The Effect of Contraceptive Pills on Coagulation Tests among Sudanese Women in Khartoum State-Sudan

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Abstract: This is a descriptive cross-sectional study which was conducted in Khartoum state in the period between February 2011 to May 2011 to evaluate the effect of contraceptive pills (combined and progestin only pills) on coagulation tests (Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT) and Fibrinogen level. One hundred women were selected as volunteers according to inclusion criteria and considered as case, and other twenty women not taken these pills, were selected and considered as control group. 79% of the cases were under Combined Oral Contraceptive (COC), and the other 21% were under Progestin Only Pills (POP), 26% of cases were used the oral contraceptives for less than six months, while 74% were used for more than six months. The age of the cases were categorized into two groups, 51% in age group less than thirty years and 49% in age group more than thirty years. 4.5 ml of fresh venous blood were collected from each volunteer, after filling the questionnaire, in plastic container containing 0.5 ml of 3.8% trisodium citrate solution for anticoagulation. Then the contents of the container were mixed and centrifuged at 3000 round/min for 15 minutes for preparation of platelets poor plasma (PPP). The PPP were tested for the PT, APTT, TT and fibrinogen level by using the coagulometer instrument (Clot). The results were analyzed by independent T test of the SPSS computer programme. The results of cases revealed that TT mean = 9.1 seconds, APTT mean= 33.4 seconds, Fibrinogen level mean= 266 mg/dl and PT mean= 14.0 seconds. The results of control group revealed that TT mean = 9.8 seconds, APTT mean= 30.7 seconds, Fibrinogen level mean= 249 mg/dl and PT mean= 13.4 seconds. Over all the results were showed significant shortened in TT and increased in APTT when compared with control group with P value < 0.05, and no significant variations were noticed in both PT and fibrinogen level with P value > 0.05 and this indicate the hypercoagulability. No significant changes were noticed between age groups, type and duration of oral contraceptives.

Keywords: Contraceptive pills, Prothrombin Time, Activated Partial Thromboplastin Time, Thrombin Time, Coagulation

1. Introduction

Contraceptive pills are intentional prevention of conception through the use of various devices, sexual practices, chemicals, drugs, or surgical procedures become a contraceptive if its purpose is to prevent a woman from becoming pregnant [1]. There are several types of contraceptives pills that have been officially labeled as such because they have shown reliability in preventing conception from occurring. The contraceptives pills are the common name for oral contraception, it's one of the safest, most effective, and popular methods of birth control. The pill is made up of synthetic forms of hormones that naturally occur in a female's body, progesterone and estrogen. The pill works by stopping the action of the hormones that trigger ovulation. There by, preventing the release of an egg, it also thickens the cervical mucus, so it makes it hard for sperm to swim. The pill comes in two forms: combination pills and progestin-only pills. The pill must be taken daily to sustain the hormone levels needed to prevent ovulation [1]. Oral contraceptives related thrombosis has become an important field of study. Women taking oral contraceptives appear to have ~3- to 4-fold increased risk of thrombosis (primarily deep venous thrombosis in the lower extremities) compared to women not taking oral contraceptives [2]. The risk is lower in young women without other risk factors for thrombosis, higher in older women or women with other risk factors. Oral contraceptives can interact with inherited thrombophilia, notably factor V Leiden. Women who are heterozygous for the mutation and take oral contraceptives have a ~30- fold increase in risk of deep venous thrombosis compared to women without the mutation and not taking oral contraceptives [2]. Women who are homozygous for the mutation and take oral contraceptives have a several hundred-fold increase in risk of thrombosis [2]. A variety of other conditions confer an increased risk of thrombosis, including pregnancy, surgery, sepsis, the nephrotic syndrome, and many others. In pregnancy, the blood levels of many clotting factors are increased. Women may be less active or confined to bed rest, and the gravid uterus presses on the veins from the lower extremities, predisposing to stasis. A “dead fetus syndrome” may induce a chronic DIC state. Surgery increases the risk of thrombosis by several means. The risk is particularly high with hip replacements and other orthopedic surgeries. Sepsis can be thrombogenic due to decreased levels of protein C and free protein S. The
nephrotic syndrome may be thrombogenic because of loss of anti thrombin in the urine [2].

Objectives
1) To evaluate the contraceptive pills effect on the coagulation tests and identifying the hypercoagulable state.
2) To measure thrombin time test.
3) To measure fibrinogen level.
4) To measure activated partial thromboplastin time test.
5) To measure prothrombin time test.
6) To compare the results of study group with control group.
7) To compare the results of these tests between different age groups.
8) To compare the results of these tests between different duration groups.

2. Materials and Methods

Study design:
A cross-sectional descriptive study conducted in Khartoum state during the period of February 2011 to May 2011 to evaluate the effects of oral contraceptive pills on the secondary hemostasis tests (Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT) and Fibrinogen level assay).

Study population:
One hundred females from Khartoum state who under oral contraceptive pills, and twenty controls.

Inclusion criteria:
All females who were under oral contraceptive pills and resident in Khartoum state were enrolled.

Exclusion criteria:
Presence of other coagulation disorders, pregnancy, and unused of oral contraceptive pills will exclude.

Ethical consideration:
The consent of the selected individuals to the study was taken after being informed with all detailed objectives of the study and its health benefit in future.

Data collection:
Data were collected using self-administered pre-coded questionnaire which was specifically designed to obtain information.

Data presentation:
The data were presented in tables and figures.

Sampling:
Non-probability sampling method was used (only who accepted study tests) (volunteers) were involved in sample.

Data analysis:
The data were analyzed using the SPSS computer programme version 11.5.

Sample:
4.5 ml of venous blood was collected in plastic container containing 0.5 ml tri sodium citrate as anticoagulant, and then the blood is centrifuged, after thoroughly mixing, for 15 minutes at 3000 rpm to obtain platelets poor plasma (PPP).

Methodology

Thrombin time:
Principle:
Thrombin is added to plasma and the clotting time is measured. The TT is affected by concentration and reaction of fibrinogen and by the presence of inhibitory substance, including fibrinogen/fibrin degradation products (FDP) and heparin. The clotting time and the appearance of clot are equally informative.

Reagent:
FIBROSCREEN reagent is a lyophilized preparation of bovine of 50 NIH/ml. Reconstituted with 1 ml of distilled water, waited for 5 minutes, not shacked but mixed gently by swirling till the solution attained homogeneity.

Fibrinogen:
Principle
Diluted sample is clotted with a strong thrombin solution; the plasma must be diluted to give a low level of any inhibitors (e. g FDP and heparin). A strong thrombin solution must be used so that the clotting time over a wide range is independent of the thrombin concentration.

Reagent:
1) Thrombin reagent, which is a lyophilized preparation from bovine source 50 NIH units per vial.
2) Fibrinogen calibrator, which is a lyophilized preparation of human plasma equivalent to stated amount of fibrinogen on mg basis (refer FIBRINOGEN graph paper supplied with each kit for the value of each lot).
3) Owren's buffer, ready to use (pH 7.35).

Procedure for fibrinogen calibration curve preparation:
The FIBRINOGEN thrombin reagent vial were reconstituted exactly with one ml of distilled water; waited for 5 minutes, not shacked but gently swirled till the solution attained homogeneity. The FIBRINOGEN calibrator vial were reconstituted exactly with one ml of distilled water; waited for 5 minutes, not shacked but gently swirled till the solution attained homogeneity. Then serial dilutions were made and these dilutions were assayed for fibrinogen assay using the automated coagulometer and the clotting time of each dilution was blotted against its corresponding concentration (dilution 1:10 were represented as 100% concentration) on log-log paper and the points were joined together to create linear line.

Test procedure for sample:
The PPP samples were diluted 1:10 with Owren's buffer solution. Then the diluted samples were assayed for fibrinogen assay using the automated coagulometer and the clotting time of each sample was blotted on the log-log paper and the corresponding concentration was gotten from the curve.
Prothrombin Time

Principle:
The PT test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. Although originally thought to measure prothrombin, the test is now known to depend also on reactions with factors V, VII, and X and on the fibrinogen concentration of the plasma [3].

Reagents:
1) Thromboplastin:
Thromboplastins were originally tissue extracts obtained from different species and different organs containing tissue factor and phospholipid. Because of the potential hazard of viral and other infections from handling human brain, it should no longer be used as a source of thromboplastin. The majority of animal thromboplastins now in use are extracts of rabbit brain or lung.
2) CaCl2: 0.025 mol/l.

Activated Partial Thromboplastin Time:
Other forms of the APTT test are known as the partial thromboplastin time with kaolin (PTTK) and the kaolin cephalin clotting time (KCCT), reflecting the methods used to perform the test.

Principle
The test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin and so indicates the overall efficiency of the intrinsic pathway. The test depends not only on the contact factors and on factors VIII and IX, but also on the reactions with factors X, V, prothrombin, and fibrinogen. It is also sensitive to the presence of circulating anticoagulants (inhibitors) and heparin [3].

Reagents:
1) Kaolin: 5 g/l (laboratory grade) in barbitone buffered saline, pH 7.4. A few glass beads were added to aid resuspension. The suspension is stable at room temperature. Other insoluble surface active substances such as silica, celite, or ellagic acid can also be used.
2) Phospholipids:Cephalin as phospholipids substitution.
3) CaCl2: 0.025 mol/l.

The coagulometer
The automatic coagulometer (Clot) is an instrument for the determination of the main parameters used in the plasma coagulation methods:

Theory and principle
The coagulometer (Clot) has an optical measurement system which detects a sudden variation in optical density when a clot is formed. The chronometer and the stirring system are activated by a sudden change of the optical density. This permits the initiation of the time measurement when the sample is added to the reagent and stop the measurement time at the moment that the clot is formed. The continuous mixing guarantees a perfect homogenization and makes the measurement possible of low concentrations of fibrinogen by grouping the fibrin filaments in the centre of the optical pass. The system has a programmable security time during which variations in optical density, when the reagent and the plasma are still in the homogenization phase, cannot activate the detection cell.

Procedure
First of all, the cuvette was placed corresponding to the determinations that were done on the thermostat. A magnetic stirrer was inserted in every cuvette and waited for the instrument to reach 37°C. After that, into the cuvette the reagent or sample volumes required were introduced.

PT = (200 μl of reagent).
APTT = (100 μl of reagent and 100 μl of plasma).
Fibrinogen = (200 μl of diluted plasma).
TT = (100 μl of plasma).

When the thermostatisation time is finished, the cuvette was placed on the reading well. The chronometer was remained inactive for some seconds and then it was showed 000:0. At this moment the reagent or plasma was added with a disposable tip pipette, the liquid was left to get down with one blow and all the reaction was started at the same time. 100 μl of the starter was added:

PT-Plasma
APTT-Calculator Chloride
Fibrinogen-Thrombin reagent
TT-Thrombin reagent.

When the reagent and the plasma were in contact an O.D. variation was produced, that automatically activated the digital chronometer and the magnetic mixer. When the clot was starts to formed, an O.D. variation was produced and stopped the chronometer and the mixer. The clotting time appeared on the display.

3. Results

When compared the mean of PT, APTT, TT and Fibrinogen results between study group and control group, insignificant difference in mean PT result between cases (14.0 seconds ± SD) and control (13.4 seconds ± SD) (P value = 0.08), significant increase of mean APTT result (33.4 seconds ± SD) when compared with mean APTT of control (30.7 seconds ± SD) (P value = 0.002), significant shortened in mean TT result in cases (9.1 seconds ± SD) when compared with control (9.8 seconds ± SD) (P value = 0.003), and insignificant difference between mean of fibrinogen in cases (266 mg/dl ± SD) and control (249 mg/dl ±SD) (P value = 0.18) in table (1). The effect of contraceptives type on mean (PT, APTT, TT, and Fibrinogen) results was measured, there were insignificant difference in mean PT result between usage of combined oral contraceptive (COC) (14.0 seconds ± SD) and usage of progestin only pills (POP) (14.0 seconds ± SD) (P value = 1.00), insignificant difference in mean APTT result between usages of combined oral contraceptive (COC) (33.5 seconds ± SD) and usage of progestin only pills (POP) (33.3 seconds ± SD) (P value = 0.87), insignificant difference in mean TT result between usage of combined oral contraceptive (COC) (9.1 seconds ± SD) and usage of progestin only pills (POP) (9.0 seconds ± SD) (P value = 0.61), and insignificant difference in mean fibrinogen result between usage of combined oral contraceptive (COC) (268 mg/dl ± SD) and usage of progestin only pills (POP) (257 mg/dl ± SD) (P value = 0.59) in table (2). When measured the effect of age group on (PT, APTT, TT, and Fibrinogen) results, there were insignificant difference in mean PT result.
in study group of age less than 30 years (14.1 seconds + SD) and of age more than 30 years (13.8 seconds + SD) (P value = 0.35), insignificant difference in mean APTT result in study group of age less than 30 years (33.7 seconds + SD) and of age more than 30 years (33.2 seconds + SD) (P value = 0.52), insignificant difference in mean TT result in study group of age less than 30 years (9.0 seconds + SD) and of age more than 30 years (9.2 seconds + SD) (P value = 0.12), and insignificant difference in mean fibrinogen result in study group of age less than 30 years (259 mg/dl + SD) and of age more than 30 years (273 mg/dl + SD) (P value = 0.42) in table (3). The effect of contraceptives duration on PT, APTT, TT, and Fibrinogen results was measured, there were insignificant difference in mean PT result between contraceptive duration of less than 6 months (14.0 seconds + SD) and of more than 6 months (14.0 seconds + SD) (P value = 0.94), insignificant difference in mean APTT result between contraceptive duration of less than 6 months (33.6 seconds + SD) and of more than 6 months (33.4 seconds + SD) (P value = 0.80), insignificant difference in mean TT result between contraceptive duration of less than 6 months (9.0 seconds + SD) and of more than 6 months (9.1 seconds + SD) (P value = 0.60), and insignificant difference in mean fibrinogen result between contraceptive duration of less than 6 months (255 mg/dl + SD) and of more than 6 months (270 mg/dl + SD) (P value = 0.46) in table (4).

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4. Discussion

This is a descriptive cross-sectional study which was conducted in Khartoum state during the period from February 2011 to May 2011 to evaluate the effect of contraceptive pills (combined & progestin only pills) on coagulation tests (Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), and Fibrinogen level. A 100 sample were collected from females according to inclusion criteria and considered as case, and 20 samples were collected from healthy females, not taken these pills, and considered as control. The results revealed the following: the mean TT result of study group was (9.1 seconds) which significantly shortened than that of control group (9.8 seconds + SD) (P value< 0.05), which consistent with the study of Babatunde (2004) [4] which conducted low-dose COC trial in Nigeria at December 2004 with significant increase (P.value 0.007). The mean fibrinogen level result of study group was (266 mg/dl ± SD) which slightly increased than that of control group (249 mg/dl ± SD) but without statistical significance (P value 0.18), which consistent with the study of Babatunde (2004) [4] which conducted low-dose COC trial in Nigeria at December 2004 with significant increase (P.value 0.007), also consistent with the study of Famodu (1997) [5] which conducted COC trial in Nigeria at 1997 with significant increase (P value < 0.001). The mean APTT result of study group was (33.4 seconds ± SD) which significantly increased than that of control group (30.7 seconds ± SD) (P value 0.002), which consistent with the study of Aldrighi et al. (2006) [6] which conducted COC trial in Sao Paulo at January 2006 with significant increase in APTT result. The mean PT result of study group was (14.0 seconds ± SD) which slightly increased than that of control group (13.4 seconds) but without statistical significance (P value 0.08), which consistent with the study of Aldrighi et al. (2006) [6] which conducted COC trial in Sao Paulo at January 2006 with significant increase in PT result. The mean fibrinogen level in study group who used combined pills was (268 mg/dl ±SD) which slightly increased than that of who used progestin only pills (257 mg/dl ± SD) but without statistical significance (P value 0.59).The mean APTT in study group who used combined pills was (33.5 seconds ± SD) which slightly increased than that of who used progestin only pills (33.3 seconds ± SD) but without statistical significance (P value 0.87). The mean TT in study group who used combined pills was (9.1 seconds ± SD) which slightly increased than that of who used progestin only pills (9.0 seconds ± SD) but without statistical significance (P value 0.61). The mean PT in study group who used combined pills was (14.0 seconds ± SD) which

**Table 1: PT, APTT, TT and Fibrinogen results in study and control group**

**Table 2: PT, APTT, TT and Fibrinogen results in different types of oral contraceptive pills**

**Table 3: PT, APTT, TT and Fibrinogen results in different age groups**

**Table 4: PT, APTT, TT and Fibrinogen results in different contraceptives duration groups**

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similar to that of who used progestin only pills (14.0 seconds) (P value 0.59). The mean TT in study group who in age group of < 30 years was (9.0 seconds ± SD) which slightly shortened than that of age group of > 30 years (9.2 seconds ± SD) but without statistical significance (P value 0.12). The mean PT in study group who in age group of < 30 years was (14.1 seconds ± SD) which slightly increased than that of age group of > 30 years (13.8 seconds ± SD) but without statistical significance (P value 0.35). The mean APTT in study group who in age group of < 30 years was (33.7 seconds ± SD) which slightly increased than that of age group of > 30 years (33.2 seconds ± SD) but without statistical significance (P value 0.52). The mean fibrinogen level in study group (who in age group of < 30) years was (259 mg/dl ± SD) which slightly decreased than that of age group of > 30 years (273 mg/dl ± SD) but without statistical significance (P value 0.42). The mean TT in study group who used the pills for < 6 months was (9.0 seconds ± SD) which slightly decreased than that who used the pills for > 6 months (9.1 seconds ± SD) but without statistical significance (P value 0.60). The mean APTT in study group who used the pills for > 6 months was (33.6 seconds ± SD) which slightly increased than that who used the pills for > 6 months (33.4 seconds ± SD) but without statistical significance (P value 0.80). The mean fibrinogen in study group who used the pills for < 6 months was (255 mg/dl ± SD) which slightly decreased than that who used the pills for > 6 months (270 mg/dl ± SD) but without statistical significance (P value 0.46). The mean PT in study group who used the pills for < 6 months was (14.0 seconds ± SD) which similar that who used the pills for > 6 months (14.0 seconds ± SD) (P value 0.94).

5. Conclusion

The results concluded that the usage of contraceptive pills (COC & POP) significantly shortening the TT result in compare with control, no significant changes occurred in fibrinogen level but slight increase. These findings were a sign of hypercoagulable state. On the other hand, significant increase in APTT result and insignificant changes in PT result. Over all the mean of these parameters were in normal ranges.

6. Acknowledgement

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References