Project summary

Background: Estrogens play a protective role during early life stages. However, endogenous 17β-estradiol (E2) can accelerate atherosclerosis progression after formation of atherosclerotic plaques.
Study design: single-center cohort study.
Purpose: Participants: adult men and women diagnosed with acute coronary syndrome, admitted to hospital who received catheter-based coronary reperfusion when appropriate.
Exclusion criteria: Acute infectious disease, any diagnosed neoplastic disease; fracture, physical trauma or surgical procedure a month before and after the inclusion period and at the end of the follow-up.
Starting date: between July 2011; recruitment period 3 years.
What does the study involve: assessment of sex steroids (E2, total testosterone [T]) and dehydroepiandrosterone-sulfate (DHEA-S) at admission and analysis for relationship oxidized low-density lipoproteins (oxLDL), extracelluar superoxide dismutase (ecSOD), high-sensitive C-reactive protein (CRP), white blood cell counts (WBC), and cardiac enzymes (creatine kinase [CK], the CK Muscle-Brain fraction [CK-MB], and high-sensitive troponin T [hsTnT]) evaluated within two hours after coronary revascularization with percutaneous angioplasty with or without stenting. The SYNTAX score gauged coronary disease severity from coronary angiography results.
Following the patients for a year, repeat measurement of CRP, oxLDL, ecSOD and recording adverse events - reinfarction, revascularizations and deaths.

General information

Protocol title
Significance of dehydroepiandrosterone-sulfate and gonadal sex hormones in acute coronary syndrome - sex-based differences
Acronym: SHACS
Protocol /serial number: 35D/2012
Name and address of the sponsor/funder:
Organisation: Medical University Sofia, Bulgaria
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Name(s) and address(es) of the clinical laboratory(ies) and department(s) involved in the research
Central Clinical Laboratory, University Hospital 'Alexandrovska'

Department of Chemistry and Biochemistry, Faculty of Medicine, Medical University, Sofia, Bulgaria
Clinic of Cardiology, University Hospital 'Alexandrovska'.

Rationale & background information

The pathophysiology of acute coronary disease remain elusive. Studies on gonadal hormones and chronic coronary disease date back from the late 50s of the last century. Conclusive results on the significance of estradiol and testosterone in the gradual progression coronary disease have been reached during the late 80s with studies of Phillips, G.B. at al.[1-5] Just recently that the results of studies on gonadal steroid hormones in relatively large cohorts with acute myocardial infarction have been published[6-8], a relationship of gonadal steroid hormones with inflammatory molecules is recognized and set grounds for hypothesis that hormones and inflammation are sex-specific indicators or co-factors of myocardial injury in acute coronary disease.

References (of literature cited in preceding sections)

[1]Phillips GB, et al. Evidence for hyperestrogenemia as the link between diabetes mellitus and myocardial infarction. The Am J Med 1984; 76 (6): 1041-1048. doi: 10.1016/0002-9343(84)90855-6
[2]Phillips GB, et al. Association of hyperestrogenemia and coronary heart disease in men in the Framingham cohort The Am J Med 1983; 74 (5): 863-869. doi: 10.1016/0002-9343(83)91078-1
[3]Sewdarsen M, et al. Abnormalities in sex hormones are a risk factor for premature manifestation of coronary artery disease in South African Indian men. Atherosclerosis 1990; 83: 111-117. doi: 10.1016/0021-9150(90)90156-D
[4]Small M, et al. Serum oestradiol and ischaemic heart disease—relationship with myocardial infarction but not coronary atheroma or haemostasis. Q J Med 1985; 57: 775-782. doi: 10.1093/oxfordjournals.qjmed.a067921
[5]Barrett-Connor E, Khaw K. Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study. Circulation 1988; 78: 539-545. doi: 10.1161/01.CIR.78.3.539
[6]Separham A, et al. Association of admission testosterone level with ST-segment resolution in male patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. Basic Clin. Androl. 2017; 27: 14 doi: 10.1186/s12610-017-0058-7
[7]Dong M , et al. Prospective Study of Effects of Endogenous Estrogens on Myocardial No-Reflow Risk in Postmenopausal Women with Acute Myocardial Infarction. J Interven Cardiol 2014;27:437–443 doi.org/10.1111/joic.12137
[8]Chen J, et al. Estrogen inhibits endoplasmic reticulum stress and ameliorates myocardial ischemia/reperfusion injury in rats by upregulating SERCA2a. Cell Commun Signal 20, 38 (2022). doi: 10.1186/s12964-022-00842-2

Study goals and objectives

Primary objective: assessment for sex-specific associations of 17 β-estradiol (E2), total testosterone (T) and dehydroepiandrosterone-sulfate (DHEA-S) with markers of oxidative stress,

inflammation, and myocardial injury, left ventricular systolic dysfunction and angiographic extent of coronary disease.
Secondary objective: association of E2, T, DHEA-S with the incidence of coronary revascularizations, re-infarction, all-cause death (as combined end-point) a year after the index ACS. Association of R213 G with E2, T, DHEA-S, serum lipids, coronary disease severity.

Study design

Study design: single-center cohort study
Primary study design: Observational
Secondary study design: Cohort study
Study setting(s): Hospital
Study type(s): Other
Overall study start date: 01/07/2011
Overall study end date: 30/06/2014
Condition: acute coronary syndrome
Age group: Adult
Lower age limit: 35 Years
Upper age limit: 95 Years
Gender: Both
Target number of participants: 200
Total final enrolment: 175
Intervention Type: Not Specified Interventions:
Sex steroids (E2, total testosterone [T]) and DHEA-S, oxidized low-density lipoproteins, high-sensitive C-reactive protein (CRP), white blood cell counts (WBC), and cardiac enzymes (creatine kinase [CK], the CK Muscle-Brain fraction [CK-MB], and high-sensitive troponin T [hsTnT]) were measured at admission. The inflammatory and myocardial injury markers were evaluated within two hours after coronary revascularization. The SYNTAX score gauged coronary disease severity from coronary angiography results.
Locations
Countries of recruitment: Bulgaria
Study participating centres:
Study Centre
Name: University Hospital 'Alexandrovska'
Address: 1 Georgi Sofyiski street
City: Sofia
Country: Bulgaria
Zip: 1431
Ethics approval required
Approved 30/05/2012, Ethics committee of Medical University (15 Acad Ivan Geshov str., Sofia, 1431, Bulgaria; 359 2 9152157; atanasova@mu-sofia.bg), ref: 81/30/05/2012
Study website: Participant information sheet:
Not available in web format; please use contact details to request a participant information sheet.

Methodology

The study is single-center cohort study. No interventions were made, all diagnostic methods that were according to medical practice,and therapeutic strategies complied with the current practice guidelines

in Europe (ESC practice guidelines) and included either coronary angiography with angioplasty with or without stent implantation and commercial drugs, or coronary angiography with commercial angina drugs.
Eligibility: adult men and women with admitted to hospital with acute coronary syndrome (unstable angina, non-ST elevation myocardial infarction, myocardial infarction with persistent ST elevation).
Exclusion criteria: diagnosed with secondary hypogonadism or diseases of the adrenal and pituitary glands were excluded. Other exclusion criteria were acute infectious disease, chronic inflammatory disease, known or suspected neoplastic processes, surgical procedures, and trauma within two weeks before hospital admission. Participants had not used hormone or immunoreactive therapies six months before or during the study.
Number of patients enrolled: 165. Full data available in: 125 patients (37% women).
All patients underwent coronary angiography (CAG); 85.6% - percutaneous coronary intervention and stent implantation.

Blood samples were drawn 48 hours after symptom onset to measure levels of sex steroids (total 17β-estradiol [E2], total testosterone [T], dehydroepiandrosterone-sulfate), while the values of oxidized low-density lipoproteins (oxLDL), high-sensitive C-reactive protein (hsCRP), white blood cell counts (WBC), and cardiac enzymes (creatine kinase [CK], Muscle-Brain fraction of CK [CPK-MB], high-sensitive troponin T [hsTnT]) were evaluated in plasma obtained within two hours after CAG and the percutaneous intervention. To measure coronary disease severity, we calculated the SYNTAX score for each patient with angiographically-defined coronary atherosclerosis.

After a 12-hour fast, venous blood samples were collected into EDTA sample tubes, centrifuged at 12,000 rpm for 20 minutes, and stored at -20°C until analysis. hsCRP concentrations were determined using a latex-enhanced immunoturbidimetric assay (Roche Diagnostics GmbH, Manheim, Germany) on the COBAS INTEGRA 700 analyzer. We assessed levels of steroid hormones and troponin T using an electrochemiluminescent immunoassay with Roche Diagnostics reagents on the Elecsys 2010 analyzer. These methods have been detailed elsewhere. Plasma levels of oxLDL were quantified using the OxiSelect Human Oxidized LDL immunosorbent assay (ELISA; MDA-LDL) kit (Cell Biolabs, San Diego, USA) and a sandwich ELISA.

Safety considerations

The study was non-interventional. The diagnostic methods used are according to medical practice; the therapeutic strategies complied with the current practice guidelines in Europe (ESC practice guidelines). This information was stated in the ethical approval. No safety issues arise during the study period.

Follow-up

Following the patients for a year, repeat measurement of CRP, oxLDL, ecSOD and recording adverse events – recurrent infarction, revascularizations and deaths.

Data management and statistical analysis

The variable distributions was tested using the Kolmogorov-Smirnov and Sapiro-Wilk tests. The associations between variables were explored by both parametric (independent samples t-test) and non-parametric ($\chi 2$ test, Fisher's exact tests, Mann-Whitney U test) methods, further validated by univariate and

multivariable analyses. Analyses were conducted using IBM SPSS Statistics for Windows, Version 19.0. (Armonk, NY: IBM Corp.). A two-tailed p-value less than 0.05 was deemed significant. The personal data as names of the patients were coded for the computer analysis. The data was analyzed by two specialist of statistics. Patients with missing or spurious data have been excluded by further analysis.

Quality assurance

The quality was ensured by an appropriate study design and gathering of adequate data analysis (bias avoided). To assure completeness and validity of the data case record forms (CRF) were designed and double data entry was provided. A source data verification of was conducted by a comparison of the data between the CRF and the medical records. The patients were documented consecutively. An assessment of the representativeness was done during the data analysis by comparing the investigated patient population with other studies and literature.

The study was conducted in a large-sized tertiary hospital, where significant number of patients are admitted on an emergency basis. The hospital was university institution with high professional qualifications of the medical personnel. The medical personnel that took part in the study (nurses, laboratory personnel) of the institute were selected carefully and trained on the project specific aspects.

The Ethics Committee build by the medical faculty was asked about the ethical and legal questions connected to the objectives by submission of the study plan (the study title, objectives, methods, sample size, a statistical analysis plan) for ethical approval. The transparency was repeatedly assured by reporting a summary of the results in a written form to a medical faculty meeting after completion of the study. All subjective influences on the interpretation of the results eliminate (Objectivity) avoided by using "Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Initiative".The report and all other documents were self-archived for later access and analyses for a minimum of 10 years.

Expected outcomes of the study

The results of the study are important to scientific theory beyond the pathophysiology of coronary disease. The incomplete gonadal hormone suppression is involved in the resistance to adjuvant therapy of different hormone-sensitive tumors (breast cancer, prostate cancer). The knowledge that variation in the endogenous levels of 17β-estradiol, testosterone and aromatase activity index in acute disease is related to increased activity of inflammatory pathways in acute myocardial is important for developing methods for overcoming the resistance to hormone-inhibition based antitumor therapy.

Dissemination of results and publication policy

Publication of the results will be made by the chief investigator. As many as colleagues that have significant contribution to the conception, realization, supervision, funding and publication of the study results will be acknowledged in publication.

Duration of the project

According to the protocol the patients should undergo re-evaluation at six months with physical examination and at the end of the 1st year with physical examination, repeated measurement of complete blood count, serum creatinine, calculation of glomerular filtration rate, serum lipids, the levels of C-reactive

protein, oxLDL, ecSOD activity. Infromation about adverse events: reinfarction, revascularizations and deaths for a year following the index ACS was recorded.

Problems anticipated

Not applicable.

Project management

Conceptualization, Niya Semerdzhieva and Adelina Tsakova; methodology, Mariana Gospodinova, Valentin Lozanov, Vesela Lozanova, and Adelina Tsakova, Julieta Hristova, Simeon Dimitrov, Peter Atanasov, Maria Chaneva; software, Niya Semerdzhieva; validation, Valentin Lozanov, and Vesela Lozanova; formal analysis, Niya Semerdzhieva; investigation, Niya Semerdzhieva, Mariana Gospodinova, Valentin Lozanov, Vesela Lozanova, and Adelina Tsakova, Julieta Hristova, Simeon Dimitrov, Peter Atanasov, Maria Chaneva; resources, Niya Semerdzhieva; data curation, Niya Semerdzhieva; writing—original draft of the project, Niya Semerdzhieva; writing—review and editing, Mariana Gospodinova, Valentin Lozanov, Vesela Lozanova, Adelina Tsakova, Julieta Hristova, Simeon Dimitrov, Peter Atanasov, Maria Chaneva; visualization, Niya Semerdzhieva; supervision, Mariana Gospodinova, Valentin Lozanov, Vesela Lozanova, Adelina Tsakova, Julieta Hristova, Simeon Dimitrov, Peter Atanasov, Maria Chaneva; project administration, Mariana Gospodinova; funding acquisition, Niya Semerdzhieva.

Informed consent forms

See Attachment 3

Research protocol: part 2

Budget

The funds requested for the study were spent for buying reagents (91%) and for payment for the statistical analysis (9%).

Other support for the project

Servier Medical Bulgaria provided financial support used for supply of a reagent for the study.

Collaboration with other scientists or research institutions

No collaborations with other scientists or research institutions.

Links to other projects

Curriculum Vitae of investigators

See Attachment 4

Other research activities of the investigators

The Principal investigator is not involved in current research projects at present.

Financing and insurance

No financing and insurance was planned for patients and medical personnel.