# IMPACT OF ESTROGENS AND PROGESTAGENS ON COAGULATION INHIBITORY PROTEINS AMONG USERS OF COMBINED HORMONAL THERAPY

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## Abstract

Hormonal therapy increases the risk of venous thromboembolism, through a negative effect on coagulation inhibitors. Studies of the oral contraceptives use show that the coagulation effects depend on the dosage of estrogen and the type of progestogen. The objective of this study was to determine the effect of two combined oral contraceptives on two important inhibitory coagulation proteins. The study included 56 women between ages 20 and 55, twenty six of them taking 30μg ethinyloestradiol (EE) and 75μg gestodene (GSD) and thirty taking 30 μg ethinyloestradiol and 75 μg levonogestrel (LNG) for two months. The subjects were healthy and they hadn't taken any other drug two months before starting the hormonal therapy. Plasma was used for measuring protein C and AT III, before using the pill and after stopping it. We evaluated protein C using ELFA (Enzyme linked fluorescence assay), and AT III using chromogenic assay. We used SPSS 20 software for the statistical analyses. Comparison of the values of protein C and AT III between two periods (before and after treatment) showed the following results: Concentrations of protein C was significantly increased while AT III level was decreased after treatment with 30 μg EE/ 75 μg GSD ($p<0.05$). Treatment with 30 μg EE/ 75 μg LNG showed no significant change in the level of AT III ($p>0.05$) and significantly increased protein C concentration ($p<0.05$). We conclude that treatment with 30 μg EE/ 75 μg GSD induces more changes in the inhibitory coagulation proteins than treatment with 30 μg EE/ 75 μg LNG, indicating an increased risk for thrombotic diseases.

***Key words***: *Combined hormonal therapy, protein C, antithrombin III, thrombosis risk*

## 1. Introduction

Epidemiologic studies published in 1995 reported a slightly higher risk of venous thromboembolism among users of oral contraceptives (OCs) containing desogestrel or gestodene as the progestogenic component (third generation OCs) as than among users of OCs containing levonorgestrel or norgestimate as the progestogenic component (second-generation OCs), (WHO, 1995; Jick H *et* al., 1995).

Oral oestrogen use in women has been associated with increased risk of venous thromboembolism. In premenopausal women, the risk of venous thromboembolism has been shown to increase by about two to six-fold during the use of combined oral contraceptives, (Rosendal FR *et* al., 2003).

Due to the observed differences in the risk of VT induced by OC containing the same dose of estrogen but different progestogen compounds, the prothrombotic effect of the pill was considered to be not strictly dependent on the dose of estrogen but rather on the "total estrogenicity" of the formulation (Odlind V *et* al., 2002). The "total estrogenicity" rises with increasing dose of estrogen but decreases with increasing antiestrogenic activity of progestogen compound. It was suggested that third generation progestogens, as well as drospirenone and cyproterone acetate possess a weaker anti-estrogenic activity than levonorgestrel and, therefore, are less potent in the counterbalancing the prothrombotic effects of estrogen (Kemmeren JM et al., 2004; Odlind V *et* al., 2002). Consequently, OC containing third generation progestogens (desogestrel or gestodene), drospirenone or cyproterone acetate have a higher "total estrogenicity" as compared to second generation OC which may explain why users of these formulations are exposed to a higher thrombotic risk (Kemmeren JM et al., 2004).

Heritable thrombophilia is another thrombotic factor that include deficiencies of antithrombin, protein C and protein S; and common genetic mutations such as factor V Leiden and the prothrombin G20210A mutation and the thermolabile variant (C677T) of the methylene tetrahydrofolate reductase (MTHFR) gene, (Walker ID *et* al., 2001). Other relatively common thrombophilias with a combination of heritable and acquired components include elevated plasma factor VIIIc (Koster T *et* al., 1995), hyperhomocysteinaemia (Heijer M *et* al., 1996) and acquired activated protein C resistance ( Clark P & Walker ID, 2001).

The further increased thrombotic risk in third generation OC users was, however, questioned in later publications (Baillargeon *et al.*, 2005; van Hylckama Vlieg *et al.*, 2009; Parkin *et al.*, 2011). The discussion that followed was hampered by the fact that there was neither a good biological explanation for the thrombotic effect of the pill nor for the difference between second and third generation oral contraceptives (Lidegaard *et al.*, 2009).

Therefore the aim of this study was to determine the effect of two combined oral contraceptives (containing: ethinylestradiol/levonogestrel and ethinylestradiol/gestodene) on protein C and antithrombin III concentrations in a group of healthy Albanian women.

## 2. Material and Methods

### a- Study design

The study was carried out at Ana Diagnostic Center Gynecologic Clinic in Tirana. Healthy women requesting contraception were included and followed up for two months. Twenty six women were instructed to use COC with 30 µg EE and 75 µg GSD and thirty were instructed to use COC with 30 µg EE and 75 µg LNG, initiating pill intake on the first day of the cycle. Clinical and laboratory assessments were carried out prior to initiation of medication (pre-treatment) and after 2 months of COC use.

### b- Laboratory methods

All participants were submitted to a blood collection to perform the antithrombin III and protein C laboratory test. Blood samples were centrifuged at 1500 rev/min for 15 min to extract plasma. We used chromogenic assay (Cobas 6000, Roche) to evaluate antithrombin III while protein C was evaluated via ELFA (Vidas, Biomerieux ).

### c- Statistical analyses

Student's *t*-test for paired samples was used for numerical variables to compare levels of inhibitory coagulation proteins at two time intervals (pre-treatment and after 2 months of COC use). Statistical significance ($p < 0.05$) was determined using SPSS 20 software.

## 3. Results

Comparison of the values of protein C and AT III between two periods (before and after treatment) showed the following results: concentrations of protein C was significantly increased while AT III level was reduced after treatment with 30µg EE/75µg GSD (p=0.001). Treatment with 30µg EE/75µg LNG showed no significant change in the level of AT III (p=0.058) and significantly increased protein C concentration, (Table. 1; Fig. 1, 2).

**Table.1** Effects of COCs on Protein C and Antithrombin III

| Parameters | 30 µg EE/75 µg GSD | | | 30 µg EE/75 µg LNG | | |
|---|---|---|---|---|---|---|
| | Baseline | COC use | p-value | Baseline | COC use | p-value |
| AT III (%) | 96.2154 ± 1.77 | 91.1615 ± 1.72 | 0.001 | 103.9 ± 1.4 | 98.6 ± 2.0 | 0.058$^{ns}$ |
| Protein C (%) | 117.2 ± 1.86 | 131.8 ± 5.0 | 0.0001 | 115.5 ± 2.5 | 128.6 ± 1.84 | 0.001 |

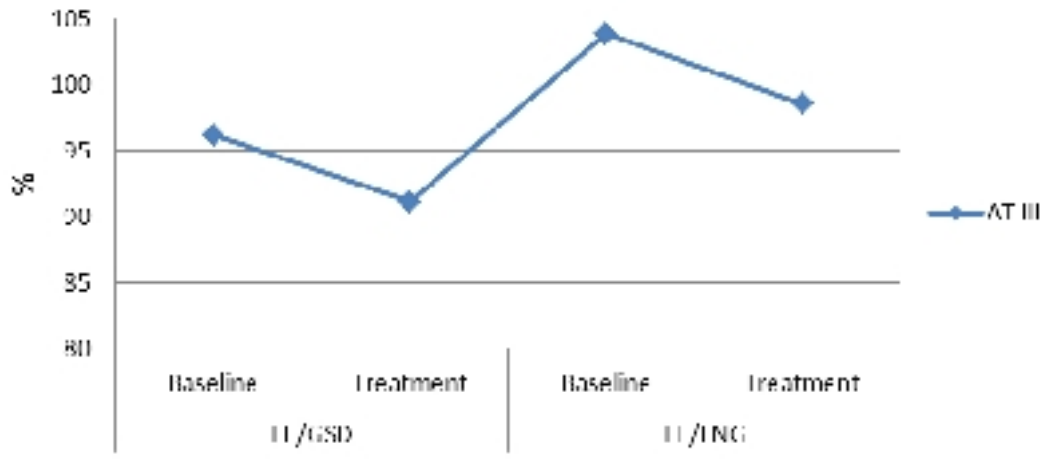


**Figure 1.** Changes in AT III level after two COCs use.

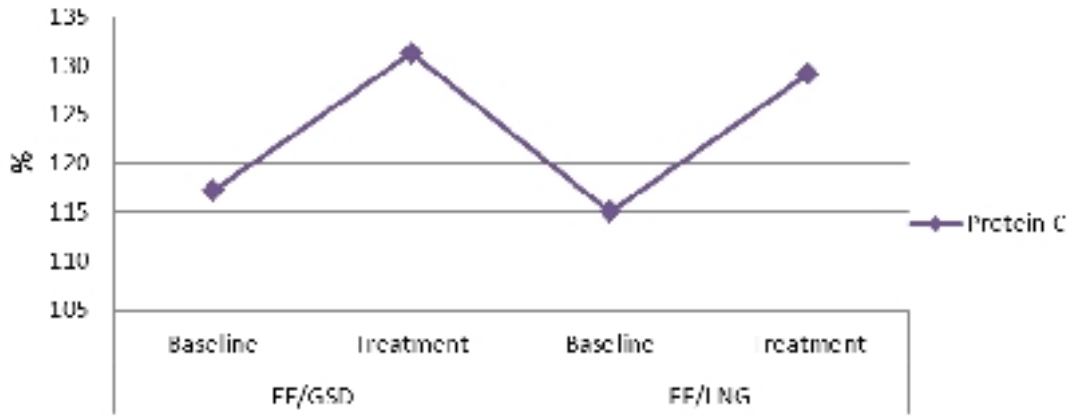


**Figure 2.** Changes in protein C level after two COCs use.

## 4. Discussions

In the present study, users of the two combined oral contraceptives showed significant changes in protein C plasma concentration, as reported in the literature (for references see below). The changes were within the normal range and are not associated with an increase in VTE risk. The results found a large effect of oestrogen and progestogen on this haemostatic variable. A greater change in protein C level was observed in the GSD group compared with the LNG group. Nevertheless, antithrombin III activity was slightly reduced by the combination containing 30 µg EE/75 µg LNG. The results agree with those of several studies with various low-dose formulations that have revealed no change or only a slight decrease in antithrombin III activity (J Spona *et* al., 1997; Jespersen J *et* al., 1991; Winkler U.H *et* al., 1999).

Our results of a rise in protein C activity during use of both formulations confirm those of previous OC studies (Spona J *et* al., 1997; Jespersen J *et* al., 1991; Parkin *et al.*, 2011).

Nevertheless, it is important to note that changes observed in inhibitory proteins of coagulation during the use of combined OCs by healthy women cannot explain the increased risk of thromboembolic disease; hence caution should be taken in considering results from this kind of clinical trial.

In conclusion, the use of a COCs containing 30 µg EE/75 µg GSD for a period of two months in healthy women with no associated risk factors caused significant changes in protein C and AT III levels. Treatment with 30 µg EE/75 µg LNG showed no significant change in AT III plasma concentration and significantly increased protein C level. The clinical significance of these findings should be evaluated in a larger cohort of women with associated risk factors, such as smoking, and over a longer period of COC use.

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