohort sites, aims to investigate the prevalence of NCD and their risk factors, as well as eye and dental health problems in Rafsanjan city, which

Table 3 Baseline characteristics of the RCS participants and non-participants

Participants characteristics	Total (n = 9990)	Men (n = 4655)	Women (n = 5335)	*P* value
Age at baseline, no. (%)				0.009
35–44 years	3466 (34.69)	1615 (34.69)	1851 (34.70)	
45–54 years	3039 (30.42)	1373 (29.50)	1666 (31.23)	
55–64 years	2759 (27.62)	1288 (27.67)	1471 (27.57)	
≥ 65 years	726 (7.27)	379 (8.14)	347 (6.50)	
Age (mean ± SD)	49.91 ± 9.56	50.11 ± 9.75	49.74 ± 9.39	< 0.001
Marriage, no. (%)				< 0.001
Single	129 (1.29)	60 (1.29)	69 (1.29)	
Married	9207 (92.16)	4547 (97.68)	4660 (87.35)	
Widow	538 (5.39)	21 (0.45)	517 (9.69)	
Divorce	116 (1.16)	27 (0.58)	89 (1.67)	
Education level, no. (%)				< 0.001
No schooling	948 (9.50)	296 (6.36)	652 (12.23)	
1–5 years of school	2547 (25.52)	850 (18.28)	1697 (31.84)	
6–12 years of school	4847 (48.56)	2533 (54.46)	2314 (43.41)	
University/college	1639 (16.42)	972 (20.90)	667 (12.51)	
Employment status, no. (%)				< 0.001
Unemployed	152 (1.52)	120 (2.58)	32 (0.60)	
Self-employed	3441 (34.45)	2768 (59.48)	673 (12.61)	
Employed	1302 (13.03)	944 (20.28)	358 (6.71)	
Retired	1081 (10.82)	821 (17.64)	260 (4.87)	
Homemaker	4013 (40.17)	1 (0.02)	4012 (75.20)	
Pistachio farmers[a], no. (%)	1260 (12.61)	1208 (25.95)	52 (0.97)	< 0.001
Copper miners[a], no. (%)	362 (3.6)	350 (7.52)	12 (0.22)	< 0.001
Smoking[b], no. (%)	2542 (25.45)	2408 (51.73)	134 (2.51)	< 0.001
Opium use[c], no. (%)	2378 (23.81)	2150 (46.20)	228 (4.27)	< 0.001
Alcohol consumption[d], no. (%)	995 (10.02)	987 (21.36)	8 (0.16)	< 0.001
BMI (mean ± SD)	27.83 ± 4.89	26.13 ± 4.31	29.31 ± 4.89	< 0.001
Non-participants, no. (%)	4827 (100.00)	2709 (56.12)	2118 (43.88)	< 0.001
Age, no. (%)				0.023
35–44 years	1565 (33.57)	873 (33.37)	696 (33.82)	
45–54 years	1503 (32.24)	879 (33.60)	624 (30.50)	
55–64 years	1063 (22.80)	594 (22.71)	469 (22.92)	
≥ 65 years	531 (11.39)	270 (10.32)	261 (12.76)	
Age (mean ± SD)	50.79 ± 9.73	50.56 ± 9.55	51.08 ± 9.95	< 0.001
Participation rate (%)	67.44	63.21	71.58	

[a]These items overlap with employed

[b]Smoking was defined as having smoked more than 100 cigarettes in lifetime

[c]Opium use was defined as having used opium at least once per week for 6 months

[d]Alcohol consumption is defined as drinking approximately 350 mL of beer OR 150 mL of wine OR 45 mL of liquor, once per week for at least 6 months [40]

is distinguished in its population's life style habits, as well as environmental and occupational exposures. This heterogeneous and large database, covering a broad array of exposures and outcomes along with a standard biobank and 15-year follow-up, enables us and other researchers to have a comprehensive assessment of many factors and exposures affecting health outcomes, accompanied by valid intra- and inter-cohort site comparisons.

Many paraclinical findings in RCS participants, such as the mean values of cholesterol, TG, LDL-cholesterol, HDL-cholesterol, FBS, and liver enzymes (AST, ALT and alkaline phosphatase) were in line with the findings of other population-based studies conducted in different cities of Iran [17–26]. Interestingly, several cardiovascular disease (CVD) risk factors such as total cholesterol, LDL and BMI were significantly higher in the women participants, which is also

Table 4 Major clinical and laboratory findings of RCS participants

	Total (n = 9990)	Men (n = 4655)	Women (n = 5335)	*P* value
Clinical findings				
Cardiac disease[a], no. (%)	870 (8.76)	446 (9.65)	424 (7.98)	0.003
Myocardial infarction, no. (%)	296 (2.98)	211 (4.56)	85 (1.60)	< 0.001
Systolic blood pressure (mean ± SD)	107.18 ± 17.56	108.49 ± 17.11	106.04 ± 17.87	< 0.001
Diastolic blood pressure (mean ± SD)	71.13 ± 10.83	72.04 ± 10.96	70.34 ± 10.65	< 0.001
	Total (n = 9941)	Men (n = 4637)	Women (n = 5304)	
Blood analysis				
Fasting blood sugar (mg/dL) (mean ± SD)	113.27 ± 39.11	111.47 ± 36.33	114.83 ± 41.31	< 0.001
Cholesterol (mg/dL) (mean ± SD)	198.78 ± 41.89	194.20 ± 37.65	202.77 ± 44.89	< 0.001
Triglycerides (mg/dL) (mean ± SD)	168.88 ± 109.22	179.81 ± 125.37	159.36 ± 91.83	< 0.001
LDL cholesterol (mg/dL) (mean ± SD)	108.04 ± 30.50	105.51 ± 29.93	110.24 ± 30.82	< 0.001
HDL cholesterol (mg/dL) (mean ± SD)	57.90 ± 12.45	54.29 ± 10.33	61.05 ± 13.26	< 0.001
S.G.O.T (AST) (U/L) (mean ± SD)	19.87 ± 11.81	21.22 ± 10.37	18.69 ± 12.82	< 0.001
S.G.P.T (ALT) (U/L) (mean ± SD)	21.55 ± 15.37	24.67 ± 16.47	18.83 ± 13.78	< 0.001
Alkaline phosphatase (IU/L) (mean ± SD)	225.48 ± 66.81	229.03 ± 64.74	222.39 ± 68.43	< 0.001
Urine analysis				
Blood (positive), no. (%)	1896 (19.07)	760 (16.41)	1136 (21.39)	< 0.001
Protein (positive), no. (%)	489 (4.92)	258 (5.57)	231 (4.35)	0.005
Glucose (positive), no. (%)	889 (8.94)	414 (8.94)	475 (8.95)	0.995

Blood pressure was measured two times in each arm, with a 10 min resting interval in between the measurements. The average of the second measurement in the right and left arms was used to report blood pressure. Blood pressure readings are expressed in millimeters of mercury (mm Hg)

LDL low-density lipoprotein, *HDL* high-density lipoprotein, *SGOT* serum glutamic oxaloacetic transaminase, *SGPT* serum glutamic pyruvic transaminase, *AST* aspartate aminotransferase, *ALT* alanine transaminase

[a]Cardiac disease is defined as having ischemic heart disease or heart failure

in accordance with the results of other studies [17–26]; but in contrast, the prevalence of self-reported cardiac disease and myocardial infarctions were significantly higher in men, again, consistent with the results of other studies in Iran and worldwide about higher prevalence of CVD in males [17, 27].

Growing rates of overweight and obesity have become a great public health concern worldwide over the past decades, and likewise, these conditions are very common among both RCS men and women, with the average BMI being 26.1 ± 4.3 and 29.3 ± 4.8, respectively. Given the impact obesity on many NCDs—CVD, diabetes, cancers, etc. [28]—important public health measures must be taken to reduce these rates. Other previous reports have also shown alarming increases in the prevalence rates of abdominal and generalized obesity in Iran [29].

Our results also indicated that more than 16% of the participants, mostly men (33.47%), are pistachio farmers or copper miners with expected high levels of exposure to different toxic chemicals or dusts. Although both of these groups have healthier lipid profiles, their blood pressure is higher compared to those engaging in other occupations. Other independent studies being performed by RUMS are measuring annual concentrations of inhalable atmospheric particulate matters (PM), more specifically, PM2.5, PM5 and PM10 as well as certain pesticides, copper, cadmium, arsenic and lead as the main local air pollutants Merging RCS follow up data and data from these studies may yield interesting findings in the future about the effects of these toxins on the health status of RCS participants, especially the pistachio farmers and copper miners.

As previously described, Rafsanjan city, along with some other cities of Kerman province, particularly Jiroft and Bam, are geographically situated in the transit route of opioids. As a result, the availability and use of opioids is more feasible for vulnerable individuals. In a study in Bam, Aflatoonian and colleagues indicated that 226 (15.26%) of 1481 individuals with a mean age of 25.7 years, were addicted to opium [30]. In a recent study on high school students in Jiroft, Seiedi and colleagues reported that among 626 students, 70% of boys and 50.7% of girls had used opium, alcohol, or a psychedelic drug at least once in their lifetime [31]. Based on our results, 23.81% of RCS participants have used opium at least once per week for 6 months. Surprisingly, mean serum levels of cholesterol and LDL-cholesterol in opium users were lower, in comparison to non-users. However,

Table 5 Comparison of major clinical and laboratory findings among pistachio farmers, copper miners and other occupations in RCS participants

	Pistachio farmer (n = 1260)	Copper miner (n = 362)	Other occupation (n = 8368)	*P* value
Clinical findings				
Cardiac disease[a], no. (%)	159 (12.68)	40 (11.08)	671 (8.07)	< 0.001
Myocardial infarction, no. (%)	72 (5.74)	16 (4.43)	208 (2.50)	< 0.001
Blood pressure systolic (mean ± SD)	110.23 ± 17.30	107.34 ± 16.66	106.71 ± 17.60	< 0.001
Blood pressure diastolic (mean ± SD)	72.43 ± 10.97	71.26 ± 10.86	70.93 ± 10.80	< 0.001
Blood analysis				
Fasting blood sugar (mg/dL) (mean ± SD)	113.92 ± 38.19	111.48 ± 39.82	113.25 ± 39.21	0.573
Cholesterol (mg/dL) (mean ± SD)	194.83 ± 37.99	192.52 ± 37.25	199.64 ± 42.58	< 0.001
Triglycerides (mg/dL) (mean ± SD)	178.55 ± 119.59	177.78 ± 148.42	167.04 ± 105.43	0.001
LDL cholesterol (mg/dL) (mean ± SD)	105.43 ± 30.08	104.97 ± 30.12	108.56 ± 30.55	< 0.001
HDL cholesterol (mg/dL) (mean ± SD)	55.13 ± 10.32	53.68 ± 9.26	58.50 ± 12.771	< 0.001
S.G.O.T (AST) (U/L) (mean ± SD)	20.44 ± 9.66	20.68 ± 8.26	19.75 ± 12.23	0.067
S.G.P.T (ALT) (U/L) (mean ± SD)	22.56 ± 14.18	24.35 ± 15.64	21.27 ± 15.52	< 0.001
Alkaline phosphatase (IU/L) (mean ± SD)	227.84 ± 63.18	231.61 ± 69.78	224.86 ± 67.20	0.070
Urine analysis				
Blood (positive), no. (%)	229 (18.28)	61 (16.90)	1606 (19.29)	0.392
Protein (positive), no. (%)	82 (6.54)	28 (7.76)	379 (4.55)	< 0.001
Glucose (positive), no. (%)	131 (10.45)	33 (9.14)	725 (8.71)	0.129

Blood pressure was measured two times in each arm, with a 10 min resting interval in between the measurements. The average of the second measurement in the right and left arms was used to report blood pressure. Blood pressure readings are expressed in millimeters of mercury (mm Hg)

LDL low-density lipoprotein, *HDL* high-density lipoprotein, *SGOT* serum glutamic oxaloacetic transaminase, *SGPT* serum glutamic pyruvic transaminase, *AST* aspartate aminotransferase, *ALT* alanine transaminase

[a]Cardiac disease is defined as having ischemic heart disease or heart failure

cardiac disease and myocardial infarction are considerably more prevalent among opium users. Previously, different studies have reported the association of opium use with an increased risk of cardiovascular diseases [32–35]. Currently, collaborative clinical and epidemiological studies on drug abusers are proceeding in Kerman province of Iran.

Alcohol consumption rate is 21% among RCS male participants (aged 35–70). On the other hand, the preliminary results of another PERSIAN Cohort Study component—the PERSIAN Youth Cohort—performed in Rafsanjan and three other sites in Iran in individuals 15–35 years of age, has revealed an approximate 30% prevalence rate of alcohol consumption (unpublished data). Notably, the majority of Rafsanjani alcohol consumers (around 73%), use homemade drinks [36], which due to a lack of surveillance over their production, can lead to dangerous consequences similar to the methanol mass poisoning that occurred in Rafsanjan in 2013 [37].

RCS has many strengths. The large sample size captures men and women with various environmental, socioeconomic and occupational exposures, making this cohort ideal for future analyses and nested studies. The inclusion of ophthalmological and dental diseases in RCS is also a remarkable strength and unique characteristic of this cohort that will yield valuable information about these conditions, particularly poor dental health, which has been shown to be associated with the occurrence of certain cancers [38, 39]. The RCS biobank is also a valuable asset that may be used in the future for the identification of new disease biomarkers.

As with all cohort studies, minimizing loss to follow up is one of the main challenges and concerns. We have taken various steps to minimize losses and to encourage long term participations. As incentives, the results of the dental and ophthalmological examinations, as well as the laboratory findings were provided to all participants and free referrals to volunteer physicians were given if needed/requested. In addition, areas with lower migration rates were chosen to be included in this study, reducing attrition due to immigration. Furthermore, given that the RCS follow-up is carried out by phone, cooperation is more plausible.

In conclusion, due to the high rates of farming and industrial activities (environmental and occupational exposures), common use of opium in Rafsanjan city, further, detailed assessment of NCD and their associated risk factors are warranted. RCS sets a valuable foundation for studying various exposures and risk factors and their resultant outcomes. Effective analysis of this data will shed a better light on NCD's epidemiology and will provide a better

Table 6 Comparison of major clinical and laboratory findings among opium users and non-opium users in RCS participants

	Opium use (n = 2378)	Non-opium use (n = 7612)	*P* value
Clinical findings			
Cardiac disease[a], no. (%)	313 (13.16)	557 (7.37)	< 0.001
Myocardial infarction, no. (%)	154 (6.48)	142 (1.88)	< 0.001
Blood pressure systolic (mean ± SD)	107.72 ± 18.27	107.08 ± 17.33	0.083
Blood pressure diastolic (mean ± SD)	70.27 ± 11.22	71.41 ± 10.69	< 0.001
Blood analysis			
Fasting blood sugar (mg/dL) (mean ± SD)	114.16 ± 42.51	112.99 ± 37.98	0.204
Cholesterol (mg/dL) (mean ± SD)	192.73 ± 38.43	200.66 ± 42.74	< 0.001
Triglycerides (mg/dL) (mean ± SD)	178.57 ± 114.71	165.86 ± 107.28	< 0.001
LDL cholesterol (mg/dL) (mean ± SD)	104.21 ± 30.61	109.23 ± 30.36	< 0.001
HDL cholesterol (mg/dL) (mean ± SD)	54.24 ± 10.95	59.04 ± 12.67	< 0.001
S.G.O.T (AST) (U/L) (mean ± SD)	20.36 ± 12.38	19.72 ± 11.63	0.022
S.G.P.T (ALT) (U/L) (mean ± SD)	20.95 ± 15.32	21.74 ± 15.38	0.030
Alkaline phosphatase (IU/L) (mean ± SD)	236.50 ± 68.66	222.05 ± 65.86	< 0.001
Urine analysis			
Blood (positive), no. (%)	493 (20.89)	1403 (18.51)	0.010
Protein (positive), no. (%)	136 (5.76)	353 (4.66)	0.030
Glucose (positive), no. (%)	227 (9.62)	662 (8.73)	0.188

Blood pressure was measured two times in each arm, with a 10 min resting interval in between the measurements. The average of the second measurement in the right and left arms was used to report blood pressure. Blood pressure readings are expressed in millimeters of mercury (mm Hg)

LDL low-density lipoprotein, *HDL* high-density lipoprotein, *SGOT* serum glutamic oxaloacetic transaminase, *SGPT* serum glutamic pyruvic transaminase, *AST* aspartate aminotransferase, *ALT* alanine transaminase

[a]Cardiac disease is defined as having ischemic heart disease or heart failure

understanding of their burden. Additionally, the results of these studies raise greater awareness for local and national health authorities and policy makers to manage their limited resources based on health priorities.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests.

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