




Study protocol to compare the influence of the use of Contraceptive Injection in the levels of C Reactive Protein and Oxidized Low-density Lipoprotein

Protocolo de estudo para comparar a influência do uso de contraceptivo injetável nos níveis de proteína C reativa e lipoproteína de baixa densidade oxidada

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ABSTRACT | INTRODUCTION: Women broadly use hormonal contraceptives to avoid an unintended pregnancy. Nevertheless, researchers suggest that its continued use can cause adverse effects, as a variation in the lipid profile and levels of C-reactive Protein (CRP), which lead to subclinical inflammation and, consequently, higher cardiovascular risk. **OBJECTIVE:** Test the hypothesis that the continuous use of contraceptive injection (CI) affects C-reactive Protein levels and Oxidized Low-density Lipoprotein of apparently healthy women. **METHODS:** Comparative observational cross-sectional study, which will include women that have made (and have not made) use of contraceptive injection for at least six months. These women will be recruited by invitations on social networks, doctors' offices, and health care units. There will be collected anthropometric and blood to analyze criteria for exclusion and blood collection to measure C-reactive Protein (CRP) and Oxidized Low-density Lipoprotein (Ox-LDL). The Term of Free and Informed Consent will be given to the volunteers. A pilot study was held with 14 participants, and the calculation of sample sufficiency was done for the primary outcome CRP, in which it was considered an alpha of 0,05 and beta 0,80 for the relationship between samples 1:1 and, then, the number of 82 participants had been estimated. The Project was submitted to the Committee of Ethics in Research with the Certificate of Presentation for Ethical Consideration (CPEC): 37695620.5.0000.0042. **ESTIMATED RESULTS:** This study may suggest a redirection of health workers' decision making, regarding the use of contraceptives in women with cardiovascular risk.

KEYWORDS: Contraceptive Injection. C-reactive Protein. Oxidized low-density Lipoprotein.

RESUMO | INTRODUÇÃO: Contraceptivos hormonais são amplamente utilizados em todo o mundo por mulheres para evitar gestação indesejada. Entretanto, pesquisadores sugerem que seu uso contínuo pode provocar efeitos colaterais como em alteração no perfil lipídico e níveis de Proteína C-reativa (PCR), o que leva à inflamação subclínica e, conseqüentemente, maior risco cardiovascular. **OBJETIVO:** Testar a hipótese de que o uso contínuo de contraceptivo injetável (CI) altera os níveis de Proteína C reativa e Lipoproteína de Baixa Densidade – Oxidada de mulheres aparentemente saudáveis. **MÉTODOS:** Estudo observacional comparativo de corte transversal, que incluirá mulheres que usam e não usam contraceptivo injetável por pelo menos 6 meses. Serão recrutadas a partir de convites por redes sociais, convites em consultórios médicos e convites em unidades de atendimento em saúde. Serão coletados dados antropométricos e sanguíneos para análise dos critérios de exclusão e a coleta de sangue para mensurar a Proteína C-reativa (PCR) e a Lipoproteína de Baixa Densidade oxidada (LDL-ox). Para as voluntárias, serão apresentadas o Termo de consentimento livre e esclarecido. Foi realizado estudo piloto em que foi obtido 14 participantes e foi feito o cálculo de suficiência amostral para o desfecho primário PCR em que foi considerado um alfa de 0,05 e beta 0,80 para relação entre amostras 1:1 e assim foi estimado 82 participantes. Projeto foi submetido ao Comitê de Ética em Pesquisa com CAAE: 37695620.5.0000.0042. **RESULTADOS ESTIMADOS:** Este estudo poderá sugerir um redirecionamento de tomadas de decisão de trabalhadores da saúde no tocante uso de contraceptivos a mulheres com risco cardiovascular.

PALAVRAS-CHAVE: Contraceptivo Injetável. Proteína C-reativa. Lipoproteína de Baixa Densidade – Oxidada.

Submitted 03/23/2021, Accepted 11/177/2021, Published 01/13/2022

J. Évid-Based Healthc., Salvador, 2022;4:e3744

<http://dx.doi.org/10.17267/2675-021Xevidence.2022.e3744>

ISSN: 2675-021X

Assigned editor: Luis Claudio Correia

How to cite this article: Muniz DL, Santos PA, Barbosa JS, Leite JM,

Petto J. Study protocol to compare the influence of the use of Contraceptive Injection in the levels of C Reactive Protein and Oxidized Low-density Lipoprotein. J Évid-Based Healthc. 2022;4:e3744. <http://dx.doi.org/10.17267/2675-021Xevidence.2022.e3744>



Introduction

The use of Hormonal Contraceptives (HC) by around 40 million women worldwide has as its main objective the prevention of unintended pregnancies.¹ Moreover, the use of HC is also indicated for non-contraceptive benefits, such as the improvement of secondary pain, endometriosis, and treatment of acne.^{2,3} However, their adverse effects were observed, and they suggest that their continuous use has influenced physiological processes, as in the formation of atherosclerotic plaques.⁴⁻⁶

Researches made by our group observed alterations in the levels of postprandial Lipemia⁷, of the C- reactive protein of high sensitivity (CRP-as)⁵, in the lipid profile and the marker of Oxidized low-density lipoprotein (Ox-LDL)⁶ in women that make use of Combined Oral Contraceptives (COC), if compared to women who do not make use of them. Moreover, other researchers observed associations between the continuous use of COC with different outcomes, like mood changes and body composition.¹

Regarding Hormonal Contraceptive Injection (HCI), there were found studies that evaluated their relationship with the increase of central obesity, protection to cancer, and changes in libido⁸; however, in the relationship between the continuous use of CHI with inflammatory parameters as CRP and Ox-LDL, there were not found studies in our literature review.

Given the above, the objective of this study is to test the hypothesis that the continuous use of HCI alters the level of the markers of CRP and Ox- LDL in women who are, apparently healthy and irregularly active.

Methods

Study Design

The research is a comparative observational cross-sectional study.

Target Population

Women who use or who do not use Contraceptive Injections.

Inclusion Criteria

Women who use continuous CI, or not, for at least six months, which are irregularly active, aged 18 to 30 years old.

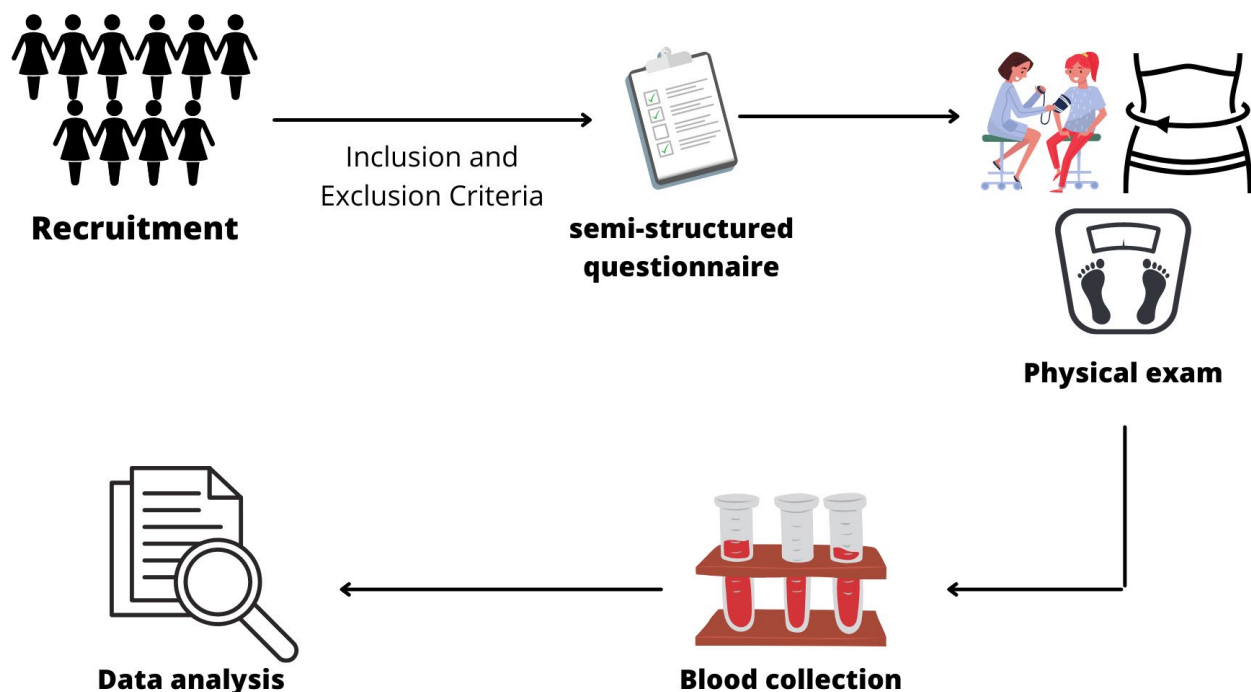
Exclusion Criteria

There were excluded the volunteers who showed medical conditions such as familial dyslipidemia, hepatic impairment, diabetes, hypo or hyperthyroidism, renal diseases, hypo or hypercaloric diet, history of alcoholism, smoking, use of hypolipidemic, corticoids, diuretics or beta-blockers, and BMI higher than 30 kg/m².

Study Protocol

The summarized flow of the study protocol can be found in Figure 1.

Figure 1. Recruitment



The participants will be recruited through invitations in specialized centers, such as clinics, basic health units, and social networks. The volunteers who made part of the inclusion criteria will answer a semi-structured questionnaire, which has the role of collecting general information on the characteristics of the sample (Annex I) and will undergo a physical exam to measure the Blood Pressure at rest, body mass, height, waist circumference (WC) and calculation of Body Mass Index (BMI) through Quetelet's equation: $\text{mass (kg)} / \text{height}_2 \text{ (cm)}$. Waist Circumference will be obtained with a metallic and inelastic tape measure – a Starrett® - with a definition of a measure of 0,1 in the smallest cm curvature between the last rib and the iliac crest – these tests will work as exclusion criteria.

All of them will be directed not to alter the diet on the week of the test, not to make any physical effort that is different from the usual ones, and not to drink alcoholic beverages 24 hours before the exam.

After fasting of 12h, there will be held an only blood collection, by an experienced professional in Clinical Analysis laboratories in the cities of Ibicarai-Ba and Salvador-Ba, in which there were dosed glycemia, glutamate pyruvic, and oxidative transaminase (SGPT and SGOT), as well as Ox-LDL and CRP. To determine Ox-LDL in the serum samples, the kit ELISA will be used. In this analysis, the values of Ox-LDL to be considered normal will be between 100 and 700 mU/mL. CRP will be determined by nephelometry with plasma serum and precision of the 0,1 mg/L.

Calculation of Sample Size

For the sample calculation there were considered means of 0,6 mg/L and pattern deviations of 0,73 mg/L (GCI) and in the control group 1,04 mg/L, both extracted from the previous pilot study (n=7)- considering that an $\alpha = 0,05$ (two-way) and $\beta = 0,80$. This way, there were studied 88 volunteers, i.e., 44 volunteers in each group. For the sample calculation, the software BioStat was used.

Plan of Statistical Analysis

The two-way, non-paired t Student test is to be used. The further variables that show parametric distribution will be described on mean and pattern deviation, and the non-parametric ones in average and interquartile deviation. The statistical program SPSS version 22.0 for Windows will be used.

Statistical Hypotheses

Null Hypothesis (NH): Women who use contraceptive injection did not show significant differences in results in the levels of C- reactive protein compared to women who do not make use of them.

Alternative Hypothesis (AH): Women who use contraceptive injection showed significant differences in results in the levels of C- reactive protein compared to women who do not make use of them.

Ethical Aspects

The project will be submitted to the Committee of Ethics in Research with the Certificate of Presentation for Ethical Consideration (CPEC) CAEE: 37695620.5.0000.0042.

All participants will receive detailed information on the objectives of this study, and they will sign a free and consented to inform (Annex II). In addition, twofold documents will be signed – one for the volunteer and another one for the researcher, according to Resolution n. 466/2012, from the National Health

Council, all the legal and ethical provisions in research with human beings were respected.

Risks

Probable risks may be psychological, intellectual, and emotional factors of the volunteers because of constraints, fear, or stress when answering the questionnaires. In this sense, the questionnaire will be passed out individually by a skilled professional and with due safety to minimize these risks and not cause any psychological pressure.

During the blood collection, because it is an invasive procedure, volunteers may feel nauseated or dizzy, but the professionals are skilled ones, so that, in these cases, they will follow the host protocol to the volunteer, to minimize the risks, and then, do not cause damages to the volunteer.

Benefits

The results in this study may contribute to better intervention and prevention measures, with the use of contraceptive injections aiming to minimize cardiovascular risks and promote a better quality of life to the population.

Expected Results

This study's results may contribute to other clinical thinking when it refers to decision-making about the use of contraceptive injections, considering the characteristics of the women who will undergo the treatment of hormonal contraception, aiming to minimize cardiovascular risks.

Few studies analyzed the use of CI in subclinical inflammatory markers as C-reaction protein. Petto⁵ and Santos⁴ published studies that observed subclinical inflammation in women who used Combined Oral Contraceptive, suggesting a higher cardiovascular in them.

This way, the study will allow the understanding of one more type of hormonal contraceptive, one with a higher hormonal load, because its use is done quarterly. Nevertheless, this study does not aim to show the cause and effect of the use of CI in cardiovascular risk and the cost-benefit of using this contraceptive method.

Authors' contribution

All the authors effectively contributed to the idea and structuring of the methodology. Muniz DLC and Leite JM wrote the manuscript, Santos PA, Barbosa JS, and Petto J reviewed the important intellectual content.

Competing interests

No financial, legal, or political competing interests with third parties (government, commercial, private foundation, etc.) were disclosed for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.).

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ANNEX I

STANDARD QUESTIONNAIRE AND PHYSICAL EXAMINATION

Date: ___/___/___

1.

♥ Identification:

Name: _____

Date of birth: ___/___/___

Education level: () 1st degree () 2nd degree () 3rd degree Other: ___

2.

♥ Drugs

A. () Does not use

B. () Uses:

➤ Qual(is): _____

➤ Purpose: _____

➤ Dosage: _____

♥ Smoking

A. () Non-smoker

B. () Smoker Quantity: _____

C. () Ex-smoker Use time: ___

3.

♥ Injectable Contraceptive

A. () Does not use

B. () Uses:

➤ Which uses: _____

➤ Usage time: _____

4.

♥ Limitations to exercise

A. () Gonartrose C. () Labyrinthitis

B. () Reports of hypoglycemia D. () Postural hypotension

Polycystic Ovary Syndrome?

() Yes

() No

5.

♥ **Body Mass:** _____ kg **Height:** _____ cm **BMI:** _____ **IC:** _____

♥ **At rest ATE:**

#####	TA in supine (mmHg)	TA in sedstation (mmHg)	TA in orthostasis (mmHg)
Arm D.			
Arm E.			

ANNEX II

FREE AND INFORMED CONSENT TERM

Project Title: **Inflammatory profile and injectable contraceptive.**

Responsible Researcher: **Dr. Jefferson Petto**

Collaborating Researcher: **Daniell Muniz**

Institution to which the Responsible Researcher belongs: **Bahian School of Medicine and Public Health**

Contact phones: **(71) 9 9378-8370**

Volunteer name: _____

Age: _____

Legal guardian (where applicable): _____

R.G: _____

You _____ are

__está invited to participate in the research project "**Inflammatory profile and injectable contraceptive.**", the responsibility of researcher Dr. Jefferson Petto.

Justification and Purpose

The main objective of this study is to evaluate whether the use of injectable contraceptives chronically influences the magnitude of the inflammatory profile of. This work is justified in the fact that it is investigating the influence of continuous and uninterrupted use of injectable contraceptive on the inflammatory and physiological profile in order to prevent arteriosclerosis. We know that the continuous use of combined oral contraceptives is a potent factor of cardiovascular risk-related diseases, but still, the effect of injectable contraceptive on inflammatory profile and women is not known.

Study Steps

First of all, it is necessary to say that all personal information (name, address, photos and personal data) will not be exposed in the search. **In addition, participants will not have any financial expenses related to the survey.**

The first step in our work is to collect clinical data through a standard questionnaire and a physical examination. After data collection, the participants will be submitted to a blood collection in which PCR, LDL-ox will be dosed, later, All test results will be stored and passed on to the volunteers at the end of the research.

This study may present minimal risk of worsening of the clinical condition of the participant when the blood collection protocol is applied, because it uses a syringe as a basic measure for collection, the participant may feel dizziness, so the team will be able and ready to take care of the same if this fact happens, taking all appropriate measures. All material used is sterilized and disposable and the tests will be carried out in a specialized laboratory and by qualified and experienced professionals. Any questions of the volunteer regarding any procedure can be answered directly with the responsible researcher.

The right of the volunteer, at any time of the study, to give up participating in the research is guaranteed.

I, _____

Salvador, _____ of _____ of 2022.

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Name and signature of the volunteer

Name and signature of the person responsible for obtaining consent