SERUM HOMOCYSTEINE LEVEL IN HORMONAL CONTRACEPTIVE USERS IN KANO METROPOLIS

[1]Abdullahi, H. L., and [2]Muhammed, L.
[1]Department of Medical Laboratory Science, Bayero University, Kano, Nigeria
[2] Department of Medical Laboratory Science, Bayero University, Kano, Nigeria
Correspondence: Email: hadeezahlawal@gmail.com; Tel: 234-803-705-2469

ABSTRACT
Background: Homocysteine is a sulfur containing amino acid that is normally present in all cells of the body. The homocysteine level is linked to folate and Vitamin B-12 levels.
Aim: The aim of this study was to investigate the effect of hormonal contraceptives (HCPs) on homocysteine levels among healthy women using the HCPs.
Method: A total of 90 participants were recruited in this study; 60 women on hormonal contraceptives served as the case group and 30 non-HCP users were considered as control. Blood samples were collected, and serum was analyzed for homocysteine levels in both groups. The mean ± standard deviation of the homocysteine levels were then determined and compared using student t-test.
Results: The mean ± standard deviation of Serum Homocysteine in the case group, (14.14 ± 7.56µmol/L, was found to be significantly higher than the mean ± standard deviation of the control group which was 9.59 ± 5.87µmol/L (p-value of 0.005). The study also found statistically significant positive correlation ($r<1$) between duration of hormonal contraceptives use and serum homocysteine levels of HCPs users with r-value of 0.058.
Conclusion:This study observed significant relationship between HCPs and increased serum homocysteine. Significantly increased homocysteine levels were observed in HCPs users compared to non-users. There was also positive correlation between the duration of HCP usage and increased homocysteine levels.
Keywords: Contraception, Folate, Homocystinuria, Oral contraceptives

INTRODUCTION

Contraception (birth control or family planning) refers to the prevention of pregnancy by interfering with the normal process of ovulation, fertilization, and implantation (Nwachukwu and Obasi, 2008). Birth control has been used since ancient times, but effective and safe methods of birth control only became available in the 20th century (Hanson, *et al.,* 2010). Different modes of contraception exist including Hormonal methods (Implant, injection or shot); Reversible Methods (Copper T intrauterine device (IUD), Levonorgestrel intrauterine system (LNG IUD)); Combined oral contraceptives, (Progestin only pill, Patch, Hormonal vaginal contraceptive ring); Barrier Methods (Diaphragm or cervical cap, Sponge, Male condom, Female condom); Spermicides; and Fertility Awareness-Based Methods. Permanent Methods of Birth Control include Female Sterilization, Tubal ligation and Male Sterilization/Vasectomy(Truswell*et al.,* 2012).

Homocysteine is a sulfur containing amino acid that is normally present in all cells of the body. It is vital for normal cellular functions; however, excess homocysteine may posea credible threat to the cardiovascular system. High levels of homocysteine is related to increased risk of venous thrombosis, cardiovascular diseases, (CVD), thrombotic, neurodegenerative, pregnancy-associated diseases and disorders of the central nervous system(Soudabeh, *et al.,* 2012).

Homocysteine accepts a methyl group from 5-methyltetrahydrofolate to form methionine in a vitamin B12-dependent reaction (JannatulFerdous*et al*., 2016).

Methylenetetrahydrofolate reductase catalyzes the formation of 5-methyltetrahydrofolate, the source of the methyl group needed for homocysteine to be converted to methionine. Premature arteriosclerosis and thromboembolism are life-threatening complications of increased homocysteine levels. This is explained by homocysteine-induced endothelial cell injury, which may initiate the development of typical arteriosclerotic lesions (Wouters, *et al.,* 1993). There has always been debates about the effect of hormonal contraceptives on cardiovascular biomarkers, including homocysteine.

The increase in the acceptance of hormonal contraceptives in north western part of Nigeria precludes the importance of further research. Potentially, a detailed knowledge of serum concentrations of this biomarker in hormonal contraceptive users may allow the development of effective early primary preventive strategies for cardiovascular disease.There is no or few documented data on the risk of hyperhomocysteineamia caused by hormonal contraceptives in Northwestern part of Nigeria. This study aimed to fill that void.

MATERIALS AND METHODS

Study Population

A total of 60 females on hormonal contraceptives attending the family planning clinics of Murtala Muhammad Specialist Hospital and Nuhu Bamalli Hospital, Kano were recruited as the study group and 30 women who were not on any form of contraceptive were recruited as the control group.

Inclusion Criteria

Only apparently healthy women on active hormonal contraceptive users were recruited as part of the case group.

Exclusion Criteria

- Women who had withdrawn from hormonal contraceptives in the last three months were not recruited
- Women with diagnosed anemias especially nutritional anemias were not recruited
- Women with history of Cardiovasculardisease were not recruited
- Women with history of kidney disease were not recruited.

Ethical Consideration

Permission to conduct the research was sought and received from the Research Ethics Committee, Ministry of Health Kano. The participants were randomly selected, informed and counseled about the study. Only those who gave written consent were included. A questionnaire was given to the participants to obtain information on their personal data.

Sample Collection and Laboratory Analysis

About 5ml of venous blood was collected aseptically from the antecubital fossa using a sterile vacutainer tube and needle. The collected sample was then allowed to clot and spun at 3500rpm for 5minutes to extract serum which was then stored at -20^{o}c until ready for assay. Then, the samples were thawed and assayed

This samples were assayed using a double-sandwich Enzyme linked immunosorbent assay(ELISA) technique (Accubind). The pre-coated antibody was human Homocysteine monoclonal antibody and the detecting antibody was a polyclonal antibody with biotin label. Samples and biotin-labeling antibody were added into ELISA plate wells and washed out with PBS. Then Avidin-peroxidase conjugates were added to ELISA wells in order, substrate for colouring after reactant thoroughly washed out by PBS.The solution turned into blue, and finally into yellow under the action of acid. The colour depth and the testing factors in samples were positively correlated.

Statistical Analysis

All data was analyzed using SPSS version 20 software. Results were expressed as mean and standard deviation (SD), and a student t-test was performed.$p<0.05$ was considered as a statistically significant difference.

RESULTS

Table 1 shows the characteristic of the study population.A total of 90 females were recruited for the study with 60 hormonal contraceptives users as the study group and 30 non-contraceptive users as control. Their mean age and standard deviation were 28.88 ± 5.78 and24.23 ± 7.51 for the case and control groups respectively.

The contraceptive methods used by the cases group had frequencies of 29(48%), 14(23.3%), 11(18.3%) and 6(10%) for implant, injectables, oral and intrauterine contraceptives devices respectively. Most of the study participants had been on hormonal contraceptives for 1-5years, with a frequency of 36(60%); 12(20%) had been on contraceptives for 6-11months; 6(10%) had been using contraceptives for 6-10years; 1 (1.7%) had used them for 11-15years and only 5(8.3%) fell within the 1-5months period.

The mean concentration ± standard deviation of serum homocysteine in case and control groups were 14.14 ± 7.56µmol/L and 9.59 ± 5.87µmol/L respectively. The mean homocysteine concentration of the case group was found to be higher than that of control group. This difference in mean values was found to be statistically significant with a p-value of 0.005, and is represented in Table 2 below.

The findings indicated Mean ± standard deviation values of 12.85 ± 7.46µmol/L for 1-5month, 13.67± 7.12µmol/L for 6-11month, 15.00 ± 10.03µmol/L for 1-5years, 16.20 ± 8.86 µmol/L for 6-10years and 27.21 ± 0.00µmol/L for 11-15years of contraceptive use. The relationship shows a significant difference with p-value of 0.000 and a positive r - value (0.058) shows a positive relation between the duration of HCP usage and homocysteine concentration.

TABLE 1: General Characteristics

	Case	Control
Age (years)	28.88 ± 5.78	24.23 ± 7.51
Age group(years)		
20-29	33(55%)	24(80%)
30-39	24(40%)	4(13.3%)
40-49	3(5%)	2(6.7%)
Total	60(100%)	30(100%)
Contraceptive methods		
Oral	11(18.3%)	
Implant	29(48.3%)	
Injectables	14(23.3%)	
IUCD	6(10%)	
Total	60(100%)	
Duration of contraceptive Usage		
1-5month	5(8.3%)	
6-11month	12(20%)	
1-5years	36(60%)	
6-10years	6(10%)	
11-15years	1(1.7%)	
Total	60(100%)	

TABLE 2: Comparison of mean serum Homocysteine levels between hormonal contraceptive users and non-users

	Case	Control	P-value
Serum homocysteine level (µmol/L)	14.142 ± 7.555	9.586 ± 5.867	0.005*

*Statistically Significant

TABLE 3: Effect of duration of hormonal contraceptives use on serum homocysteine levels

	Serum homocysteine level	r-value	P-value
1-5month	12.8525 ± 7.4630	0.058	0.000
6-11month	13.6684 ± 7.12241		
1-5years	15.000 ±10.0305		
6-10years	16.2020 ± 8.8564		
11-15years	27.2100		
Total	14.142 ± 7.555		

Key: r-value=coefficient of correlation (Paired T-test)

DISCUSSION

In the present study the level of homocysteine were assessed in hormonal contraceptive users and non-users and the effect of contraceptive usage duration on homocysteine levels.

The findings showed that the level of homocysteine is significantly elevated in women hormonal contraceptives users compared to non-users.

The changes in homocysteine concentration observed in our study is in agreement with a study conducted by Norouzi*et al.* (2011) in Tehran, Iran,that reported that there was significant increase in serum homocysteine level in females using hormonal contraceptives without any risk factors.Soudabeh, *et al.* (2012) also reported a significant increase in serum homocysteine level in women on hormonal contraceptives.In addition, elevated levels of homocysteine has been implicated as risk biomarker for coronary heart disease and other cardiovascular disorders (Soudabeh, *et al.,* 2012).

Ridker*et al.* (1999) in a study conducted on healthy postmenopausal American women without coronary disease and cancer, reported that cardiovascular risk was increased more than twice in women with moderately elevated homocysteine levels, irrespective of the conventional risk factors.

A groundbreaking research also showed that long-term (3) cycles treatment with two HCPs of EE/LVG (30µg ethyinylestrsdiol/150µg levonorgestrel) and EE/GSD (30µg ethynylestradiol/75µg gestodene) caused an increase in Homocysteine levels (Gabriele *et al*., 2002).

However, our findings are contrary to the results generated by Du *et al.* (2011) which did not find evidence for an association between hormonal contraceptives use and homocysteine levels. This difference might have been due to differences in techniques applied, and/or the ethnicity of the study participants.

The elevation reported in this study might be due to the effect of hormonal contraceptives in decreasing the bioavailability of group B vitamins for homocysteine metabolism. The homocysteine metabolic pathway strongly depends on folate, vitamin B_{12} and $B_{6,}$ and a deficiency of one or more of these vitamins can cause moderately elevated homocysteine level in adult.

Our study also found a positive correlation between long-term use of contraceptives and increased homocysteine levels. This further supports the theory that hormonal contraceptives can cause an increase in serum homocysteine levels.

CONCLUSION

This study concludes that contraceptive usage is linked to increased homocysteine levels in hormonal-contraceptive users. It also found that homocysteine level is affected by the duration of hormonal contraceptives usage.

Therefore, it is recommended that regular assessment of cardiovascular risk biomarkers should be considered for HCPs users.Group Bvitamins supplementation could also help HCP users to compensate for their decreased bioavailability and improve homocysteine metabolism.

REFERENCES

Du, Y., Rosner, B.M., Knopf, H., Schwarz, S., Dören, M., Scheidt-Nave, C. (2011)Hormonal contraceptive use among adolescent girls in Germany in relation to health behaviorand biological cardiovascular risk factors. *Journal of Adolescent Health*; **48(4)**: 331-337

Gabriele, S., Merki, F., Bruno, I., Paul, J.K. (2002). Effects of oral contraceptives on plasma levels of nitric oxide, homocysteine and lipid metabolism. *Metabolism*; **51**:1216–1221.

Hanson, S.J. Burke, Anne, E. (2010). "Fertility control: contraception, sterilization, and abortion".In: Hurt K et al, editors. *The Johns Hopkins Manual of Gynecology and Obstetrics*. 4th ed. Wolters Kluwer Health, Philadelphia, USA: Lippincott Williams & Wilkins; 2010. p. 382–395.

Jannatul, F., Sabera, K., Subrata, K. B., Latifa, A. and Kashfia, A. K. (2016). Serum concentration of folate, vitamin B12 and homocysteine in patients of cervical cancer. *Bangabandhu Sheikh Mujib Med university Journal;***1016** (9): 188-192.

Norouzi, V., Morteza S., Soudabeh F., Mohsen, K., Ali S. (2011). Effect of oral contraceptive therapy on homocysteine and C-reactive protein levels in women: an observational study. *Anatolian Journal of Cardiology*; **11**: 698-702

Nwachukwu, I. and Obasi, O. (2008). USE OF Modern Birth control Methods among Rural Communities in Imo State Nigeria. *African Journal of Reproductive Health*; 101-108

Ridker, P.M. (1999) Evaluating novel cardiovascular risk factors: can be we better predict heart attacks? *Annual Internal Medicine*; **130**: 933-7.

Soudabeh, F., VajiheNouroozi, M., Seifi, Ali S. and Elaheh M. A. (2012). Influence of Oral Contraceptive Pills on Homocysteine and Nitric Oxide Levels: As Risk Factors for Cardiovascular Disease. Journal of Clinical Laboratory Analysis; 26: 120–123

Truswell, A.S., Mann, T. (2012). The B-Vitamins. In *Essentials of Human Nutrition*, 4th ed. Oxford University Press: New York, USA; 217–235.

Wouters, M.D., Maurice, G. A. J., Godfried, H. J., Boers, M.D.II., Chris, M. G., Thomas, George, F., Borm, Henk, J., Blom, Regine, P. M., Steegers-Theunissen, M.D., Frans, J. M., Trijbels, Tom, K. A. B., Eskes, M.D.(1993). Hyperhomocysteinemia: a risk factor in women with unexplainedrecurrent early pregnancy loss. *Fertility and Sterility*;**60** (5), 820-825