Study Protocol

PolyPill for Prevention of Cardiovascular Disease in an Urban Iranian Population with Special Focus on Nonalcoholic Steatohepatitis: A Pragmatic Randomized Controlled Trial within a Cohort (PolyIran - Liver) – Study Protocol

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Abstract

Background: Cardiovascular disease (CVD) is among the most common causes of mortality in all populations. Nonalcoholic steatohepatitis is a common finding in patients with CVD. Prevention of CVD in individual patients typically requires periodic clinical evaluation, as well as diagnosis and management of risk factors such as hypertension and hyperlipidemia. However, this is resource consuming and hard to implement, especially in developing countries. We designed a study to investigate the effects of a simpler strategy: a fixed-dose combination pill consisting of aspirin, valsartan, atorvastatin and hydrochlorthiazide (PolyPill) in an unselected group of persons aged over 50 years.

Design: The PolyIran-Liver study was performed in Gonbad city as an open label pragmatic randomized controlled trial nested within the Golestan Cohort Study. We randomly selected 2,400 cohort study participants aged above 50 years, randomly assigned them to intervention or usual care and invited them to participate in an additional measurement study (if they met the eligibility criteria) to measure liver related outcomes. Those agreeing and randomized to the intervention arm were offered a daily single dose of PolyPill. We will follow participants for 5 years. The primary outcome is major cardiovascular events, secondary outcomes include all-cause mortality and liver related outcomes: liver stiffness and liver enzyme levels. Cardiovascular outcomes and mortality will be determined from the cohort study and liver-related outcomes in those consenting to follow up. Analysis will be by allocated group.

Trial status: Between October and December 2011, 1,320 intervention and 1,080 control participants were invited to participate in the additional measurement study. For all these participants, the major cardiovascular events will be determined using blind assessment of outcomes through the cohort study. In the intervention and control arms, 875 (66%) and 721 (67%) respectively, met the eligibility criteria and agreed to participate in the additional measurement study. Liver related outcomes will be measured in these participants. Of the 1,320 participants randomized to the intervention, 787 (60%) accepted the PolyPill.

Conclusion: The PolyIran-liver urban study will provide us with important information on the effectiveness of PolyPill on major cardiovascular events, all-cause mortality and liver related outcomes. (ClinicalTrials.gov ID: NCT01245608).

Keywords: Atherosclerosis, Iran, liver, primary prevention, secondary prevention

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Introduction

As control and characterization of infectious diseases improve in the developing world, non-communicable diseases (NCD), particularly cardiovascular diseases (CVD), become more important. In Iran, cardiovascular disease (CVD) causes over 50% of non-communicable disease mortality in middle and old age.[1] Nonalcoholic fatty liver disease is currently the most common liver disease in Iran and the majority of subjects with the condition will die of CVD.

The current approach to clinical prevention of CVD is to identify patients with elevated risk factors through regular clinic visits and periodic clinical assessment and to offer treatment. However, the seemingly simple task of periodic clinical assessment requires substantial clinical resources and is not always an option in developing countries. Furthermore, it is not always easy to achieve patient and physician commitment which is required for this type of prevention.

One alternative which has recently gained major attention is providing individuals with a fixed dosed combination pill regardless of the presence of current risk-factors.[2] This eliminates the need for periodic assessment and greatly simplifies the treatment.

There is good evidence that for primary and secondary preven-

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tion of CVD, aspirin reduces the incidence of CVD and statins reduce both incidence and mortality from CVD.[3–6] Reducing elevated blood pressure using antihypertensive agents such as diuretics, angiotensin enzyme inhibitors and angiotensin receptor blockers can be also beneficial in reducing CVD risk.[7]

Nonalcoholic fatty liver disease (NAFLD) and the more severe non-alcoholic steatohepatitis (NASH) are also common in populations at high risk of CVD.[8–10] Mechanisms have already been suggested for the causal relationships between NASH and atherosclerosis.[11] Thus, preventive cardiovascular measures, such as PolyPill, might be more effective in participants with NASH or NAFLD than in the general population and might also have beneficial effects on liver disease.[12–15]

We have already conducted a pilot study of a fixed-dose combination pill (PolyPill) containing aspirin, atorvastatin, hydrochlorthiazide and enalapril in the general population.[16] We are also studying the long term effects of this combination (or valsartan if participants are intolerant of enalapril) in a cluster randomized controlled trial in a rural population.[17] In the present study, we will evaluate the effect of this fixed-dose combination pill on major cardiovascular events, mortality and liver outcomes in a pragmatic individually randomized controlled trial in an urban population. We will also investigate the effects of the PolyPill on CVD and liver outcomes in a subgroup of patients with presumed NASH (pNASH) or NAFLD (pNAFLD) at baseline.

Materials and Methods

The PolyIran-Liver study is designed as an open-label, pragmatic, parallel individually randomized controlled trial with a 110:90 allocation ratio (PolyPill: Control). It is nested in the Golestan Cohort Study.[18] The aim is to study the effects of a fixed-dose combination pill (PolyPill) comprising aspirin, atorvastatin, hydrochlorothiazide and valsartan on CVD and liver-related outcomes linked to non-alcoholic fatty liver disease.

Trial design

This randomized controlled trial is nested within the Golestan cohort study, and consists of two parts. The first is a Zelen design[19] and includes all randomly selected participants with outcomes assessed through the cohort study. The second is an additional measurement study investigating liver related outcomes in those who agree to liver related measurements and meet the eligibility criteria. Those in the intervention arm are offered a PolyPill if agreeing to participate in the additional measurement study and meeting the eligibility criteria.

Participants

The Golestan Cohort Study was originally designed to study upper gastrointestinal cancers and includes 50,045 healthy participants who are being followed up for major health outcomes, including cardio-vascular outcomes.[18] There are few exclusion criteria for the Golestan Cohort Study; participants are permanently resident in Golestan province, aged 40 to 70 years, without a previous diagnosis of upper gastrointestinal cancer. Participants were eligible for the Zelen design trial if they were a member of the cohort study participants, older than 50 years and resident in Gonbad city. A random sample of eligible participants from the cohort study was selected for the Zelen trial. Participants were eligible for the additional measurement study if they met further eligibility criteria and agreed to participate. The eligibility criteria included no recent upper gastro-intestinal bleeding, hypotension or important morbidities (e.g., debilitating psychological illness) and the ability to make an informed treatment decision (Table 1).

Intervention

The intervention (PolyPill) is a combination pill including 80 mg aspirin, 12.5 mg hydrochlorthiazide, 20 mg atorvastatin and 40 mg valsartan (PolyPill 4–2, Alborz-Darou, Ghazvin, Iran), taken once a day. The timing of the dose was at participants' discretion although we recommended taking the dose at bedtime.

Each participant was given a single PolyPill and asked to return

Table 1. Criteria making participants ineligible for the additional measurement study.

Proven moderate or severe asthma
Rhinitis
Nasal polyp
Tinnitus or hearing loss
Bleeding diathesis such as hemophilia
Using anticoagulants
Hepatitis B or C
Anemia (Hemoglobin levels less than 10g/dL for women and less than 11g/dL for men)
Renal failure (Creatinine over 2mg/dL or GFR < 30 mL/min)
Upper GI bleeding in the recent 3 months
Clinical cirrhosis or active hepatitis
Pregnancy or willing to become pregnant in the next 5 years
Lactation
Uncontrolled convulsion
Uric acid greater than 7 mg/dL for women and greater than 8.5 mg/dL for men
Angioedema
Hypotension (systole less than 90mmHg or diastole less than 60mmHg in at least two separate measurements)
Debilitating psychological illness
Physical disability hindering mobility and ability to cooperate
Other severe debilitating conditions

the next day for a brief examination. Participants returning the next day without any immediate adverse drug reactions continued to receive the PolyPill. Those not returning were contacted and the reason recorded. These participants were regarded as having discontinued treatment or withdrawn consent accordingly.

Participants who were already taking aspirin, a statin or a low-dose diuretic had it discontinued and replaced by the PolyPill. If other antihypertensive medications were being taken, they were either discontinued or the dose was adjusted by the study physician. All participants for whom antihypertensive medications were modified were also advised to consult their own doctor within the following week in case further adjustments were required.

Eligible participants were provided with a supply of PolyPill and asked to return for two additional visits at months one and two to check for important adverse drug reactions.

Control

Participants allocated to the control arm received no intervention and were followed up as explained below. The usual medical practice in Gonbad is that people refer to their doctor only if they have symptoms or if they wish to undergo medical examination. Regular check-ups are not performed unless asked for.

Baseline characteristics

In the Zelen trial, baseline measurements are not available for all randomized participants because only those included in the additional measurement study underwent baseline assessment.

In the additional measurement study, baseline assessments including height, weight, waist and hip circumference and blood pressure are measured along with additional blood and liver related measurements. In brief, 10cc blood was drawn for laboratory tests including liver enzymes, lipid levels, renal function, complete blood count, measures of glycemia, insulin and insulin resistance. Ultrasonography and Doppler examination were performed as explained below. Liver stiffness was measured using the FibroScan machine (Echosens, Paris, France) using the M or XL probes as appropriate.

Ultrasound assessments were performed using an Accuvix XQ ultrasound unit (Medison, Seoul, Korea) equipped with a 3–7 MHz curved-array and a 5–12 MHz linear-array transducer for evaluation of liver, abdominal fat and carotid arteries. Presence of fatty liver was determined using the ultrasonographic scoring system, which provides high sensitivity (91.7%) and specificity (100%) for the histological diagnosis of fatty liver.[20] Ultrasonographic findings scored in this protocol included hepatorenal echo contrast and/or liver brightness (0 to 3), deep attenuation (0 to 2), and vascular blurring (0 to 1). The fatty liver diagnosis (pNAFLD) requires a total score of at least 2, which includes the hepatorenal echo contrast and/or bright liver score of at least 1. The severity of steatosis is classified according to the total fatty liver score as 0 to 1 (no fatty liver), 2 to 3 (mild fatty liver), or 4 to 6 (moderate to severe fatty liver).[21]

Measurement of Visceral Adipose Tissue thickness (VAT) and Subcutaneous Adipose Tissue thickness (SAT) used previously described techniques.[21,22] VAT (mm) was defined as the distance between the anterior wall of the aorta and the internal face of the rectus abdominis muscle perpendicular to the aorta, which has been shown to have strong correlations with visceral fat area measured by computed tomography.[23] SAT (mm) was determined as the thickness of the fat tissue between the skin-fat interface and the linea alba, perpendicular to the skin, avoiding any compression. Ultrasonographic assessment of SAT has been described as a valid and reliable technique.[24] VAT and SAT measurements were obtained 1 cm above the umbilicus. Participants were assessed in the supine position following at least 6 hours of fasting.

The common and internal carotid arteries on both sides were also evaluated. Participants were examined in the supine position with the neck extended and the head turned 45° to the right or left. Carotid Intima-Media Thickness (mm) (IMT) was determined as the distance between the lumen–intima interface and the media–adventitia interface, measured at its thickest point on the distal (far) wall of the common carotid arteries, 1.5–2 cm proximal to the carotid bulb. The average of right and left sides was considered as common carotid artery IMT.[25] A localized thickening of >1.2 mm in common and internal carotid arteries, not involving the whole circumference of the lumen, was defined as atherosclerotic plaque.[26]

Liver stiffness was measured by transient elastography using the FibroScan® 502 (EchoSense, Paris, France, 5MHz). According to the manufacturer's guidelines, the M probe was used for participants with a thoracic perimeter less than 110 cm and the XL probe for 110 cm and above. With the patient lying in the dorsal decubitus position with maximal abduction of the right arm, the probe was placed on the patient's skin, overlying the right lobe of the liver, through the intercostal spaces. At least 10 measurements were done for each patient and the median value was recorded. Values were considered valid if the inter-quartile range (IQR) was less than 30% of the median reading and the success rate was at least 60%.

A single experienced radiologist performed ultrasound examinations without knowledge of participants' demographic and clinical data but was not blinded to the participants' allocation status. The examiner, when performing carotid assessment in a separate session, was also not blinded to the allocation status but was unaware of liver and abdominal fat ultrasound findings.

Outcomes

The primary outcome is the occurrence of a major cardiovascular event defined as hospitalization for acute coronary syndrome (non-fatal myocardial infarction and unstable angina), fatal myocardial infarction, sudden death, new-onset heart failure, coronary artery revascularization procedures and stroke (fatal or non-fatal).

Secondary outcomes include all-cause mortality and the individual components of the primary outcome and also liver related secondary outcomes: changes in liver stiffness, liver enzyme levels, Visceral Adipose Tissue thickness (VAT), Subcutaneous Adipose Tissue thickness (SAT) and carotid Intima-media thickness (IMT). Additional secondary outcomes include the proportion of patients with pNASH and pNAFLD. Compliance and adverse events will also be assessed.

Measurement of outcomes

Outcomes will be assessed at 2.5 and 5 years of follow up, with outcome at five years being the primary end point. Primary and secondary cardiovascular outcomes and all-cause mortality are independently determined by routine annual follow up as part of the Golestan cohort study. Therefore, cardiovascular outcomes will be available for all randomized participants irrespective of whether they participated in the additional measurement study. In the Golestan cohort study, mortality and significant medical

events are recorded in detail by investigating all available medical records. The cohort study follow up team is blind to the allocation status of participants in this trial.

In participants included in the additional measurement study, liver related secondary outcomes will be assessed as described above.

Sample size justification

The sample size includes 1,320 in the intervention arm and 1,080 in the control arm. For the primary outcome, the expected follow-up through the cohort study is around 80% over 5 years. The expected cumulative incidence in the control arm over 5 years is anticipated to be in the region of 0.0826 (=1-(1-0.0171)^5) where 0.0171 is the yearly per-person incidence of major cardiovascular events in the cohort study (unpublished data) and we count first events only. A clinically important effect size was pre-specified to be in the region of 0.60 (relative risk scale). Assuming therefore that outcome data are available on 1,920 individuals, this trial is in the region of 80% power (assuming a chi-squared test for proportions with continuity correction). This power calculation was carried out in Stata 13 using the power function.

We are not powered to detect differences in subgroup analyses but based on our experience in this population, we anticipated a 25% prevalence of increased liver enzymes, which is one of our definitions for pNASH.[27,28]

Randomization

The Golestan cohort study includes 7,351 participants aged older than 50 years and living in Gonbad. From these, 2,400 were randomly selected of which 1200 were males and 1200 were females. This was to achieve a 50/50 sex ratio. The 2,400 participants thus selected were randomized into two arms; PolyPill (intervention) group and control group. We chose to randomize into PolyPill and control arms with a 110:90 ratio, using unrestricted randomization without blocking or stratification. The randomization was performed by a statistician from the Digestive Disease Research Institute of Tehran University of Medical Sciences independent from the study group.

Invitation and enrollment

Following randomization, the participants were contacted by telephone by the Golestan cohort invitation team which was blind to the allocation status of participants. Participants were asked if they would be willing to take part in an additional measurement study. They were informed that this would consist of additional laboratory tests, ultrasonography and liver stiffness measurements. Participants randomized to the intervention arm were informed about the trial after baseline measurements were made and then offered the PolyPill.

At the time when the patients were asked to participate, their allocation was known, as is the case in a Zelen design.[19] However, all participants allocated to the PolyPill and control arms will be followed up within the cohort study for the primary outcome, all-cause mortality and the individual components of the primary outcome. Therefore, from the perspective of an intention to treat analysis of these outcomes, allocation was concealed. Liver-related outcomes were assessed only in those participants who met the eligibility criteria and consented to the additional measurement study and therefore allocation was not concealed for these outcomes.

Blinding

Because this is a pragmatic trial, participants are not blind to their treatment status. However, outcome assessment for the primary outcome (major cardiovascular events), all-cause mortality and the individual components of the primary outcome will be blind to allocation. Liver outcomes will also be assessed blind to allocation status.

Follow up

All selected participants are followed up through the Golestan Cohort Study to which they had previously consented. Participants who consented to the additional measurement study are also followed up every 6 months for 5 years. On each follow up visit, a short questionnaire is completed; weight, waist circumference, and hip circumference are measured; blood pressure is measured and the participant is advised to visit their doctor if the systolic blood pressure is found to be greater than 140 mmHg or the diastolic greater than 90 mmHg. The questionnaire includes adverse events, additional medicines used by the participant and events leading to medical attention since the last follow-up.

Analysis plan

For participants selected from the cohort study and randomly allocated to the PolyPill or control, cohort characteristics from their date of cohort study enrollment will be compared to confirm that allocation was random. Additionally, the baseline characteristics of those enrolled in the additional measurements study will be summarized. These characteristics will be summarized using appropriate summary statistics: numbers and percentages for categorical variables; means and standard deviations or medians and interquartile ranges for continuous variables.

Analyses of primary outcome and the secondary cardiovascular outcomes will be by intention to treat: all participants for whom outcomes are available will be analyzed in the arm to which they were allocated. The primary aim of the study is to evaluate whether there is a difference in the proportion of people in intervention and control arms experiencing these outcomes. As these outcomes are binary, we will report the risks of the events in both arms, the relative risks and also report the risk differences along with their 95% confidence intervals. Tests of statistical significance will be performed using Fisher's exact test. This analysis will be unadjusted because we do not have baseline covariates on all of the participants allocated to the intervention and control arms.

Secondary liver-related outcomes will be analyzed only in participants included in the additional measurement study. All participants for whom outcomes are available will be analyzed in the arm to which they were allocated, but it is recognized that outcomes will not be available for a large proportion of participants. Secondary liver-related outcomes will be analyzed in two different ways, the first analysis is only adjusted for baseline values of that outcome and the second analysis additionally adjusted for baseline values of a pre-specified set of clinically important covariates. The covariates to be included in the adjustment will be pre-specified and will include age, sex, diabetic status and previous history of cardiovascular disease. For the secondary analysis, adjusting for covariates, we will use the generalized linear model with an appropriate transformation to accommodate non-normality to obtain an adjusted estimate of the treatment effect. We do not anticipate any missing baseline covariate data; therefore, no missing data methods will be needed. Secondary binary outcomes

will be analyzed with adjustment for baseline presence of that outcome. For continuous outcomes, we will fit an appropriately transformed generalized linear model and report mean differences. For binary outcomes, we will fit Poisson regression models with robust standard errors and report relative risks and risk differences.

Finally, to explore the effect of the treatment in those who adhered to the study medication, we will also estimate the complier average causal effect.[29] The instrument used in this will be either whether or not the patient accepted the Polypill or another measurement of compliance using the data assessed through the pill-counts.

Interim analyses will occur after 2.5 years for review by the monitoring committee. Interim analyses may provide an opportunity to examine, and confirm or adapt, the assumptions used in sample size estimation. Formal statistical methods will be used as guidelines rather than absolute rules. Differences between major endpoints of at least equivalent to $P < 0.001$ (similar to Haybittle-Peto boundary) will be considered the order of magnitude necessary to consider any study modification or stopping the trial.[30]

Subgroups

Ideally, to evaluate the effect of the PolyPill on cardiovascular outcomes in participants with NASH, we will identify the subset of patients with fatty liver disease at baseline. However, both NASH and NAFLD can only be diagnosed by liver biopsy which is invasive and carries risks.[31] We therefore identified three subgroups for secondary analysis of outcomes: pNAFLD, diagnosed by ultrasonography as explained above; pNASH defined as pNAFLD and any elevation of alanine transaminase (ALT) levels above the upper limit of normal (30 and 20 IU/L for men and women, respectively); and elevated ALT levels only. In each case, the participant should not have a diagnosis of hepatitis B or C. Although there is no consensus on this definition, we believe it is the closest approximation that can be made in a population-based study.[32,33] Further subgroup analyses will investigate outcomes by sex and by BMI category normal weight (<25 kg/m^2), overweight (25 to 29.9 kg/m^2) and obese (≥30 kg/m^2).

Ethics

The study protocol was approved by the institutional review board of the Digestive Diseases Research Institute of Tehran University of Medical Sciences and ethics committees at Tehran University of Medical Science and Ministry of Health and Medical Education. Written informed consent was obtained from all participants and it was explained that they will be free to leave the study at any time. The protocol is registered at ClinicalTrials.gov, ID: NCT01245608.

Important changes after trial commencement

Initially this trial was intended to investigate the effects of a PolyPill on liver related outcomes in participants with pNASH selected from the Golestan Cohort Study. The original design was therefore the additional measurement study with liver related outcomes as the primary outcomes. However, this design suffered from lack of allocation concealment and a risk of selection bias and imbalance between the intervention and control arms. We therefore changed the primary outcome to major cardiovascular events which could be ascertained for all randomized participants through the Golestan Cohort Study.

All patients in the Golestan Cohort Study have previously given consent to be followed up for cardiovascular and other major disease outcomes; therefore, their previous consent includes follow up for the changed primary outcome and the wider group in which outcomes are to be assessed. We sought and received ethical committee approval to confirm this.

Results

The flow of participants is shown in Figure 1. There were 2400 participants selected from Golestan cohort study for participation in this study: 1320 participants were randomized to the intervention arm and 1080 to the control arm. Of these, 21 participants (13 intervention, 8 control) had moved and 113 (58 intervention, 55 control) had died before they could be invited to participate in this study (Table 2). These participants will not be included in the Zelen trial. Cohort characteristics from their date of cohort study enrollment (2004–2008) are available for the remaining 1249 intervention and 1017 control participants in the Zelen trial. The mean age of participants in 2011 was 59.8 years and 49.7% were male. Socio-economic, demographic and measurement characteristics of the Zelen intervention and control trial groups were very similar at their date of cohort study enrollment (Table 3).

After invitation by telephone, an additional 218 in the intervention arm and 186 in the control arm refused to participate in the additional measurement study, could not attend, could not be contacted or failed to attend for baseline assessment (Table 2). For these 538 participants (289 plus 249) no baseline measurements were taken. This left 1031 participants in the intervention group and 831 in the control group who attended baseline assessment for the additional measurements study. Baseline history, physical examination and laboratory were undertaken for these 1862 participants. Liver stiffness measurement, ultrasonography and Doppler examination were also performed as explained above. Some of the baseline ultrasonography findings have been published separately.[34] The mean age of participants in the additional measurement study was 58.9 years and 51.2% were male. The intervention and control groups had similar height, weight, hip circumference, waist circumference and measures of livers stiffness. Participants in the intervention group had higher lipid levels than the control group (total cholesterol 218.5 and 206.9 mg/dL, respectively) and lower mean blood pressures (130.8/78.2 and 135/82.4 mm Hg, respectively) (Table 4).

Following baseline measurements, 156 participants in the intervention group and 110 in the control group were found to have contraindications to the PolyPill. The most common contraindications are given in Table 5. A further 88 participants in the intervention group did not complete the run in period. This left 787 (59.6%) of those randomized to PolyPill and 721 (66.8%) of those allocated to control to be included in the additional measurements study.

Discussion

This study design selects patients from within an existing cohort study for inclusion in a randomized controlled trial in order to determine whether use of PolyPill in largely unselected population has an effect on cardiovascular outcomes. The study has a Zelen design because participants randomized to the control group have already consented to follow up within the cohort study. A subset

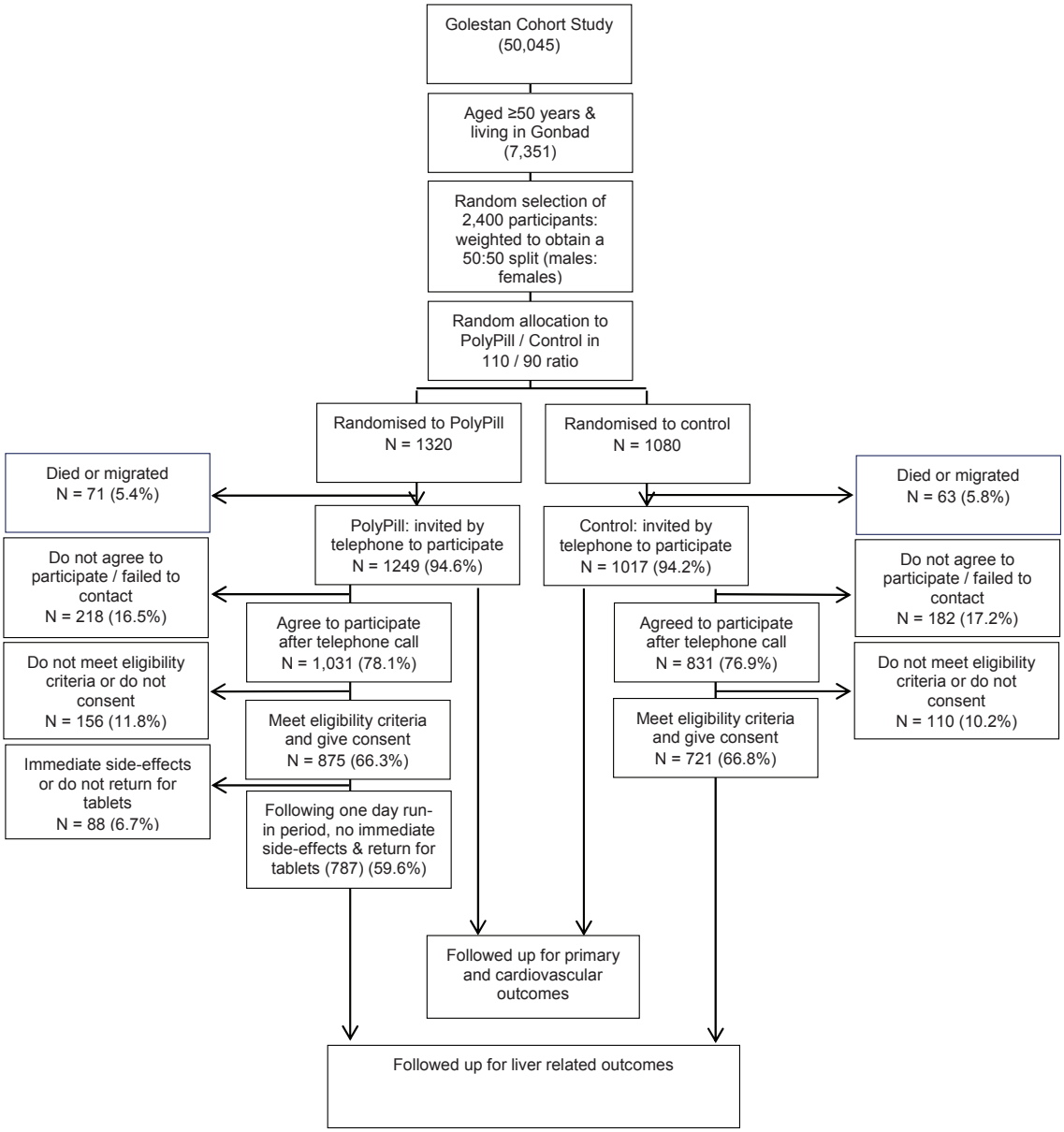

Figure 1. CONSORT diagram of participant flow through the study.

Table 2. Participants lost after randomization.

	Intervention	Control	Total
Total randomised	1320	1080	2400
Migrated	13	8	21
Died	58	55	113
Total remaining in Zelen trial (cardiovascular outcomes)	1249	1017	2266
Refused to participate	135	108	243
Could not attend	55	60	115
Could not be contacted by telephone	22	12	34
Failed to attend baseline assessment	6	6	12
Total attending baseline assessment for the additional measurements study (liver related outcomes)	1031	831	1862

Table 3. Baseline characteristics of participants included in the Zelen trial collected during cohort enrollment at 2004–2008.

	PolyPill arm (N = 1249)	Control arm (N = 1017)
Male	608 (48.7%)	495 (48.7%)
Age at 2011 years, mean (SD)	59.5 (7.5)	60.1 (7.6)
Height cm, mean (SD)	160.6 (9.1)	160.8 (9.5)
Weight kg, mean (SD)	73.2 (13.3)	73.0 (14.2)
BMI kg/m2, mean (SD)	28.4 (5.1)	28.3 (5.3)
Waist circumference cm, mean (SD)	99.9 (12.2)	99.6 (12.5)
Hip circumference cm, mean (SD)	103.2 (9.0)	103.1 (9.6)
Systolic blood pressure mmHg, mean (SD)	128.1 (23.0)	129.0 (23.6)
Diastolic blood pressure mmHg, mean (SD)	78.1 (12.0)	78.6 (12.8)
Smoking history, ever	264 (21.2%)	224 (22%)
Diabetes mellitus	115 (9.2%)	96 (9.4%)
Car ownership	443 (35.5%)	321 (31.6%)
House ownership	1169 (93.6%)	953 (93.7%)
Computer ownership	332 (26.6%)	259 (25.5%)
Values are numbers and percentages unless otherwise specified.		

Table 4. Baseline characteristics of participants included in the additional measurements study.*

	Polypill	Control
Variable	N = 1031	N = 831
Male (%)	50.3%	52.2%
Age (years)	58.5 (6.5)	59.4 (7.0)
Cardiovascular variables		
Height (cm)	161.6 (9.0)	161.8 (9.6)
Weight (kg)	74.1 (13.2)	73.9 (14.0)
Hip circumference (cm)	101.7 (8.3)	101.3 (9.0)
Waist circumference (cm)	99.9 (12.3)	99.6 (12.4)
Body Mass Index (kg/m^2)	28.4 (4.9)	28.2 (5.0)
Total Cholesterol (mg/dL)	218.5 (42.7)	206.9 (40.1)
High Density Lipoprotein cholesterol (mg/dL)	59.9 (15.4)	58.0 (14.3)
Low Density Lipoprotein cholesterol (mg/dL)	128.5 (34.4)	120.7 (32.9)
Triglycerides (mg/dL)	152.4 (82.6)	143.7 (81.4)
Systolic Blood Pressure (mmHg)	130.8 (22.9)	135.0 (21.4)
Diastolic Blood Pressure (mmHg)	78.2 (10.3)	82.4 (10.9)
Liver related variables		
Liver stiffness (KPa), *n*=1580	5.1 (3.5)	5.1 (3.6)
Aspartate aminotransferase (IU/L)	21.1 (10.6)	21.9 (11.4)
Alanine aminotransferase (IU/L)	22.2 (15.3)	25.2 (18.9)
Alkaline phosphatase (IU/L)	260.5 (82.6)	251.7 (100.5)
Gamma-glutamyltransferase (IU/L)	31.1 (32.5)	35.6 (45.6)
pNAFLD (%), *n*=1614	39.6%	40.6%
Increased alanine aminotransferase (%)	28.1%	35.3%
pNASH (%), *n*=1614	18.4%	22.5%
Haematological variables		
Haemoglobin (gr/dL)	13.6 (1.5)	12.7 (1.5)
White blood count (1000/mm^3)	6.4 (1.8)	6.3 (1.7)
Platelets (1000/mm^3)	255.4 (62.1)	240.5 (66.4)
Renal related variables		
Creatinine (mg/dL)	1.2 (0.3)	1.1 (0.3)
Estimated Glomerular Filtration Rate (ml/min)	66.7 (17.0)	73.5 (20.0)
Urea (mg/dL)	30.0 (9.2)	29.4 (8.1)
Glucose metabolism variables		
Fasting Blood Sugar (mg/dl)	112.1 (44.2)	109.7 (45.0)
Insulin (mU/L)	12.1 (8.4)	10.7 (9.0)
HOMA-IR, *n*=1822	3.5 (3.6)	2.9 (2.7)
Glycosylated haemoglobin (mg/L), *n*=473**	7.4 (1.7)	6.6 (2.9)
*Figures are means and Standard Deviations unless stated otherwise. HOMA-IR: homeostatic model assessment insulin resistance. **Performed only for subjects with fasting blood sugar > 110 mg/dL.		

Table 5. Frequency of contraindications in patients selected for the PolyPill and control arms*.

Contraindication to PolyPill	PolyPill arm (N = 1320)		Control arm (N = 1080)	
Anemia	15	1.1%	22	2.0%
Moderate or severe asthma	19	1.4%	9	0.8%
Renal failure	8	0.6%	6	0.6%
Hypotension	20	1.5%	9	0.8%
Physical disability	49	3.7%	32	3.0%
Hepatitis B or C	24	1.8%	27	2.5%
Other reasons	25	1.9%	15	1.4%
Total	**156**	**11.8%**	**110**	**10.2%**

*Percentages are from participants selected from Golestan cohort study (1320 for PolyPill group, 1080 for controls). Some participants had more than one contraindication.

of randomized participants is included in an additional measurements study to investigate the effects of the PolyPill on liver related outcomes.

Strengths

By selecting participants from an existing cohort study, the study makes use of existing arrangements for follow up and is therefore a highly efficient trial design. Using the follow up arrangement of the cohort study should minimize losses to follow up. The primary outcome and other cardiovascular outcomes are independently assessed through the cohort study.

Limitations

Allocation was not concealed because cases and controls were invited separately. Fewer randomized participants entered the additional measurements study in the PolyPill group than the control group (59.6% vs. 66.8%). This may affect the interpretation of the effects of the PolyPill on liver related outcomes. The study is also open label and this may influence the assessment of secondary outcomes. On the other hand, the liver related outcomes are measured objectively; therefore, there is less scope for measurement bias.

The Zelen design study has fewer limitations. Including all randomized participants in the Zelen design means that the low numbers entering the additional measurements study will not influence the validity of the results with respect to cardiovascular endpoints. Nevertheless, the effects of the PolyPill may be attenuated by the fact that 40.4% of randomized participants did not receive the PolyPill. However, if the PolyPill is to be used in primary care, physicians may make similar judgements about which patients should receive treatment as is the case in our study. But some randomized participants may have declined the PolyPill because they did not agree to additional measurements and ideally we would have offered all randomized participants a PolyPill.

Baseline characteristics for randomly allocated participants in the Zelen study were obtained from the date of their enrollment into the Golestan Cohort Study, which was about 5 to 10 years previous to allocation. This means that they cannot be used for baseline adjustment. However, as distribution of these differences is consistent with chance, it allows us to confirm that allocation was random.

References

1. Merat S, Malekzadeh R. Prevention of non-communicable diseases: what can be done? *Arch Iran Med*. 2013; 16(3): 136-7.
2. Wald NJ, Law MR. A strategy to reduce cardiovascular disease by more than 80%. *BMJ*. 2003; 326(7404): 1419.
3. Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, et al. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 2013; 1: CD004816.
4. Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet*. 2009; 373(9678): 1849-60.
5. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005; 366(9493): 1267-78.
6. ulbulia R, Bowman L, Wallendszus K, Parish S, Armitage J, Peto R, et al. Effects on 11-year mortality and morbidity of lowering LDL cholesterol with simvastatin for about 5 years in 20,536 high-risk individuals: a randomised controlled trial. *Lancet*. 2011; 378(9808): 2013-20.
7. Law MR, Morris JK, Wald NJ. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ*. 2009; 338: b1665.
8. Karbasi-Afshar R, Saburi A, Khedmat H. Cardiovascular disorders in the context of non-alcoholic Fatty liver disease: a literature review. *J Tehran Heart Cent*. 2014; 9(1): 1-8.
9. Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G, Marmur J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology*. 2010; 51(2): 595-602.
10. Stepanova M, Younossi ZM. Independent association between nonalcoholic fatty liver disease and cardiovascular disease in the US population. *Clin Gastroenterol Hepatol*. 2012; 10(6): 646-50.
11. Min HK, Kapoor A, Fuchs M, Mirshahi F, Zhou H, Maher J, et al. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. *Cell Metab*. 2012; 15(5): 665-74.
12. Lai SW, Liao KF, Lai HC, Muo CH, Sung FC, Chen PC. Statin use and risk of hepatocellular carcinoma. *Eur J Epidemiol*. 2013; 28(6): 485-92.
13. Eslami L, Merat S, Malekzadeh R, Nasseri-Moghaddam S, Aramin H. Statins for non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Cochrane Database Syst Rev*. 2013; 12: CD008623.
14. Paschos P, Tziomalos K. Nonalcoholic fatty liver disease and the renin-angiotensin system: Implications for treatment. *World J Hepatol*. 2012; 4(12): 327-31.
15. Sahasrabuddhe VV, Gunja MZ, Graubard BI, Trabert B, Schwartz LM, Park Y, et al. Nonsteroidal anti-inflammatory drug use, chronic liver disease, and hepatocellular carcinoma. *J Natl Cancer Inst*. 2012; 104(23): 1808-14.
16. Malekzadeh F, Marshall T, Pourshams A, Gharravi M, Aslani A, Nateghi A, et al. A pilot double-blind randomised placebo-controlled trial of the effects of fixed-dose combination therapy ('polypill') on cardiovascular risk factors. *Int J Clin Pract*. 2010; 64(9): 1220-7.
17. Ostovaneh MR, Poustchi H, Hemming K, Marjani H, Pourshams A, Nateghi A, et al. Polypill for the prevention of cardiovascular disease (PolyIran): study design and rationale for a pragmatic cluster randomized controlled trial. *Eur J Prev Cardiol*. 2014.
18. Pourshams A, Khademi H, Malekshah AF, Islami F, Nouraei M, Sadjadi AR, et al. Cohort Profile: The Golestan Cohort Study--a prospective study of oesophageal cancer in northern Iran. *Int J Epidemiol*.

2010; 39(1): 52-9.
19. Zelen M. A new design for randomized clinical trials. *N Engl J Med*. 1979; 300(22): 1242-5.
20. Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol*. 2007; 102(12): 2708-15.
21. Ayonrinde OT, Olynyk JK, Beilin LJ, Mori TA, Pennell CE, de Klerk N, et al. Gender-specific differences in adipose distribution and adipocytokines influence adolescent nonalcoholic fatty liver disease. *Hepatology*. 2011; 53(3): 800-9.
22. Kim SK, Kim HJ, Hur KY, Choi SH, Ahn CW, Lim SK, et al. Visceral fat thickness measured by ultrasonography can estimate not only visceral obesity but also risks of cardiovascular and metabolic diseases. *Am J Clin Nutr*. 2004; 79(4): 593-9.
23. Ribeiro-Filho FF, Faria AN, Azjen S, Zanella MT, Ferreira SR. Methods of estimation of visceral fat: advantages of ultrasonography. *Obes Res*. 2003; 11(12): 1488-94.
24. Haymes EM, Lundegren HM, Loomis JL, Buskirk ER. Validity of the ultrasonic technique as a method of measuring subcutaneous adipose tissue. *Ann Hum Biol*. 1976; 3(3): 245-51.
25. Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaides AN, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. *Stroke*. 1999; 30(4): 841-50.
26. Belhassen L, Carville C, Pelle G, Monin JL, Teiger E, Duval-Moulin AM, et al. Evaluation of carotid artery and aortic intima-media thickness measurements for exclusion of significant coronary atherosclerosis in patients scheduled for heart valve surgery. *J Am Coll Cardiol*. 2002; 39(7): 1139-44.
27. Sohrabpour AA, Rezvan H, Amini-Kafiabad S, Dayhim MR, Merat S, Pourshams A. Prevalence of nonalcoholic steatohepatitis in Iran: A population based study. *Mid East J Digest Dis*. 2010; 2: 14-9.
28. Jamali R, Khonsari M, Merat S, Khoshnia M, Jafari E, Bahram Kalhori A, et al. Persistent alanine aminotransferase elevation among the general Iranian population: prevalence and causes. *World J Gastroenterol*. 2008; 14(18): 2867-71.
29. Emsley R, Dunn G, White IR. Mediation and moderation of treatment effects in randomised controlled trials of complex interventions. *Stat Methods Med Res*. 2010; 19(3): 237-70.
30. Schulz KF, Grimes DA. Multiplicity in randomised trials II: subgroup and interim analyses. *Lancet*. 2005; 365(9471): 1657-61.
31. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology*. 2002; 123(3): 745-50.
32. Verma S, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). *Liver Int*. 2013; 33(9): 1398-405.
33. Sorrentino P, Tarantino G, Conca P, Perrella A, Terracciano ML, Vecchione R, et al. Silent non-alcoholic fatty liver disease-a clinical-histological study. *J Hepatol*. 2004; 41(5): 751-7.
34. Radmard AR, Merat S, Kooraki S, Ashrafi M, Keshtkar A, Sharafkhah M, et al. Gallstone disease and obesity: A population-based study on abdominal fat distribution and gender differences. *Ann Hepatol*. 2015; 14(5): 702-9.